JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2004, p. 4154–4157 Vol. 42, No. 9
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.9.4154–4157.2004

Diagnosing Invasive Aspergillosis during Antifungal Therapy by PCR Analysis of Blood Samples

Cornelia Lass-Flörl,[1]* Eberhard Gunsilius,[2] Günther Gastl,[2] Hugo Bonatti,[3] Martin C. Freund,[4] Andreas Gschwendtner,[5] Gabriele Kropshofer,[6] Manfred P. Dierich,[1] and Andreas Petzer[3]

Department of Hygiene and Social Medicine[1] and Department of Pathological Anatomy,[5] University of Innsbruck, and Department of Haematology and Oncology,[2] Department of Surgery,[3] Department of Radiology I,[4] and Department of Pediatric Oncology,[6] University Hospital Innsbruck, Innsbruck, Austria

Received 4 March 2004/Returned for modification 11 April 2004/Accepted 6 June 2004

We evaluated the value of *Aspergillus* PCR as a tool for diagnosing invasive aspergillosis from whole-blood samples during antifungal therapy. In a 3-year study, 36 patients receiving antifungal therapy due to chest radiographic findings highly suggestive of fungal pneumonia were evaluated. The PCR results from whole-blood samples were compared to those obtained from bronchoalveolar lavage fluids and/or tissue specimens. A total of 205 whole-blood samples, 15 fine-needle aspirations or tissue biopsy specimens, and 21 bronchoalveolar lavage fluids and tracheal secretions were analyzed using PCR. Of the 36 patients, 15 had proven, 9 had probable, and 12 had possible invasive *Aspergillus* infection according to European Organization for Research and Treatment of Cancer/Mycosis Study Group definitions. For patients with proven infection the sensitivity values of PCR in lung and blood samples were 100 and 40%, respectively. The negative predictive value of blood monitoring under conditions of antifungal treatment was 44%. Clearance of fungal DNA from blood was associated with resolution of clinical symptoms in six of nine patients with proven infection. Repeated positive PCR results for *Aspergillus* were associated with fatal outcome, as three of six patients died. For patients with probable infection the sensitivity values of PCR in lung fluid and blood were 66 and 44%, respectively. The benefit of PCR diagnosis using whole-blood samples is limited when sampling takes place after treatment has been started. Performance of *Aspergillus* PCR using tissue samples is recommended in addition to microscopic examination and culture technique for sensitive detection of fungal infection.

Invasive aspergillosis (IA) is increasingly recognized in immunocompromised hosts (9). Patients with prolonged and deep granulocytopenia following chemotherapy for hemato-oncologic disorders or allogeneic bone marrow transplant recipients are particularly at risk (2, 21). The case fatality rate of IA approaches 100% and results at least partly from difficulties in obtaining a reliable diagnosis at an early stage of the disease, often leading to a fatal delay in adequate therapy (1). No method has proven sufficiently sensitive and specific to allow diagnosis at an early stage. Culture detection is often delayed, and blood culture results are rarely positive for patients with IA (10). The *Aspergillus* galactomannan (GM) enzyme-linked immunosorbent assay is presently the most promising test for diagnosis of IA (20). However, Herbrecht et al. (14) observed that the GM assay seems less sensitive than previous studies have suggested. Buchheidt et al. (8) compared PCR to the GM assay in a prospective study and found that PCR was superior to the GM assay with respect to sensitivity (63.6 versus 33.3%). So far, the detection of circulating fungal DNA has been advocated as a promising, rapid, and more sensitive diagnostic tool for overcoming these drawbacks (5–7, 11, 12, 25).

In a previous study Lass-Flörl et al. evaluated the value of twice-weekly screening for circulating fungal DNA by means of PCR of whole-blood samples (15). Our results indicate the potential usefulness of PCR when screening for *Aspergillus* spp. in patients at risk. Hence, positive results became negative shortly after commencement of antifungal therapy and did not correlate with clinical responsiveness to treatment.

