

# Galactomannan in Bronchoalveolar Lavage Fluid

## A Tool for Diagnosing Aspergillosis in Intensive Care Unit Patients

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**Rationale:** Invasive aspergillosis (IA) is an important cause of mortality in patients with hematologic malignancies. However, IA appears to be gaining a foothold in the intensive care unit (ICU) in patients without classical risk factors. A recent study described 89 cases of IA in patients in a medical ICU without leukemia or cancer. The diagnosis of IA remains difficult and is often established too late. Galactomannan (GM) is an exo-antigen released from *Aspergillus* hyphae while they invade host tissue.

**Objectives:** This prospective single-center study was conducted to investigate the role of GM in bronchoalveolar lavage (BAL) fluid as a tool for early diagnosis of IA in the ICU.

**Methods:** All patients with risk factors identified in our earlier study were evaluated. BAL for culture and GM detection, serum GM levels, and computed tomography scan were obtained for all included patients with signs of pneumonia. Patients were classified as having proven, probable, or possible IA.

**Measurements and Main Results:** A total of 110 patients out of 1,109 admissions were eligible. There were 26 proven IA cases. Using a cutoff index of 0.5, the sensitivity and specificity of GM detection in BAL fluid was 88 and 87%, respectively. The sensitivity of serum GM was only 42%. In 11 of 26 proven cases, BAL culture and serum GM remained negative, whereas GM in BAL was positive.

**Conclusions:** IA is common in immunocompromised, critically ill patients. GM detection in BAL fluid seems to be useful in establishing or excluding the diagnosis of IA in the ICU.

**Keywords:** *Aspergillus*; bronchoscopy; intensive care unit; galactomannan; immunosuppression

Invasive aspergillosis (IA) has emerged as an important cause of morbidity and mortality in transplant recipients and in patients with hematologic disorders (1–3). Recent data indicate that IA may be an underestimated opportunistic fungal infection in critically ill patients, even in the absence of hematologic malignancy (4–8). Several autopsy studies from intensive care unit (ICU) patients confirm that invasive fungal infections are among the most common missed diagnoses (9, 10). Patients with chronic obstructive pulmonary disease (COPD), patients with cirrhosis, and patients receiving steroids are especially at risk and were not included in the original criteria for definition of proven and probable IA according to the widely accepted European Organization for Research and Treatment of Cancer/Mycoases Study Group guidelines (11, 12).

(Received in original form April 20, 2007; accepted in final form September 14, 2007)

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This article has an online supplement, which is accessible from this issue's table of contents at [www.atsjournals.org](http://www.atsjournals.org)

Am J Respir Crit Care Med Vol 177, pp 27–34, 2008

Originally Published in Press as DOI: 10.1164/rccm.200704-606OC on September 20, 2007  
Internet address: [www.atsjournals.org](http://www.atsjournals.org)

### AT A GLANCE COMMENTARY

#### Scientific Knowledge on the Subject

Aspergillosis is an increasingly recognized problem in ICU patients, but diagnosis remains challenging.

#### What This Study Adds to the Field

The use of galactomannan in bronchoalveolar lavage fluid as a means of establishing early diagnosis of invasive aspergillosis in critically ill patients at risk is promising.

Establishing a diagnosis of IA at an early stage of disease is necessary for successful treatment but challenging in patients with hematologic diseases, and even more difficult to achieve in the poorly defined, broad group of critically ill patients at risk. Definite diagnosis is rarely established before death or before overwhelming fungal proliferation. The utility of radiology (e.g., high-resolution computed tomography [CT] scan with the “halo” sign) is of limited value in mechanically ventilated patients (13).

Tissue biopsy as a means of making a definite diagnosis is not without risk in the critically ill patient, and its sensitivity is unknown. Conventional diagnostic tests, such as culture and microscopy of respiratory tract samples, have only a sensitivity and specificity of around 50% (14, 15).

