Optimization of the detection of microbes in blood from immunocompromised patients with haematological malignancies

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Abstract

The present study aimed to improve the rate of detection of blood-borne microbes by using PCRs with pan-bacterial and *Candida* specificity. Seventeen per cent of the blood samples (n = 178) collected from 107 febrile patients with haematological malignancies were positive using standard culture (BacT/Alert system). *Candida* PCR was positive in 12 patients, only one of whom scored culture-positive. Bacterial PCR using fresh blood samples was often negative, but the detection rate increased when the blood was pre-incubated for 2 days. These data indicate that PCR assays might be a complement for the detection of blood-borne opportunists in immunocompromised haematology patients.

Keywords: Bacteraemia, blood cultures, *Candida albicans*, *Candida glabrata*, fungaemia, haematological malignancy, immunocompromised, neutropenic fever, opportunistic infection, PCR

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Persistent fever despite treatment with broad-spectrum antibiotics in patients with haematological malignancies represents a major diagnostic challenge because it could signal tumour recurrence, a drug-related reaction, or severe infection. Blood cultures are positive in only one in five febrile haematological patients [1,2], and even less often in patients who have received antibiotics [3]. False-negative cultures may result from the presence of antibiotics or infections as a result of opportunists that grow poorly in standard automated blood culture systems [4,5]. The present study aimed to improve the detection rate of blood-borne microbes in this patient group by using PCRs with pan-bacterial and *Candida* specificity in addition to culture.

Blood cultures (n = 178) were drawn between September 2003 and February 2007 from 107 hospitalized patients with haematological malignancies presenting with fever (≥38°C) and/or chills. Two-thirds (119/178) were neutropenic (blood leukocytes: $<0.5 \times 10^9$/L) and 29% (52/178) were receiving prophylactic ciprofloxacin. Blood was drawn at the onset of fever, and also later if symptoms suggested infection, via a central venous catheter in most cases.

Blood was cultured according to standard procedure (BacT/Alert system, two bottle pairs; for details, see Supporting Information, Appendix S1). Figure 1 shows how the culture bottles were processed. Bottle contents were subcultured on solid media designed for fastidious microbes, and re-inoculated into fresh blood culture bottles to dilute antimicrobial drugs. Further, 3 mL of blood was assayed directly by pan-bacterial and *Candida* PCR. PCR was also performed using the bottle contents obtained after 2 and 6 days of incubation. The PCR methods are fully described in the Supporting Information (Appendix S1).

Automated blood cultures yielded 17% (30/178) positive samples (growth in ≥1 bottle) (Table 1). Gram-positive bacteria dominated, especially coagulase-negative staphylococci (CoNS) (Table 1).

To dilute antimicrobial agents, fresh culture bottles were re-inoculated with the culture bottle contents before introduction into the culture cabinet. Three additional samples turned out to be positive, yielding CoNS, *Bacillus licheniformis* and *Micrococcus* spp. However, 24 of 30 isolates that appeared in the original culture bottles were not detected in the diluted bottles. Only six cultures were positive according to both methods and gave identical results.

Subculture of bottle contents onto solid media yielded 11 additional positive samples, with *Propionibacterium acnes* being the most common finding. Among the 30 samples positive according to standard culture, only seven were also identified by subculture.

Pan-bacterial PCR was performed with 172 of 178 freshly-drawn blood samples. Only four were positive according to PCR, two of which were also detected by culture (Table 1). All positive blood cultures could be confirmed by