This study examined the diagnostic value of PCR of whole-blood samples during antifungal therapy. Evaluation was performed prospectively over a 3-year period for patients with proven, probable, or possible aspergillosis. The PCR results of whole-blood samples were compared to those obtained from bronchoalveolar lavage (BAL) fluid and/or tissue specimens.

MATERIALS AND METHODS

Patients. A prospective study conducted between January 2000 and September 2003 evaluated 36 patients with hematological malignancies or solid-organ transplantations (median age, 44 years [range, 19 to 78 years]) who were undergoing antifungal therapy due to chest radiographic findings highly suggestive of *Aspergillus* pneumonia. Blood samples, fine-needle aspirations, tissue biopsies, BAL, and tracheal secretion (TS) fluid specimens were investigated by culture technique, histopathologic examination, and PCR to confirm invasive fungal disease. Blood samples were taken twice a week, and at least three samples were required for inclusion in the study. Patients received intensive myelosuppressive or immunosuppressive chemotherapy for hematological malignancies ($n = 25$) or long-term therapy with corticosteroids ($n = 2$) or had had an organ transplantation ($n = 9$).

All patients received amphotericin B (Bristol Meyer Squibb, Vienna, Austria) (1.0 to 1.5 mg/kg of body weight/day) or intravenous voriconazole (Vfendt; Pfizer, Austria) (4 mg/kg/12 h or 200 mg/day orally), liposomal amphotericin B (Ambisome; Gilead, Vienna, Austria) (3 to 5 mg/kg/day), caspofungin (Cancidas; MSD, Vienna, Austria) (70 mg/day), itraconazole (Sporanox; Jannson Cilag, Vienna, Austria) (200 mg/12 h), or several combinations of the above-mentioned antifungal drugs at the time of blood monitoring. For all patients dying from pulmonary infections, autopsy samples were collected for microscopy, fungal culture, and histopathologic evaluation.

In addition, before starting antifungal treatment blood samples were drawn

* Corresponding author. Mailing address: Department of Hygiene and Social Medicine, Medical University Innsbruck, Fritz Pregl Str. 3, 6020 Innsbruck, Austria. Phone: 43 512 507 3425. Fax: 43 512 507 2870. E-mail: Cornelia.Lass-Floerl@uibk.ac.at.

TABLE 1. PCR results for patients with proven aspergillosis and who were undergoing antifungal therapy

Patient no.	Age/sex	Underlying disease or cause[a]	Outcome due to infection	Result of[e]:					
				culture of lung specimens	Microscopic examination	PCR		HRCT[d]	PCR before start of antifungal therapy[c]
						Blood[b]	Specimen[c]		
1	27/M	ALL	Dead	*A. fumigatus*	Pos	Neg	Pos	Pos	Not done
2	47/M	AML	Alive	*A. fumigatus*	Pos	Neg	Pos	Pos	Not done
3	31/M	ALL	Dead	*A. terreus*	Pos	Pos (2)	Pos	Pos	Pos (2)
4	51/M	AML	Alive	Neg	Pos	Neg	Pos	Pos	Not done
5	59/F	Organ transplantation	Dead	*A. fumigatus*	Pos	Pos (1)	Pos	Pos	Pos (1)
6	61/M	Organ transplantation	Alive	Neg	Pos	Neg	Pos	Pos	Pos (1)
7	51/M	Organ transplantation	Alive	*A. terreus*	Pos	Neg	Pos	Pos	Not done
8	63/F	Organ transplantation	Alive	Neg	Pos	Pos (2)	Pos	Pos	Not done
9	49/M	NHL	Dead	Neg	Pos	Neg	Pos	Pos	Pos (2)
10	70/F	MDS	Alive	Neg	Pos	Pos (1)	Pos	Pos	Neg
11	29/F	ALL	Dead	Neg	Pos	Neg	Pos	Pos	Pos (1)
12	67/M	Aplastic anemia	Dead	*A. terreus*	Pos	Pos (1)	Pos	Pos	Not done
13	53/M	Solid tumor	Alive	*A. fumigatus*	Pos	Pos (1)	Pos	Pos	Pos (1)
14	27/M	ALL	Alive	*A. terreus*	Pos	Neg	Pos	Pos	Neg
15	69/F	MDS	Alive	Neg	Pos	Neg	Pos	Pos	Not done