Galactomannan (GM) is a polysaccharide fungal cell wall component that is released during tissue invasion by *Aspergillus* hyphae and that can be detected in body fluids. The presence of  $\beta$ -lactam antibiotics, such as piperacillin–tazobactam may give rise to false-positive results. GM serum levels have been evaluated most extensively in allogeneic bone marrow or stem cell transplant recipients and/or neutropenic patients (16, 17). Data on the performance of GM detection in serum are sparse in nonneutropenic patients but suggest that serum GM is probably not a good marker for IA in these patients (18, 19).

Sandwich ELISA methods have also been used to detect GM in bronchoalveolar lavage (BAL) fluid of neutropenic patients, yielding sensitivities between 76 and 100% (20, 21). The use of GM detection in BAL samples may be also useful in the subset of patients without neutropenia, as demonstrated in an experimental model of IA (22).

To investigate the role of GM in BAL for the diagnosis of IA in the ICU, we conducted a prospective trial in critically ill patients at risk for IA and compared the diagnostic performances of GM detection in BAL, radiologic signs, culture results, and serum GM detection.

### METHODS

#### Study Population and Data Collection

Between July 2005 and December 2006, all patients older than 18 years admitted to our medical ICU were screened for inclusion in a prospective study.

Eligible patients displayed at least one of the following host factors:

1. A hematologic malignancy, unless they were already treated with antifungals for a presumed or proven IA
2. Cancer and receiving chemotherapy within the last 3 months before admission
3. Solid organ transplant recipient
4. Steroid use: at least 4 mg methylprednisolone (or equivalent) a day for at least 7 days in the past 3 weeks before admission *or* during the course of the ICU stay for at least 5 days *or* a cumulative dose of at least 250 mg of methylprednisolone (or equivalent) in the past 3 months before enrollment
5. Recipient of any other immunosuppressive treatment (tacrolimus, cyclosporine, methotrexate, cyclophosphamide, sirolimus)
6. Child C cirrhosis
7. HIV

In addition, eligible patients could only be enrolled if they had at least two of the three following features:

- Fever refractory to at least 3 days of appropriate antibiotics or fever relapsing after a period of defervescence of at least 48 hours while still receiving antibiotics
- Clinical signs and/or symptoms suggestive of invasive mycosis: pleuritic chest pain or physical finding of pleural rub, or one of the following symptoms of lower respiratory tract infection (new sputum secretions, dyspnea, or hemoptysis)
- Development of new pulmonary infiltrates on chest X-ray

Three patients who did not have any of the prespecified host factors were also included in the study because of a positive tracheal surveillance culture (performed once weekly) for *Aspergillus* spp.

The following information was stored in a data file: patients' characteristics, including age, sex, medical history, and reason for ICU admission; standard ICU laboratory findings; presence or absence of fever and/or neutropenia (if applicable); and use of immunosuppressive drugs (steroids and others). Disease severity at admission in the ICU was assessed by the Simplified Acute Physiology Score II (SAPS II).

The sandwich ELISA assay for GM detection (Platelia *Aspergillus*; Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France) was performed as per the manufacturer's instructions and modified to a semi-automated protocol (23) (see the online supplement).

Fiberoptic bronchoscopy with BAL (2 × 20 ml) was performed upon inclusion and weekly thereafter if feasible. The sampling area was selected based on the infiltrate location on the chest radiograph. The presence of any tracheal or bronchial lesions was recorded by the endoscopist (W.M.). Lavage samples were submitted for direct microscopic examination, GM detection, and bacterial, fungal, and mycobacterial cultures. An optical density (OD) ratio of 0.5 or greater for GM in serum and BAL was considered positive.

Serum sampling for GM detection was done twice weekly. Clinicians remained blinded throughout the study from the BAL results for GM, but serum indices of 0.5 or more were reported.

Antifungal treatment was started at the discretion of the attending physician and was not protocol defined.

Positive GM levels in patients taking piperacillin-tazobactam or amoxicillin-clavulanate were analyzed separately.

A pulmonary CT scan was done (if feasible) between Day 1 and 3 after inclusion or, in case of clinical deterioration, 1 week after enrollment.

Autopsies were pursued in all fatal cases unless there was an explicit refusal of the family.

