

Prospective Evaluation of a Polymerase Chain Reaction–ELISA Targeted to *Aspergillus fumigatus* and *Aspergillus flavus* for the Early Diagnosis of Invasive Aspergillosis in Patients with Hematological Malignancies

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Background. Current laboratory and radiological methods for diagnosis of invasive aspergillosis (IA) lack sensitivity and specificity.

Methods. We prospectively evaluated the diagnostic value of twice-weekly screening for circulating *Aspergillus fumigatus* and *A. flavus* DNA with a polymerase chain reaction–enzyme-linked immunosorbent assay (PCR-ELISA).

Results. Among the 201 adult patients with hematological malignancies who were included in the study, 55 IA cases were diagnosed. On the basis of the analysis of 1205 serum samples from 167 patients, the sensitivity, specificity, and positive and negative predictive values of the PCR-ELISA for proven and probable IA cases were 63.6%, 89.7%, 63.6%, and 89.7%, respectively, when samples with 2 consecutive positive results were used. The use of a combination of the PCR-ELISA and a galactomannan (GM) assay increased the sensitivity to 83.3%, increased the negative predictive value to 97.6%, and decreased the specificity to 69.8%. In most patients with IA, PCR-ELISA positivity anticipated or was simultaneous with the initiation of antifungal therapy, the abnormalities found by computed tomography, the mycological/histological diagnosis, and the GM positivity. Overall, 56.3% of the patients had at least 1 positive sample, and the false single-positive rate was 44.8%.

Conclusions. In addition to serial screening for GM antigenemia and radiological surveillance, PCR-ELISA may improve the rates of early diagnosis of IA and the management of patients with hematological malignancies.

Invasive aspergillosis (IA) has emerged as a major cause of death in patients with hematological malignancies and in stem cell transplant recipients [1–3]. Early diagnosis and early antifungal therapy are critical in improving the prognosis for these patients [4, 5]. However, no method has proven to be sufficiently sensitive and specific to allow for an early diagnosis. The ref-

erence standard for diagnosis requires invasive procedures, so that histological examination or culture can be performed [6]. The other diagnostic tools are imperfect: high-resolution computed tomography (CT) of the lungs is very helpful but does not provide proof of IA, culture of body fluids such as the fluid collected during bronchoalveolar lavage (BAL) has low sensitivity, and fungemia due to *Aspergillus* species is rarely detected [5, 7, 8]. Thus, in recent years, interest has focused on nonculture, noninvasive methods. An ELISA (Platelia *Aspergillus*; Bio-Rad) for the detection of galactomannan (GM), a circulating fungal antigen, has been developed, and its sensitivity ranges from 60.0% to 92.6% [9]. This assay produces some false-positive results [9], and, more recently, cross-reactivity with piperacillin-tazobactam therapy—which was prob-

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ably the result of contamination with GM during the production of the antibiotic—has been reported [10–12]. The detection of GM in 2 blood samples has been approved by the European Organization for Research and Treatment of Cancer (EORTC) and the Mycoses Study Group (MSG) of the National Institute of Allergy and Infectious Diseases as the criterion for the diagnosis of probable IA [6]. The development of polymerase chain reaction (PCR) assays for use in the diagnosis of IA has shown promising but discrepant results, and most studies that evaluated PCR assays in clinical samples were retrospective or involved limited numbers of patients [7, 13–26]. The selection of the target sequence and the end stage of amplicon detection can have a major influence on the performance of a PCR assay [27, 28]. BAL samples had a high rate of false-positive results, and this was likely due to the contamination of the samples by ubiquitous *Aspergillus* conidia or colonization without true invasive *Aspergillus* infection of the patients' lungs [29]. Because earlier studies have shown that serum was superior to plasma or white cells for amplification of *Aspergillus* DNA, we developed a PCR-ELISA to detect *Aspergillus* DNA in serum samples [30–32]. PCR-based methods need to be standardized and adapted for use in the daily clinical routine. Therefore, we used a combination of commercially available kits for DNA extraction and ELISA detection of the amplicons. We conducted a prospective trial in a large cohort of patients with hematological malignancies, to validate the diagnostic potential of the PCR-ELISA.