[a] ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; NHL, non-Hodgkin's lymphoma; MDS, myelodysplastic syndrome.
[b] Values in parentheses are numbers of positive PCR samples.
[c] Specimens originated from lung biopsy or fine-needle aspiration.
[d] Positive high-resolution computer tomography results indicate findings consistent with invasive aspergillosis.
[e] Pos, positive; Neg, negative.

from 15 patients (with proven, probable, or possible aspergillosis) in case fever developed during broad-spectrum antibacterial therapy. Four BAL samples, three lung specimens, and seven blood samples from immunocompetent patients with proven bacterial infection served as a negative control.

Definitions. IA was assessed on the basis of EORTC/MSG criteria (3). Proof of infection was based on histopathologic or cytopathologic examinations showing hyphae from needle aspirations or biopsy specimens with evidence of associated tissue damage or a positive culture for a sample obtained by sterile procedure from a normally sterile site and a clinically or radiologically abnormal site consistent with infection. Patients with a probable invasive infection were characterized by at least one host factor criterion, one microbiological criterion, and one major clinical criterion (or two minor clinical criteria).

Patients with a possible invasive infection were characterized by at least one host factor criterion and one microbiological criterion or one major clinical criterion (or two minor clinical criteria).

PCR assay. DNA extraction of 10 ml of EDTA-anticoagulated blood was performed using recombinant lyticase (Sigma, Vienna, Austria) and a QIAmp tissue kit (QIAGEN, Vienna, Austria) as previously described (11). In addition, biopsy specimens were treated with lyticase and proteinase K and beaten with glass beads. A highly conserved sequence of the multicopy 18S rRNA of various fungal pathogens was amplified by PCR using specific primers. Primers (Roth, Graz, Austria) (5′-ATT GGA GGG CAA GTC TGG TG and 5′-CCG ATC CCT AGT CGG CAT AG) bind to conserved regions of this 18S rRNA. A total of 34 cycles of repeated denaturation (94°C for 30 s), annealing (62°C for 1 min), and extension (72°C for 2 min) were applied. Amplicons were detected by PCR–enzyme-linked immunosorbent assay using digoxin-labeled oligonucleotide (Roth) (5′-CAT GGC CTT CAC TGG CTG TGG GGG GAA CCA) for *Aspergillus* spp. and antidigoxigenin antibodies conjugated with alkaline phosphatase (Boehringer Mannheim, Vienna, Austria) as described earlier (18). *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus*, and *Aspergillus nidulans* were detected with these genus-specific oligonucleotide probes. Negative and positive probes were routinely used for quality control. Several PCR-negative blood samples from patients with proven aspergillosis were retested by adding 20 pg of *A. fumigatus* bacteria to the samples to detect PCR inhibition.

RESULTS

A total of 205 whole-blood samples (mean, 4.7 samples/patient; range, 3 to 8), 15 fine-needle aspirations or tissue biopsy specimens, and 21 BAL or TS fluid specimens from 36 patients were analyzed using PCR. Of the patients, 15 showed proven, 9 showed probable, and 12 showed possible invasive *Aspergillus* infection, as shown in Table 1, Table 2, and Table 3.

In patients with proven infection the sensitivity of PCR in lung and blood samples was 100 and 40%, respectively. Specificity was 100%. The negative predictive value of blood monitoring with antifungal treatment was 44%. In patients with probable infection the sensitivity of PCR in lung fluid and blood was 66 and 44%, respectively. Specificity was 100%. The negative predictive value of blood monitoring with antifungal therapy was 58%. Positive PCR results correlated to 100% with the microscopic presence of fungal hyphae in patients with proven infection. Three patients with culture-positive samples showed negative BAL-TS PCR results. By contrast, seven patients showed positive lung specimen PCR results, and yet culture results were negative.

All patients received antifungal therapy for at least 3.1 days (mean; range, 2 to 15 days) before diagnosis of blood by PCR was performed. Biopsy specimens were taken between 5 and 31 days after the start of antifungal therapy.