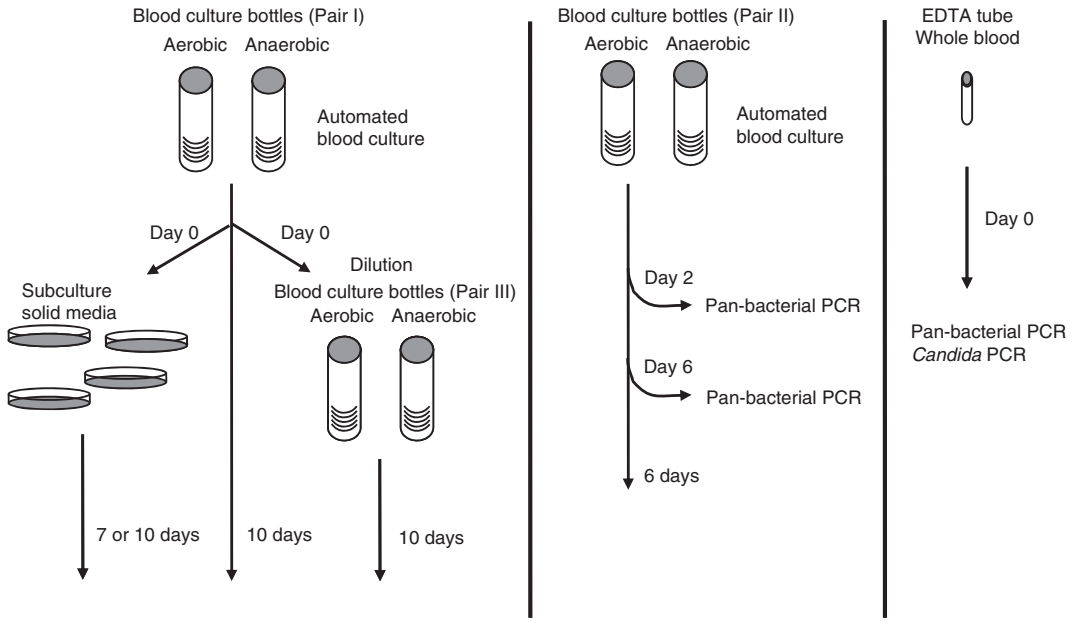

FIG. 1. Scheme used to enhance the detection rate of microbes in the bloodstream of febrile patients with haematological malignancies. Upon arrival at the Bacteriological Laboratory, the membranes of the blood culture bottles designated Pair I were punctured after thorough disinfection, and 2 mL were removed from each bottle, using a Monovette syringe (Sarstedt, Nümbrecht, Germany). Of this portion, 200 μL were subcultured on solid media (for details, see Supporting Information, Appendix S1), whereas 1 mL per bottle was transferred to a new pair of aerobic and anaerobic bottles (Pair III). The four culture bottles were incubated in the blood culture system at 36°C for 10 days. The remaining intact bottles, Pair II, were incubated in the blood culture cabinet for 6 days. Between 0.5 and 1 mL of bottle content was removed using Monovette syringes from each bottle of this latter pair after 2 and 6 days of culture, respectively, and was processed for pan-bacterial PCR (for details, see Supporting Information, Appendix S1). Finally, *Candida* and pan-bacterial PCR were directly performed with DNA extracted from EDTA-anticoagulated blood (for details, see Supporting Information, Appendix S1).

pan-bacterial PCR and the same bacterial species were unanimously identified.

Pan-bacterial PCR run on aliquots drawn from the aerobic and anaerobic blood culture bottles after 2 and 6 days of incubation, were positive in 14 and eight cases, respectively, and several of these were negative according to culture (Table 1).

Direct *Candida* PCR was performed with EDTA-blood (n = 128). PCR products were hybridized with probes specific for *Candida albicans* and *Candida glabrata*. Four samples were positive for *C. albicans* and eight for *C. glabrata* by PCR (Table 1). *Candida albicans* was recovered in only a single sample after culture.

Blood samples from three healthy controls were negative according to all methods. In addition, eight afebrile haematological patients without signs of invasive infection were negative according to our pan-bacterial and *Candida* PCRs.

The present study aimed to enhance the detection rate of microbes in the blood of febrile patients with haematological malignancies. Neither dilution, nor subculture of blood culture bottle contents increased the rate of positive cultures. However, pan-bacterial PCR performed with the contents of pre-incubated blood culture bottles led to the identification of a number of bacterial isolates that were not detected using the automated blood culture system. In our hands, PCR performed with freshly-drawn blood was seldom positive. This is in disagreement with a previous study performed by Mancini *et. al.*, [2] who identified 33% positive samples using real-time PCR for the same category of patients.

