

Effects of Blood Volume Monitoring on the Rate of Positive Blood Cultures from the Emergency Room

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Background: Blood cultures are essential in diagnosing and treating sepsis. There are several factors that affect the diagnostic yield of blood cultures such as the number of blood sampling episodes, the incubation period, the type and volume of culture media, and the amount of blood drawn. This study aimed to elucidate whether monitoring the volume of blood drawn with an educational intervention could affect the diagnostic quality of blood cultures.

Methods: We implemented quality monitoring for the blood volume drawn during blood culture testing for adults in an emergency room. We instructed the nurses in the emergency room to draw the optimal amount of blood and to reduce the number of blood culture sets from three to two. We analyzed and compared the amount of blood drawn, the rate of positive blood cultures, the contamination rate, and time to positivity (TTP) between 908 patients pre-intervention and 921 patients post-intervention.

Results: The amount of blood drawn increased from 0.7±0.3 mL per bottle (pre-intervention) to 6.5±1.7 mL

per bottle (post-intervention) ($P<0.0001$). The rate of positive blood culture post-intervention (12.14%) was higher than that pre-intervention (6.65%) ($P<0.0001$). The contamination rate post-intervention (1.82%) was also significantly greater than that pre-intervention (0.60%) ($P<0.0001$). Except for anaerobes, there was no significant difference in the distribution of microorganisms between the pre- and post-intervention periods. The TTP for anaerobe bottles post-intervention was significantly shorter than that of pre-intervention (16.1±16.3 versus 18.6±18.3 h).

Conclusion: This study suggests that continuing education about adequate blood volume and aseptic techniques is needed to increase the rate of positive blood cultures and reduce the contamination rate of blood cultures. (*Ann Clin Microbiol* 2016;19:70-76)

Key Words: Blood culture, Bloodstream infection, Blood volume monitoring, Rate of positive blood culture

INTRODUCTION

Blood culture is a critical tool for the diagnosis and treatment of bacteremia, which is a major cause of morbidity and mortality [1]. Increasing the identification accuracy of true pathogens that cause bacteremia by reducing false negatives can prevent mortality among patients. Potential causes of false-negative blood cultures include inadequate blood volume or insufficient number of collected sets, collection of samples after antibiotic therapy, and infections caused by microorganisms that are not readily recovered using routine blood culture methods.

Since the bacterial density in the blood of most patients with

a bloodstream infection is very low, drawing an optimal volume of blood is the single most important parameter for maximizing the detection rate of true pathogens [2,3]. Several clinical studies have reported that the yield of microorganisms increases in direct proportion to the blood culture volume [4,5]. In a comparison between standard-volume (mean, 8.7 mL) and low-volume (mean, 2.7 mL) blood cultures, the detection rate of standard-volume cultures was substantially higher than that of low-volume cultures (92% vs. 69%) [6]. Therefore, the detection rate of cultured blood could be maximized with an adequate inoculation volume.

We recently discovered that, in the emergency room (ER) at

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our hospital, blood culture specimens were drawn at less than 1 mL per blood bottle. Our findings highlighted the need to improve the blood culture techniques used in the ER. A quality improvement intervention was conducted in April 2016 to establish the procedures for an adequate blood culture volume. Subsequently, the volume of blood specimens submitted from the ER for blood culture was monitored using an automated system. In addition, we evaluated the effects of our quality improvement intervention on improving blood culture yields in the clinical setting.

MATERIALS AND METHODS

We analyzed the data of patients in the ER who were over 18 years of age and had blood cultures ordered by attending physicians between 1 January 2016 and 30 June 2016. Blood culture data from 1 January 2016 to 30 March 2016 were used as the baseline data (pre-intervention data). In our hospital, phlebotomy for blood culture is carried out by nurses, not by dedicated phlebotomy teams. Therefore, initially, the educational intervention was conducted for a head nurse of ER, and then the head nurse took responsibility to educate the intervention to all ER nurses who conduct phlebotomies during last week of March. Following the intervention, all adult blood cultures in the ER were obtained by the trained nurses during a 3-month period from 1 April 2016 and 30 June 2016 (post-intervention) according to the new protocol, which emphasized the importance of an optimal volume for blood culture. For each venipuncture, we recommended a total collection of 20 mL of blood to be divided into aerobic and anaerobic blood culture bottles (BD BACTEC Plus Aerobic/F and BD BACTEC Plus Anaerobic/F; Becton Dickinson, Sparks, MD, USA). In addition, the protocol called for two sets of blood cultures instead of the routine three.