The study was approved by the ethics committee and written, informed consent was obtained from the patient or next of kin.

### Case Definitions of IA

Patients were classified as having proven, probable, or possible IA, based on the standardized Invasive Fungal Infections Group of the European Organization for the Research and Treatment of Cancer/

Mycoses Study Group case definitions (12), with the modification that cirrhosis, COPD, and steroids were added to the host factor section. Detection of GM in serum and BAL was not included as a microbiological criterion. Proven IA referred to the histopathologic evidence of tissue invasion by septated, acutely branching filamentous fungi together with a positive culture. Probable IA referred to the presence of a positive culture or cytology for *Aspergillus* species from BAL fluid together with one major (halo sign, air-crescent sign, or cavity within an area of consolidation on CT scan) or two of three minor clinical criteria (symptoms of lower respiratory tract infection, pleural rub, new infiltrate without an alternative diagnosis). The same criteria for probable IA were used to classify cases as "probable invasive fungal infection [IFI]" with the modification that the culture and/or cytology was positive for non-*Aspergillus* molds. Possible IA was defined by the presence of a host factor and either a positive culture or one major (or two minor) clinical criteria. Fatal cases who showed no histopathologic signs of IA upon autopsy were classified as "no IA" and patients with proven non-*Aspergillus* mold infections were classified as "proven IFI." Patients without a host factor and with positive culture for *Aspergillus* from a nonsterile site, but without any other evidence of fungal infection, were considered to be colonized.

### Statistical Analysis

Data are expressed as means ± SD, and were compared as follows: continuous variables with the Mann-Whitney U test; categorical variables with the chi-square test; *P* values less than 0.05 were considered significant. A *t* test was used to compare paired data (culture, serum, and BAL GM values). A Kruskal-Wallis test was used to compare GM values between the different groups (proven, probable, possible, no IA). Sensitivity of diagnostic techniques was calculated from proven cases. The specificity was calculated from a biopsy- or necropsy-verified negative group (including those with proven non-*Aspergillus* mold infections). Receiver operating characteristic (ROC) curves were constructed to illustrate differences in performance of BAL GM, and serum GM. An additional ROC analysis has been performed in the group of patients who underwent an autopsy (see paragraph "Autopsy verified cases" in the online supplement).

## RESULTS

### Patient Characteristics

From July 2005 to December 2006, 1,109 patients were admitted to our ICU. All 110 patients (10%) fulfilling the inclusion criteria were enrolled. The characteristics of the overall study group are shown in Table 1. Thirty-six (33%) patients suffered from a hematologic malignancy, whereas 74 (67%) had other immunocompromising factors. Only 24 patients (22%) were neutropenic. Mean SAPS II score was 53 (±15). The ICU mortality rate of 66% was higher than the predicted risk of death based on the SAPS II score (52%). After *post mortem* examination in 69 (95%) of 73 fatalities, study patients were classified as proven IA (n = 26, 24%), probable IA (n = 8, 7%), possible IA (n = 27, 24%), proven non-*Aspergillus* mold infection (n = 3, 3%), colonization (n = 3, 3%), and no IA (n = 43, 39%). Only 4 of the 26 proven cases were diagnosed as proven IA before death (Figure 1).

### Test Characteristics

**BAL GM levels.** On average, bronchoscopy with BAL was performed 6 days after admission to the ICU. A total of 156 procedures were performed. All 26 patients with proven IA had at least one BAL GM index ≥ 0.5 (range, 0.6–7.9). In 23 patients, the first BAL sample yielded a positive value, while in the remaining 3 patients, only the second BAL GM index was ≥ 0.5 (Table 2). The number of BAL GM-positive patients in the truly negative group (n = 46) was 6. As depicted in Figure 2A, the levels of GM in BAL were significantly higher in the