PATIENTS, MATERIALS, AND METHODS

Study population and design. From April 2001 through November 2002, all patients with hematological malignancies who were routinely screened for GM detection and ≥ 15 years old were included in the study. Official French directives on the conduct of clinical trials and human experimentation were followed. Patients were stratified into 3 IA risk groups—high, intermediate, and low—as defined by Prentice et al. [33].

During the high-risk periods for infection and until absolute neutrophil counts increased to >500 cells/ μL , all patients were hospitalized in protected facilities with high-efficiency particulate air filtration associated with laminar air flow for patients undergoing allogeneic stem cell transplantation. Antifungal prophylaxis consisted of 400 mg/day itraconazole in patients with acute lymphoblastic leukemia and of 400 mg/day fluconazole in allogeneic stem cell transplant recipients. Empirical antifungal therapy (0.7–1.0 mg/kg/day amphotericin B or 3 mg/kg/day liposomal amphotericin B) was initiated if fever persisted for 3 days despite receipt of broad-spectrum antibacterial therapy. Diagnostic procedures included daily physical examination, weekly bacterial and fungal cultures on stool and urine samples, weekly chest radiography, and twice-weekly testing for GM. Blood cul-

tures were performed when patients presented with fever. When IA was suspected, an additional serum sample was collected to test for the presence of GM, and high-resolution CT of the lungs and, when possible, culture of BAL fluid were performed. PCR results were not used in the management of the patients.

Case definition and PCR-ELISA precocity analysis. We used the consensus criteria proposed by the EORTC/MSG [6]. To evaluate the performance of the GM assay either alone or in combination with the PCR-ELISA, the results of the GM assay were not included in the microbiological criteria for the diagnosis of probable IA. The date of diagnosis of proven IA was defined as the day on which the initial positive histopathological sample was obtained, the date of diagnosis of probable IA was defined as either the day on which the initial sample that yielded *Aspergillus* species was obtained or the day on which the second blood sample that was positive for GM was obtained, and the date of diagnosis of possible IA was defined as the onset of clinical symptoms. PCR-ELISA precocity in diagnosing IA was assessed in comparison with the timing of the clinical suspicion of IA, the results of CT, and histological and microbiological criteria as defined by the EORTC/MSG [6].

DNA extraction. DNA was extracted from both serum and fungal cultures by use of the QIAamp DNA Mini Kit (Qiagen), in accordance with the manufacturer's recommendations. Two negative controls were used in each DNA extraction experiment. The PCR-ELISA was performed using the serum sample that was collected for GM detection, which was stored at -20°C until processing.

Amplification and detection of *Aspergillus* DNA. The following primer set was used to amplify a sequence of the *A. fumigatus* mitochondrial gene (GenBank accession number L37095 [14]): 5'-digoxigenin-labeled forward primer at position 780–803 (5'-TGGTAGAGCTGTGCGCTTTACACGC-3') and reverse primer at position 915–936 (5'-TTGGTTGCGGGTTTAGGGATTG-3'). PCR was performed with 50 μL of PCR solution (dUTP [Roche Molecular Diagnostics] and HotStarTaq DNA Polymerase [Qiagen]) and 15 μL of nonconcentrated DNA in a PE 2400 thermocycler (Perkin Elmer). Annealing was 45 cycles for 30 s at 55°C . Each amplification step included at least 2 negative controls with sterile water. As inhibition positive controls, 1 pg of *Streptococcus pyogenes* DNA was added to all samples, and they were analyzed in a second PCR assay for amplification of the M protein gene of *S. pyogenes* (the sensitivity of this PCR assay was 1 pg) [34]. To control the sensitivity of the PCR-ELISA, *A. fumigatus* genomic DNA from 10 conidia was amplified as a positive control in each assay. DNA extraction, PCR, and amplicon detection were performed in separate rooms, to avoid amplicon contamination. The PCR-ELISA that we used is commercially available as the plate assay GEN-ETI-K DEIA (Diasorin) and is based on ELISA techniques to detect the PCR product after probe hybridization. The probe was labeled with

Table 1. Characteristics of the patients.