All blood cultures in pre- and post-intervention periods underwent standard incubation and processing using the BACTEC FX system (Becton Dickinson). Each blood culture was kept in the system until microbial growth was detected, for a maximum of 5 days (at which time samples were classified as “negative”). A blood culture was considered positive if a microorganism was isolated from at least one media of each set. All positive cultures were identified using the VITEK2 microbial ID/AST testing system (bioMérieux, Inc., Hazelwood, MO, USA). To monitor blood volume, we used the BACTEC FX blood system, which analyzed samples in batches of 25 Aerobic/F media bot-

tles [7]. The culture processing and identification of microorganism were conducted identically between pre-and post-intervention periods.

Total positive rates, true positive rates, contamination rates, and time to positivity (TTP) were evaluated by comparing the period before intervention with the period after intervention (post-intervention). The total positive rate was determined by dividing the total number of positive blood culture sets by the total number of cultured sets. The identification of contaminant bacteria among the positive blood culture sets was in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (2007) [8]. Blood cultures were considered to be contaminated when microorganisms from common skin flora, such as *Corynebacterium* spp., *Bacillus* spp., *Propionibacterium* spp., *Micrococcus* spp., and coagulase-negative *Staphylococcus* spp. (CoNS), were identified. Because CoNS might represent a true infection, cultures with two or more positive sets were not considered to be contaminated with CoNS. The contamination rate was determined by dividing the number of culture sets that were considered to be contaminated by the total number of cultured sets. The true positive rate was calculated by subtracting the contamination rate from the total positive rate. The TTP, which is the length of time from the beginning of incubation to the detection of bacteria growth, was calculated by the BACTEC FX system. Statistical analysis included comparing proportions by the Chi-square test, Student's t-test, and Mann-Whitney test. A *P*-value less than 0.05 was considered statistically significant.

RESULTS

In the preliminary inspection, we analyzed inoculated blood volumes of blood cultures requested from each department in our hospital from January to March 2016. During the study period, the volume of 5,481 aerobic blood culture bottles was analyzed, and 49% of those bottles were submitted from the ER. The overall mean volume during the period was 2.4 ± 1.5 mL. The ER department had the lowest mean volume, at 0.7 ± 0.3 mL (Table 1). Based on these preliminary results, we focused the intervention on the ER department, which submitted the highest number of blood cultures and collected the lowest blood volume. To improve blood culture quality, we carried out an educational intervention for ER nurses who conduct phlebotomies. Following the intervention, we analyzed the data of all adult blood cultures submitted from the ER in the 3 months both pre- and post-intervention.

Table 1. Analysis of the blood culture volumes submitted from each department prior to intervention (January-March 2016)

Department	Mean±SD (mL)	N
Emergency	0.7±0.3	2,630
Hematology	1.3±0.8	928
Neurological Surgery	2.7±1.5	453
Pulmonology	3.1±1.8	413
Nephrology	3.0±1.8	205
Gastroenterology	2.0±1.4	170
Cardiology	2.9±1.8	141
Hepatobiliary Surgery	2.3±1.5	58
Oncology	2.7±1.5	54
Neurology	2.7±2.1	39
Neurology ICU	3.5±1.9	39
Transplantation Surgery	2.8±1.7	38
Orthopedic Surgery	2.3±1.6	37
Thoracic and Cardiovascular Surgery	2.5±1.7	32
Infectious Disease	2.1±1.3	31
Breast and Thyroid Surgery	1.9±1.2	28
Urology	2.7±1.5	27
All groups	2.4±1.5	5,481

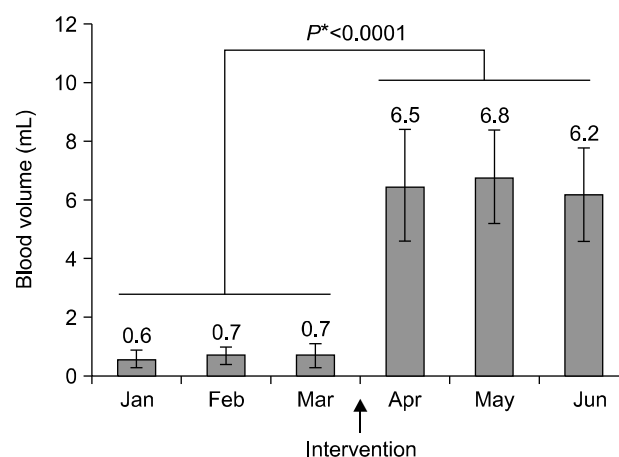
Departments that submitted fewer than 25 blood culture sets from January-March 2016 are not shown in the table.

