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Full Length Research Paper

Correlation between inoculum volume, positivity rates and microorganisms isolated from blood cultures

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The study aimed to assess the volume of blood inoculum in each culture bottle and its effect on the rate and time to positivity, types of microorganisms isolated and contamination rate. During a period of six months, two sets of blood cultures (aerobic and anaerobic: 3006 bottles each) were collected. Of the bottles collected, only 2916 aerobic and 2898 anaerobic culture bottles were analysed by categorising the samples into three different blood volume groups: < 8 ml, 8-10 ml and >10 ml, where ≥8 ml is considered an adequate volume. In the aerobic bottles, there was no significant difference in the rate of positivity among the three groups: 8-10 ml and < 8 ml (*p* = 0.226) or > 10 ml (*p* = 0.282). However, there was a significant decrease in positivity in the anaerobic bottles when comparing the > 10 ml blood group with the < 8 ml group (*p* = 0.032). The contamination rate was lower in the > 10 ml volume category in aerobic blood cultures (*p* = 0.008) and the isolation rate of Gram-negative bacilli was higher in this category compared with the other two categories (*p* = 0.05). Our study shows that the volume of blood cultured was a variable that influences the positivity rate and contamination rate of a blood culture sample.

Key words: Blood culture, blood volume, positivity rates, bacterial isolates.

INTRODUCTION

Proper collection of blood cultures has been reported to be the cornerstone for the diagnosis of bacteraemia and sepsis. In addition, it also depends on the site where the blood is drawn from and on the volume of blood inoculated in the culture bottles (Connell et al., 2007; Gonsalves et al., 2009; Shafazand and Weinacker, 2002). Correct interpretation of the culture results is vital to initiate appropriate antimicrobial therapy in a patient, as contamination from skin flora is a common occurrence leading to misinterpretation of the results in certain clinical situations (Shafazand and Weinacker, 2002). Although the most common contaminants are coagulase-negative staphylococci (CoNS); in the majority of studies worldwide these organisms are considered as primary

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pathogens in immuno-compromised patients as well as in those with indwelling intravascular devices (Huebner and Goldman, 1999; Von Eiff et al., 2002). The uncertain clinical significance of isolation of potential contaminants may result in extended hospital stay, unnecessary antibiotic therapy and additional laboratory investigations. This leads to increased expenditures of 39% in antibiotic charges and 80% in microbiology laboratory charges (Bates and Goldman, 1991). By contrast, isolation of a potential pathogen or even exclusion of bacteraemia has a direct impact on the management of the patient, with inclusion of optimal antibiotic therapy in the former and cessation of unnecessary antibiotic use in the latter (Shafazand and Weinacker, 2002).

Among the many factors that are known to influence the yield of microorganisms from blood cultures, the single most important factor is the blood volume (Shafazand and Weinacker, 2002). Evidence from both adult and paediatric studies show that the rate of isolation from blood culture increases with the volume of blood collected (Jonsson et al., 1993). The ability to exclude bacteraemia on the basis of negative blood culture results depends on the sensitivity and on the negative predictive value of the test. The interpretation of negative blood culture results is based on studies using adequate volumes of blood in research settings. When the volume of blood submitted for culture is inadequate, the negative blood culture result is potentially misleading, as it can falsely exclude significant bacteraemia (Connell et al., 2007). Although the value of blood cultures in the management of septic patients has been well documented in several studies, there are recent reports that have questioned this practice, particularly in patients with pneumonia (Abe et al., 2009). This current study aimed to determine the correlation between inoculum size and its effect on the positivity rates, time to positivity and isolation rates of different microorganisms from blood cultures received in our laboratory at the Farwania Hospital. Furthermore, we looked at the effect of inoculum size in relation to contamination rates.