Characteristic	Proven IA (n = 4)	Probable IA (n = 29)	Possible IA (n = 18)	No IA (n = 116)	Total (n = 167)
IA risk group ^a					
High	2 (50.0)	17 (58.6)	8 (44.4)	46 (39.7)	73 (43.7)
Intermediate	2 (50.0)	10 (34.5)	10 (55.6)	62 (53.4)	84 (50.3)
Low	...	2 (6.9)	...	8 (6.9)	10 (6.0)
Underlying condition ^b					
Acute leukemia	3 (75.0)	15 (51.7)	13 (72.2)	68 (58.6)	99 (59.3)
Chronic leukemia	...	7 (24.1)	...	8 (7.0)	15 (9.0)
Lymphoma	...	2 (6.9)	4 (22.2)	22 (19.0)	28 (16.8)
Multiple myeloma	...	4 (13.8)	...	15 (12.9)	19 (11.4)
Miscellaneous	1 (25.0)	1 (3.5)	1 (5.6)	3 (2.6)	6 (3.6)
Stem cell transplantation	1 (25.0)	11 (37.9)	5 (27.8)	55 (47.4)	72 (43.1)
Neutrophil count <500 cells/ μ L	4 (100.0)	24 (82.8)	17 (94.4)	102 (87.9)	147 (88.0)
Episodes, ^c no.	6	42	29	147	224
Duration, median, days	19.5	19	17.0	17.0	17.0
Corticosteroid therapy ^c	1 (25.0)	13 (44.8)	7 (38.9)	28 (24.1)	49 (29.3)

NOTE. Data are no. (%) of patients, unless otherwise indicated. IA, invasive aspergillosis.

^a Defined by Prentice et al. [33].

^b Patients may have >1 underlying condition.

^c During the whole study period.

biotin and was specific for *A. fumigatus* and *A. flavus* (5'-GTAAT-AGTAACATAGATGGC-3'; position 873–892) [14]. The optical density (OD) was measured at 450 nm and 630 nm. Negative and positive cutoff values were calculated using 100 serum samples from healthy donors. OD values ≥ 4 SD and < 2 SD above the mean OD of all healthy donors were considered to be positive and negative results, respectively. Thus, the positive and negative cutoff values were 0.323 and 0.211 OD values, respectively, and results within these 2 cutoff values were considered to be equivocal. The PCR-ELISA was performed by an investigator who was blinded to the results of the other diagnostic procedures.

GM detection. The GM assay was an ELISA (Platelia *Aspergillus*; Bio-Rad). As is currently recommended, the results were analyzed using the 0.5 OD index (ODI) cutoff value [35]. However, for management of patients, a positive sample was defined as one with ≥ 1.5 ODI, which followed the manufacturer's instructions that were available in Europe at the time of the study.

Statistical analysis. Before comparisons were made that involved continuous variables, tests of normality using the Shapiro-Wilks method were applied, so that the 2-sample *t* and the Wilcoxon rank sum tests could be properly used. The χ^2 test—or the Fisher exact test, if it was more appropriate—was used for comparisons of categorical variables. Single-positive results were defined as at least 1 positive result, and consecutive-positive results were defined as at least 2 positive results obtained consecutively within 1 week. Thus, patients who did not have consecutive data obtained within 1 week were excluded from the final analysis. Because of the uncertainty of the diagnosis in patients with possible IA, we performed 3 separate

analyses: the first included only proven and probable IA cases; the second included proven and probable IA cases, and possible cases were considered to be proven IA cases; and the third included proven and probable IA cases, and possible cases were not considered to be IA.

RESULTS

Study patients. A total of 201 patients were enrolled in the study and had 256 consecutive episodes of neutropenia (neutrophil count, < 500 cells/ μ L). In accordance with EORTS/MSG criteria, 55 patients (27.4%) were diagnosed with IA [6]. During the study period plus 3 months, 106 patients (52.7%) died. Mortality was significantly higher in patients with IA than in patients without IA (65.4% vs. 47.9%; $P = .03$).

