Effect of sample volume on yield of positive blood cultures from adult patients with haematological malignancy

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Abstract

Six hundred and sixteen blood samples from patients with haematological malignancy were each distributed equally among three identical cells in a Malthus Microbiological Growth Analyser. The mean (SD) volumes inoculated into sets in which one, two, or three of the three bottles were positive were 37.7 (10.1) ml, 37.4 (12.9) ml, and 37.7 (10.5) ml, respectively. Overall, clinically important organisms were isolated from one bottle only with 18 cultures, from two bottles only with 19 cultures, and from all three bottles in a set with 104 cultures. If the yield from a single bottle inoculated with a mean volume of 12.6 ml blood is taken as 100%, the yield from 25.2 ml in two bottles was 110.7% and the yield from 37.7 ml in three bottles was 115.6%. The increased yield from increased volume was considerably lower than that reported from unselected groups of patients, which suggests that the magnitude of bacteraemia is greater in patients with neutropenia. The isolation of infecting organisms from the blood of patients with neutropenia is, however, particularly important in directing chemotherapy and consequently 45 ml blood samples from these patients continue to be requested.

In adults around 40% of blood samples taken from peripheral veins for culture and assessed by pour plate or lysis methods contain less than one colony forming unit (cfu) per ml.¹⁻⁷ It might therefore be expected that the isolation rate from blood cultures would increase significantly with the volume of the blood sample, and work with a variety of blood culture systems with various media has shown this to be the case.⁸⁻¹⁶ These studies have shown an increase in the isolation rate with increasing volume of blood sample up to 30 ml. With samples greater than 30 ml there is little information available.

Patients with haematological malignancy have a high risk of bacteraemia when neutropenic. Blood cultures are usually taken when the patient has been feverish for two hours or less and immediately before chemotherapy is given. Consequently clinical illness is at a defined early stage. The reduced number of phagocytic cells in neutropenia might affect

the numbers of organisms present in blood samples taken for culture when compared with other groups of patients. Larger numbers of organisms are present in the blood of patients with bacteraemia whose spleens have been removed,¹⁷ and the same might be expected with neutropenic patients. Alternatively, the higher risk of bacteraemia in neutropenic patients, and earlier treatment, may result in low level bacteraemia occurring more often. In one study of bone marrow transplant recipients 62% of blood cultures had organisms present at less than 1 cfu/ml.¹⁸ We investigated the effect of blood sample volume on the isolation rate of clinically important organisms from patients with haematological malignancy and neutropenia.

Methods

BLOOD CULTURE PROCEDURE

Between October 1986 and December 1987 clinicians were requested to take 45 ml of blood at a single venepuncture from adult patients with haematological malignancy. Patients with clinically important isolates were included in the analysis if their neutrophil counts were $<1.0 \times 10^9/l$ when blood cultures were taken. Blood cultures were taken when the patient was feverish (temperature higher than 38°C on two occasions or greater than 38.5°C on one occasion), hypotensive, or failing to respond to antimicrobial agents already administered.

Cultures from central lines and peripheral veins were not distinguished by clinicians. Blood samples were divided equally among three identical Malthus conductivity cells,¹⁹ each containing 85 ml of medium. The medium was supplemented brain heart infusion broth as described previously,¹⁹ except that the saponin was replaced by 0.5% weight/volume dextran (Sigma D5251). The dextran eliminates the early signal from blood which may prevent detection of early signals from bacteria.²⁰ All bottles were weighed before distribution from the laboratory and, after inoculation with a blood sample, on receipt back in the laboratory. As blood has a density of about 1.055, blood volumes could be estimated from the weight of the samples. The blood cultures were monitored continuously on the Malthus apparatus and processed as described previously.^{19,21} The medium, atmosphere of incubation, and screening procedure was identical for all three bottles in each set.

