Clinical Evaluation of Diagnostic Hemoperfusion for In Vivo Enrichment of Bacteria and Fungi in Comparison with a Conventional Blood Culture Technique

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The hemoperfusion module, a newly developed technique for recovering pathogenic microorganisms from patients suffering from septicemia, was compared with conventional blood cultures. The module was interconnected with the extracorporeal circulation of 92 predominantly hemodialyzed patients. Nearly 12 liters of flowing blood (up to 200 ml min⁻¹; mean running time, 60 min) came in contact with the coated charcoal. Of 99 modules examined, 44 (44.7%) yielded positive cultures and contained 54 potentially pathogenic bacteria or fungi (22 species). Only 32 of 190 (16.8%) conventional blood cultures were positive and contained 37 microorganisms (10 species). Even when patients were receiving antibiotic treatment, the frequency of isolation was significantly higher in hemoperfusion (21 of 44 modules positive, 47.7%) than in conventional blood cultures (10 of 88 cultures positive, 11.4%). In contrast, 23 of 55 modules (41.8%) and 22 of 102 conventional blood cultures (21.6%) were positive when patients were not treated with antibiotics prior to blood sampling. Altogether, hemoperfusion modules appeared to be superior to and more sensitive than the conventional blood cultures used and seemed to be a valuable tool for detecting septicemia.

Even nowadays, life-threatening septicemia is without any doubt a dangerous condition which requires rapid diagnosis and specific treatment. Effective therapy is based upon the nature of the expected or culturally proven causative agent and its susceptibility to antibiotics. Unfortunately, pathogens are usually isolated from the bloodstream in low frequencies, i.e., only up to 20% of cultures examined yield microbial growth (5). In part this is due to false clinical indications (drug, tumorous, and central fever) or inadequate sampling when blood culturing is done.

Recently, new and innovative methods have been introduced (19, 20, 23, 29). These include filtration (27), lysis-centrifugation (Isolator 1.5 microbial tube; E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) (1-3), automated methods such as radiometric (BACTEC; Johnston Laboratories, Inc., Towson, Md.) and impedance (6, 25) systems, the Antimicrobial Removal Device (Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.) (15), agar slide blood culture bottles (Septi-Chek; Roche Diagnostics, Div. Hoffmann-La Roche Inc., Nutley, N.J.), chromatography (18), and more sensitive stains such as acridine orange (13, 14), ethidium bromide (12), and 4-6-diamidino-2-phenylindole (23, 26). Although these advanced technologies may represent progress, they are all based upon the demonstration of pathogens in comparatively small amounts of blood. The "in vivo" enrichment of bacteria and fungi (7) by hemoperfusion may, therefore, have the advantage of allowing larger volumes of blood to be tested over a longer period of time.

The purpose of this study was to evaluate the clinical

efficiency of diagnostic hemoperfusion in comparison with a commercially available blood culture system.

Patients and materials. A total of 92 patients (48 females and 44 males; 20 to 86 years old; mean age, 55.2 years) were included in the study, 7 of them two times. Because of underlying severe diseases, nearly all patients had to be routinely hemodialyzed or treated by continuous arteriovenous hemofiltration. Patients showed clinical signs of septicemia when hemoperfusion modules were applied. A total of 44 patients received various antibiotics or antimycotics, whereas in 55 patients antimicrobial therapy was not used at all or was stopped 3 days before hemoperfusion was performed.

Hemoperfusion. A total of 99 hemoperfusion modules originating in nine clinical departments in the Federal Republic of Germany (Berlin, two departments, $n = 31$; Würzburg, $n = 25$; Bonn, $n = 22$; Homburg/Saar, $n = 10$; Bochum, $n = 8$; Hamburg, Aachen, and Cologne, each $n = 1$) were examined.