**TABLE 1. BASELINE CHARACTERISTICS OF 110 CRITICALLY ILL PATIENTS AT RISK FOR INVASIVE ASPERGILLOSIS**

Characteristics	Patients with						Total
	Proven IA	Probable IA	Possible IA	No IA	Proven IFI	Colonization	
No. of patients (%)	26 (24)	8 (7)	27 (25)	43 (39)	3 (3)	3 (2)	110
No. of deaths (%)	24 (92)	1 (12)	3 (11)	43*	2 (66)	0 (0)	73 (66)
No. of autopsies (%)	24 (100)	0 (0)	0 (0)	43*	2 (100)	—	69 (95)
Age, mean, yr	62	57	55	64	59	50	60
Sex, no. male/no. female	15/11	6/2	17/10	32/11	2/1	2/1	74/36
SAPS II, mean	57	49	48	55	58	30	53
Length of stay, mean, d	13	5	19	16	13	30	16
Mechanical ventilation (%)	25 (96)	5 (63)	23 (85)	40 (93)	3 (100)	3 (100)	99 (90)
Neutropenia (%)	10 (38)	1 (12)	2 (7)	9 (21)	2 (66)	0 (0)	24 (22)
Steroids (%)	15 (58)	4 (50)	10 (37)	21 (49)	3 (100)	—	53 (48)
No. with disease							
Hematologic (%)	11 (42)	3 (38)	7 (26)	13 (30)	2	—	36 (33)
GVHD	5	1	2	3	2	—	13
Nonhematologic (%)	15 (60)	5 (62)	20 (74)	30 (70)	1	3	74 (67)
Cirrhosis	3	1	10	9	—	—	23
COPD	2	2	5	6	—	—	15
Solid organ transplants	2	—	2	1	1	—	6
Systemic diseases	4	2	—	10	—	—	16
Solid cancer	4	—	1	3	—	—	8
Other	—	—	2	1	—	3	6
BAL culture: <i>Aspergillus</i>	15 (60)	6 (75)	0 (0)	11 (26)	—	3 (100)	35 (26)
No. of samples tested							
GM serum	125	16	97	129	15	12	397
Mean no. of samples (per patient)	4.8	2	3.6	3.1	5	4	3.6
GM BAL	38	10	41	59	6	4	158
Mean no. of samples (per patient)	1.5	1.25	1.5	1.37	2	1.3	1.4

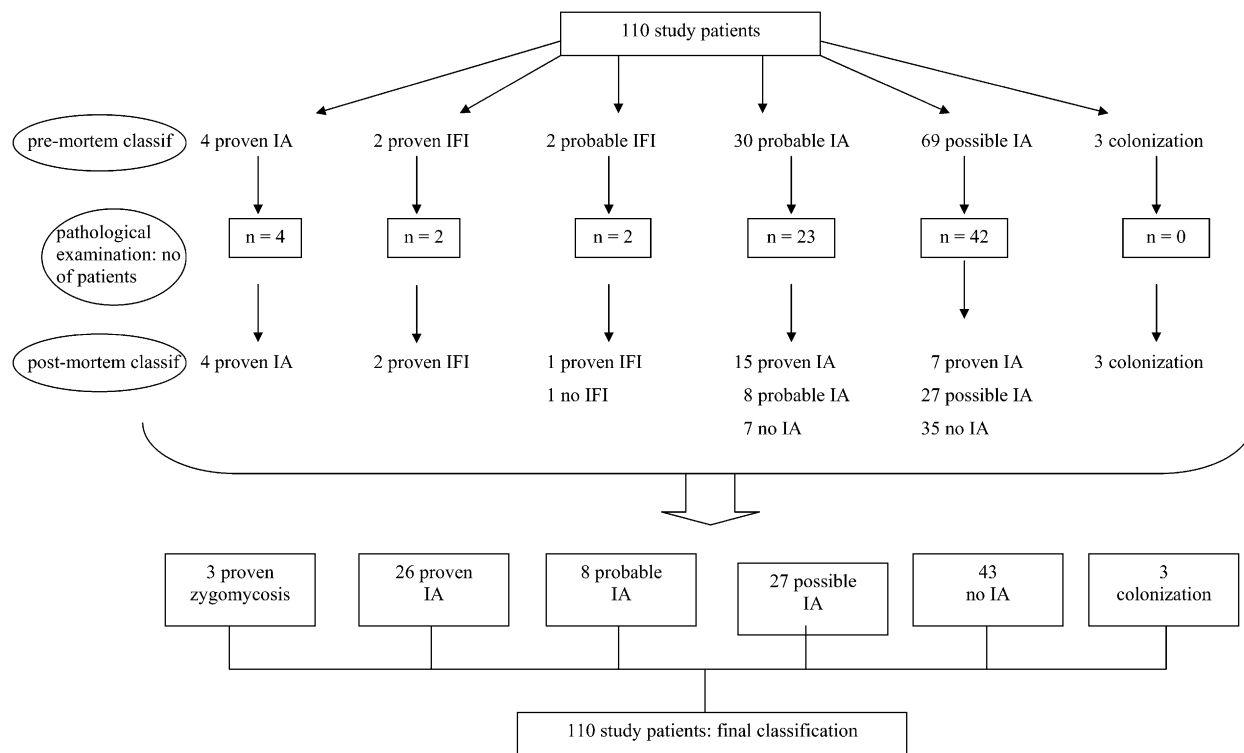
Definition of abbreviations: BAL = bronchoalveolar lavage; COPD = chronic obstructive pulmonary disease; GM = galactomannan; GVHD = graft-versus-host disease; IA = invasive aspergillosis; ICU = intensive care unit; IFI = invasive fungal infection (non-*Aspergillus* molds); SAPS II = Simplified Acute Physiology Score II.