Thirty-four patients did not have consecutive serum samples that were collected within 1 week, and therefore they were excluded from the final analysis; this left 167 patients, 51 of whom had IA (4 proven cases, 29 probable cases, and 18 possible cases). The distribution of underlying conditions and risk groups for IA are shown in table 1.

PCR-ELISA results. Inhibitors were detected in 18 serum samples, and these samples were excluded from the analysis. In total, 1205 serum samples from 167 patients were analyzed (median, 6 samples/patient). The mean number of samples per patient was similar in all IA groups ($P = .3$). Patients with proven or probable IA had significantly more positive samples than did patients without IA (mean, 3.0 vs. 1.4 positive samples; $P < .0001$). Ninety-four patients (56.3%) and 38 patients (22.7%) had single-positive results and consecutive-pos-

itive results, respectively. Among the latter patients, 26 (68.4%) were diagnosed with IA (table 2). Results for 105 samples (8.7%) from 52 patients without IA (44.8%) were considered to be false single-positive results. The rate of false consecutive-positive results was 10.3% (table 2). Sensitivity, specificity, and predictive values of the PCR-ELISA, the GM assay, and the combination of these 2 assays are detailed in table 3. Both the PCR-ELISA and the GM assay showed low specificity when samples with single-positive results were tested. When only the samples with consecutive-positive results were tested, the specificity increased to 89.7% for the PCR-ELISA and 80.2% for the GM assay, whereas the sensitivity decreased from 87.9% to 63.6% and from 75.0% to 66.7%, respectively (table 3). Both tests showed high negative predictive values (89.7% and 95.9%, respectively), which reached 97.6% when the tests were combined (table 3).

PCR-ELISA positivity precocity: comparison with other diagnostic criteria. PCR-ELISA positivity predated all other criteria of IA, including clinical suspicion of IA, initiation of antifungal therapy (either empirical or targeted antifungal therapy), abnormalities on CT scan, mycological/histological diagnosis of IA, and GM positivity in 11 of 51 IA cases, including in 3 of 4 proven IA cases, in 5 of 29 probable IA cases, and in 3 of 18 possible IA cases. PCR-ELISA positivity preceded the initiation of antifungal therapy in 24 of 50 patients by a median of 8 days (range, 1–62 days) and coincided in 2 patients. PCR-ELISA positivity preceded the mycological/histological diagnosis in 8 patients (a total of 12 patients had mycological/histological diagnoses) by a median of 16 days (range, 1–110 days). For the radiological diagnosis, 33 patients underwent high-resolution chest CT, and 31 patients presented with radiological features that are suggestive of IA (12 had halo signs, 2 had air-crescent signs, 3 had cavities within consolidations, and 14 had new infiltrates). Among these 31 patients, PCR-ELISA positivity preceded the abnormalities on CT scan in 19 patients by a median of 8 days (range, 1–84 days), and these events coincided in 2 patients. Among the 38 patients with IA who had at least 1 positive GM assay result, PCR-ELISA positivity preceded the positive GM assay result in 12 patients by

a median of 17 days (range, 4–63 days), these events were simultaneous in 11 patients, and PCR-ELISA positivity followed the positive GM assay result in 11 patients.

Mycological and histological results. Mycological or histological confirmation of IA was obtained for 12 patients. *Aspergillus* species were found in 9 patients: *A. fumigatus* in 5 patients, *A. flavus* in 3 patients, and both *A. fumigatus* and *A. nidulans* in 1 patient. All of these patients had positive PCR-ELISA results.