The hemofiltration capsule (polypropylene) consists of a cylindrical flask (8-ml volume) with screw caps at both ends (Fig. 1). The pore size of the included filters allows particles with up to a 200- μ m diameter to pass. The adsorption medium (Hemocol; Smith & Nephew, Pharmac. Ltd., Hertfordshire, England) is prepared from coconuts. Each module contains less than 4 g of dried medium. The active surface is more than 5,000 m². Single granules have a mean diameter of 500 μ m. The medium is coated with polyhydroxy-ethyl-methyl-acrylate dissolved in 0.9% NaCl. Activation of coated charcoal is achieved by heating at 1,000°C under water vapor, which optimizes adsorptive capacities and hemocompatibility. Additionally, the procedure eliminates microparticles which might cause an embolism. For the

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FIG. 1. Hemoperfusion module containing 4 g of coated charcoal (Hemocol) as the adsorbing material.

same reason, the module was washed with 500 ml of NaCl immediately before use. Clotting of blood was prevented by adding 2,500 IU of heparin to the module. Patients not taking anticoagulants received 5,000 IU of heparin, which was sufficient for 1 h of hemoperfusion.

The hemoperfusion module was connected to the extracorporeal circuit as a bypass to the routinely used hemodialysis system. A flow rate of 200 ml min^{-1} was adequate and could safely be reduced to 10 ml min^{-1} to maintain a sufficient flow. No additional pump was necessary. The operating time of the 99 modules was 60 min on average, i.e., up to 12 liters of blood passed the coated charcoal h^{-1} .

Collection of blood cultures. A total of 95 duplicates of conventional blood culture bottles were inoculated at the beginning of hemoperfusion, and 95 were inoculated immediately after hemoperfusion. Blood was collected directly from the bypass system with a three-way valve after thorough disinfection. The duplicates consisted of a vented bottle (supplemented peptone broth, 45 ml; Becton Dickinson and Co., Paramus, N.J.) and an unvented bottle (thioglycolate broth, 45 ml; Becton Dickinson) to which 10 ml of venous blood had been added.

Microbiological assay. Conventional blood cultures and hemoperfusion modules were transported to the microbiological laboratory as rapidly as possible (mean time, 12.3 h). The hemoperfusion modules were opened under strongly aseptic conditions (laminar airflow cabinet). The coated charcoal was then distributed equally in two VACUTAINER blood culture bottles (supplemented peptone broth) for aerobic cultures and thioglycolate broth for anaerobic cultures, each 45 ml; Becton Dickinson Vacutainer Systems, Rutherford, N.J.), i.e., each bottle contained 2 g of wet charcoal. The blood transferred with the charcoal into the broth amounted to about 0.2 ml, in contrast to the 10 ml of venous blood added to each conventional culture. Vented and unvented blood cultures were incubated at 37°C . Bottles were inspected daily for turbidity, hemolysis, and gas production. Gram stains and subcultures were performed at days 2 and 7, even when there was no macroscopic evidence of growth. Subcultures were performed by spreading 0.2-ml volumes of the incubated media on Columbia blood agar, Endo agar, and Sabouraud agar. Pathogenic bacteria and fungi were identified by established procedures.

Comparison of culture techniques. Pathogenic bacteria and fungi were only recovered from 32 of 190 conventional blood cultures (16.8%). The frequency of positive hemoperfusion

modules was much higher. Of 99 hemoperfusion modules, 44 were positive (44.4%) and so were 2.6-fold more effective than conventional cultures. Neither procedure revealed microbial growth in 51 cases. Both procedures were positive in 19 cases when growth in one of the conventional blood cultures was considered as a positive result. Identical pathogens were found in 15 cases. In 24 cases only the hemoperfusion modules were positive; in 5 cases only conventional blood cultures were positive. There were five possible contaminants in hemoperfusion modules.

While 54 microorganisms (22 species) could be recovered from 44 hemoperfusion modules, only 37 microorganisms (10 species) could be recovered from 32 conventional blood cultures. The gram-positive isolates from blood cultures represented 5 species (26 isolates), but those from hemoperfusion modules represented 11 species (29 isolates) (Table 1).