\* By definition, all cases died and were autopsied.

proven group (median value, 4.1) than in the truly negative group (median value, 0.1;  $P < 0.0005$ ).

**Serum GM levels.** A total of 397 serum samples were tested for GM (mean, 3.6 samples per patient). A total of 125 samples were

analyzed from 26 patients with proven IA; 44% of these serially analyzed samples tested positive. The median value of GM in serum in the proven cases was 0.3 (Figure 2B). Fourteen patients did not have a serum index  $\geq 0.5$  at the time of BAL positivity.



**Figure 1.** Classification of study patients before and after *post mortem* examination. *classif* = classification.

TABLE 2. PATIENT CHARACTERISTICS AND DOCUMENTATION OF PROVEN IA CASES

Patient No.	Patient characteristics								Culture and/or Microscopic Examination. (BAL)*	HRCT Lesions	Macro Lesions†	ELISA (ng/ml)				Antifungal R/‡	Histo Evidence
	Age	LOS	Predicted Mortality (%)	Host Factor	Admission Diagnosis	Neutropenia (<500/mm <sup>3</sup> )	Outcome	Di				BAL D1	Serum D1	BAL D8	Serum D8		
1	50	17	60	AML, GVHD	Encephalopathy	No	Death	4	No	NA	No	4.3	0.1	NA	NA	No	Autopsy
2	35	13	41	CML, GVHD	ARDS	No	Death	6	No	ARDS	No	0.3	0	3.9	0.7	No	Autopsy
3	74	7	77	CLL	ARDS	Yes	Death	4	Yes	ARDS	Yes	6.9	0.7	NA	NA	Caspo	Biopsy + aut
4	67	7	48	Cirrhosis	Peritonitis	No	Survived	6	Yes	NA	Yes	3.9	0	NA	NA	Ampho	Biopsy
5	79	4	95	ALL	Pneumonia	Yes	Death	2	Yes	NA	No	4.1	1.7	NA	NA	No	Autopsy
6	49	17	41	Steroids	Encephalopathy	No	Death	4	Yes	NA	No	3.4	0.5	NA	NA	Vorico	Autopsy + LP
7	77	15	70	Steroids, ILD	ARDS	No	Death	2	No	ARDS	No	0.1	0.1	2.2	0.1	No	Autopsy
8	53	68	55	Kidney Tx, steroids	Renal failure + peritonitis	No	Survived	6	Yes	Necrotizing	Yes	6.2	0.1	5.7	0.1	Caspo	Biopsy
9	64	14	60	AML	Pneumonia	Yes	Death	4	No	NA	No	0.1	0.6	2.7	0.1	Vorico	Autopsy
10	74	25	37	Steroids	Stevens-Johnson	No	Death	10	Yes	Nodular	No	3.4	0.1	6.2	0.1	No	Autopsy
11	47	21	87	Solid cancer, neutropenia	Enterocolitis	Yes	Death	8	Yes	Nodular	Yes	5.3	1.7	NA	NA	Caspo + Vorico	Autopsy
12	68	12	74	Solid cancer, neutropenia	Enterocolitis	Yes	Death	9	Yes	NA	No	5.7	3.2	NA	NA	Ampho	Autopsy
