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Original Article

Comparison of Blood Culture Parameters between 2 and 10 mL Aerobic Bottles from Patients with Sepsis

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패혈증 환자에서 2 mL과 10 mL 투입 호기병의 혈액배양 지표 비교

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ABSTRACT

Background: Blood volume is the most important parameter for an optimal blood culture; however, the effect of blood volume on blood culture is not clearly understood from patients with sepsis.

Methods: Blood cultures were obtained from 1,049 patients (≥ 15 years old) who visited the emergency department (ED). Two sets of 20 mL each was collected from each patient, 12 mL of which was transferred to 2 and 10 mL FA Plus (aerobic) bottles (bioMérieux, USA) and the remaining into an FN Plus (anaerobic) bottle. Medical records were reviewed to confirm the diagnosis and clinical significance of the blood culture isolates. The positive rate and time-to-detection (TTD) were compared between the 2 and 10 mL groups.

Results: Among the 2,098 sets collected, 612 sets (29.2%) were excluded due to inadequate (either too much or too little) blood volume. The positive rate of clinically significant pathogens was lower in the 2 mL group (6.1%) than in the 10 mL group (7.5%) (P = 0.003) among the 1,486 sets. However, there was no significant difference in the positive rate (11.0% vs. 12.5%, P = 0.152) and TTD (15.7 hours vs. 14.2 hours, P = 0.299) among the 585 (39.4%) patients with sepsis.

Conclusion: The positive rate and TTD were similar between the 2 and 10 mL groups from patients with sepsis who visited the ED, suggesting a high concentration of bacteremia in this group. Therefore, a smaller blood volume should be carefully considered in patients with sepsis in the ED.

Keywords: Bacteremia, Blood culture, Blood volume, Detection, Sepsis

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INTRODUCTION

Blood amount is the most important parameter for optimal blood culture. About 20-30 mL should be collected for each set and be divided into aerobic and anaerobic bottles evenly [1,2]. The main reason to collect such a large volume of blood is based on the most important theory that bacteremia level is very low,

sometimes less than < 1 CFU/mL [2,3]. However, we found out there are several pitfalls in this theory. First, the data supporting this dogma are too old, more than 30 years. There were no currently available automated continuous monitoring of blood culture systems (CMBCS) at that time. It is possible to collect 20-30 mL when they used the manual method, whereas 20 mL would be the maximum volume while using the current CMBCS. There are only a few studies regarding the effect of blood volumes in the era of CMBCS [4,5]. Second, there is a question whether the study design or statistical method was adequate for the previous studies [4,6-8]. We found the flaws that yield was exaggerated for the studies comparing 2 vs. 5 mL [6], or 5 vs. 10 mL [8]. When both-positive data is added, the difference between two groups becomes rather similar in both studies. Third, most of the previous studies did not focus on the sepsis patients. They just analyzed the positive cases of blood cultures in the laboratory, not by clinical diagnosis. If we include only the clinically diagnosed sepsis group, the result might be different.

The most serious limitation of blood cultures is the very low detection rate of pathogens even though we collect a large amount of blood volume. Second, the treatment modality is the same in many cases, regardless of the pathogens isolated [9,10]. Third, emotional stress is very high both for the phlebotomists and patients due to such a large blood volume sampling. Practically, it is very difficult to collect a large amount of blood in the emergency situation.

With these problems of the previous studies and poor clinical utility of blood cultures, we evaluated whether a smaller blood volume (2 mL) may replace the currently recommended blood volume (10 mL) for the sepsis patients group. The rationale to adopt 2 mL was based on the assumption that bacteremia level might be higher in the sepsis group than the other groups.

MATERIALS AND METHODS

1. Patients

The patients (≥ 15 years old) who visited the emergency department (ED) of Gyeongsang National University Hospital during January to June, 2015 and were requested for blood cultures were enrolled for this study. This study was approved by the Institutional Review Board (IRB No. GNUH 2014-12-015), and written consent was exempted.

2. Blood cultures

After disinfection of skin with 0.5% chlorhexidine-alcohol, medical technicians drew 20 mL using a syringe for each set and put 2 and 10 mL into FA Plus (aerobic) bottles (bioMérieux, Durham, NC, USA) separately and the remaining into FN Plus (anaerobic) bottle. Two sets were collected for each episode in one patient. Blood culture bottles were incubated for 5 days at BacT/Alert 3D (bioMérieux) or until there was a positive signal. Bacterial identification was carried out using Vitek-2 system (bioMérieux, Marcy l'Etoile, France). Medical technicians were educated before or during this study for the proper skin disinfection and distribution of blood into each bottles.

3. Underlying infections and clinical significance of the isolates

Sepsis patients were screened and their underlying infections were registered by the database of electronic medical record (EMR) system retrospectively for the enrolled patients. Sepsis was defined bythe previous guideline [11]. All-cause-mortality in hospital was compared between blood culture-positive and -negative groups.