Gram-negative bacteria were not encountered so frequently (Table 1). Only 4 species (9 isolates) were isolated from blood cultures, and 10 species (20 isolates) were isolated from hemoperfusion modules. Yeasts were rarely isolated from blood cultures (one species, two isolates) but were isolated more often from hemoperfusion modules (three species, seven isolates). One *Propionibacterium* strain was recovered from a hemoperfusion module, but three *Propionibacterium* strains and one *Bacteroides* strain were recovered from conventional blood cultures. *Staphylococcus aureus* (8 isolates) and coagulase-negative staphylococci (13 isolates), some of which were identified as *Staphylococcus epidermidis* and *Staphylococcus capitis*, were the species most frequently encountered.

Influence of antibiotics. Microorganisms were recovered (21 isolates) from 21 hemoperfusion modules, although the respective patients were receiving antibiotics (44 patients, 47.7% positive; Fig. 2). From 25 modules for 55 patients not receiving antibiotic treatment, 23 positive cultures (28 isolates) were obtained (41.8%). The rate of isolation from conventional blood cultures appeared worse. Isolation of bacteria or fungi was only possible (11 isolates) for 10 patients receiving antibiotics (11.4% positive cultures), despite the fact that two blood cultures from the same patient were investigated, and for 22 patients not receiving antibiotics (21.6% positive cultures).

It should also be noted that most isolates obtained under chemotherapy were not susceptible to the corresponding antibiotics. *S. aureus*, *Pseudomonas aeruginosa*, *Acinetobacter lwoffii*, and coagulase-negative staphylococci were

TABLE 1. Distribution of pathogens ($n = 93$) isolated from hemoperfusion modules ($n = 99$) and conventional blood cultures ($n = 190$)

Organism	No. of organisms isolated from:	
	Conventional blood cultures	Hemoperfusion modules
Gram-positive bacteria		
<i>Staphylococcus aureus</i>	10	8
Coagulase-negative staphylococci ^a	9	8
<i>Staphylococcus epidermidis</i>		4
<i>Staphylococcus capitis</i>		1
<i>Micrococcus luteus</i> ^b		1
<i>Eubacterium lentum</i> ^b		1
<i>Streptococcus viridans</i>		1
<i>Streptococcus agalactiae</i>	2	1
<i>Enterococcus faecalis</i> subsp. <i>liquefaciens</i>		1
<i>Streptococcus</i> spp.		1
<i>Corynebacterium</i> spp.	2	1
<i>Propionibacterium</i> spp.	3	1
Gram-negative bacteria		
<i>Escherichia coli</i>	3	3
<i>Klebsiella</i> spp.		1
<i>Proteus mirabilis</i>	1	2
<i>Morganella morganii</i>		1
<i>Pseudomonas aeruginosa</i>	4	5
<i>Pseudomonas fluorescens</i>		1
<i>Acinetobacter anitratus</i>		2
<i>Acinetobacter lwoffii</i>		1
<i>Flavobacterium</i> spp. ^b		2
<i>Bacteroides</i> spp.	1	2
Filamentous fungi and yeasts		
<i>Candida albicans</i>		5
<i>Candida tropicalis</i>	2	
<i>Torulopsis glabrata</i>		1
<i>Penicillium</i> spp. ^b		1

^a Species not determined.

^b Questionable clinical relevance; isolated from only three hemoperfusion modules.

resistant to ampicillin, mezlocillin, and azlocillin, which were used in these cases. The same was true for *Proteus mirabilis* and *Escherichia coli* isolated from patients treated with doxycycline.

