British Journal of Haematology, 2003, **121**, 448–457

Galactomannan detection in computerized tomography-based broncho-alveolar lavage fluid and serum in haematological patients at risk for invasive pulmonary aspergillosis

Martin J. Becker,[1] Elly J. Lugtenburg,[2] Jan J. Cornelissen,[2] Cindy van der Schee,[1] Henk C. Hoogsteden[3] and Siem de Marie[1] [1]*Department of Medical Microbiology and Infectious Diseases,* [2]*Department of Haematology, and* [3]*Department of Pulmonology, Erasmus Medical Centre Rotterdam, the Netherlands*

Received 18 September 2002; accepted for publication 17 January 2003

Summary. We determined the value of galactomannan (GM) detection in computerized tomography (CT)-based broncho-alveolar lavage (BAL) fluid and serum for the diagnosis of invasive pulmonary aspergillosis (IPA) in haemato-oncological patients with neutropenia. CT of the thorax and BAL were performed systematically at predefined clinical indications. GM was determined by sandwich enzyme-linked immunosorbent assay; the clinicians were unaware of the results. Of 160 patients, 17 patients (10·6%) presented with proven, probable or suspected IPA. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of GM detection in CT-based BAL fluid were all 100%. For GM detection in serially sampled serum, the sensitivity was 47%, the specificity 93%, the PPV 73% and the NPV 82%. A non-blinded follow-up study was performed to validate these results. In this study, 22 of 198 patients (11·1%) presented with IPA, and the sensitivity, specificity, PPV and NPV of GM detection in CT-based BAL fluid were 85%, 100%, 100% and 88% respectively. None of BAL fluids obtained after antifungal treatment of 3 d or more were positive. These results indicate that, when CT is used systematically and at an early stage, GM detection in CT-based BAL fluid has a high PPV for diagnosing IPA early in untreated patients.

Keywords: aspergillosis, galactomannan, BAL, serum, CT.

Invasive pulmonary aspergillosis (IPA) remains a major challenge in the management of immunocompromised patients. In neutropenic patients, mortality rates range from 50% to 90% in different settings (Denning, 1996; Lin *et al,* 2001). This is probably due to the difficulty in obtaining a reliable diagnosis at an early stage and the relatively poor efficacy of the currently available antifungal armamentarium (Denning, 1996; De Marie 2000). The gold standard for the diagnosis of IPA is the histological demonstration of the fungus in a lung biopsy with concomitant fungal growth from the same specimen. However, biopsies are often precluded by thrombocytopenia or by the critical condition of the patient. Therefore, the diagnosis of IPA before death is mostly based on clinical signs, computerized tomography (CT) scan findings and culture of respiratory specimens. The general symptoms, primarily fever refractory to antibacterial therapy, chest pain, cough and dyspnoea, are variable and non-specific. CT of the chest has been advocated for the early diagnosis of IPA as it often shows a 'halo sign' in the early phase of the disease in neutropenic patients with IPA (Caillot *et al*, 1997, 2001; Denning *et al*, 1997). However, the halo sign is not specific for IPA as it is also seen in a number of other entities, including mucormycosis, organizing pneumonia and pulmonary haemorrhage (Won *et al*, 1998). The 'air-crescent sign' and other signs of cavitations are highly suggestive for invasive pulmonary fungal infection, but they often appear in a late stage of the disease, after bone marrow recovery. Microscopical examination or culture of respiratory specimens such as broncho-alveolar lavage fluid (BAL fluid) and sputum have limited sensitivity, and do not discriminate between invasive disease, colonization and contamination (Horvath & Dummer, 1996; Perfect *et al,* 2001). Hence, attention has focused on other ways to demonstrate IPA. In this respect, the detection of *Aspergillus* galactomannan (GM) in the serum, using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) (Stynen *et al*, 1995), has shown promising results

Correspondence: Martin J. Becker, Department of Medical Microbiology and Infectious Diseases, Erasmus Medical Centre Rotterdam, Dr Molewaterplein 50, 3015 GE Rotterdam, the Netherlands. E-mail: becker@kmic.fgg.eur.nl

(Maertens *et al*, 1999, 2001; Sulahian *et al*, 2001). The antigen can also be detected in urine (Dupont *et al*, 1987; Ansorg *et al*, 1994) or BAL fluid (Andrews & Weiner, 1982; Verweij *et al*, 1995a; Salonen *et al*, 2000). Few studies have compared GM detection in serum and BAL fluid in neutropenic patients (Verweij *et al*, 1997; Salonen *et al*, 2000).

In our institute, CT and BAL are routinely used for evaluating haemato-oncological patients at risk for IPA. In this clinical setting, we performed a study in two parts. In the first part, a blinded study investigated the value of GM detection in serum and BAL fluid, and their relation to CT, fungal culture, histopathology and antifungal treatment. In the second part, GM detection in CT-based BAL fluid was evaluated as a tool for diagnosing IPA in a non-blinded study.

PATIENTS, MATERIALS AND METHODS

Study population and design. The study consisted of two parts. First, between February 1999 and April 2000, we performed a prospective, blinded study. Thereafter, between June 2000 and October 2001, a prospective, non-blinded study was carried out. Both studies included haemato-oncological patients that had an expected neutropenia (less than $0{\cdot}5 \times 10^9$ cells/l) for at least 10 d and were aged at least 18 years. All patients received oral ciprofloxacin (500 mg twice daily) for selective bowel decontamination. In addition, all patients received either fluconazole (200 mg/d) or itraconazole (200 mg twice daily) as antifungal prophylaxis. During hospitalization, patients were evaluated for the development of fever and respiratory signs and symptoms. Physical examinations were carried out daily. Chest radiographs were performed at admission and once/twice weekly during hospitalization or every other day during periods of fever (temperature $> 38{\cdot}3$°C). In case of fever, broad-spectrum antibiotic treatment was administered. Chest CT's were performed when fever of unknown origin had lasted for 5 d of antibacterial treatment or when chest radiographs showed abnormalities. Broncho-alveolar lavage (BAL) was performed, if feasible, as soon as the CT showed abnormalities.

