Original Article

''Evaluation of Flora Bacteria Grown in Blood Cultures: Are They Etiologic Agent of Infection or Only Contaminants?"

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Abstract

***Objective*:** In this study, it was aimed to determine whether the flora bacteria growing in blood cultures were infectious agents or only contaminants, for this purpose the hemogram parameters and other demographic characteristics of the patients were evaluated. ***Materials and Methods*:** We evaluated 11.579 blood culture results using the BacT/ALERT® microbial detection system. The skin flora bacteria was detected in the blood cultures during one year and the rates of infectious agents among these bacteria were investigated retrospectively. The blood culture results, which were accepted as true bacteremia and contamination were compared in terms of positive blood culture flask count and inflammation markers (white blood cell count, neutrophil ratio, lymphocyte ratio and C-reactive protein levels). ***Results*:** The total number of blood culture test was 11.579. Out of this number, 8205 (70.87%) was free of microbial growth and there was 3374 (29.13%) with microbial growth. 2609 (77.3%) of the positive cultures represented skin flora bacteria, 2510 (96.2%) of them were coagulase negative staphylococci. Only 50 (1.9%) of the flora bacteria were considered as infectious agents in terms of clinical and laboratory findings in addition to culture. A statistically significant correlation was found between true bacteremia and white blood cell (WBC) count and C-reactive protein (C-RP) levels ($p < 0.05$). ***Conclusion*:** Peripheral blood collection instead of catheter and using special phlebotomy teams should be taken into consideration in order to reduce contamination rates precautions such as adequate skin preparation, preparation of blood culture bottles and using single needle instead of double needle.

Keywords: Blood culture; contamination; flora bacteria

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Introduction

The blood culture is still considered as "gold standard'' for the diagnosis of bacteremia. However, the detection of growth in the blood culture does not always indicate the presence of an infection. Positive blood culture results must be determined whether the organism represents a clinically significant infection or a false positive result of no clinical consequence.

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Contaminant bacterial growth has been recognized as a problem for clinical and also laboratory staff in terms of the decision of true bacteremia. Contamination with skin flora bacteria may be encountered during the collection of blood and inoculation into culture bottles.

The most isolated contaminants in blood cultures are microorganisms that are found in natural microbial flora. Contaminant bacteria isolated in blood cultures are coagulase negative Staphylococcus (CNS), *Corynebacterium* spp*., Propionibacterium* spp*., Micrococcus* spp., viridans streptococci and *Bacillus* spp. other than *Bacillus anthracis*. CNS are the most common blood culture contaminants, typically representing a percentage between 70% and 80%. In the evaluation of blood culture contamination, it is recommended to evaluate the clinical characteristics of the patient as well as the implementation of laboratory-based algorithm[1,2].

The successful isolation of microorganisms from the blood in the laboratory depends on the type of bacteremia, the sampling method, the volume of the blood sample taken, the number and timing of the blood cultures, the interpretation of the results, and the patient population served by the laboratory[3]. Number of positive blood culture sets and bottles within a set, time to growth, clinical and laboratory data, source of culture are important clues [4].

CDC's Laboratory Medicine Best Practices are followed in distinction of blood cultures as pathogen/ contaminants [5].

Materials and methods

The blood cultures results obtained from the clinical microbiology laboratory of Kahramanmaraş SutcuImam University Training and Research Hospital between January 2018-June 2019, were evaluated retrospectively. Blood samples were collected from the patients using the standard microbial detection systems available in the hospital (BacT/ALERT®, Biomérieux Inc. Durham, N.C., EUA and BD BACTEC™, Becton, Dickinson and Company, Shannon, Ireland).Conventional methods and BD Phoenix™ automated bacterial identification system (Becton Dickinson, USA) were used for routine identification. Certain microbial species were investigated in natural microbial flora such as coagulase negative Staphylococcus (CNS), *Corynebacterium* spp*, Propionibacterium* spp*,, Micrococcus* spp.*,*viridians Streptococcus growths and *Bacillus* spp other than *Bacillus anthracis.*. The decision about the growth as being a pathogen or contamination was made by considering CDC'S Laboratory Medicine Best Practices[5].

Fifty blood culture results which were considered to be true bacteremia were compared with another 50 blood culture results that were evaluated as contamination with similar demographic characteristics.