DISCUSSION

Serial screening with the PCR-ELISA showed high sensitivity (87.9%) in the 33 proven and probable IA cases when at least 1 positive serum sample was considered (table 3). In most patients, PCR-ELISA positivity preceded or was simultaneous with the initiation of antifungal therapy, the abnormalities on CT scan, the mycological/histological diagnosis, and positive GM assay result. However, this high sensitivity was hampered by rather low specificity and a low positive predictive value, although they could be improved by using samples with consecutive-positive results—a criterion that is also required for GM detection. Our results showed that this assay, in combination with other techniques—such as CT and GM assay—is reliable for clinical management of patients with hematological malignancies. PCR-ELISA positivity should suggest a diagnosis of IA and support physicians in testing a different sample and using technologies such as CT, bronchoscopy, and GM assay for confirmation of the IA diagnosis. However, because of the high rate of false single-positive results, the first positive PCR-ELISA result cannot be used as the sole criterion to initiate a specific therapy targeted to *A. fumigatus* or *A. flavus* or to switch from empirical antifungal therapy. Despite this, the high negative predictive value of the combined PCR-ELISA and GM assay (97.6%) make a diagnosis of IA very unlikely and should lead clinicians to investigate a non-*Aspergillus* fungal infection or another diagnosis in patients who repeatedly test negative with the PCR-ELISA and GM assay. Thus, our PCR-ELISA appears to be very helpful for diagnostic purposes, not only by

Table 2. Results of polymerase chain reaction (PCR)-ELISA and galactomannan (GM) assay in 167 patients with hematological malignancies.

IA	PCR-ELISA		GM assay		PCR-ELISA or GM assay	
	Single-positive results	Consecutive-positive results	Single-positive results	Consecutive-positive results	Single-positive results	Consecutive-positive results
Proven	4/4 (100.0)	4/4 (100.0)	4/4 (100.0)	3/4 (75.0)	4/4 (100.0)	4/4 (100.0)
Probable	25/29 (86.2)	17/29 (58.6)	5/8 (62.5)	5/8 (62.5)	8/8 (100.0)	6/8 (75.0)
Possible	13/18 (72.2)	5/18 (27.8)	29/39 (74.4)	16/39 (41.0)	35/39 (89.7)	24/39 (61.5)
None	52/116 (44.8)	12/116 (10.3)	90/116 (77.6)	23/116 (19.8)	103/116 (88.8)	35/116 (30.2)

NOTE. Data are no. of patients/total no. of patients (%). Single-positive results were defined as at least 1 positive result, and consecutive-positive results were defined as at least 2 positive results obtained consecutively within 1 week. IA, invasive aspergillosis.

Table 3. Sensitivity, specificity, and predictive values of the polymerase chain reaction (PCR)–ELISA and galactomannan (GM) assay.