Antifungal treatment was started when new abnormalities emerged on chest radiograph under antibiotic treatment, when abnormalities compatible with invasive fungal infection were found on CT, when moulds were cultured from the respiratory tract, when blood cultures revealed fungi or when fever persisted under antibiotic treatment for 7 d.

In the first part of the study, serum samples were taken from all patients twice weekly, starting at the beginning of neutropenia. Sampling was stopped at the end of neutropenia or, in case of (possible) fungal infection, until the patient was discharged from hospital. Serum and BAL fluid samples were stored at -20°C. After the patients' discharge, the obtained BAL fluids and serum samples were tested for the presence of GM by a researcher who was unaware of the identity and clinical status of the patient. Clinicians were blinded from the laboratory results in this part of the study.

In the first part of the study, BAL's were performed in about half of the patients with IPA, because of cautiousness of the clinicians in performing the procedure in this fragile group of patients. To exclude selection bias, a second part of the study was performed in which BAL's were performed in all patients with abnormalities on CT, unless there was a strong contraindication. In this non-blinded investigation, BAL fluid samples were tested for GM within 3 d after the sample was obtained, and results were immediately reported back to the clinicians.

The study concerned the routine development of new methodologies in the laboratory and did not involve investigational drugs or additional sampling. Therefore, the study was considered by the ethics committee to be a quality-control investigation of the hospital and not experimentation with human beings that would require formal ethics review and informed consent. However, all patients signed a declaration that they allowed their clinical data and specimens to be used for research. The first part of the study was blinded and data regarding galactomannan detection were not used for patient management. After careful analysis of the data of the first part of the study, CT-based BAL's and galactomannan detection in BAL fluids were incorporated into the routine work-up of suspected patients, independent of the planning of the second part of the study.

Case definitions and classification. Invasive fungal infections were classified according to the European Organization for Research and Treatment of Cancer (EORTC) case definitions, with some modifications (Ascioglu *et al*, 2002). Results of GM detection were excluded from the criteria.

1. Proven IPA was defined as histopathological or cytopathological evidence of acutely branched, septated hyphae from a needle aspiration or biopsy with evidence of associated tissue damage (either microscopically or unequivocally by imaging) and a positive culture for *Aspergillus* sp. from sputum or BAL fluid. The isolation of *Aspergillus* sp. by a sterile procedure (transthoracic or open lung biopsy/needle aspiration from lungs) showing radiological abnormalities consistent with infection was also defined as proven IPA.
2. Probable IPA was defined as a positive culture for *Aspergillus* sp. from sputum or BAL fluid or cytopathology showing acutely branched, septated hyphae together with one major or two minor clinical criteria. Major clinical criteria included: (a) halo sign, (b) air-crescent sign or (c) cavitation on CT. Minor clinical criteria included: (a) symptoms of lower respiratory tract infection (cough, chest pain, haemoptysis or dyspnoea), (b) physical finding of pleural rub, (c) any new infiltrate not fulfilling a major criterion.
3. Suspected IPA was defined as one major clinical criterion together with negative bacterial and fungal cultures from specimens related to lower respiratory tract infection and no evidence for viral disease. Although this category is not included in the EORTC/Mycoses Study Group (MSG) criteria, there was consensus between the clinicians in our department that these patients should be classified as a separate group as they were more likely

to have IPA than the patients defined in the 'possible' IPA category and less likely than the patients defined in the 'probable' IPA category.

4. Possible IPA was defined as either (a) a positive culture or cytology for *Aspergillus* sp., (b) at least two minor clinical criteria together with negative bacterial and viral cultures from specimen related to lower respiratory tract infection.
5. Proven and probable other invasive fungal infections (IFI) were defined as analogous to the respective IPA categories, with the identification of filamentous fungi by culture or cytology, other than *Aspergillus* sp.

CT and classification. CT scans were performed using a Siemens Somaton Plus 4 scan. A scanning protocol was used in which the whole lung fields were scanned with 3-mm thick sections. All CT scans were evaluated after the study by two observers who were unaware of the clinical status of the patients.

Galactomannan (GM) detection. The sandwich ELISA was performed as described by Stynen *et al* (1995). Briefly, 300 μl of each serum or BAL fluid sample was used in the sandwich ELISA (Platelia Aspergillus, Sanofi Diagnostics Pasteur, Belgium). Positive and negative controls were included in each assay. All doubtful or positive samples were retested in parallel with recent samples in the next assay. The optical density (OD) index was calculated as the OD of the clinical sample divided by the OD of a control sample containing 1 ng/ml GM. As previously recommended (Verweij *et al*, 1998; Maertens *et al*, 2001), two subsequent serum samples with an index larger than 1·0 were considered positive. A BAL fluid sample was considered positive when the index was larger than 1·0, as recommended by others (Salonen *et al*, 2000; Siemann & Koch-Dorfler, 2001).

Cytology and histopathology. Cytology on BAL fluids was performed using calcofluor white (CFW) stain (Monheit *et al*, 1986). Histopathology was performed by staining tissue sections with haematoxylin–eosin (H&E) or Grocott's methenamine silver Grocott stain, with haematoxylin–eosin contra-stain (Grocott, 1955).

Statistical analysis. We defined the total group of patients with IPA as the sum of patients with proven IPA, probable IPA and suspected IPA. The total group of patients without IPA was defined as the patients that were not classified in any IPA category and did not receive empirical antifungal treatment. Calculations of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were based on these two groups.