Streptococcus pneumoniae, β-hemolytic streptococci, Enterobacteriaceae, *Neisseria meningitidis*, *Haemophilus influenzae*, *Pseudomonas aeruginsa*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus* species, and *Salmonella* species were primarily regarded as true pathogens. *Staphylococcus epidermidis*, other coagulase-negative staphylococci, α-hemolytic streptococci, non-hemolytic streptococci, diphtheroids, *Bacillus* species, and *Micrococcus* species were generally regarded as skin contaminants [1]. Final decision was made by an ED physician based on clinical manifestations and diagnosis in the EMR whether the isolates were true pathogens or skin contaminants. When the patient's condition was improved by using the antibiotics effective for the isolates, or if the same pathogens were isolated in the other body site cultures, or if diagnosis was compatible with the isolates, then these isolates were considered as clinically significant pathogens.

4. Data analysis

All aerobic bottles were measured for weight. Then, we subtracted the weight of original bottle from the weight of the bottle after blood culture and divided it by the density of blood (1.055 g/mL) to calculate blood volume. Less than 1 mL or > 3 mL in the 2 mL group; < 8 mL or > 12 mL in the 10 mL group were regarded as inappropriate volume and excluded for the data analysis, because these cases would have significantly affected to the results [2].

Four different parameters were compared between 2 and 10 mL groups: first, the proportion of sepsis among the blood culture-requested patients; second, concomitant infections of these sepsis patients; third, the isolation rate of clinically significant pathogens or contaminants; fourth, time to detection (TTD). TTD is defined as the time interval between the entry of bottles into BacT/Alert 3D to the positive signal.

5. Statistical analysis

All data are presented as mean and standard deviation (SD). The independent samples t-test was used to test the difference of means by the independent two groups. Chi-square test was applied for calculating the difference of proportions by groups. McNemar test was carried out to observe the difference of positive rate and contamination rate between 2 and 10 mL groups. The paired t-test was used to analyze the difference of TTD. Statistical significance was defined as $P < 0.050$. IBM SPSS Statistics version 24.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis.

RESULTS

1. Blood volume

A total of 1,049 patients were enrolled for this study, which included 2,098 sets of blood cultures. After 612 sets were excluded due to the criteria of over- or under volume, 1,486 sets were included for analysis. Mean age was 64.1 ± 17.5 years, and males were 56.1%. The average blood volume revealed 2.3 ± 0.4 mL and 9.8 ± 0.8 mL in each group, respectively.

2. Positive rate

Microorganisms grew in 11.6%. The mortality was significantly higher in the 'growth' group compared with the 'no growth' group (12.8% vs. 7.7%, $P = 0.022$). Microorganisms were less frequently isolated in the 2 mL group compared with the 10 mL group (6.5% vs. 8.1%, $P = 0.005$) (Table 1). There was no significant difference in the contamination rate (0.5% vs. 0.6%, $P = 0.804$). Clinically significant pathogens were less commonly isolated in the 2 mL group compared with the 10 mL group (6.1% vs. 7.5%, $P = 0.003$).

Table 1. Positive rate, isolation of clinically significant pathogens, and time to detection of blood cultures between 2 and 10 mL groups

Variable	No.	2 mL group	10 mL group	*P*-value
Positive rate				
in total	1,486	(6.5)	(8.1)	0.005
in sepsis patients	585	(11.0)	(12.5)	0.152
Isolation of clinically significant pathogens	1,486	(6.1)	(7.5)	0.003
Contamination rate	1,486	(0.5)	(0.6)	0.804
Time to detection (hr)				
in total	1,486	15.0 ± 12.9	13.1 ± 7.0	0.090
in sepsis patients	585	15.7 ± 14.9	14.2 ± 8.0	0.299

Values are presented as number (%) or mean±standard deviation.

3. TTD

There was no significant difference in TTD between the 2 mL group and 10 mL group (15.0 ± 12.9 hours vs. 13,1 ± 7.0 hours, $P = 0.090$) (Table 1). Among the sepsis group, there was also no significant difference of TTD (15.7 ± 14.9 hours vs. 14.2 ± 8.0 hours, $P = 0.299$).

4. Pathogens in the sepsis

A total of 585 sets (39.4%) were taken from sepsis patients. Interestingly, there was no statistically significant difference of positive rate in this sepsis patients between 2 mL group and 10 mL group (11.0% vs. 12.5%, $P = 0.152$) (Table 1). A total of 101 sets grew pathogens among sepsis patients. Primary sites of infections were most common in gastrointestinal tract (37.6%), followed by urinary tract (24.8%), respiratory tract (15.8%), and musculoskeletal system (5.9%). Primary focus was indeterminate in 15.8% (Table 2). *Escherichia coli* (41.8%) was the most commonly isolated pathogen, followed by *Klebsiella pneumoniae* (8.9%), *Enterococcus* species (7.9%), and *Streptococcus pneumoniae* (5.9%) causing sepsis.