From January to June 2016, 4,741 blood culture sets were drawn from 1,829 patients in the ER at our hospital. The mean blood culture volume in the aerobic bottles was significantly increased from 0.7±0.3 mL/bottle pre-intervention to 6.5±1.7 mL/bottle post-intervention ($P<0.0001$) (Fig. 1). The mean number of blood culture sets per person was 3.09 pre-intervention and 2.10 post-intervention.

Of the 2,813 blood cultures collected before the intervention, 6.65% were identified as positive. Post-intervention, the total positive culture rate was 12.14%, which was a significant increase ($P<0.0001$) (Table 2). Although the contamination rate also increased from 0.60% to 1.82% ($P<0.0001$), the true positive rate significantly increased from 6.04 to 10.32% ($P<0.0001$).

Because the TTP in blood cultures can be influenced by the volume of blood drawn, the TTP values of aerobic and anaerobic blood bottles were compared pre- and post-intervention (Table 3). The TTP of anaerobic bottles at post-intervention was significantly shorter than that of those at pre-intervention (18.6±18.3 vs. 16.1±16.3 h; $P<0.037$). The difference in mean TTP of aerobic bottles between pre- and post-intervention was not statistically significant (18.8±15.2 vs. 22.7±22.3 h; $P=0.528$).

The distribution of microorganisms detected pre- and post-intervention is shown in Table 4. The five most common micro-

**Fig. 1.** Blood volumes in aerobic bottles submitted for blood culture during pre- and post-intervention periods. Error bars represent the standard deviation. *Comparison between pre- and post-intervention.

organisms during pre-intervention were *Escherichia coli*, coagulase-negative staphylococcus (CoNS), *Klebsiella* spp., *Staphylococcus aureus*, and anaerobes. The most common microorganisms post-intervention were *E. coli*, CoNS, *Klebsiella* spp., *Streptococcus* spp., and other *Enterobacteriaceae*. Except for anaerobes, there was no significant difference in the distribution of microorganisms pre-intervention versus post-intervention. The detection frequency of anaerobes between pre- and post-intervention was significantly different ($P=0.009$).

DISCUSSION

ER patients with a suspected underlying infection should be evaluated for the presence of bloodstream infection using blood cultures. Patients with positive blood culture results should be treated with adequate antibiotics to reduce morbidity and mortality. False negative results caused by an inadequate blood culture technique can have devastating consequences for patients with bacteremia. Therefore, blood cultures should be performed according to the protocols of an effective quality control program to avoid false negatives.

Blood culture volume is the most important factor for reducing false negative results. Several studies have reported that the sensitivity of detecting bacteria in blood cultures can be volume-dependent [4,6]. Although the CLSI recommends blood draw volumes of 20 mL per set for adult patients, incidences of low-volume blood draws still exist in the clinical setting. A survey conducted by Shin et al. showed that the mean blood draw volume per set for adults among nine university hospitals in South Korea was 7.7 mL [9]. In comparison, the mean volume

Table 2. Comparison of the true positive rate and contamination rate in bottles submitted for blood culture pre- and post-intervention

Period	Total No. of blood culture sets	No. (%) of		
		Positive culture sets	Contaminant culture sets	True positive culture sets
Pre-intervention	2,813	187 (6.65)	17 (0.60)	170 (6.04)
Post-intervention	1,928	234 (12.14)	35 (1.82)	199 (10.32)

Table 3. Comparison of the time to positivity (TTP) values between pre- and post-intervention

Parameter	Aerobic bottles			Anaerobic bottles		
	Pre-intervention	Post-intervention	<i>P</i> -value*	Pre-intervention	Post-intervention	<i>P</i> -value*
TTP of total positive (h)	18.8±15.2	22.7±22.3	0.5283	18.6±18.3	16.1±16.3	0.0370
TTP of contaminant (h)	43.5±22.4	32.8±22.5	0.0487	60.8±36.4	49.7±36.7	0.5283

*Calculated by the Mann-Whitney test.