RESULTS

Study part one: comparison of value of GM detection in serum and CT-based BAL fluid for diagnosing IPA

Patients' characteristics and diagnosis of IPA. Between February 1999 and April 2000, 160 patients were included, with a total of 249 neutropenic episodes. The median age of the patients was 49 years (range 18–79 years). Patients' characteristics are shown in Table I. A total of 17 patients (10·6% ± 2·5%) were diagnosed as having IPA. Two of these patients had proven IPA, 11 probable IPA and four suspected IPA. In nine patients, the causative organism was *Aspergillus fumigatus*, in one patient *A. flavus* and in one patient *A. niger*. In two patients, acutely branched, septated hyphae were seen in sputum and biopsy, indicating Aspergillus sp. In four patients with suspected IPA, clinical and radiological signs were strongly suggestive for IPA, whereas repeated cultures from respiratory specimens remained negative. Six out of 17 patients (35%) with IPA died during hospitalization. The cause of death in these patients was pulmonary bleeding due to aspergillosis ($n = 3$), cerebral aspergillosis ($n = 2$) and concomitant infection with *Pneumocystis carinii* ($n = 1$). In four patients, other invasive fungal infections were diagnosed: Rhizopus species ($n = 1$), Mucor species ($n = 1$), *Saccharomyces cerevisiae* ($n = 1$) and *Candida kruseï* ($n = 1$). One hundred and seventeen patients did not have a diagnosis of IFI.

CT scan. A total of 475 CT's were performed in all patients, of which 111 were performed in the 17 patients with IPA. Sixteen of 17 IPA patients showed halo signs on CT, most of them in the earliest stage of disease (Fig 1). In eight of the 17 IPA patients, a crescent or cavitation sign was observed, mostly during bone-marrow recovery. In 15 of the IPA patients, multiple nodular or wedge-shaped lesions were seen, mostly after several weeks of antifungal therapy. The first CT in the neutropenic episode, in which the IPA was diagnosed, showed a halo sign in 13 patients, multiple nodular lesions in two patients, non-specific abnormalities in one patient and no abnormalities in one patient.

In 74 of 117 patients without IFI, CT showed non-specific abnormalities, which were caused by bacterial or viral infections ($n = 27$), malignant lymphomas ($n = 24$), other ($n = 19$), or unknown aetiologies ($n = 4$). A halo sign was seen on the CT's of four patients, but IFI was discarded because culture or histopathology revealed lymphoma cells ($n = 2$), influenza ($n = 1$) and bacterial infection ($n = 1$). Nodular or wedge-shaped lesions were seen in two patients with pulmonary lymphoma. The CT's showed no abnormalities or were not performed in the remaining 43 patients.

GM detection in serum. A total of 1145 samples were tested for GM (mean 12·9 samples/patient). Of the 270 samples taken from patients with IPA, 26 (9·6%) were positive, with a median index of 1·891 (range 1·000–4·326). Eight of 17 IPA patients had two or more subsequent GM-positive samples ($2 \times \text{index} \geq 1{\cdot}0$). Only one patient with IPA had more than three subsequent positive sera. GM was detected in this patient for a period of more than 3 months; the antigenaemia eventually disappeared and the patient recovered (Fig 1). In the other seven patients, the antigenaemia was transient, lasting no longer than 1 week. In six of these seven patients, the antigenaemia disappeared under antifungal treatment, whereas in one patient antigenaemia disappeared spontaneously. GM was relatively more often detected in patients that died (four out of six, 66%) than in patients that survived (four out of 11, 36%), but this difference was not significant ($P = 0{\cdot}33$).

Table I. Characteristics and serum samples, BAL's and CT distribution of neutropenic patients included in a blinded study between February 1999 and April 2000.

						No IFI		
	Proven IPA	Probable IPA	Suspected IPA	Possible IPA	Other IFI*	Empiric Ampho B	No Ampho B	Total
Number of patients	2	11	4	18	4	4	117	160
Number of neutropenic episodes	3	20	6	28	9	6	177	249
Mean duration of neutropenic episode (d)	35	26	32	29	24	22	22	24
Underlying disorder (number of patients)								
Acute myeloid leukaemia	1	6	3	15	4	2	40	71
Acute lymphoid leukaemia	–	2	–	–	–	–	9	11
Chronic myeloid leukaemia	–	1	–	–	–	1	7	9
Myelodysplastic syndrome	1	1	1	1	–	1	2	7
Non-Hodgkin's lymphoma	–	1	–	1	–	–	29	31
Hodgkin's disease	–	–	–	–	–	–	5	5
Multiple myeloma	–	–	–	–	–	–	12	12
Other	–	–	–	1	–	–	13	14
Bone marrow transplant (number of patients)					–			
Autologous BMT	–	1	–	0	1	1	28	30
Allogeneic BMT	–	2	1	3	–	0	30	33
Number of patients with CT's of the chest	2	11	4	18	4	3	77	101
Mean number of CT's per patient	12	4·9	6·6	3·5	7·3	1·1	1·6	2·1
Number patients with halo/crescent or cavity on CT	2	11	4	–	3	–	4	21
Number of patients with nodular, wedge-shaped or pleura-based abnormalities on CT	2	9	4	18	4	–	2	39
Number of patients with CT's with non-specific abnormalities	–	1	1	5	–	3	74	84
Number of patients with no abnormalities on CT	–	–	1	3	–	2	42	48
Number of patients with BAL's	1	6	2	2	2	1	16	30
Mean number of BAL's/patient	2·0	0·6	0·5	0·2	0·5	0·3	0·1	0·2
Number of patients tested for serum GM	2	11	4	18	4	4	44	88
Mean number serum samples/patient	18·5	18·0	15·0	12·8	17·3	14·0	10·4	12·9

*Invasive pulmonary fungal infections other than IPA, proven and probable.