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CRITERIA FOR DETERMINING CLINICAL IMPORTANCE OF ISOLATES

All patients had clinical features of systemic infection and were neutropenic. None was judged to be clinically important on bacteriological criteria alone. Isolates commonly found as contaminants in blood cultures—that is, coagulase negative staphylococci, *Bacillus* spp and diphtheroid organisms—were considered to be clinically important if there was evidence of line or tunnel infection with the same organism, if a cannula tip on removal yielded more than 15 colony forming units,²² or if the same organism was isolated from more than one blood culture bottle in a set or from more than one set over 24 hours.

Results

COMPLIANCE WITH REQUEST FOR 45 ML BLOOD SAMPLES

Six hundred and sixteen blood cultures were examined. Clinically important organisms were isolated from 141 blood cultures and data on volumes of blood inoculated into bottles were available for 108 of these. The mean (SD) total volumes of blood inoculated into sets in which one, two, or three bottles were positive were 37.7 (10.1) ml, 37.4 (12.9) ml, and 37.7 (10.5) ml, respectively. There was reasonable compliance with the request for 45 ml samples and there was no evidence of bias in volumes of blood inoculated into sets in which different numbers of bottles were positive. In sets where only one or two of the three bottles were positive the mean (SD) volumes inoculated into positive and negative bottles were 12.6 (4.6) ml and 12.5 (3.9) ml, respectively, indicat-

ing equal distribution of blood among bottles in a set.

ISOLATES FROM BLOOD CULTURES

The isolates from only one out of three bottles, from two out of three bottles, and from all three bottles in a set are shown in the table. In total, clinically important organisms were isolated from one bottle only in 18 cultures (20 isolates), from two bottles only in 19 cultures (21 isolates), and from all three bottles in 104 cultures (114 isolates). Ten of the 141 positive cultures were polymicrobial and from these 10 cultures two isolates were from one bottle only, two were from two bottles only, and 20 were from all three bottles.

Discussion

In our study the effect of blood sample volume on the yield of positive cultures may be estimated by comparing the yield of positive cultures from one, two, or three identical bottles in a set in which each of the bottles was inoculated with a similar volume of blood.

The yield from one bottle may be calculated as one third of the cultures positive in a single bottle, plus two thirds of the cultures positive in two bottles, plus all cultures positive in three bottles—that is, $6 + 12 + 104 = 122$. Similarly, the yield from two bottles is two thirds of the cultures positive in a single bottle plus all cultures positive in two and three bottles—that is, $12 + 19 + 104 = 135$; and the yield from all three bottles is the number of cultures positive in any bottle—that is, $18 + 19 + 104 = 141$. A mean volume of 12.6 ml blood was added to each bottle in a set. Hence if the yield from culture of 12.6 ml in one bottle was