MATERIALS AND METHODS

Farwania Hospital is a general hospital with 1200 beds serving mainly an urban population. It houses medical and surgical specialties and subspecialties. However, cancer, HIV and burn patients are referred to specialised institutions. All blood cultures received from patients during a 6-month period (March to August, 2009) were analysed. As a routine practice, blood cultures were ordered as a set, which contained one aerobic (BACTEC Plus Aerobic/F) and one anaerobic bottle (BACTEC Plus Anaerobic/F) (Becton Dickinson Diagnostics, Sparks, MD, USA). A minimum of two sets of blood cultures, drawn at least one hour apart, were received in the laboratory. The total blood volume collected was assumed to have been distributed equally in the two bottles at the same time. These bottles require a minimum inoculum size of 5 ml, although ideally, 8-10 ml is recommended according to the manufacturer's instructions. Each inoculated bottle was weighed to two decimal places using a digital balance. The volume of blood in each bottle was calculated by subtracting the weight of the un-inoculated bottle from the weight of inoculated bottle as described previously (Bouza et al., 2007), and the density of blood was assumed to be 1 (Lingwood et al., 2006). All bottles were incubated at 35°C in asemi-automated BACTEC system (BD Diagnostics) and monitored for a positive signal over a five-day period. When the system detected growth in an individual blood culture bottle, the time to positivity was recorded and the broth was examined by microscopy and further cultured on appropriate agar plates. Of the 3006 sets of blood cultures received, 2916 aerobic and 2898 anaerobic blood cultures were analysed. Bottles for which the weight measurement or other data, such as patient's age or culture results were missing were excluded from analysis. True bacteraemia was considered when Gram-positive cocci, Gram-negative bacilli (GNB), and yeast were isolated from one or more blood cultures from the same patient. Coryneforms, *Bacillus*, *Micrococcus* spp. and non-pneumococcal α-haemolytic streptococci (except if multiple bottles were positive) were classified as contaminants. Additionally, CoNS were classified as contaminants if they grew out of only one blood culture with an extended time to positivity (> 24 h). However, they were considered to represent true bacteraemia if the same organism grew on multiple consecutive cultures. For the purpose of analysing the data, inoculated blood volumes in aerobic and anaerobic blood culture bottles were grouped into three categories based on blood volume (< 8 ml, 8-10 ml, and > 10 ml) on a similar pattern as previously described (Lingwood et al., 2006). Inadequate volume was regarded as < 8 ml and adequate volume was regarded as ≥8 ml. Furthermore, >10 ml was given a separate grouping entity to ascertain whether this increase in volume affects positivity and or contamination rates.

Statistical analysis

The data were analysed using the computer software Statistical Package for Social Sciences, Windows version 19.0 (SPSS, Chicago, IL, USA). The descriptive statistics are presented as the number and the percent of positive results. The Z-test was used to test whether any significant differences exist in the proportion of positive samples in the two groups as compared with the standard group, which was considered as the group with highest number of positive results. The odds ratio (OR) and 95% Confidence Interval (CI) were also calculated. A Chi-square 'χ^2' test was applied, as appropriate, to assess the statistical significance of the association between proportions. The two-tailed probability value (p< 0.05) was considered statistically significant.

RESULTS

Aerobic bottles

Volume

Of the 2916 aerobic cultures received in the 6-month period, 297 (10.2%) were positive, with 129 (43.4%) being in the inadequate volume category (<8 ml) and 168 (56.6%) in the adequate volume category (≥8 ml) as shown in Table 1 and Figure 1.

Time to positivity

Of the 297 positive blood cultures from the aerobic cultures, 224 (75.4%) signalled positive result within 24 h. There was no difference in the time to positivity rate

Table 1. Positivity rates among aerobic and anaerobic culture bottles in the three inoculated blood volume categories

Blood volume (ml)	Blood culture bottle							
	Aerobic (2916)				Anaerobic (2898)			
	Positive	Negative	% Positive	Positivity rate	Positive	Negative	% Positive	Positivity rate
< 8	129	1196	9.7	4.4	139	1508	8.4	4.8
8-10	80	611	11.6	2.7	55	511	9.7	1.9
> 10	88	812	9.8	3.0	43	642	6.3	1.5

% Positive = Number of positive cultures / total number of cultures in corresponding blood volume category. Positivity rate = number of positive cultures / total number of cultures in all blood volume categories.