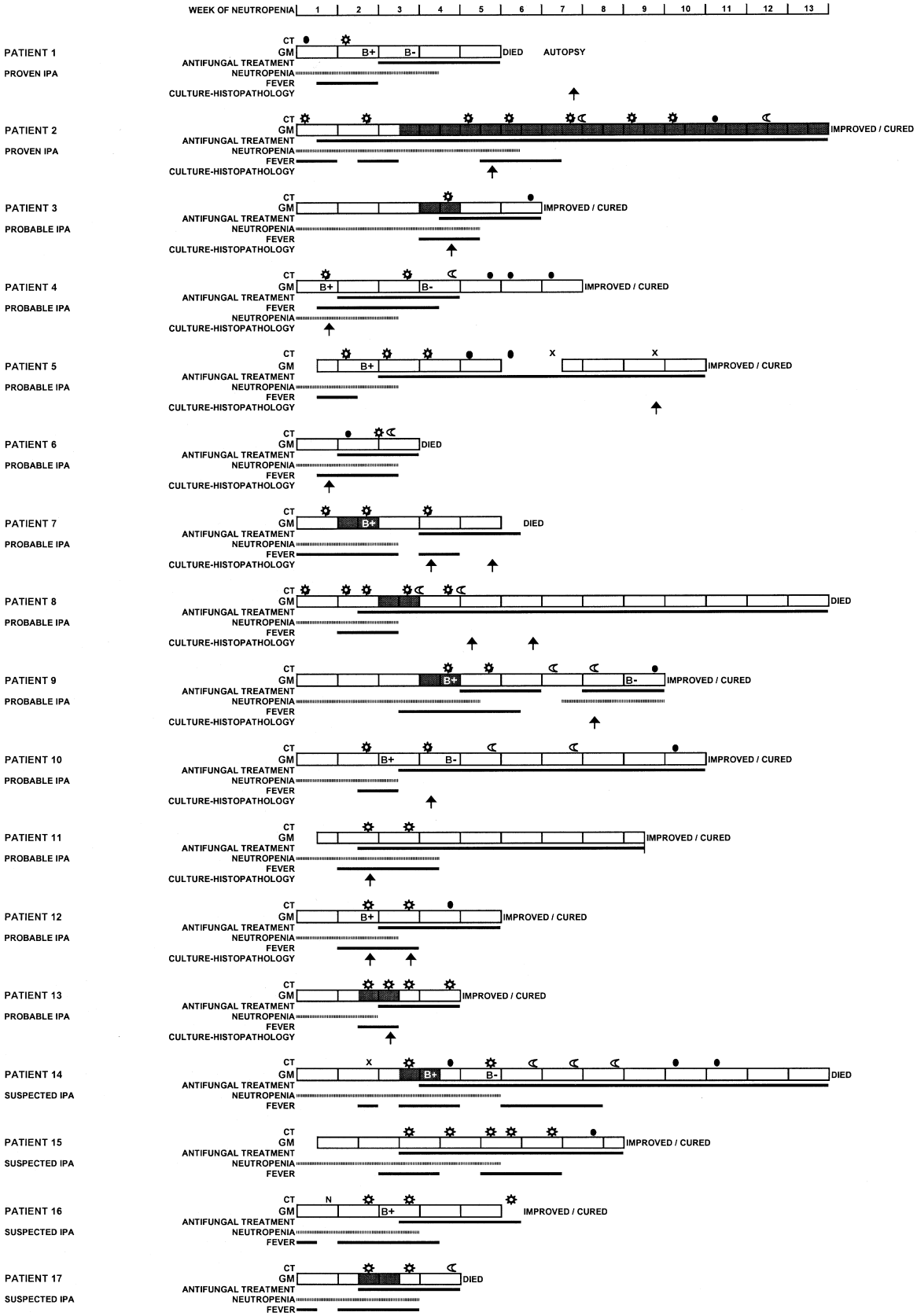
WEEK OF NEUTROPENIA
1
2
3
4
5
6
7
8
9
10
11
12
13
PATIENT 1
PROVEN IPA
PATIENT 2
PROVEN IPA
PATIENT 3
PROBABLE IPA
PATIENT 4
PROBABLE IPA
PATIENT 5
PROBABLE IPA
PATIENT 6
PROBABLE IPA
PATIENT 7
PROBABLE IPA
PATIENT 8
PROBABLE IPA
PATIENT 9
PROBABLE IPA
PATIENT 10
PROBABLE IPA
PATIENT 11
PROBABLE IPA
PATIENT 12
PROBABLE IPA
PATIENT 13
PROBABLE IPA
PATIENT 14
SUSPECTED IPA
PATIENT 15
SUSPECTED IPA
PATIENT 16
SUSPECTED IPA
PATIENT 17
SUSPECTED IPA
CT
GM
ANTIFUNGAL TREATMENT
NEUTROPENIA
FEVER
CULTURE-HISTOPATHOLOGY
B+
B-
N
X
DIED
AUTOPSY
IMPROVED / CURED

Fig. 1 Timing of CT, GM detection, antifungal treatment, fever and culture/histopathology in patients with IPA. Symbols: ☼ = halo sign, ☾ = crescent sign or cavitation, ⬮ = wedge-shaped or nodular abnormalities, × = non-specific abnormalities, N = no abnormalities. GM detection: □ = serum negative, ■ = serum positive, B+ = BAL fluid positive, B– = BAL fluid negative. Arrows: positive findings in culture/histopathology.

Of 18 patients with possible IPA, four patients were GM positive.

Of 44 patients without IFI that were tested for GM, three (7%) were positive. In a patient-based analysis, the sensitivity of the test using the above criterion was 47 ± 7% (SEM), the specificity 93 ± 5%, the PPV 73 ± 6% and the NPV 82 ± 5%. When other criteria were used, the results were less favourable (Table II).