Of the patients with proven aspergillosis and PCR positivity during treatment, 50% (three of six) died; of those, 33% (three of nine) exhibited a negative PCR signal. Similar results were observed in patients with probable infection, as shown in Table 2. Of patients with proven aspergillosis, six showed positive PCR results before starting antifungal therapy; four of those patients died from IA. No inhibition of PCR signal was observed in the various samples tested.

DISCUSSION

We evaluated the value of *Aspergillus* PCR as a tool for diagnosing IA from whole-blood samples during antifungal therapy. The sensitivity of sequential blood samples in patients with proven infection was 40% and in patients with probable infection was 44%; the negative predictive values were 44%

TABLE 2. PCR results in patients with probable aspergillosis and undergoing antifungal therapy

Patient no.	Age/sex	Underlying disease or cause[a]	Outcome due to infection	Result of[d]:					
				Culture of BAL and/or TS	Microscopic examination	PCR		HRCT[c]	PCR before start of antifungal therapy[b]
						Blood[b]	BAL or TS		
1	25/M	AML	Dead	*A. fumigatus*	Not done	Neg	Pos	Pos	Not done
2	39/M	CLL	Alive	*A. fumigatus*	Pos	Pos (1)	Pos	Pos	Not done
3	77/M	MDS	Dead	*A. terreus*	Pos	Neg	Pos	Pos	Pos (2)
4	31/F	Solid tumor	Alive	*A. fumigatus*	Not done	Neg	Neg	Pos	Not done
5	65/M	Aplastic anemia	Dead	*A. terreus*	Pos	Pos (1)	Pos	Pos	Pos (1)
6	47/F	Organ transplantation	Dead	*A. fumigatus*	Not done	Pos (2)	Neg	Pos	Not done
7	51/M	Organ transplantation	Alive	*A. terreus*	Not done	Neg	Neg	Pos	Pos (2)
8	38/F	Organ transplantation	Alive	*A. fumigatus*	Pos	Pos (2)	Pos	Pos	Not done
9	28/F	ALL	Dead	*A. terreus*	Pos	Neg	Pos	Pos	Not done

[a] AML, acute myeloid leukemia; CLL, chronic lymphoid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoid leukemia.
[b] Values in parentheses are numbers of positive PCR samples.
[c] Positive high-resolution computer tomography results indicate findings consistent with invasive aspergillosis.
[d] Pos, positive; Neg, negative.

and 58%, respectively. Similar data were found by others when using nested PCR (8, 13, 26). Because PCR can be inhibited by the presence of antifungals, we spiked several PCR-negative blood samples and showed positive PCR results. Thus, it can be assumed that antimycotic treatment is in part responsible for the clearance of fungi from blood to nondetectable levels whereas clearance from tissue does not occur (15). This assumption is supported by PCR-negative blood sample results combined with positive-testing tissue sample results for our patients. In addition, the half-life of circulating DNA is short, probably less than 5 min, and a positive signal is observed only when the fungal burden is large enough (23). Yet Loeffler et al. (17) postulate a correlation between high fungus load in tissue and the presence of fungal DNA in blood. In their study, *Aspergillus* DNA was detected in only 25% of the blood samples from infected animals. Mice whose blood became PCR positive showed a mean fungus load in the lung 10 times higher than that in mice whose blood remained PCR negative. Similar differences in mean fungus loads could matter in humans.

A PCR assay that detects circulating DNA in serum can possibly give a better correlation with fungal load and severity of disease and thus more consistent results (5). Loeffler et al. (19) reported that the sensitivity of plasma PCR was lower than that of PCR performed with whole-blood samples. Analyzing blood samples is clinically applicable, because the samples can be obtained repeatedly and by noninvasive means. So far, repeated positive PCR results for *Aspergillus* have been associated with fatal outcome, as three out of six patients died, and a finding of several negative PCR blood results does not exclude the presence of aspergillosis. Clearance of fungal DNA from blood was associated with resolution of clinical symptoms in six of nine patients with proven infection. However, to assess this PCR assay as a means of monitoring responding and nonresponding patients, further studies are needed. Screening for and diagnosis of aspergillosis prior to treatment gives better sensitivity, as shown in the literature (13, 16). Four patients showing PCR positivity prior to antifungal treatment died; whether such individuals might benefit from immediate antifungal therapy remains to be investigated in more detail. However, one of the major drawbacks to this application of *Aspergillus* PCR is the high rate of transient *Aspergillus* fungemia without evidence of IA (4).