Samples, IA type	PCR-ELISA	GM assay	PCR-ELISA or GM assay
Single-positive samples^a			
Proven and probable IA vs. no IA (possible IA was excluded)			
Sensitivity	87.9 (71.8–96.6)	75.0 (42.8–94.5)	100.0 (73.5–100.0)
Specificity	55.2 (45.7–64.4)	22.4 (15.2–31.1)	11.2 (6.1–18.4)
Positive predictive value	35.8 (25.4–47.2)	9.1 (4.2–16.6)	10.4 (5.5–17.5)
Negative predictive value	94.1 (85.6–98.4)	89.7 (72.6–97.8)	100.0 (75.3–100.0)
Proven, probable, and possible IA vs. no IA (possible IA was considered to be proven IA)			
Sensitivity	82.4 (69.1–91.6)	74.5 (60.4–85.7)	92.2 (81.1–97.8)
Specificity	55.2 (45.7–64.4)	22.4 (15.2–31.1)	11.2 (6.1–18.4)
Positive predictive value	44.7 (34.4–55.3)	29.7 (21.9–38.4)	31.3 (24.0–39.4)
Negative predictive value	87.7 (77.9–94.2)	66.7 (49.8–80.9)	76.5 (50.1–93.2)
Proven and probable IA vs. no IA and possible IA (possible IA was considered to be no IA)			
Sensitivity	87.9 (71.8–96.6)	75.0 (42.8–94.5)	100.0 (73.5–100.0)
Specificity	51.5 (42.7–60.2)	23.2 (16.8–30.7)	11.0 (6.5–17.0)
Positive predictive value	30.9 (21.7–41.2)	7.0 (3.3–12.9)	8.0 (4.2–13.6)
Negative predictive value	94.5 (86.6–98.5)	92.3 (79.1–98.4)	100.0 (80.5–100.0)
Consecutive-positive samples^b			
Proven and probable IA vs. no IA (possible IA was excluded)			
Sensitivity	63.6 (45.1–79.6)	66.7 (34.9–90.1)	83.3 (51.6–97.9)
Specificity	89.7 (82.6–94.5)	80.2 (71.7–87.0)	69.8 (60.6–78.0)
Positive predictive value	63.6 (45.1–79.6)	25.8 (11.9–44.6)	22.2 (11.2–37.1)
Negative predictive value	89.7 (82.6–94.5)	95.9 (89.8–98.9)	97.6 (91.6–99.7)
Proven, probable, and possible IA vs. no IA (possible IA was considered to be proven IA)			
Sensitivity	51.0 (36.6–65.2)	47.1 (32.9–61.5)	66.7 (52.1–79.2)
Specificity	89.7 (82.6–94.5)	80.2 (71.7–87.0)	69.8 (60.6–78.0)
Positive predictive value	68.4 (51.3–82.5)	51.1 (36.1–65.9)	49.3 (37.0–61.6)
Negative predictive value	80.6 (72.7–87.0)	77.5 (69.0–84.6)	82.7 (73.7–89.6)
Proven and probable IA vs. no IA and possible IA (possible IA was considered to be no IA)			
Sensitivity	63.6 (45.1–79.6)	66.7 (34.9–90.1)	83.3 (51.6–97.9)
Specificity	87.3 (80.5–92.4)	74.8 (67.2–81.5)	61.9 (53.8–69.6)
Positive predictive value	55.3 (38.3–71.4)	17.0 (7.6–30.8)	14.5 (7.2–25.0)
Negative predictive value	90.7 (84.3–95.1)	96.7 (91.7–99.1)	98.0 (92.8–99.8)

NOTE. Data are % (95% confidence interval). IA, invasive aspergillosis.

^a Samples with at least 1 positive result.

^b Samples with at least 2 positive results obtained consecutively within 1 week.

suggesting IA, in the case of a positive result, but also—and principally—by almost excluding IA due to *A. fumigatus* or *A. flavus*, in the case of a negative result.

GM detection with an ELISA is recognized as a useful diagnostic tool [6, 9]. Studies that compared the performance of a PCR assay with that of the GM assay showed contradictory results, and the variability in the GM cutoff values that were used hampers comparisons between studies [14, 19, 23–26, 31, 36,

37]. In the present study, whereas the performance of the PCR-ELISA appeared to be better than that for the GM assay when samples with single-positive results were used, the 2 assays showed comparable results when samples with consecutive-positive results were used—a criterion that is recommended for reliable clinical use of such an assay (table 3). Thus, either one of these assays was superior for diagnostic purposes, and either one could be substituted for the other. Furthermore, the com-

bined use of the 2 assays increased the sensitivity and negative predictive value of each individual test to 83.3% and 97.6%, respectively. The combined use of DNA and GM detection assays appears to be very attractive, because of the different targets concerned, and many authors have recommended it [14, 19, 24, 26]. Our study is the first, to our knowledge, that provides evidence of the actual benefit of this combination.

The results of this prospective evaluation showed that the PCR-ELISA may favorably be incorporated into the IA diagnosis strategy, in association with serial screening for GM antigenemia and surveillance by use of chest CT. Use of the PCR-ELISA may not only improve the ability to offer an early diagnosis of IA, when positive results are obtained, but also provide more confidence to exclude a diagnosis of IA, when negative results are obtained. Correlation of its results with clinical outcome and its value in monitoring the effectiveness of antifungal therapy will require further specific evaluation.