To investigate the interlaboratory reproducibility of our results, 125 sera taken randomly from patients with IPA and 75 sera taken from patients with no IFI were retested in an external laboratory (Department of Medical Microbiology, University Medical Center Nijmegen, the Netherlands). The same results were found in 94% of the sera. In the external laboratory, one patient with IPA and one patient without IFI were found to be positive (2 × index ≥ 1·0) that had been tested negative in our own laboratory. In both patients, the disconcordance was based on one serum sample.

GM detection in CT-based BAL fluid. In part one of the study, a total of 36 BAL fluids were tested for GM. Of these, 14 BAL fluids were obtained from nine patients with IPA (see Table III). In all nine patients with IPA, at least one BAL fluid was positive for GM (index ≥ 1·0). In all patients, this was the first BAL fluid that was obtained in the neutropenic episode in which IPA was diagnosed (Fig 1). The five BAL fluids from the patients with IPA that were GM negative were either obtained after 1 or more weeks of antifungal treatment (amphotericin B or lipid formulation of amphotericin B in all patients) or obtained in another neutropenic episode than in the one in which IPA was diagnosed. Of the 14 BAL fluids obtained from patients with IPA, three produced a positive culture for *A. fumigatus* and one for *A. niger*. All these four culture-positive BAL fluids were also positive for GM. One BAL fluid was positive for both CFW and GM. Conversely, four BAL fluids were positive for GM, and both the culture and CFW were negative. Of the two patients with possible IPA that were tested, one had a BAL fluid positive for GM.

Nineteen BAL fluids were taken from 18 patients without IPA. GM was not detected in any of these BAL fluids. In the patient-based analysis, the sensitivity, specificity, PPV and NPV were all 100%, using the criterion that at least one BAL fluid should have an index ≥ 1·0.

Time interval between onset of fever, signs of infection on CT, antifungal treatment and GM detection. In patients with IPA, CT scans (halo/crescent or cavitation sign), serum GM, BAL fluid GM and culture/histopathology were positive on an average of 4·0 ± 0·8, 4·6 ± 1·6, 6·9 ± 1·1 and 14·8 ± 5·0 d, respectively, after the onset of fever relative to the emergence of IPA. Antifungal treatment was started an average 6 ± 1·4 d after the onset of fever.

Study part two: prospective investigation to confirm the value of GM detection in CT-based BAL fluid for diagnosing IPA

A total of 198 patients were included in this part of the study, between June 2000 and October 2001. The median age of the patients was 47 years (range 18–74 years). Twenty-two patients (11·1%) presented with IPA: three proven, 10 probable and nine suspected cases. *A. fumigatus* was the causative organism in eight patients, whereas *A. flavus* was found in the other culture-positive patient with IPA. A total of 453 CT's were performed, of which 95 were for 22 patients with IPA. Thirty of these 95 CT's showed a halo sign, 12 a crescent sign or cavitation, and 29 nodular or wedge-shaped lesions.

A total of 80 CT-based BAL fluids were tested for GM. Of these, 31 BAL fluids were obtained from 20 patients with IPA. In 17 of these 20 patients, at least one BAL fluid was positive for GM (Table IV). In 16 of these 17 patients, GM was detected in the first BAL fluid sample that was obtained in the neutropenic episode in which the IPA diagnosis was made, and in the second BAL fluid sample in the other patient. Four patients had more than one positive BAL fluid sample. However, all BAL fluids obtained after more than 2 d of antifungal treatment were GM negative. In all these patients, antifungal treatment consisted of amphotericin B or lipid formulations of amphotericin B. Of the 21 BAL fluids from patients with IPA that were GM positive, only six had cultures that were positive for *Aspergillus* sp. No BAL fluids were GM negative and culture or CFW positive. GM was not detected in any BAL fluid samples from three patients with IPA: one patient with probable and two with suspected IPA.

Thirteen patients had possible IPA. A total of 56 CT's were made in these patients, 32 of these showed nodular or wedge-shaped lesions, 21 showed abnormalities that were not suggestive for fungal infection and three showed no abnormalities. Twelve of 13 patients had nodular or wedge-shaped lesions on CT at some stage of the disease. The BAL fluid was GM positive in five of 12 patients. In three of these patients, the CT preceding the positive BAL fluid showed nodular or wedge-shaped lesions, in one patient abnormalities not suggestive for fungal infection and in one patient no abnormalities. Of 12 patients with possible IPA, five patients had a BAL fluid positive for GM. Three patients were diagnosed with pulmonary fungal infections other than IPA: two with *Candida albicans* and one with *C. kruse*. BAL fluids taken from these patients were all negative for GM.

A total of 33 BAL fluids were obtained from 23 patients without IFI. GM was not detected in any of these BAL fluids. In a patient-based analysis, the sensitivity of the test was 85 ± 8% SEM, the specificity and PPV were 100%, and the NPV was 88 ± 7%, using the criterion of at least one BAL fluid with an index ≥ 1·0.

Table II. Sensitivity, specificity and predictive values of GM detection in serum according to different criteria for assessment for positivity.

	Proven, probable and suspected IPA			
	Sensitivity ± SEM	Specificity ± SEM	PPV	NPV
Criterion positive				
1 × index > 0·7	59%	61%	36%	80%
2 × index > 0·7	53%	89%	64%	84%
1 × index > 1·0	59%	75%	48%	83%
2 × index > 1·0	47%	93%	73%	82%
1 × index > 1·5	18%	84%	30%	73%
2 × index > 1·5	12%	95%	50%	74%

Table III. GM detection in serum and BAL fluid from neutropenic patients included in a blinded study between February 1999 and April 2000.