Statistical Analysis

In the evaluation of the data, the suitability of the variables to the normal distribution was examined by Kolmogorov-Smirnov test. Group comparisons of variables not showing normal distribution were performed by Mann-Whitney U test. $p < 0.05$ was considered statistically significant. Statistical

Table1.The relationship between demographic characters and true positive

			True Bacteremia	Contamination	*p*
Age		Median (Q1-Q3)	**67.00(28.00-82.00)**	**64.50(18.00-76.00)**	**0.367**
Gender	Male	*n(%)*	26.00(52.00)	25(50.00)	0.841
	Female	*n (%)*	24.00(48.00)	25(50.00)	
Clinic	Anesthesiology and reanimation	*n(%)*	10.00(20.00)	10.00(20.00)	1.00
	Neurosurgery	*n(%)*	4.00(8.00)	4.00(8.00)	
	Chest diseases	*n(%)*	3.00(6.00)	3.00(6.00)	
	Emergency	*n(%)*	2.00(4.00)	2.00(4.00)	
	Surgery	*n(%)*	2.00(4.00)	2.00(4.00)	
	Hematology	*n(%)*	3.00(6.00)	3.00(6.00)	
	Infectious diseases	*n(%)*	2.00(4.00)	2.00(4.00)	
	Internal medicine	*n(%)*	11.00(22.00)	11.00(22.00)	
	Neurology	*n(%)*	4.00(8.00)	4.00(8.00)	
	Newborn	*n(%)*	7.00(14.00)	7.00(14.00)	
	Pediatrics	*n(%)*	2.00(4.00)	2.00(4.00)	

Mann-Whitney U test; Chi-Square test; Exact test;α:0,05;Median(Q1-Q3):Median(quartile %25-quartile %75)

C-RP and WBC values were found to be statistically significant in terms of pathogen / contaminant discrimination ($p < 0.05$) (Table 2).

Table 2. Comparison of groups for hemogram parameter

	True Bacteremia	**Contamination**		
	Median(Q1-Q3)	*Median(Q1-Q3)*	MW-U	*p*
C-RP	98.80(54.20-189.00)	73.65(18.50-150.00)	**951.500**	**0.040***
WBC	13.63(9.15-18.68)	10.39(7.34-14.78)	**954.500**	**0.042***
Neutrophil %	79.20(65.50-86.20)	74.45(63.50-85.60)	1155.500	0.515
Neutrophil	10.24(5.48-13.40)	7.89(5.20-12.48)	1054.000	0.177
Lymphocyte %	11.85(5.80-19.70)	12.75(7.70-23.10)	1108.500	0.329
Lymphocyte	1.29(0.79-2.86)	1.31(0.73-1.70)	1194.000	0.699

Mann-Whitney U test; α: 0.05; Median (Q1-Q3): Median (quartile %25-quartile %75); *The difference between the groups was statistically significant.

parameters were expressed as Median (1st quarter-3rd quarter).Distribution relationship of categorical variables was examined by Chi-square test and Exact test. Results were expressed in ratio (*%*) and frequency (*n*). Data were evaluated in IBM SPSS version 22 (IBM SPSS for Windows version 22, IBM Corporation, Armonk, New York, United States).

Ethical clearance: Ethics Committee approval for this study was obtained by Kahramanmaras Sutcu Imam University, Turkey (CAAE no.23/03/2019/05-11).

Results

Demographic characters of the patients were not found significant in terms of true bacteremia or contamination (Table 1). The total number of tests accepted for blood culture was 11.579; 8205 (70.87%) of which were free of microbial growth whereas 3374 (29.13%) yielded microbial growth. Out of the ones with microbial growth (*N* = 3374), 2609 (77.3%) of them were found to be the positive cultures representing skin flora bacteria. 2510 (96.2%) of these positive cultures were coagulase negative staphylococci, and only 50(1.9%) of the flora bacteria were considered as true bacteremia in terms of clinical and laboratory findings in addition to culturel examination.

Table3. The presence of flora microorganisms in the blood culture

Microorganism	**True Bacteremia *n* (%)**	**Contaminant *n* (%)**	**Total Skin Flora Bacteria Growth *n* (%)**
Coagulase negative staphylococci	45 (%1.7)	2465 (%98.2)	2510 (%100)
Corynebacterium spp.	1 (%5.5)	17 (%94.4)	18 (%100)
Aerococcusviridans	4 (%13.7)	25 (%86.2)	29 (%100)
Micrococcus spp.	0 (%0)	8 (%100)	8 (%100)
Viridans Streptococcus	0 (%0)	44 (%100)	44 (100%)
Total number of skin flora bacteria	50(%1.9)	2559 (%98.0)	2609(%100)

Of the microorganisms with true bacteremia, 45(1.7%) were identified as coagulase negative staphylococci and 4(8%) as *Aerococcus viridans* and the rest 1 (2%) as *Corynebacterium* spp. (Table 3).

Discussion

The blood cultures represent an important diagnostic tool though they detect bacteremia in only about 50% of patients who are clinically suspected of having sepsis [6].