13	74	6	48	Liver Tx, steroids	Pneumonia	No	Death	3	Yes	Necrotizing	No	4.1	0.1	NA	NA	Liposomal ampho	Autopsy
14	78	19	8	Steroids	COPD	No	Death	4	Yes	Necrotizing	No	3.5	0.2	1.2	0.2	Caspo	Autopsy
15	48	8	53	Solid cancer, steroids	Cardiac arrest	No	Death	3	No	Necrotizing	No	0.6	0.1	NA	NA	no	Autopsy
16	48	5	53	AML	ARDS	Yes	Death	2	No	ARDS	No	3.6	0.1	NA	NA	No	Autopsy
17	76	11	94	CLL	Septic shock	Yes	Death	2	No	NA	Yes	7.4	0.2	NA	NA	No	Autopsy
18	60	9	20	Steroids (myasthenia)	Pneumonia	No	Death	3	Yes	Necrotizing	No	8.0	7.5	NA	NA	Vorico	Autopsy
19	70	3	37	Steroids, COPD	COPD	No	Death	4	Yes	NA	No	6.8	0.1	NA	NA	No	Autopsy
20	61	7	81	Cirrhosis	Septic shock, peritonitis	No	Death	4	Yes	NA	No	6.6	5	NA	NA	Caspo	Autopsy
21	74	8	46	Solid cancer	Pneumonia	No	Death	3	No	Necrotizing	No	4.6	0.1	NA	NA	No	Autopsy
22	60	7	72	Cirrhosis	Hepatorenal syndrome	No	Death	6	Yes	NA	No	5.6	0.1	NA	NA	No	Autopsy
23	60	6	44	CLL, GVHD	GVHD lung, liver	Yes	Death	3	Yes	Necrotizing	Yes	5.8	2.6	NA	NA	Caspo	Autopsy
24	44	16	99	AML, GVHD	GVHD liver, skin	Yes	Death	8	No	ARDS	No	7.9	0.6	NA	NA	Caspo	Autopsy
25	60	4	27	AML	Pneumonia	Yes	Death	5	No	Nodular	No	1.0	0.4	0.4	0.4	No	Autopsy
26	66	13	64	AML, GVHD	GVHD, liver, skin, lung	No	Death	5	No	NA	Yes	2.6	1.4	1.4	0.4	Vorico	Autopsy

Definition of abbreviations: ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; ampho = Amphotericin B; ARDS = adult respiratory distress syndrome; Aut = autopsy; BAL = bronchoalveolar lavage; Caspo = caspofungin; CLL = chronic lymphatic leukemia; COPD = chronic obstructive pulmonary disease; D1 = day of inclusion; D8 = 8 days after inclusion; Di = inclusion on Day x after admission; F = female; GVHD = graft-versus-host disease; HRCT = high-resolution computed tomography; ILD = interstitial lung disease; LOS = length of stay; LP = lumbar puncture; M = male; NA = not available; Tx = transplant recipient; Vorico = voriconazole.

\* Yes/no: denotes the presence/absence of *Aspergillus* in culture or branching hyphae visible through Gomori stain.

† Macro lesions: lesions seen during bronchoscopy and compatible with invasive aspergillosis.

‡ Antifungal R/ = antifungal treatment.