Enhancement of recovery of pathogens and acceleration of their detection are the general requirements which modern blood culture techniques should meet. Unfortunately, the widely used traditional blood culture systems often fail to detect pathogenic microorganisms. This fact deprives the physician of both a reliable diagnosis and a carefully directed antibiotic treatment. Various factors may be responsible for false-negative cultures. These include the use of small volumes of blood, the presence of low numbers of microbes at the time of collection, the inability of fastidious pathogens to grow in simple media, preceding antibiotic treatment, and, not least, the collection of blood at the wrong time and under false indications. Therefore, negative blood cultures do not definitely rule out bacteremia or fungemia.

Some new and innovative methods (19, 23) appear to be more sensitive. The total yield of isolates is predominantly a function of specimen volume (4, 24, 28), particularly as far as anaerobes and yeasts are concerned (17). In this respect, hemoperfusion has the advantage of screening higher volumes of blood over a longer period of time. Bacteremic episodes may therefore be detected more easily.

FIG. 2. Comparison of positive hemoperfusion modules ($n = 99$) (bars labeled 1) and positive conventional blood cultures ($n = 190$) (bars labeled 2). A total of 92 patients were tested; 44 were treated with antibiotics, and 55 were not treated or were treated with antibiotics until 3 days before hemoperfusion was performed (see text).

The spectrum of pathogens in septicemic episodes mainly depends on predisposing factors such as age, immunity, consumptive illness, and location of infection. In this study, the patients were not representative of all these circumstances. The number of hemoperfusions performed was too low to necessarily detect rare pathogenic bacteria or fungi. A good number of the species encountered, such as coagulase-negative staphylococci, *Micrococcus luteus*, *Eubacterium lentum*, *Propionibacterium* spp., *Flavobacterium* spp., and, not least, *Penicillium* spp., are often considered to be contaminants (20). However, with regard to the clinical symptoms of patients, it seemed likely that at least some of these isolates represented the causative organisms of infection. Therefore, it may be possible that transient low-level bacteremia or fungemia caused by these organisms occurs with greater frequency than previously thought and is detected more easily by the in vivo enrichment of the microbes used in this study. Nevertheless, omission of these microorganisms of questionable pathogenicity (three hemoperfusion modules) only reduced the percentage of positive hemoperfusion modules from 44.4 to 41.4%.

The application of the hemoperfusion module increased the numbers of both isolates and species. Nearly every second hemoperfusion module detected bacteremia (22 species), in contrast to every sixth conventional blood culture (10 species). Safer detection of filamentous fungi and yeasts may be an additional benefit, as disseminated fungal infections are often difficult to prove (8, 10). In this study 1 hemoperfusion module was sufficient to detect *Candida albicans*, whereas only 3 of 15 conventional blood cultures were positive.

It should be mentioned that there is a species-dependent loss of CFU when bacteria and fungi adsorb to coated charcoal (9). *S. aureus* and *E. coli*, both labeled with L-[³⁵S]methionine, were bound up to 95% (7). In subcultures, the numbers of CFU decreased to 50% of the original count for *S. aureus* and to 1% for *E. coli*. On the other hand,

no preference for any of the pathogens investigated was observed, nor did any bacterial species not adsorb (9).

The risk of airborne contamination, which may occur during the handling of hemoperfusion modules, advocates careful and aseptic processing in a laminar airflow cabinet. The handling is easy and safe, and needs neither specialized personnel nor materials. Such simplicity allows direct application to clinical and microbiological settings.

Rapid transport to the laboratory is not a major problem with the hemoperfusion device. *S. aureus* could be recovered from the module even when the module was stored for 5 days in a refrigerator. Nevertheless, the ability to detect fastidious organisms, such as *Haemophilus influenzae* or *Neisseria meningitidis*, is higher when the hemoperfusion module is processed as soon as possible (9; unpublished data).