The most considerable improvement was obtained by analysis of blood samples according to *Candida*-specific PCR. *Candida* DNA was detected in 8.6% of the blood specimens, *C. glabrata* three times more often than *C. albicans*. The PCR method for detection of *C. glabrata* was 100-fold more sensitive than the method for *C. albicans*, which might explain this superiority. However, culture may underestimate *C. glabrata* as a septic agent because this species grows less well than *C. albicans* [6]. Accordingly, *C. glabrata* was recovered in 40% of blood samples that were negative according to conventional culture, when selective fungal broths were used [7]. In the absence of detailed data concerning the clinical course of the patients,

TABLE 1. Microbial species detected using the automated blood culture system and PCR performed with direct blood or medium drawn from culture bottles after 2 and 6 days

	BacT/Alert[a]	PCR		
Microbial species	Direct blood No. isolates	2-day incubation No. isolates	6-day incubation No. isolates	No. isolates
Gram-positive bacteria				
Actinomyces spp.	–	–	1	1
Bacillus spp.[b]	4	–	2	1
CoNS[c]	11	1	2	–
Enterococcus faecalis	2	–	1	–
CoNS + *Enterococcus faecium*	1	–	–	–
CoNS × 2[d] + *Propionibacterium acnes*	1	–	–	–
Diphteroid rods	1	–	–	–
Enterococcus faecium	1	–	–	–
Gemella hemolysans	1	–	–	–
Group B streptococci	1	–	–	–
Micrococcus spp.	–	–	1	–
Propionibacterium acnes[e]	1	1	–	2
Rhodococcus spp.	–	–	4	1
Rhodococcus spp. + *P. acnes*	–	–	–	1
Sporosarcina spp.	1	–	–	–
Streptococcus oralis/mitis/sanguinis	1	–	–	–
Streptococcus gordonii	1	–	–	–
Gram-negative bacteria				
Aquabacterium spp.	–	1	–	–
Enterobacteriaceae spp.	–	–	–	2
Fusobacterium + *Leptotrichia* spp.[f]	1	–	1	–
Klebsiella pneumoniae[g]	1	1	x	x
Moraxella osloensis	–	–	1	–
Stenotrophomonas maltophilia	–	–	1	–
No. positive	30	4	14	8
Yeasts				
Candida albicans	1	3	Non-relevant	Non-relevant
Candida glabrata	–	7	Non-relevant	Non-relevant
Candida albicans + *Candida glabrata*	–	1	Non-relevant	Non-relevant
No. positive	1	11	Non-relevant	Non-relevant

[a]Traditional automated culture of two pairs of bottles at 36°C for 6 and 10 days, respectively.
[b]One *Bacillus* spp. was found by both traditional blood culture and PCR after 2 days of incubation, and one *Bacillus* spp. was found by PCR after 2 and 6 days of incubation, respectively, but not by the automated blood culture.
[c]In five cases, coagulase-negative staphylococci (CoNS) with identical resistance pattern was found in more than one culture bottle. In four of these cases, all culture bottles were removed prior to day 2 and were not analysed by PCR.
[d]Two different kinds of CoNS were identified according to antimicrobial resistance data.
[e]The bacterium was identified from four different patients.
[f]*Leptotrichia* spp. was identified both by culture and PCR after 2 days of incubation.
[g]All culture bottles were removed prior to day 2 and were not analysed by PCR.

we do not know whether patients who score positive for *Candida* DNA had true infection, or had transient and clinically irrelevant fungaemia. One patient positive according to PCR for both *C. albicans* and *C. glabrata* was diagnosed with hepatosplenic candidiasis; this patient was positive according to culture for *C. albicans* only.