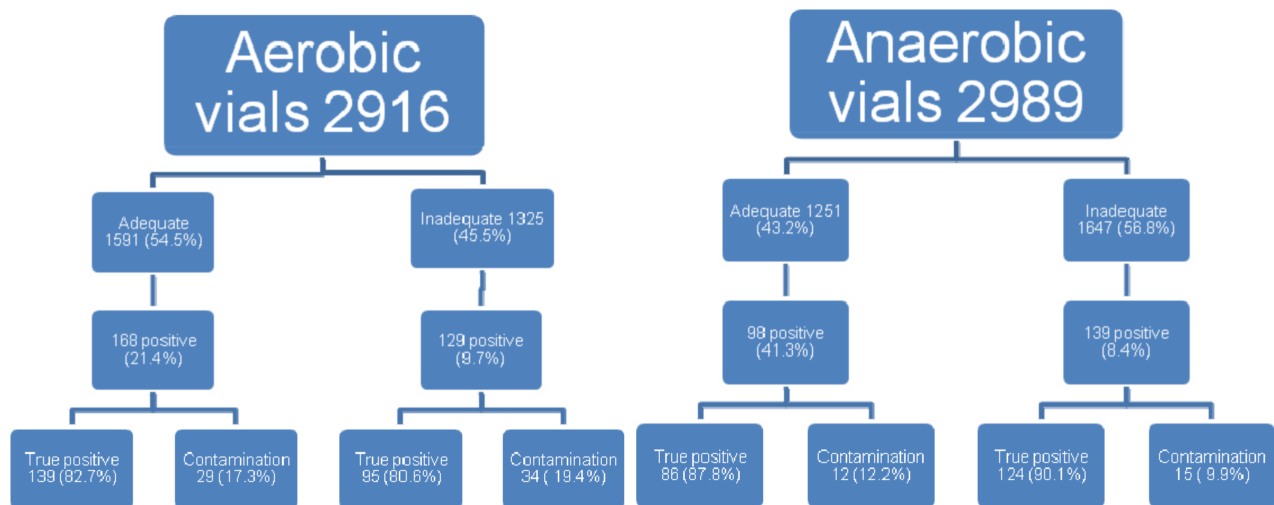

Figure 1. Distribution of blood cultures based on results and volumes.

between the adequate and inadequate blood volume cultures, even in the samples with > 10 ml blood volume.

Organisms isolated

There was no difference in the isolation rate of GNB (mainly members of *Enterobacteriaceae*, *Pseudomonas*, and *Acinetobacter* spp.), *S. aureus* and *Candida* spp. between the adequate 29 (17.3%) and inadequate 34 (19.4%) blood culture volume groups. However, the isolation rate of GNB was significantly higher in the > 10 ml category compared with the other two categories in the aerobic blood cultures (61.5%) (p = 0.021). In addition, the contamination rate (7.3%) was significantly lower in the > 10 ml category (p = 0.008) (Table 2).

Anaerobic bottles

Volume

Of the 2898 anaerobic cultures, 237 (8.2%) were positive and included facultative organisms, with 98 (41.3%) having adequate blood volume (Table 1 and Figure 1).

Time to positivity

Time to positivity was similar in both the adequate and inadequate blood volume cultures. However, 45 vials (81.8%) in the 8-10 ml blood volume category signalled positive in ≤ 24 h. Additionally, when the 8-10 ml category was compared with the < 8 ml and the > 10 ml categories, the resulting ORs were 2.5 (95% CI 1.14 to 5.3; p = 0.022) and 1.4 (95% CI 0.51 to 3.7; p = 0.54), respectively. A statistically significant difference (p < 0.01) was found when the samples that signalled positive within the first 24 h in the 8-10 ml volume category were compared with samples in the < 8 ml category signalling positive during a similar time period.

Organisms isolated

There was no statistically significant difference in the

Table 2. Microorganisms isolated from blood culture in each category.

Blood culture vial	Blood volume (ml)	Positive samples (n)	Microorganism[a] isolated number (%)*							
			Total[b] (n)	GNB	STREP	SAUR	CONS	CONT	CAND	ANAE
Aerobic	< 8	129	175	82 (46.9)	9 (5.1)	20 (11.4)	26 (14.9)	34 (19.4)	4 (2.3)	0
	8-10	80	94	47 (50.0)	6 (6.4)	5 (5.3)	12 (12.8)	22 ((23.4)	2 (2.1)	0
	> 10	88	96	59 (61.5)[c]	8 (8.3)	12 (12.5)	8 (8.3)	7 (7.3)[c]	2 (2.1)	0
Anaerobic	< 8	139	152	80 (52.6)	12 (7.9)	15 (9.9)	24 (15.8)	15 (9.9)	1 (0.7)	5 (3.3)
	8-10	55	59	31 (52.5)	6 (10.2)	7 (11.9)	6 (10.2)	8 (13.6)	0	1 (1.7)
	> 10	43	45	32 (71.1)[c]	2 (4.4)	4 (8.9)	2 (4.4) [c]	4 (8.9)	0	1 (2.2)

[a]GNB, Gram-negative bacilli; STREP-,*Streptococci* (including *S. pneumoniae*); SAUR,*S. aureus*; CONS, Coagulase-negative *Staphylococci*; CONT, contaminants; CAND, *Candida* spp.; ANAE, anaerobes, .[b] Includes mixed growth of microorganisms, [c] $p < 0.05$ (comparing yield from > 10 ml volume to other two categories); *No. of isolates / total number of microorganisms in respective category

isolation rate of other microorganisms from both blood culture categories. However, the isolation rate of GNB was significantly higher in the > 10 ml category (71.1%) ($p = 0.027$). Furthermore, in the >10 ml volume category, the isolation rate of CoNS (usually considered contaminants unless proven otherwise) was lower, with a value of 4.4% ($p = 0.05$). Concerning the total number of samples categorised as true anaerobes; it was 7 (2.9%), with 5 (71.4%) being in the inadequate volume category (Table 2).