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References

- Denning DW. Invasive aspergillosis. *Clin Infect Dis* **1998**; 26:781–805.
- Groll AH, Shah PM, Mentzel C, Schneider M, Just-Nuebling G, Huebner K. Trends in the postmortem epidemiology of invasive fungal infections at a university hospital. *J Infect* **1996**; 33:23–32.
- Cornet M, Fleury L, Maslo C, Bernard JF, Brucker G. Invasive Aspergillosis Surveillance Network of the Assistance Publique-Hopitaux de Paris. Epidemiology of invasive aspergillosis in France: a six-year multicentric survey in the greater Paris area. *J Hosp Infect* **2002**; 51:288–96.
- Denning DW. Therapeutic outcome in invasive aspergillosis. *Clin Infect Dis* **1996**; 23:608–15.
- Caillot D, Casasnovas O, Bernard A, et al. Improved management of invasive pulmonary aspergillosis in neutropenic patients using early thoracic computed tomographic scan and surgery. *J Clin Oncol* **1997**; 15: 139–47.
- Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* **2002**; 34:7–14.
- Bretagne S, Costa JM, Marmorat-Khuong A, et al. Detection of *Aspergillus* species DNA in bronchoalveolar lavage samples by competitive PCR. *J Clin Microbiol* **1995**; 33:1164–8.
- Duthie R, Denning DW. *Aspergillus* fungemia: report of two cases and review. *Clin Infect Dis* **1995**; 20:598–605.
- Yeo SF, Wong B. Current status of nonculture methods for diagnosis of invasive fungal infections. *Clin Microbiol Rev* **2002**; 15:465–84.
- Sulahian A, Touratier S, Ribaud P. False positive test for *Aspergillus* antigenemia related to concomitant administration of piperacillin and tazobactam. *N Engl J Med* **2003**; 349:2366–7.
- Viscoli C, Machetti M, Cappellano P, et al. False-positive galactomannan Platelia *Aspergillus* test results for patients receiving piperacillin-tazobactam. *Clin Infect Dis* **2004**; 38:913–6.
- Adam O, Aupérin A, Wilquin F, Bourhis J-H, Gachot B, Chachaty E. Treatment with piperacillin-tazobactam and false-positive *Aspergillus* galactomannan antigen test results for patients with hematological malignancies. *Clin Infect Dis* **2004**; 38:917–20.
- Einsele H, Quabeck K, Müller KD, et al. Prediction of invasive pulmonary aspergillosis from colonization of lower respiratory tract before marrow transplantation. *Lancet* **1998**; 352:1443.
- Bretagne S, Costa JM, Bart-Delabesse E, Dhedin N, Rieux C, Cordonnier C. Comparison of serum galactomannan antigen detection and competitive polymerase chain reaction for diagnosing invasive aspergillosis. *Clin Infect Dis* **1998**; 26:1407–12.
- Skladny H, Buchheidt D, Baust C, et al. Specific detection of *Aspergillus* species in blood and bronchoalveolar lavage samples of immunocompromised patients by two-step PCR. *J Clin Microbiol* **1999**; 37:3865–71.
- Hebart H, Löffler J, Meisner C, et al. Early detection of *Aspergillus* infection after allogeneic stem cell transplantation by polymerase chain reaction screening. *J Infect Dis* **2000**; 181:1713–9.
- Hebart H, Löffler J, Reitze H, et al. Prospective screening by a panfungal polymerase chain reaction assay in patients at risk for fungal infections: implications for the management of febrile neutropenia. *Br J Haematol* **2000**; 111:635–40.
- Buchheidt D, Baust C, Skladny H, et al. Detection of *Aspergillus* species in blood and bronchoalveolar lavage samples from immunocompromised patients by means of 2-step polymerase chain reaction: clinical results. *Clin Infect Dis* **2001**; 33:428–35.
- Kami M, Fukui T, Ogawa S, et al. Use of real-time PCR on blood samples for diagnosis of invasive aspergillosis. *Clin Infect Dis* **2001**; 33:1504–12.