An interpretation of microbial findings is difficult in immunocompromised patients who are susceptible to infections with opportunistic microbes, normally considered as contaminants. We detected several species of low pathogenicity, according to both conventional culture and PCR. Because clinical information was scant, clinically relevant isolates could not be separated from contaminants. Although extensive precautions were taken at the laboratory to avoid contamination, and the laboratory has a long-standing experience with performing pan-bacterial PCR with clinical specimens [8,9], contamination cannot be completely ruled out. *Propionibacterium acnes* and *Aquabacterium* spp. may be contaminants because these species have not been described as blood-borne pathogens. Moreover, the three species detected in the diluted bottles were probably contaminants.

In summary, the most important finding of the present study is that the incidence of candidaemia appears to be grossly underestimated according to current blood culture practices, and of that attributable to *C. glabrata* in particular. The clinical relevance of *Candida* spp. detected by PCR will be investigated in additional clinical studies. These preliminary findings suggest that direct *Candida* PCR could be an

adjunct to conventional blood culture in the assessment of febrile patients with haematological malignancies. By contrast, pan-bacterial PCR with direct blood is currently less sensitive than standard culture.

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Transparency Declaration

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Materials and methods.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

References

1. Xu J, Moore JE, Millar BC *et al.* Improved laboratory diagnosis of bacterial and fungal infections in patients with hematological malignancies using PCR and ribosomal RNA sequence analysis. *Leuk Lymphoma* 2004; 45: 1637–1641.
2. Mancini N, Clerici D, Diotti R *et al.* Molecular diagnosis of sepsis in neutropenic patients with haematological malignancies. *J Med Microbiol* 2008; 57: 601–604.
3. Glerant JC, Hellmuth D, Schmit JL, Ducroix JP, Jounieaux V. Utility of blood cultures in community-acquired pneumonia requiring hospitalization: influence of antibiotic treatment before admission. *Respir Med* 1999; 93: 208–212.
4. Peters RP, van Agtmael MA, Danner SA, Savelkoul PH, Vandenbroucke-Grauls CM. New developments in the diagnosis of bloodstream infections. *Lancet Infect Dis* 2004; 4: 751–760.
5. Klaerner HG, Eschenbach U, Kamereck K, Lehn N, Wagner H, Miethke T. Failure of an automated blood culture system to detect nonfermentative gram-negative bacteria. *J Clin Microbiol* 2000; 38: 1036–1041.
6. Fidel PL Jr, Vazquez JA, Sobel JD. *Candida glabrata*: review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*. *Clin Microbiol Rev* 1999; 12: 80–96.
7. Meyer MH, Letscher-Bru V, Jaulhac B, Waller J, Candolfi E. Comparison of Mycosis IC/F and plus Aerobic/F media for diagnosis of fungemia by the Bactec 9240 system. *J Clin Microbiol* 2004; 42: 773–777.
8. Welinder-Olsson C, Dotevall L, Hogevik H *et al.* Comparison of broad-range bacterial PCR and culture of cerebrospinal fluid for diagnosis of community-acquired bacterial meningitis. *Clin Microbiol Infect* 2007; 13: 879–886.
9. Fritzell P, Bergstrom T, Welinder-Olsson C. Detection of bacterial DNA in painful degenerated spinal discs in patients without signs of clinical infection. *Eur Spine J* 2004; 13: 702–706.

Clinical practice of obtaining blood cultures from patients with a central venous catheter in place: an international survey

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Abstract

Several aspects of the procedure for obtaining blood cultures are rather controversial. An international survey among 386 experts was performed to investigate the clinical practice of obtaining blood cultures from patients with a central venous catheter (CVC). Among respondents, 64.5% obtain one set of blood cultures from the CVC and one set from the peripheral vein (PV). Other participants answered 'two sets from PV', 'two sets from CVC', 'one from PV', 'one from CVC' and 'other' (9.1%, 4.1%, 8.3%, 7%, and 7%, respectively). Clinicians who, according to the survey, demonstrated that they know the diagnostic performance characteristics of cultures more often obtain one culture from the CVC and one from the PV (73.9% vs. 61.7%, p 0.037).

Keywords: Bacteraemia, blood stream infection, diagnosis, fungaemia, negative predictive value, positive predictive value, sensitivity, specificity

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