**Culture results and direct microscopic examination of BAL fluid.** Overall, 35 patients (26%) had a positive culture for *Aspergillus* and/or a positive direct examination. Among the proven cases, 15 of 26 (58%) had a positive culture and/or direct examination. In 10 proven cases, both tests were positive, whereas in 4 other proven cases only direct examination was positive, and in 1 only *Aspergillus* growth was present. An average of 72 hours were required for laboratory processing before definitive culture results were available.

**Diagnostic value of GM assay in BAL fluid.** The performance characteristics of GM detection in BAL compared with serum GM detection, culture, and direct examination results were assessed with an ROC curve analysis (Figure 3). The area under the ROC, when a cutoff OD index of 0.5 for GM in BAL on Day 1 was used to diagnose IA, was 0.898 (95% confidence interval, 0.811 to 0.985). At an OD index cutoff value of 0.5, GM detection in BAL had a sensitivity of 88% and a specificity of

87%. Overall, on Day 1, GM in BAL fluid performed significantly better in diagnosing IA than GM in serum and BAL culture or direct examination ( $P < 0.0005$  and  $P = 0.003$ , respectively). GM in BAL was positive in 11 of the 26 proven cases, whereas the BAL culture and the direct examination remained negative (Table 3). Moreover, the median value of GM in BAL on Day 1 for the proven cases was significantly higher than the serum value on the same day (4.1 vs. 0.3,  $P < 0.005$ ). In proven cases with a positive culture, mean BAL GM value was 5.0, compared with 3.3 in proven cases with a negative culture.

**False positivity of GM in serum and/or BAL.** Overall, six patients had false-positive tests in the truly negative group (13%). Four of these six patients were treated with antifungals before death. GM values were higher in BAL than in serum.

When an index cutoff of 0.5 was applied, 4 of 33 patients treated with piperacillin-tazobactam had a positive serum and

**TABLE 3. GALACTOMANNAN AND CULTURE RESULTS IN 72 PATHOLOGY-CONTROLLED CASES\***

	No. of Patients		
	Invasive Aspergillosis (n = 26)	No Invasive Aspergillosis <sup>†</sup> (n = 46)	Total
Serum galactomannan, no. <sup>‡</sup>			
Positive	11	3	14
Negative	15	43	58
Total	26	46	72
BAL galactomannan, no. <sup>‡</sup>			
Positive	23	6	29
Negative	3	40	43
Total	26	46	72
BAL culture, direct examination, no. <sup>§</sup>			
Positive (%)	15 (58)	14 (30)	29
Negative (%)	11 (42)	32 (70)	43
Total	26	46	72

Definition of abbreviation: BAL = bronchoalveolar lavage.

\* Seventy-two cases subdivided in 69 deceased patients who underwent an autopsy (24 proven invasive aspergillosis [IA] cases, 2 proven zygomycosis cases, and 43 "no IA" cases) and three survivors with a lung biopsy (2 proven IA cases and 1 proven zygomycosis).

<sup>‡</sup> Cutoff value for positivity: 0.5 ng/ml, only the galactomannan value of the first bronchoscopy and the serum value at the same day was taken into account.

<sup>†</sup> No invasive aspergillosis = no hyphal tissue invasion with *Aspergillus* spp or tissue invasion with non-*Aspergillus* molds.

<sup>§</sup> Fourteen patients in the proven "no IA" category had positive culture and/or direct examination results. Among those were two patients in which *Zygomycetes* were seen on direct microscopic examination.

BAL GM result. The false-positive values in BAL ranged from 1.3 to 5.8, and from 0.7 to 2.8 in serum. Results remained positive until 4 days after the antibiotic had been stopped. No patients were treated with amoxicillin-clavulanate.