		GM detection in serum		GM detection in BAL fluid	
	No. patients	No. patients tested	No. patients positive (2 × index > 1·0)	No. patients tested	No. patients positive (1 × index > 1·0)
Proven IPA	2	2	1 (50%)	1	1 (100%)
Probable IPA	11	11	5 (45%)	6	6 (100%)
Suspected IPA	4	4	2 (50%)	2	2 (100%)
Possible IPA	18	18	4 (22%)	2	1 (50%)
Empiric ampho B	4	4	1 (25%)	1	0 (0%)
Other IFI*	4	4	0 (0%)	2	0 (0%)
No IFI	117	44	5 (11%)	16	0 (0%)

*Invasive pulmonary fungal infections other than IPA, proven and probable.

Table IV. GM detection in BAL fluid from neutropenic patients included in a non-blinded study between June 2000 and October 2001.

	GM detection in BAL fluid		
	No patients	No patients tested	No patients positive (1 × index > 1·0)
Proven IPA	3	3	3 (100%)
Probable IPA	10	9	8 (89%)
Suspected IPA	9	8	6 (75%)
Possible IPA	13	12	5 (42%)
Empiric ampho B	2	2	0 (0%)
Other IFI*	3	2	0 (0%)
No IFI	158	21	0 (0%)

*Invasive pulmonary fungal infections other than IPA, proven and probable.

Combined calculation of values of GM detection in BAL fluid in part one and two of the study

Analysing the combined data of all 108 patients included between February 1999 and October 2001 in which one or more BAL's were performed, the sensitivity for GM detection in CT-based BAL fluid was 90 ± 5·6%, the specificity and PPV were 100%, and the NPV was 93 ± 4%.

DISCUSSION

The diagnosis of IPA remains a difficult task, especially in neutropenic patients. Early diagnosis is of great importance as early start of antifungal treatment improves survival (von Eiff *et al*, 1995). CT has been recently recognized as an important tool for diagnosing IPA. Caillot *et al* (1997) suggested that the systematic use of CT scans enabled the earlier diagnosis of IPA and significantly improved survival when combined with early antifungal therapy. However, the use of CT scans seems to differ between different centres. For example, in some studies, CT's were performed in only a minority of IPA patients (Williamson *et al,* 2000; Siemann & Koch-Dorfler 2001), whereas Caillot *et al* (1997) performed CT's in all 25 patients with IPA, with a mean of almost three CT's per patient. In our study, CT's were performed systematically and early in all patients with IPA, with a mean of 5·3 CT's per patient, and starting within 4 d after onset of fever.

GM detection for diagnosing IPA has been investigated in several studies (Dupont *et al*, 1987; Verweij *et al*, 1995a,b; Rohrlich *et al*, 1996; Maertens *et al*, 1999, 2001; Salonen *et al,* 2000; Siemann & Koch-Dorfler 2001; Sulahian *et al,* 2001). The antigen can be detected by a latex agglutination test or by a sandwich ELISA, the latter of which has shown the higher sensitivity (Verweij *et al*, 1995b; Sulahian *et al*, 1996).

Recently, several large, prospective studies in neutropenic patients reported sensitivities, specificities and predictive values above 90% for GM detection by ELISA in serum (Maertens *et al*, 1999, 2001; Sulahian *et al,* 2001). In our study, these characteristics were less favourable: we found a sensitivity of GM detection in serum of 47%, a specificity of 93%, a PPV of 73% and a NPV of 82%. Differences in definitions of IPA may partly explain this disparity. Another reason for the observed difference may be that early and systematic use of CT in our study produced less advantageous characteristics of the test. Early detection of suspected lesions on CT might have resulted in an earlier start of antifungal treatment. As antifungal treatment has been shown to suppress circulating GM levels (Rohrlich *et al*, 1996) and we observed that GM-positive serum often became negative as soon as antifungals were started, the early start of treatment may have prevented detectable antigenaemia in a number of patients. With a PPV of 73% and a NPV of 82%, GM detection in serum may still have some additional value for diagnosing IPA in the individual patient in our clinical setting. However, these values were only achieved when serum was sampled serially twice per week, and using the optimal criterion of two subsequent indexes $\geq 1{\cdot}0$.

A very recent, large study in oncological patients also reported less favourable test characteristics of GM detection in serum (Herbrecht *et al,* 2002), i.e. a sensitivity of 32%, a specificity of 95%, a PPV of 58% and a NPV of 85%. In this report, the presence in patients of circulating anti-Aspergillus antibodies was suggested as a possible cause for the relatively low sensitivity and PPV.

In contrast to GM detection in serum, we found a surprisingly high sensitivity (100%) and specificity (100%) for GM detection in CT-based BAL fluid in the first part of our study. To confirm these figures, a second investigation was started, in which BAL's were performed more frequently. Overall, the sensitivity of GM detection in CT-based BAL fluid was 90%, the specificity 100%, the PPV of 100% and NPV of 93%. However, these figures were based on BAL fluids obtained before the start of antifungal treatment. All BAL fluids obtained from patients that had received less than 2 d of antifungal treatment, and most BAL fluids obtained after ceasing antifungal treatment were negative. This indicates that BAL for GM detection should be performed early, promptly after CT and before the start of antifungal treatment. Possibly, the use of antifungal treatment could explain the somewhat lower sensitivities of GM detection in BAL fluid found in other studies, in which the use of antifungals before or during BAL was not reported (Verweij *et al*, 1995a; Salonen *et al,* 2000). In accordance with our findings, Caillot *et al* (1997, 2001) found higher sensitivities for GM detection in BAL fluid (73–83%) than in serum (41–45%), using the latex agglutination test, in a clinical setting with early and systematic CT use.

A number of studies have reported the use of polymerase chain reaction (PCR) in BAL fluid for the diagnosis of IPA (Verweij *et al*, 1995a; Hayette *et al,* 2001; Buchheidt *et al,* 2002; Raad *et al,* 2002). However, the PPV of this test for diagnosing IPA may be compromised by the fact that it does not distinguish between infection and colonization (Hayette *et al,* 2001; Raad *et al,* 2002). This problem may be less when GM detection is used, as was suggested by the high PPV in our study. In addition, our animal model of IPA showed that GM detection in BAL fluid was more often positive during invasive fungal growth than during colonization, in contrast to PCR (Becker *et al,* 2000).