Isolates from blood cultures

Organisms isolated	Number with isolates from:		
	One bottle	Two bottles	Three bottles
<i>Escherichia coli</i>	6	3	21
<i>Klebsiella</i> sp	1	1	4
<i>Enterobacter</i> sp	1	0	4
<i>Citrobacter</i> sp	0	0	2
<i>Salmonella</i> sp	0	1	0
<i>Acinetobacter</i> sp	0	0	1
<i>Pseudomonas</i> sp	1	1	8
<i>Campylobacter</i> sp	0	0	2
<i>Capnocytophaga ochracea</i>	0	1	0
<i>Staphylococcus aureus</i>	3	0	9
Coagulase negative staphylococci	2	6	22
<i>Streptococcus pneumoniae</i>	0	0	1
<i>Streptococcus mitis</i> , <i>sanguis</i> , <i>salivarius</i>	0	3	6
<i>Streptococcus faecalis</i>	1	2	3
<i>Streptococcus faecium</i>	0	0	2
Group A or C streptococci	0	0	2
<i>Listeria monocytogenes</i>	0	1	0
Unidentified diphtheroid organism	0	0	1
<i>Bacillus cereus</i>	0	0	1
<i>Clostridium</i> sp	2	0	5
<i>Fusobacterium necrophorum</i>	1	0	0
<i>Escherichia coli</i> and <i>Klebsiella</i> sp (and <i>Clostridium tertium</i> in two bottles)	0	0	1
<i>Escherichia coli</i> and <i>Klebsiella</i> sp (and <i>Streptococcus sanguis</i> in one bottle)	0	0	1
<i>Escherichia coli</i> and <i>Streptococcus faecium</i> (and <i>Clostridium tertium</i> in two bottles)	0	0	1
<i>Escherichia coli</i> and <i>Streptococcus sanguis</i>	0	0	1
<i>Klebsiella</i> sp and <i>Streptococcus sanguis</i>	0	0	1
<i>Enterobacter</i> sp <i>Streptococcus sanguis</i>	0	0	1
<i>Pseudomonas</i> sp and <i>Staphylococcus epidermidis</i>	0	0	1
<i>Staphylococcus aureus</i> (and <i>Escherichia coli</i> in one bottle)	0	0	1
<i>Streptococcus faecium</i> and <i>Staphylococcus epidermidis</i>	0	0	1
<i>Clostridium</i> sp and <i>Streptococcus mitis</i> and <i>Bacillus</i> sp	0	0	1
Total Gram negative monomicrobials	9	7	42
Total Gram positive monomicrobials	6	12	47
Total anaerobic monomicrobials	3	0	5
Total polymicrobials	0	0	10
Overall totals	18	19	104

taken as 100%, then the yield from 25.2 ml in two bottles was 110.7% and the yield from 37.7 ml in three bottles was 115.6%. Ilstrup and Washington found that the yields from culture of 20 ml in two bottles and 30 ml in three bottles compared with 10 ml in one bottle were 138% and 162%, respectively.¹⁰ In our study the increased yield with increased volume was substantially lower. The inference is that the magnitude of bacteraemia in patients with neutropenia, managed with indwelling catheters, is greater than in other groups of patients. This could be directly studied with a quantitative blood culture system.

Assessment of the clinical importance of seven isolates of coagulase negative staphylococci and streptococci was based on their isolation from more than one of three bottles in a set. This is a different criterion than would be applied to isolates of such organisms from most groups of patients, but necessary with these patients as early treatment before repeat culture is essential. The criterion of isolation from more than one bottle in a set is affected by the number of bottles in the set and some of the seven isolates might be judged not to be clinically important if a two bottle set had been used. Even if these isolates were eliminated from our assessment, however, the increased isolation rate with increased volume would change only marginally and our conclusions would not be affected.

In unselected patients low numbers of organisms in blood are more common with Gram negative bacteraemias.¹⁻⁷ There is also evidence that the number of bacteria present in samples drawn from intravascular cannulae is greater than that from peripheral vein samples in patients with lines infected with Gram positive organisms.²³⁻²⁶ Sepsis associated with cannulae in our unit is common and is also usually caused by Gram positive organisms. Isolation of Gram negative bacilli from a single bottle would therefore be expected to be much more common than isolation of Gram positive organisms from a single bottle. Our overall results with neutropenic patients do not show this difference, despite the probable inclusion of some cultures taken using intravascular devices. It may be that higher counts of Gram negative bacteria in the blood are more common in patients with haematological malignancy and neutropenia than in unselected patients. The benefit to be gained from increasing the volume of blood cultured is consequently reduced.

In polymicrobial bacteraemia in our study the increase in isolation rate with increasing volume was less evident than with monomicrobial bacteraemia, suggesting that the numbers of organisms in the blood are greater in polymicrobial bacteraemia. The higher mortality in neutropenic patients with polymicrobial bacteraemia is well recognised.²⁷

In this study the rate of isolation of clinically important organisms increased with increased volume of blood sample up to 37.7 ml, but the increase seemed to be less than that reported for

unselected groups of patients. The isolation of infecting organisms from the blood of patients with neutropenia is, however, particularly important in directing chemotherapy and consequently we continue to request 45 ml blood samples from these patients.

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