Identifying the real factors and reporting all positive findings to the clinician as quickly as possible reduces morbidity and mortality directly by reducing the transition time from empirical to causative treatment. Volume of blood obtained is the most important factor affecting the detection of the causative microorganism. As blood volume increases, the likelihood of isolating the causative agent increases, the frequency of contamination decreases, and the time for culture becomes positive[7,8]. Errors that lead to contamination often occur in the preanalytical process. Inadequate skin preparationis viewedto be the most common cause of blood culture contamination.

The most important factors in prevention of contamination are adequate skin preparation and no blood culture from existing central venous catheters[9]. Skin antisepsis cannot completely prevent contamination; however, up to 20% of the bacteria in the skin can survive. In addition, when povidone-iodine - an antiseptic ionophores- is used instead of iodine tincture, it can affect the contamination rate [10, 11, 12]. In addition, there are several factors

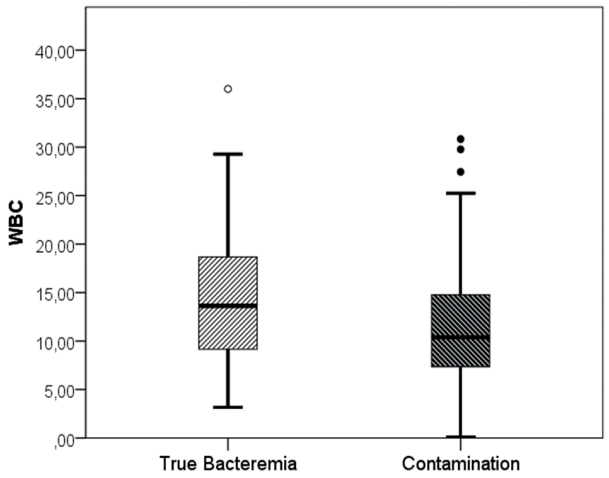

Figure 1: The impact of white blood cell count in true bacteremia / contaminant discrimination

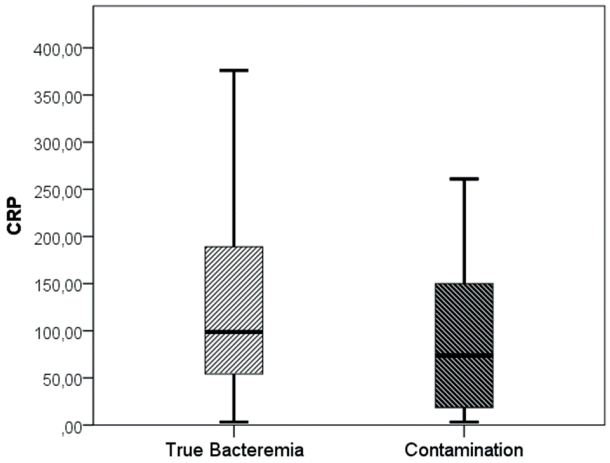

Figure 2: The impact of C-RP count in true bacteremia / contaminant discrimination

which can influence the isolation of the causative microorganism. These are the technique in which the blood culture was taken, the volume of blood obtained, the number of blood cultures, the previous antibiotic usage and the period of time for the growth of blood culture. Positive signaling in only one of two simultaneous blood culture sets suggests contamination [9, 13]. Theoretically, it will provide an earlier positive signal in patients with bacteremia due to the higher bacterial load than contaminated cultures[5].

In cases where multiple samples cannot be collected, it is emphasized that the growth time in the blood culture bottle has a critical role in predicting whether the isolated microorganism is causative or contaminant. It is widely accepted that bacteria can be considered to be pathogen as they grow in the first 24 hours. On the other hand, the bacteria are evaluated as contaminant once they are isolated after three to five days of time[14,15]. In addition, the rate of skin flora members as causative agents is found very low (1.9%).

The clinical and hemogram parameters of the patient are as important as the time for growth and the number of positive blood culture bottles. Signs of sepsis syndrome, such as fever, hypothermia (<36 ° C) or fever (> 40 ° C),> 20,000 leukocytes / μL or <4000 leukocytes / μL, and hypotension are predictors of microorganism as causative agent[16]. A significant relationship was found between the count of WBC and the causative agent (Figure 1). C-RP values were found to be valuable in 24 hours after blood sample was sent [17-19]. Elevation of C-RP value in newborns in the first 12 hours after the onset of clinical findings was significant in the diagnosis of clinical sepsis[18]. We found C-RP values to be valuable in distinction of pathogen or contaminant ($p < 0.040$) (Figure 2). Coagulase-negative staphylococci represent the most frequent contaminant[20, 21]. Of the microorganisms grown in positive blood cultures, 2609 (77.3%) were composed of skin flora bacteria and coagulase negative Staphylococci (96.2%) were mostly identified.

Conclusion

Accurate interpretation of culture results is high of importance not only for patient treatment, but also for public health and hospital epidemiology. Hemogram parameters, clinical status and time to positivity play major role in guiding parameters to distinguish true bacteremia from contamination.

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