Table 2. Underlying infections and pathogens identified in blood cultures for 585 sepsis patients

Site of infections	Pathogens	No. (%) of sets
Gastrointestinal tract		38 (37.6)
	Escherichia coli	13
	Enterococcus spp.	8
	Enterobacter cloacae	5
	Klebsiella pneumoniae	4
	Raoultella planticola	4
	Citrobacter braakii	2
	Candida albicans	2
Urinary tract		25 (24.8)
	Escherichia coli	19
	Enterobacter aerogenes	2
	Klebsiella pneumoniae	2
	Candida albicans	2
Respiratory tract		16 (15.8)
	Streptococcus pneumoniae	6
	Escherichia coli	4
	Klebsiella pneumoniae	2
	Pseudomonas aeruginosa	2
	Streptococcus anginosus	1
	Acinetobacter baumannii	1
Musculoskeletal		6 (5.9)
	Escherichia coli	5
	Morganella morganii	1
Indeterminate		16 (15.8)
	Escherichia coli	6
	Staphylococcus aureus	4
	Streptococcus agalactiae	2
	Candida albicans	2
	Klebsiella pneumoniae	1
	Streptococcus pyogenes	1
Total		101 (100.0)

DISCUSSION

Although 20-30 mL of blood volume is recommended for each set [1,7], this amount is not easy to obtain, especially when the venous condition is not good. Taking the recommended blood volume is sometimes ignored in the very busy ED environment. The patients sometimes do not understand or cooperate to draw such a large amount of blood volume causing a serious argument. If we have a not-inferior yield with a small amount of blood volume (such as 2 mL), then we may expect many changes for the diagnosis of sepsis in the ED. This will relieve the tension between the patients and phlebotomists as well as enable faster fluid infusion and antibiotic administration, which is essential to save sepsis patient's life [12]. Although there was a significant difference of positive rate between 2 mL group and 10 mL group in total, there was no difference specifically in the sepsis group.

Several studies indicated an increasing skin contamination rate with the increased blood volume, which caused unnecessary laboratory tests or longer admission days [2,7,13]. However, there was no difference in skin contamination rate between the two groups in this study. TTD was also similar regardless of blood volume, suggesting higher bacteremia level in the sepsis group. However, clinically significant pathogens were less commonly isolated in 2 mL group. Therefore, we should be careful to challenge the dogma: the more blood volume, the higher yield.

Gastrointestinal infections were the most common underlying infections among sepsis patients, and this finding is different from the previous studies [11]. *E. coli* comprised of almost half of pathogens in the sepsis patients, and the prevalence is much higher than the previous studies [4,11,14,15]. These findings indicate that there is a large gap for underlying infections and frequency of pathogens according to the study population and severity of sepsis patients.

There are several limitations in this study. First, the sample size (585 sepsis patients) is not enough to draw a strong conclusion. Second, this is a single-center, retrospective study. A multi-center prospective study may demonstrate more comprehensive data containing more sepsis cases. Third, the data of anaerobic bottle is not included due to difficulty of obtaining a large amount of blood. Fourth, we used the old definition of sepsis. As Sepsis-3 guideline was released in 2016 [16], it is necessary to analyze the data according to this new guideline. We observed a significant difference of positive rate as well as isolation of significant pathogens between two groups (2 vs. 10 mL) in total; therefore, the dogma (the more blood volume, the higher positive rate) seems still correct.

In conclusion, the smaller amount of blood volume (2 mL in this study) demonstrated a not-inferior microbiological outcome (positive rate and TTD) from the patients with sepsis. Therefore, smaller blood volume could be carefully considered for strongly suspected sepsis group in emergency situation.

요약

배경: 채혈량은 올바른 혈액배양의 가장 중요한 요소이지만 패혈증 환자에서 채혈량이 혈액배양에 미치는 영향은 충분히 알려지지 않았다.

방법: 응급의학과를 방문하여 혈액배양을 시행한 1,049명을 대상으로 한 번에 20 mL를 채혈하여 호기병(FA Plus, bioMérieux, USA) 2개에 2 mL과 10 mL을 분주하고, 나머지를 혐기병(FN Plus)에 주입하였다. 혈액배양에서 분리된 균주의 임상적 의의를 판단하기 위해 의무기록을 검토하였다. 호기병 2 mL과 10 mL 두 그룹간에 양성률 및 균 검출시간을 비교하였다.

결과: 총 2,098세트가 채취되었는데, 612세트(29.2%)는 채혈량이 너무 적거나 많아서 분석에서 제외하였다. 채혈량이 적합한 1,486세트 중 임상적으로 의미가 있는 세균의 양성률은 2 mL군(6.1%)이 10 mL군(7.5%)보다 유의하게 낮았다($P = 0.003$). 하지만 패혈증 환자군 585명(39.4%)만 따로 떼어 분석하면, 양성률(11.0% 대 12.5%, $P = 0.152$)와 균 검출시간(15.7시간 대 14.2시간, $P = 0.299$)으로 유의한 차이가 없었다.

결론: 응급의학과를 방문한 패혈증 환자에서 2 mL군은 10 mL군과 비교하여 양성률 및 균 검출시간이 비슷하게 나타나서, 이들은 균혈증 농도가 높다는 것을 시사한다. 따라서 이들 대상군에서는 소량의 채혈을 조심스럽게 고려해볼 수 있다.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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