False-negative cultures are often due to the transfer of antibiotics into blood culture bottles (16). As outlined by Rodriguez and Lorian (21), the rates of recovery from blood cultures with antibiotics were much lower (9.5%) than those from blood cultures without antibiotics (17.6%). The same observation was made in this study. The percentage of positive conventional blood cultures ranged from 11.4% (treated patients) to 21.6% (untreated patients). The recovery rate obtained by hemoperfusion was 2.6-fold higher than that obtained by the conventional blood culture system and did not show a pronounced difference between treated (47.7%) and untreated (41.8%) patients. Microbes were predominantly adsorbed to the outer surface of the coated charcoal (10), whereas antibiotics were adsorbed to the inner surface of microparticles (unpublished data).

For the decision as to whether an isolate is to be considered an etiological agent or a contaminant, it is important to prove the identity of blood culture isolates with isolates from other lesions of the same patient at the species level or, even better, the subspecies level (11, 22). Such investigations (503 specimens from only 18 patients) clearly demonstrated that 22 hemoperfusion modules had a 1.44-fold-higher recovery rate than did 125 blood cultures collected simultaneously and independently from the hemoperfusion modules. To obtain yields similar to those obtained with the hemoperfusion modules, we had to examine 8.18 conventional blood cultures.

This study demonstrates that the recovery rates of conventional blood culture systems apparently need to be improved. The hemoperfusion technique may be considered one further step toward an improved microbiological diagnosis of septicemia and infections with concomitant bacteremia or fungemia. However, for the present, its application will have to be restricted to special cases and conditions because an extracorporeal bypass circulation to which the module can be attached is certainly not always available or feasible.