We also investigated the time interval between onset of fever, first halo sign on CT, antigen detection and culture/histopathology in the first part of our study. The CT was already positive 4 d after onset of fever and GM was detected in BAL fluid after 7 d. In comparison, culture/histopathology became positive only 15 d after onset of fever on average. This indicates that a diagnostic strategy in which GM detection in CT-based BAL is used results in an earlier confirmed diagnosis of IPA compared with classical methods.

We conclude that, when CT is used early and systematically, GM detection in CT-based BAL fluid has a high PPV for diagnosing neutropenia-associated IPA in an early phase of the disease. BAL fluids should be obtained before the start of antifungal treatment, as GM detection becomes negative under antifungal treatment. GM detection in serum seems to be of less value, as its sensitivity and PPV are limited.

ACKNOWLEDGMENTS

We thank Paul Verweij (Department of Medical Microbiology, University Medical Center Nijmegen, Nijmegen, the Netherlands) for his help with retesting the serum samples.

REFERENCES

Andrews, C.P. & Weiner, M.H. (1982) Aspergillus antigen detection in bronchoalveolar lavage fluid from patients with invasive aspergillosis and aspergillomas. *American Journal of Medicine*, **73**, 372–380.

Ansorg, R., Heintschel von Heinegg, E. & Rath, P.M. (1994) Aspergillus antigenuria compared to antigenemia in bone marrow transplant recipients. *European Journal of Clinical Microbiology and Infectious Diseases*, **13**, 582–589.

Ascioglu, S., Rex, J.H., de Pauw, B., Bennett, J.E., Bille, J., Crokaert, F., Denning, D.W., Donnelly, J.P., Edwards, J.E., Erjavec, Z., Fiere, D., Lortholary, O., Maertens, J., Meis, J.F., Patterson, T.F., Ritter, J., Selleslag, D., Shah, P.M., Stevens, D.A. & Walsh, T.J. (2002) Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clinical Infectious Diseases*, **34**, 7–14.

Becker, M.J., de Marie, S., Willemse, D., Verbrugh, H.A. & Bakker-Woudenberg, I.A. (2000) Quantitative galactomannan detection is superior to PCR in diagnosing and monitoring invasive pulmonary Aspergillosis in an experimental rat model. *Journal of Clinical Microbiology*, **38**, 1434–1438.

Buchheidt, D., Baust, C., Skladny, H., Baldus, M., Brauninger, S. & Hehlmann, R. (2002) Clinical evaluation of a polymerase chain reaction assay to detect Aspergillus species in bronchoalveolar lavage samples of neutropenic patients. *British Journal of Haematology*, **116**, 803–811.

Caillot, D., Casasnovas, O., Bernard, A., Couaillier, J.F., Durand, C., Cuisenier, B., Solary, E., Piard, F., Petrella, T., Bonnin, A., Couillault, G., Dumas, M. & Guy, H. (1997) Improved management of invasive pulmonary aspergillosis in neutropenic patients using early thoracic computed tomographic scan and surgery. *Journal of Clinical Oncology*, **15**, 139–147.

Caillot, D., Couaillier, J.F., Bernard, A., Casasnovas, O., Denning, D.W., Mannone, L., Lopez, J., Couillault, G., Piard, F., Vagner, O. & Guy, H. (2001) increasing volume and changing characteristics of invasive pulmonary Aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. *Journal of Clinical Oncology*, **19**, 253–259.

De Marie, S. (2000) New developments in the diagnosis and management of invasive fungal infections. *Haematologica*, **85**, 88–93.

Denning, D.W. (1996) Therapeutic outcome in invasive aspergillosis. *Clinical Infectious Diseases*, **23**, 608–615.

Denning, D.W., Evans, E.G., Kibbler, C.C., Richardson, M.D., Roberts, M.M., Rogers, T.R., Warnock, D.W. & Warren, R.E. (1997) Guidelines for the investigation of invasive fungal infections in haematological malignancy and solid organ transplantation. British Society for Medical Mycology. *European Journal of Clinical Microbiology and Infectious Diseases*, **16**, 424–436.

Dupont, B., Huber, M., Kim, S.J. & Bennett, J.E. (1987) Galactomannan antigenemia and antigenuria in aspergillosis: studies in patients and experimentally infected rabbits. *Journal of Infectious Diseases*, **155**, 1–11.

von Eiff, M., Roos, N., Schulten, R., Hesse, M., Zuhlsdorf, M. & van de Loo, J. (1995) Pulmonary aspergillosis: early diagnosis improves survival. *Respiration*, **62**, 341–347.

Grocott, R.G. (1955) A stain for fungi in tissue sections and smears during Gomori's methenamine-silver nitrate technique. *American Journal of Clinical Pathology*, **25**, 975–979.

Hayette, M.P., Vaira, D., Susin, F., Boland, P., Christiaens, G., Melin, P. & De Mol, P. (2001) Detection of Aspergillus species DNA by PCR in bronchoalveolar lavage fluid. *Journal of Clinical Microbiology*, **39**, 2338–2340.

Herbrecht, R., Letscher-Bru, V., Oprea, C., Lioure, B., Waller, J., Campos, F., Villard, O., Liu, K.L., Natarajan-Ame, S., Lutz, P., Dufour, P., Bergerat, J.P. & Candolfi, E. (2002) Aspergillus galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. *Journal of Clinical Oncology*, **20**, 1898–1906.

Horvath, J.A. & Dummer, S. (1996) The use of respiratory-tract cultures in the diagnosis of invasive pulmonary aspergillosis. *American Journal of Medicine*, **100**, 171–178.