TABLE 3. PCR results in patients with possible aspergillosis and undergoing antifungal therapy

Patient no.	Age/sex	Underlying disease or cause[a]	Outcome due to infection	Result of[d]:					
				Culture of BAL and/or TS	Microscopic examination	PCR		HRCT[c]	PCR before start of antifungal therapy[b]
						Blood[b]	BAL or TS		
1	44/M	HD	Alive	Neg	Neg	Neg	Neg	Pos	Pos (2)
2	32/M	Solid tumor	Alive	Neg	Neg	Neg	Pos	Pos	Not done
3	43/F	Multiple myeloma	Alive	Neg	Not done	Pos (2)	Neg	Pos	Not done
4	41/M	NHL	Alive	Neg	Neg	Neg	Pos	Pos	Pos (1)
5	47/M	HD	Alive	Neg	Not done	Pos (1)	Neg	Pos	Not done
6	62/M	Organ transplantation	Alive	Neg	Not done	Neg	Neg	Pos	Not done
7	62/F	Organ transplantation	Alive	Neg	Neg	Neg	Neg	Pos	Pos (1)
8	43/F	Solid Tumor	Alive	Neg	Neg	Pos (2)	Pos	Pos	Not done
9	51/F	Solid Tumor	Alive	Neg	Not done	Neg	Pos	Pos	Pos (2)
10	59/M	ALL	Dead	Neg	Neg	Neg	Pos	Pos	Not done
11	27/F	ALL	Alive	Neg	Not done	Neg	Neg	Pos	Not done
12	52/M	CML	Alive	Neg	Neg	Pos (1)	Neg	Pos	Not done

[a] HD, Hodgkin's disease; NHL, non-Hodgkin's lymphoma; ALL, acute lymphoid leukemia; CML, chronic myeloid leukemia.
[b] Values in parentheses represent the numbers of positive PCR samples.
[c] Positive high-resolution computer tomography results indicate findings consistent with invasive aspergillosis.
[d] Pos, positive; Neg, negative.

Until now, only limited diagnostic tools with poor sensitivity have been available for early detection of IA. Combining microscopy, culture, and PCR may improve the diagnostic outcome, as recently found (7, 24). Positive PCR results correlated up to 100% with the microscopic presence of fungal hyphae in patients with proven infection. However, some findings are ambiguous and confirm the difficulties in diagnosing invasive fungal infection. Seven patients showed PCR positivity of lung specimens, and yet culture results were negative. Whether these results should be classified as false positives is questionable, since treatment with liposomal amphotericin B and caspofungin caused typical CT lesions to shrink. Also, a possible cross-amplification could result from this panfungal PCR. By contrast, another three patients showed BAL-TS PCR negativity, and yet culture results were positive. Fungal contamination during specimen management could be one explanation; however, these results remain difficult to explain. So far, a clear benefit of the use of PCR is the identification of genus and species in cases in which culture results are negative. Identification to the species level is highly warranted to administer optimal antifungal treatment (22).

From our findings we conclude that the benefits of PCR diagnosis are limited when sampling is performed after treatment has started. *Aspergillus* PCR should be recommended in addition to the use of microscopic examination and culture technique for sensitive detection of fungal infection.