- Lass-Flörl C, Aigner J, Gunsilius E, et al. Screening for *Aspergillus* spp. using polymerase chain reaction of whole blood samples from patients with haematological malignancies. *Br J Haematol* **2001**; 113:180–4.
- Raad I, Hanna H, Sumoza D, Albitar M. Polymerase chain reaction on blood for the diagnosis of invasive pulmonary aspergillosis in cancer patients. *Cancer* **2002**; 94:1032–6.
- Yamakami Y, Hashimoto A, Tokimatsu I, Nasu M. PCR detection of DNA specific for *Aspergillus* species in serum of patients with invasive aspergillosis. *J Clin Microbiol* **1996**; 34:2464–8.
- Yamakami Y, Hashimoto A, Yamagata E, et al. Evaluation of PCR for detection of DNA specific for *Aspergillus* species in sera of patients with various forms of pulmonary aspergillosis. *J Clin Microbiol* **1998**; 36: 3619–23.
- Williamson EC, Leeming JP, Palmer HM, et al. Diagnosis of invasive aspergillosis in bone marrow transplant recipients by polymerase chain reaction. *Br J Haematol* **2000**; 108:132–9.
- Ferns RB, Fletcher H, Bradley S, Mackinnon S, Hunt C, Tedder RS. The prospective evaluation of a nested polymerase chain reaction for the early detection of *Aspergillus* infection in patients with leukaemia or undergoing allograft treatment. *Br J Haematol* **2002**; 119:720–5.
- Challier S, Boyer S, Abachin E, Berche P. Development of a serum-based Taqman real-time PCR assay for diagnosis of invasive aspergillosis. *J Clin Microbiol* **2004**; 42:844–6.
- Löffler J, Hebart H, Sepe S, Schumcher U, Klingebiel T, Einsele H. Detection of PCR-amplified fungal DNA by using a PCR-ELISA system. *Med Mycol* **1998**; 36:275–9.
- Jones ME, Fox AJ, Barnes AJ. PCR-ELISA for the early diagnosis of invasive pulmonary *Aspergillus* infection in neutropenic patients. *J Clin Pathol* **1998**; 51:652–6.
- Bretagne S. Molecular diagnostics in clinical parasitology and mycology: limits of the current polymerase chain reaction (PCR) assays and interest of the real-time PCR assays. *Clin Microbiol Infect* **2003**; 9:505–11.
- Costa C, Vidaud D, Olivi E, Bart-Delabesse E, Vidaud M, Bretagne S. Development of two real-time quantitative TaqMan PCR assays to detect circulating DNA in serum. *J Microbiol Methods* **2001**; 44:263–9.
- Costa C, Costa JM, Desterke C, Botterel F, Cordonnier C, Bretagne S. Real-time PCR coupled with automated DNA extraction and detection of galactomannan antigen in serum by enzyme-linked immunosorbent assay for diagnosis of invasive aspergillosis. *J Clin Microbiol* **2002**; 40: 2224–7.
- García ME, Blanco JL, Caballero J, Gargallo-Viola D. Anticoagulants

- interfere with PCR used to diagnose invasive aspergillosis. *J Clin Microbiol* **2002**;40:1567–8.
33. Prentice HG, Kibbler CC, Prentice AG. Towards a targeted, risk-based, antifungal strategy in neutropenic patients. *Br J Haematol* **2000**;110:273–84.
 34. Podbielski A, Melzer B, Lutticken R. Application of the polymerase chain reaction to study the M protein(-like) gene family in beta-hemolytic streptococci. *Med Microbiol Immunol* **1991**;180:213–27.
 35. Marr KA, Balajee SA, McLaughlin L, Tabouret M, Bentsen C, Walsh TJ. Detection of galactomannan antigenemia by enzyme immunoassay for the diagnosis of invasive aspergillosis: variables that affect performance. *J Infect Dis* **2004**;190:641–9.
 36. Kawamura S, Maesaki S, Noda T, et al. Comparison between PCR and detection of antigen in sera for diagnosis of pulmonary aspergillosis. *J Clin Microbiol* **1999**;37:218–20.
 37. Kawazu M, Kanda Y, Nannya Y, et al. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and (1→3)- β -D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. *J Clin Microbiol* **2004**;42:2733–41.