Table 4. Distribution of microorganisms isolated from blood culture-positive patients pre- and post-intervention

Microorganisms	No. of patients with positive results (%)		<i>P</i> -value
	Pre-intervention	Post-intervention	
<i>Escherichia coli</i>	31 (36)	60 (38)	0.656
Coagulase-negative <i>Staphylococcus</i> spp. (CoNS)	10 (12)	28 (18)	0.197
<i>Klebsiella</i> spp.	9 (10)	18 (12)	0.800
<i>Streptococcus</i> spp.	5 (6)	11 (7)	0.711
<i>Staphylococcus aureus</i>	9 (10)	8 (5)	0.121
<i>Enterococcus</i> spp.	0 (0)	4 (3)	0.135
Other <i>Enterobacteriaceae</i>	3 (3)	10 (6)	0.336
Anaerobes	8 (9)	3 (2)	0.009
<i>Candida</i> spp.	1 (1)	1 (1)	0.669
<i>Pseudomonas aeruginosa</i>	3 (3)	1 (1)	0.100
Others	7 (8)	12 (8)	0.902
Total	86 (100)	156 (100)	

per set in our hospital was less than 2 mL in the ER. The total rate of bacteria-positive blood draws was 8.0% among the nine South Korean university hospitals in the Shin et al. study [9], and the CLSI suggests that an adequate positive rate is 6-12%. In our hospital, the data from the 3-month period before intervention revealed a total positive rate of 5.49-6.44%, which was lower than average. Following intervention, the total positive rate of the ER was increased to 12.14%. As blood volume was increased, the total positive rate increased, and the rate was higher than the average of the nine South Korean university hospitals in the Shin et al. study. The data demonstrated that the detection yield of microorganisms that cause bloodstream infection was higher when the blood culture volume was increased, thus confirming the importance of blood volume mon-

itoring as part of our quality control program.

To measure the blood volume in the sample-collection bottles, previous studies have used methods that subtract the unused bottle weight (or media level) from the full bottle weight (or media level) [10,11]. These manual monitoring methods are labor-intensive and time-consuming. Recently, a virtual blood volume monitoring method using the BACTEC FX system, which is based on the metabolism of red blood cells, was introduced, and it demonstrated a linear correlation with manual blood volume measurements [7]. The BACTEC FX system provides reliable estimates of the blood volume in blood culture bottles, with a mean error of 0.2 mL. The method is very convenient for the monitoring of blood volume; thus, we used the virtual monitoring method in this study. However, a potential limitation of the

system is that it can only measure aerobic bottles. Therefore, blood volume in the anaerobic bottles was not tracked. Nevertheless, a previous study reported internal consistency of volumes within sets of aerobic and anaerobic bottles [11]. In our study, we found that positive blood culture yields in both aerobic and anaerobic bottles post-intervention were increased from 4.6 to 9.5% and 5.7 to 8.8%, respectively. Although there can still be a possible difference in blood volumes inoculated between the aerobic and anaerobic bottles, the results suggest that the intervention was successful.

To detect bacteremia, the optimal number of blood culture sets is an important factor. Over the past 40 years, several studies have attempted to determine the optimal number of blood culture sets. Previous studies have demonstrated that the cumulative detection yields of microorganisms were 65-91% from the first blood culture set, 80-99% from the first two blood culture sets, and 96-99% from first three blood culture sets [12-14]. Based on these data, the CLSI guidelines recommend collecting two to three sets per episode and strongly suggest avoiding collection of only a single blood culture set from adult patients. In the planning of our study, medical doctors and nurses from the Emergency Department, Department of Laboratory Medicine, Division of Infectious Diseases and Department of Infection Control discussed the issue of inadequate blood culture volume from the ER. We found that ER nurses who conduct phlebotomies were pressured to obtain three sets of blood culture per patient. To reduce the stress in performing phlebotomies and adapt to the current clinical situation within an acceptable guideline, we decided to reduce the number blood culture sets required from three sets to two. Even though it was reducing the number of blood sets, based on the result of this study, increasing blood volume is allowed to achieving the improvement of true positive rate.