REFERENCES

1. **Aisner, J., P. H. Wiernik, and S. C. Schimpff.** 1977. Treatment of invasive aspergillosis: relation of early diagnosis and treatment to response. Ann. Intern. Med. **86:**539–543.
2. **Anaissie, E.** 1992. Opportunistic mycoses in the immunocompromised host: experience at a cancer center and review. Clin. Infect. Dis. **14**(Suppl. 1)**:**43–53.
3. **Ascioglu, S., J. H. Rex, B. de Pauw, J. E. Bennett, J. Bille, F. Crokaert, D. W. Denning, P. Donnelly, J. E. Edwards, Z. Erjavec, D. Fiere, O. Lortholary, J. Maertens, J. F. Meis, T. F. Patterson, J. Ritter, D. Selleslag, M. Shah, D. A. Stevens, and T. J. Walsh.** 2002. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoetic stem cell transplants: an international consensus. Clin. Infect. Dis. **34:**7–14.
4. **Bialek, R., D. Moshous, J. L. Casanova, S. Blanche, and C. Hennequin.** 2002. *Aspergillus* antigen and PCR assays in bone marrow transplanted children. Eur. J. Med. Res. **7:**177–180.
5. **Bretagne, S., J. M. Costa, A. Marmor-Khuong, F. Poron, C. Cordonnier, M. Vidaud, and J. Fleur-Feith.** 1995. Detection of *Aspergillus* species DNA in bronchoalveolar lavage samples by competitive PCR. J. Clin. Microbiol. **33:**1164–1168.
6. **Bretagne, S., J. Costa, E. Bart-Delabesse, N. Dhedin, C. Rieux, and C. Cordonnier.** 1998. Comparison of serum galactomannan antigen detection and competitive polymerase chain reaction for diagnosing invasive aspergillosis. Clin. Infect. Dis. **26:**1407–1412.
7. **Buchheidt, D., C. Baust, H. Skladny, M. Baldus, S. Bräuninger, and R. Hehlmann.** 2002. Clinical evaluation of a polymerase chain reaction assay to detect *Aspergillus* species in bronchoalveolar lavage samples of neutropenic patients. Br. J. Haematol. **116:**803–811.
8. **Buchheidt, D., M. Hummel, D. Schleiermacher, B. Spiess, R. Schwerdtfeger, O. Cornely, S. Wilhelm, S. Reuter, W. Kern, T. Südhoff, H. Mörz, and R. Hehlmann.** 2004. Prospective clinical evaluation of LightCycler™-mediated polymerase chain reaction assay, a nested-PCR assay and galactomannan enzyme-linked immunosorbent assay for detection of invasive aspergillosis in neutropenic cancer patients and haematological stem cell transplant recipients. Br. J. Haematol. **125:**196–202.
9. **Denning, D. W., S. E. Follansbee, M. Scolaro, S. Norris, H. Edelstein, and D. Stevens.** 1991. Pulmonary aspergillosis in the acquired immunodeficiency syndrome. N. Engl. J. Med. **324:**654–662.
10. **Duthie, R., and D. W. Denning.** 1995. *Aspergillus* fungemia: report of two cases and review. Clin. Infect. Dis. **20:**598–605.
11. **Einsele, H., H. Hebart, G. Roller, J. Löffler, I. Rothenhöfer, C. Müller, R. Bowden, J. A. Burik, D. Engelhard, L. Kanz, and U. Schumacher.** 1997. Detection and identification of fungal pathogens in blood by using molecular probes. J. Clin. Microbiol. **35:**1353–1360.
12. **Einsele, H., K. Quabeck, K. D. Müller, H. Hebart, I. Rothenhöfer, J. Löffler, and U. W. Schaefer.** 1998. Prediction of invasive pulmonary aspergillosis from colonization of lower respiratory tract before marrow transplantation. Lancet **358:**1443.
13. **Ferns, R. B., H. Fletcher, S. Bradley, S. Mackinnon, C. Hunt, and R. S. Tedder.** 2002. The prospective evaluation of a nested polymerase chain reaction assay for the early detection of *Aspergillus* infection in patients with leukaemia or undergoing allograft treatment. Br. J. Haematol. **119:**720–725.