LITERATURE CITED

1. Bille, J., R. S. Edson, and G. D. Roberts. 1984. Clinical evaluation of the lysis-centrifugation blood culture system for the detection of fungemia and comparison with a conventional biphasic broth blood culture system. *J. Clin. Microbiol.* 19:126-128.
2. Brannon, P., and T. E. Kiehn. 1985. Large-scale clinical comparison of the lysis-centrifugation and radiometric systems for blood culture. *J. Clin. Microbiol.* 23:951-954.
3. Dorn, G. L., J. R. Haynes, and G. G. Burson. 1976. Blood culture technique based on centrifugation: development phase. *J. Clin. Microbiol.* 3:251-257.
4. Hall, M. M., D. M. Ilstrup, and J. A. Washington II. 1976. Effect of volume of blood cultured on detection of bacteremia. *J. Clin. Microbiol.* 3:643-645.
5. Heym, B., I. Just, U. Brüdigam, and R. Ringelmann. 1986. Die Blutkultur: Leistungsfähigkeit des Untersuchungsmaterials bei Verdacht auf Bakteriämie und Sepsis. *Immun. Infekt.* 1/86:18-21.
6. Holland, R. L., B. H. Cooper, N. G. P. Helgeson, and A. W. McCracken. 1980. Automated detection of microbial growth in blood cultures by using stainless-steel electrodes. *J. Clin. Microbiol.* 12:180-184.
7. Keller, F., K. Feldmann, B. Gaugler, and B. Besenfelder. 1981. Investigation of the viability of radioactively labelled bacteria after adsorption on activated charcoal. *Zentralbl. Bakteriol. Mikrobiol. Hyg. 1 Abt. Orig. A* 250:113-121.
8. Keller, F., R. Schäffer, and H. Hennemann. 1981. Möglichkeiten zur frühzeitigen Erkennung einer Candidasepsis. *Intensivmedizin* 18:86-90.
9. Keller, F., G. Reimann, R. Hoffmann, and S. Korn. 1984. Adsorption von Bakterien an Acrylhydrogel-beschichtete Aktivkohle. *Münch. Med. Wochenschr.* 126:923-926.
10. Keller, F., H. Hennemann, and R. Barz. 1981. Adsorption of *Candida albicans* from blood of infected rats by coated charcoal. *Mykosen* 25:64-72.
11. Kühnen, E., K. Rommelsheim, and L. Andries. 1987. Combined use of phage typing, enterococci typing and species differentiation of group D streptococci as an effective epidemiological tool. *Zentralbl. Bakteriol. Mikrobiol. 1 Abt. Orig. A* 266:586-595.
12. Mansour, J. D., J. L. Schram, and T. H. Schulte. 1984. Fluorescent staining of intracellular and extracellular bacteria in blood. *J. Clin. Microbiol.* 19:453-456.
13. McCarthy, L. R., and J. E. Senne. 1980. Evaluation of acridine orange stain for detection of microorganisms in blood cultures. *J. Clin. Microbiol.* 11:281-285.
14. Messegue, M., L. de Rafael, M. Barquero, M. Martinez Ferrer, and M. Lopez-Brea. 1984. Acridine orange stain in the early detection of bacteria in blood. *Eur. J. Clin. Microbiol.* 3:113-115.
15. Moody, J. A., C. E. Fasching, C. J. Shanholtzer, D. N. Gerding, and L. R. Peterson. 1984. Evaluation of new blood culture processing systems. *J. Clin. Microbiol.* 20:351-356.
16. Pazin, G. J., S. Saul, and M. E. Thompson. 1982. Blood culture positivity. Suppression by outpatient antibiotic therapy in patients with bacterial endocarditis. *Arch. Intern. Med.* 142:263-268.
17. Plorde, J. J., F. C. Tenover, and L. G. Carlson. 1985. Specimen volume versus yield in the BACTEC blood culture system. *J. Clin. Microbiol.* 22:292-295.
18. Reig, M., D. Molina, E. Loza, M. A. Ledesma, and M. A. Messegue. 1981. Gas-liquid chromatography in routine processing of blood cultures for detecting anaerobic bacteremia. *J. Clin. Pathol.* 34:189-193.
19. Reller, L. B. 1983. Recent and innovative methods for detection of bacteremia and fungemia. *Am. J. Med.* 28:26-30.
20. Reller, L. B., P. R. Murray, and J. D. MacLowry. 1982. Cumitech 1A, Blood cultures II. Coordinating ed., J. A. Washington II. American Society for Microbiology, Washington, D.C.
21. Rodriguez, F., and V. Lorian. 1985. Antibacterial activity in blood cultures. *J. Clin. Microbiol.* 21:262-263.
22. Rommelsheim, K., and E. Kühnen. 1985. Rachenflora in der Funktion eines Referenzspektrums für Infektionserreger bei Intensivpatienten. *Anästh. Intensivther. Notfallmed.* 20:273-276.
23. Salari, S. H., and M. E. Ward. 1979. Early detection of *Chlamydia trachomatis* using fluorescent DNA-binding dyes. *J. Clin. Pathol.* 32:1155-1162.
24. Sandven, P., and A. E. Hoiby. 1981. The importance of blood volume on detection of bacteremia. *Acta Pathol. Microbiol. Scand.* 89:149-152.
25. Specter, S., R. Throm, R. Strauss, and H. Friedman. 1977. Rapid detection of bacterial growth in blood samples by a continuous-monitoring electrical impedance apparatus. *J. Clin. Microbiol.*

- 6:489-493.
26. **Steen, H. B., and E. Boye.** 1980. Bacterial growth studied by flow cytometry. *Cytometry* **1**:32-36.
27. **Sullivan, N. M., V. L. Sutter, and S. M. Finegold.** 1975. Practical aerobic membrane filtration blood culture techniques: development of procedure. *J. Clin. Microbiol.* **1**:30-36.
28. **Tenney, J. H., L. B. Reller, S. Mirrett, W.-L. L. Wang, and M. P. Weinstein.** 1982. Controlled evaluation of the volume of blood cultured in detection of bacteremia and fungemia. *J. Clin. Microbiol.* **15**:558-561.
29. **Tilton, R. C.** 1982. The laboratory approach to the detection of bacteremia. *Annu. Rev. Microbiol.* **36**:467-493.