Previous studies have reported that blood cultures with an inadequate blood volume were prone to growth of contaminants [2,15]. In our study, however, as the volume of the cultures in our study was increased, the contaminant rate also increased. Despite this increased contamination rate during the post-intervention period, the rate was within the CLSI-recommended range of less than 3%. This observation could be due to the fewer episodes of blood sampling. CoNS are typically contaminants, but they have increasingly become clinically important as a true pathogen. The clinical significance of a CoNS-positive blood sample is commonly determined by increasing the number of bottles in a series and monitoring for

CoNS growth. Result of this study demonstrated that percentages of the CoNS-positive blood samples in total contaminant sets have increased from 41.2% pre-intervention to 71.4% post-intervention. The Contamination rate except CoNS between pre- and post-intervention was not statistically significant (0.36% vs. 0.52%; $P=0.3945$) (data not shown). Therefore, reducing the number of blood culture sets can cause true CoNS infections to be considered contaminants. To prevent possible contamination in the future, appropriate education and guidelines for skin disinfection will be necessary.

While automated, continuous-monitoring blood culture systems cannot provide quantitative data on sample volume, TTP, which is detected by the system, can reflect the quantity of the inoculum. Weinstein et al. demonstrated that, with 5 mL versus 10 mL of blood culture, an increased blood volume inoculated per bottle resulted in reduced TTPs [16]. In our study, the mean TTP of anaerobic bottles post-intervention was 2.5 h shorter than that at pre-intervention. This data support the relationship between a higher blood volume and shorter TTP. We also found that the TTPs were significantly higher in contaminated bottles, indicating a higher bacterial concentration in the blood from patients with bloodstream infections than in contaminated samples [17]. However, in analysis of TTPs by the bacterial species, there was no significant difference (data not shown).

There was no significant difference in distribution among the microorganism species between pre- and post-intervention, except in the anaerobe group. The anaerobe group was detected more frequently in the pre-intervention period than in the post-intervention period. This finding is unlikely to be due to the intervention itself. Rather, it might be related to differences in the distribution of patients between the pre- and post-intervention periods.

In conclusion, blood volumes were previously obtained in a suboptimal amount, and the results of the present study demonstrates how the intervention increased the true positive rate of blood cultures from the ER. A continuous-monitoring system to ensure adequate blood volumes for blood culture would be important in improving the true positive rate and the identification of microorganisms that cause bloodstream infections which is a major cause of morbidity and mortality.

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=국문초록=

응급실에서 혈액배양 채혈량 모니터링이 혈액배양 양성률에 미치는 효과

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배경: 혈액배양은 균혈증 진단과 치료에 필수적인 검사이다. 혈액배양 양성률에 영향을 미치는 인자로는 혈액 배양량, 채혈횟수, 배양기간, 사용한 배지의 종류, 배지의 양 등이다. 본 연구는 응급실에서 혈액배양 질관리 교육 후 혈액배양 채혈량의 모니터링을 실시하여 그에 따른 효과에 대하여 알아보고자 한다.

방법: 본 연구는 응급실에서 성인 혈액배양 검사를 대상으로 혈액배양 질관리 교육 후 혈액배양 채혈량의 질관리 모니터링을 하였다. 질관리 중재로는 채혈량을 늘려 채혈하도록 교육하였으며, 채혈량 증가에 따라 채혈 횟수는 3쌍에서 2쌍으로 줄여 채혈하도록 하였다. 질관리 중재 전인 908명과 중재 후인 921명의 혈액배양을 시행한 환자를 대상으로 하여 질관리 중재 전후의 혈액채혈량, 혈액배양 양성률, 오염률, time to positivity (TTP)를 비교하여 분석하였다.

결과: 혈액채혈량은 질관리 중재 전 병당 0.7 ± 0.3 mL에서 중재 후 병당 6.5 ± 1.7 mL로 증가하였다($P < 0.0001$). 환자 당 채혈 횟수는 중재 전은 3.09회, 중재 후는 2.10회였다. 중재 전후로 혈액배양 양성률은 6.65%에서 12.14%로 증가하였으며 통계적으로 유의한 차이를 보였다($P < 0.0001$). 혈액배양 오염률은 혈액채취량의 질관리 중재 이후 0.60%에서 1.82%로 상승하였다($P < 0.0001$). 두 군간의 균종은 혐기성균주를 제외하고는 통계적 유의한 차이는 없었다. TTP는 중재 전 후를 비교 했을 때 혐기성배지에서 18.6 ± 18.3 시간에서 16.1 ± 16.3 시간으로 통계학적으로 중재 후 유의하게 단축되었다.

결론: 본 연구를 통하여 채혈하는 직원을 대상으로 충분한 권장 혈액량의 채혈과 오염방지를 위한 무균술에 대한 지속적인 질향상 교육이 필요하겠다. [Ann Clin Microbiol 2016;19:70-76]

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