14. **Herbrecht, R., V. Letscher-Bru, C. Oprea, B. Lioure, J. Waller, F. Campos, O. Villard, K. Liu, S. Natajan-Ame, P. Lutz, J. P. Bergerat, and E. Candolfi.** 2002. Aspergillus galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. J. Clin. Oncol. **20:**1898–1906.
15. **Lass-Flörl, C., J. Aigner, G. Eberhard, A. Petzer, D. Nachbaur, G. Gastl, H. Einsele, J. Löffler, M. P. Dierich, and R. Würzner.** 2001. Screening for *Aspergillus* spp. using polymerase chain reaction of whole blood samples from patients with haematological malignancies. Br. J. Haematol. **113:**180–184.
16. **Lass-Flörl, C., C. Speth, A. Mayr, R. Würzner, M. P. Dierich, H. Ulmer, and H. Dietrich.** 2003. Diagnosing and monitoring of invasive aspergillosis during antifungal therapy by polymerase chain reaction: an experimental study in mice. Diagn. Microbiol. Infect. Dis. **42:**569–572.
17. **Loeffler, J., H. Hebart, U. Brauchle, U. Schumacher, and H. Einsele.** 2000. Comparison between plasma and whole blood specimens for detection of *Aspergillus* DNA by PCR. J. Clin. Microbiol. **38:**3830–3833.
18. **Loeffler, J., K. Kloepfer, H. Hebart, L. Najvar, J. R. Graybill, W. R. Kirkpatrick, T. F. Patterson, K. Dietz, R. Bialek, and H. Einsele.** 2002. Polymerase chain reaction detection of *Aspergillus* DNA in experimental models of invasive aspergillosis. J. Infect. Dis. **185:**1203–1206.
19. **Löffler, J., H. Hebart, S. Sepe, U. Schumacher, T. Klingebiel, and H. Einsele.** 1998. Detection of PCR-amplified fungal DNA by using a PCR-ELISA system. Med. Mycol. **36:**275–279.
20. **Maertens, J., J. Van Eldere, J. Verhaegen, E. Verbeken, J. Verschakelen, and M. Boogaerts.** 2002. Use of circulating galactomannan screening for early diagnosis of invasive aspergillosis in allogeneic stem cell transplant recipients. J. Infect. Dis. **186:**1297–1306.
21. **Peterson, P. K., P. McGlave, N. K. Ramsay, F. Rhame, E. Cohen, G. S. Perry, A. J. Goldman, and J. Kersey.** 1983. A prospective study of infectious diseases following bone marrow transplantation: emergence of *Aspergillus* and *Cytomegalovirus* as the major causes of mortality. Infect. Control **4:**81–89.
22. **Ruhnke, M., A. Bohme, D. Buchheidt, K. Donhuijsen, H. Einsele, R. Enzensberger, A. Glasmacher, H. Gumbel, C. P. Heussel, M. Karthaus, E. Lambrecht, T. Sudhoff, and H. Szelenyi.** 2003. Diagnosis of invasive fungal infections in hematology and oncology—guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Oncology (DGHO). Ann. Hematol. **82**(Suppl. 2)**:**S141–S148.
23. **Rumore, P., B. Muralidhar, M. Lin, C. Lai, and C. Steinman.** 1992. Haemodialysis as a model for studying endogenous plasma DNA: oligonucleosome-like structure and clearance. Clin. Exp. Immunol. **90:**56–62.
24. **Skladny, H., D. Buchheidt, C. Baust, F. Krieg-Schneider, W. Seifarth, C. Leib-Mösch, and R. Hehlmann.** 1999. Specific detection of *Aspergillus* species in blood and bronchoalveolar lavage samples of immunocompromised patients by two-step PCR. J. Clin. Microbiol. **37:**3865–3871.
25. **Williams, E., J. P. Leeming, H. M. Palmer, C. G. Steward, D. Warnock, D. I. Marks, and M. R. Millar.** 2000. Diagnosis of invasive aspergillosis in bone marrow transplant recipients by polymerase chain reaction. Br. J. Haematol. **108:**132–139.
26. **Yamakami, Y., A. Hashimoto, E. Yamagata, P. Kamberi, R. Karashima, H. Nagai, and M. Nasu.** 1998. Evaluation of PCR for detection of DNA specific for *Aspergillus* species in sera of patients with various forms of pulmonary aspergillosis. J. Clin. Microbiol. **36:**3619–3623.