DISCUSSION

In this study, the overall incidence of positive results of 9.2% was lower than the internationally reported rate of 21.5-24.5% (Gonsalves et al., 2009; Bouza et al., 2007). A probable explanation could be due to the increased risk of mortality in patients with bacteraemia, in which there is generally a lower threshold for drawing blood culture samples, which increased our denominator (Bates and Goldman, 1991). However, the contamination rate of 1.6% in this study was within the range of 0.6-6.0% shown in previous studies (Lingwood et al., 2006; Bekeris et al., 2005). Several factors are known to influence blood culture yield, including low rates of bacteraemia, low numbers of circulating colony-forming units, intermittent bacteraemia and antimicrobial properties of blood components. Other factors include clinical and laboratory variables, such as volume of blood drawn, concurrent antibiotic use, number of blood sample cultured, timing of blood collection, length of incubation of blood cultures, atmosphere of incubation and culture media and collecting system used (Shafazand and Weinacker, 2002; Darby et al., 1997). It is conceivable that because this study was laboratory-based, interplay of any of the aforementioned clinical barriers was unknown.

BACTEC systems are the most widely used blood culture systems in microbiology laboratories worldwide and have been reported to be superior to other blood culture systems, particularly for the isolation of *S. pneumoniae*, *Pseudomonas aeruginosa*, anaerobic bacteria and other *Streptococcus* spp. (Doern, 1994; Weinstein, 1996). Although the use of a combination of BACTEC and DuPont Isolator Systems can broaden the range of detection of bacterial and fungal infections, it is not economically feasible. For routine clinical conditions, the use of BACTEC systems and the duration of incubation of blood culture vials for five days have been found to be adequate (Pohlman et al., 1995). It is generally accepted that the yield of blood cultures from adults is volume dependent, with an increase of 3-5% in the detection rate with the addition of each millilitre of blood (Mermel and Maki, 1993; Tarrand et al., 1991).

It is established that most blood stream infection (BSI) episodes in adults have a low density of microorganisms in the blood with an average of 1 CFU/ml, necessitating greater volume of blood to be cultured in order to obtain a positive yield (Darby et al., 1997; Weinstein, 1996; Pohlman et al., 1995; Tarrand et al., 1991). However, our study shows that the increased yield in the > 10 ml category was observed for GNB only ($p < 0.05$), whereas there was no significant increase in the detection rate of other microorganisms (Gram-

positive bacteria and yeasts). Similar findings were reported in an earlier study (Weinstein et al., 1996), in which another continuous-monitoring blood culture system, the BacT/Alert system (Organon Teknika Corp, Durham, NC, USA) was used. The possible explanation for the higher yield of GNB could be shorter generation times (20 min) when compared with others.

However, our data indicated that the contamination rate was lower in the >10 ml category compared with the < 8 and 8-10 ml categories (p<0.008), which is in consistent with the finding of an inverse correlation between blood culture contamination and the volume of blood, previously reported by Bekeris et al. 2005 and Gonsalves et al., 2009. Although the authors did not present any obvious explanations, they hypothesised that during venipuncture, the contaminating skin microflora are likely to be diluted and are therefore less likely to be detected during the 5 days of incubation. In our study, the occurrence of positive samples in the aerobic and anaerobic bottles within 24 h of incubation in the BACTEC system was 75.4 and 70.9%, respectively. In an analogous study, the average detection time of growth was observed to be 13.55 h (range, 0.66-89.02 h) regardless of the blood volume inoculated in the culture bottles (Weinstein et al., 1996).

To shorten and improve current laboratory procedures for detecting microorganisms causing blood stream infection, an ideal diagnostic technique is being investigated to overcome limitations of culture. Several nucleic acid-based techniques have been developed for infection diagnosis and pathogen identification (Dark et al., 2009; Paolucci et al., 2010; Ecker et al., 2010).

Our study was limited by laboratory-based diagnosis and hence clinical interpretation of blood culture result is not included. However, it was clear that a blood volume of >10 ml contributes significantly to lower contamination rates and possibly to an increase in positivity rates especially GNB.

Conclusion

Our study demonstrates that blood volume was a variable that influenced the positivity of blood cultures using the BACTEC Aerobic Plus and the Anaerobic Plus vials in the BACTEC 9240 systems. Blood culture bottles, inoculated with > 10 ml of blood are more likely to be positive for GNB than bottles with lesser volume. These vials were also less likely to be contaminated, with a significant p value.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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