Lin, S., Schranz, J. & Teutsch, S. (2001) Aspergillosis case-fatality rate: systematic review of the literature. *Clinical Infectious Diseases*, **32**, 358–366.

Maertens, J., Verhaegen, J., Demuynck, H., Brock, P., Verhoef, G., Vandenberghe, P., Van Eldere, J., Verbist, L. & Boogaerts, M. (1999) Autopsy-controlled prospective evaluation of serial screening for circulating galactomannan by a sandwich enzyme-linked immunosorbent assay for hematological patients at risk for invasive Aspergillosis. *Journal of Clinical Microbiology*, **37**, 3223–3228.

Maertens, J., Verhaegen, J., Lagrou, K., Van Eldere, J. & Boogaerts, M. (2001) Screening for circulating galactomannan as a non-invasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood*, **97**, 1604–1610.

Monheit, J.G., Brown, G., Kott, M.M., Schmidt, W.A. & Moore, D.G. (1986) Calcofluor white detection of fungi in cytopathology. *American Journal of Clinical Pathology*, **85**, 222–225.

Perfect, J.R., Cox, G.M., Lee, J.Y., Kauffman, C.A., de Repentigny, L., Chapman, S.W., Morrison, V.A., Pappas, P., Hiemenz, J.W. & Stevens, D.A. (2001) The impact of culture isolation of Aspergillus species: a hospital-based survey of aspergillosis. *Clinical Infectious Diseases*, **33**, 1824–1833.

Raad, I., Hanna, H., Huaringa, A., Sumoza, D., Hachem, R. & Albitar, M. (2002) Diagnosis of invasive pulmonary Aspergillosis using polymerase chain reaction-based detection of Aspergillus in BAL(*). *Chest*, **121**, 1171–1176.

Rohrlich, P., Sarfati, J., Mariani, P., Duval, M., Carol, A., Saint-Martin, C., Bingen, E., Latge, J.P. & Vilmer, E. (1996) Prospective sandwich enzyme-linked immunosorbent assay for serum galactomannan: early predictive value and clinical use in invasive aspergillosis. *Pediatric Infectious Diseases Journal*, **15**, 232–237.

Salonen, J., Lehtonen, O.P., Terasjarvi, M.R. & Nikoskelainen, J. (2000) Aspergillus antigen in serum, urine and bronchoalveolar lavage specimens of neutropenic patients in relation to clinical outcome. *Scandinavian Journal of Infectious Diseases*, **32**, 485–490.

Siemann, M. & Koch-Dorfler, M. (2001) The Platelia Aspergillus ELISA in diagnosis of invasive pulmonary aspergillosis (IPA). *Mycoses*, **44**, 266–272.

Stynen, D., Goris, A., Sarfati, J. & Latge, J.P. (1995) A new sensitive sandwich enzyme-linked immunosorbent assay to detect galactofuran in patients with invasive aspergillosis. *Journal of Clinical Microbiology*, **33**, 497–500.

Sulahian, A., Tabouret, M., Ribaud, P., Sarfati, J., Gluckman, E., Latge, J.P. & Derouin, F. (1996) Comparison of an enzyme immunoassay and latex agglutination test for detection of galactomannan in the diagnosis of invasive aspergillosis. *European Journal of Clinical Microbiology and Infectious Diseases*, **15**, 139–145.

Sulahian, A., Boutboul, F., Ribaud, P., Leblanc, T., Lacroix, C. & Derouin, F. (2001) Value of antigen detection using an enzyme immunoassay in the diagnosis and prediction of invasive aspergillosis in two adult and pediatric hematology units during a 4-year prospective study. *Cancer*, **91**, 311–318.

Verweij, P.E., Latge, J.P., Rijs, A.J., Melchers, W.J., De Pauw, B.E., Hoogkamp-Korstanje, J.A. & Meis, J.F. (1995a) Comparison of

antigen detection and PCR assay using bronchoalveolar lavage fluid for diagnosing invasive pulmonary aspergillosis in patients receiving treatment for hematological malignancies. *Journal of Clinical Microbiology*, **33**, 3150–3153.

Verweij, P.E., Stynen, D., Rijs, A.J., de Pauw, B.E., Hoogkamp-Korstanje, J.A. & Meis, J.F. (1995b) Sandwich enzyme-linked immunosorbent assay compared with Pastorex latex agglutination test for diagnosing invasive aspergillosis in immunocompromisedpatients. *Journal of Clinical Microbiology*, **33**, 1912–1914.

Verweij, P.E., Dompeling, E.C., Donnelly, J.P., Schattenberg, A.V. & Meis, J.F. (1997) Serial monitoring of Aspergillus antigen in the early diagnosis of invasive aspergillosis. Preliminary investigations with two examples. *Infection*, **25**, 86–89.

Verweij, P.E., Erjavec, Z., Sluiters, W., Goessens, W., Rozenberg-Arska, M., Debets-Ossenkopp, Y.J., Guiot, H.F. & Meis, J.F. (1998) Detection of antigen in sera of patients with invasive aspergillosis: intra- and interlaboratory reproducibility. The Dutch Inter-university Working Party for Invasive Mycoses. *Journal of Clinical Microbiology*, **36**, 1612–1616.

Williamson, E.C., Oliver, D.A., Johnson, E.M., Foot, A.B., Marks, D.I. & Warnock, D.W. (2000) Aspergillus antigen testing in bone marrow transplant recipients. *Journal of Clinical Pathology*, **53**, 362–366.

Won, H.J., Lee, K.S., Cheon, J.E., Hwang, J.H., Kim, T.S., Lee, H.G. & Han, J. (1998) Invasive pulmonary aspergillosis: prediction at thin-section CT in patients with neutropenia: a prospective study. *Radiology*, **208**, 777–782.