**Comparison of proven cases with and without neutropenia (n = 26).** We determined whether the diagnostic tools for IA performed equally well in proven cases with and without classical risk factors (Table 4). Neutropenia was present in 10 patients (38%). The other 16 proven cases were treated with steroids (n = 13) for various reasons (graft-versus-host disease, COPD, solid organ transplantation, acute respiratory distress syndrome, septic shock, or systemic diseases) or had cirrhosis (n = 3) as underlying risk factor (Table 1). Patients with neutropenia tended to have higher baseline SAPS II score upon admission (60 vs. 55,  $P = 0.063$ ) and were at greater risk of dying (100 vs. 88%,  $P = 0.18$ ). The length of stay was significantly shorter for patients with neutropenia (median, 7 vs. 13 d;  $P = 0.014$ ). The sensitivity of mycological culture and/or direct examination of BAL was lower in the proven cases with neutropenia (50%) versus nonneutropenic patients (63%). GM testing on BAL (on Day 1) performed equally well in the two groups (GM index  $\geq 0.5$  in 90% of patients with neutropenia vs. 88% of patients without neutropenia,  $P = 0.53$ ). GM levels in serum performed significantly better in patients with neutropenia (GM index  $\geq 0.5$  in 70 vs. 25%;  $P < 0.05$ ).

Thoracic CT scan performed in 15 proven cases displayed no halo or air-crescent sign, not even in neutropenic patients. Most patients had nonspecific radiologic findings (nodular lesions, necrotizing cavities) (see the online supplement for illustrations).

All deceased patients in the proven group underwent autopsy (n = 24). The histopathologic pattern of IA in steroid-treated patients and patients with cirrhosis differed from neutropenic patients. In the latter group, inflammation in the lung was less pronounced and fungal burden was higher than in patients without neutropenia.

**TABLE 4. CLINICAL CHARACTERISTICS OF NEUTROPENIC AND NONNEUTROPENIC PATIENTS WITH PROVEN INVASIVE ASPERGILLOSIS**

Characteristics	Neutropenic Patients (n = 10)	Nonneutropenic Patients (n = 16)	All Proven IA Cases (n = 26)
No. of males (%)	4 (40)	10 (63)	14 (54)
Age, mean yr	62	62	62
Clinical characteristics			
Fever, no. of patients (%)	8 (80)	10 (63)	18 (69)
Respiratory failure requiring MV, no. (%)	10 (100)	15 (94)	25 (96)
Length of stay, no. of days (range)	7 (4–21)	13 (3–68)	13 (3–68)
Macroscopic lesions,* no. of patients (%)	4 (40)	3 (19)	7 (27)
CT rate, no. of patients (%)	6 (60)	9 (56)	15 (58)
Necrotizing pneumonia on CT scan <sup>†</sup> (%)	1 (17)	6 (67)	7 (47)
Halo sign on CT scan (%)	0 (0)	13 (0)	0 (0)
Steroids, no. of patients (%)	2 (20)	3 (81)	15 (58)
Cirrhosis (%)	0 (0)	55 (19)	3 (12)
SAPS II <sup>‡</sup>	60	55	57
Predicted mortality, %	71	62	
Outcome			
Survival, no. of patients (%)	0 (0)	2 (12)	2 (8)
Lung autopsy (n = 25) and/or biopsy results			
Strong inflammation, low fungal burden	1/10 (10)	13/16 (81)	14/26 (54)
Scant inflammation, high fungal burden	9/10 (90)	3/16 (19)	12/26 (46)
Sensitivity of test, n/N (%) <sup>§</sup>			
BAL culture or direct examination positive	5/10 (50)	10/16 (63)	15/26 (60)
<i>Aspergillus</i> GM BAL	9/10 (90)	14/16 (88)	23/26 (88)
<i>Aspergillus</i> GM serum	7/10 (70)	4/16 (25)	11/26 (42)
Median GM value in BAL Day 1 (range)	5.5 0.1–7.9	4.0 0.1–8.0	4.3 0.1–8.0
Median GM value in serum Day 1 (range)	0.7 0.1–5.0	0.1 0.0–7.5	0.3 0.0–7.5

Definition of abbreviations: BAL = bronchoalveolar lavage; CT = computed tomography; GM = galactomannan; IA = invasive aspergillosis; MV = mechanical ventilation; SAPS II = Simplified Acute Physiology Score II.

\* Macroscopic lesions were defined as ulcerative or pseudomembranous lesions in the trachea or the bronchi visible during the bronchoscopy.

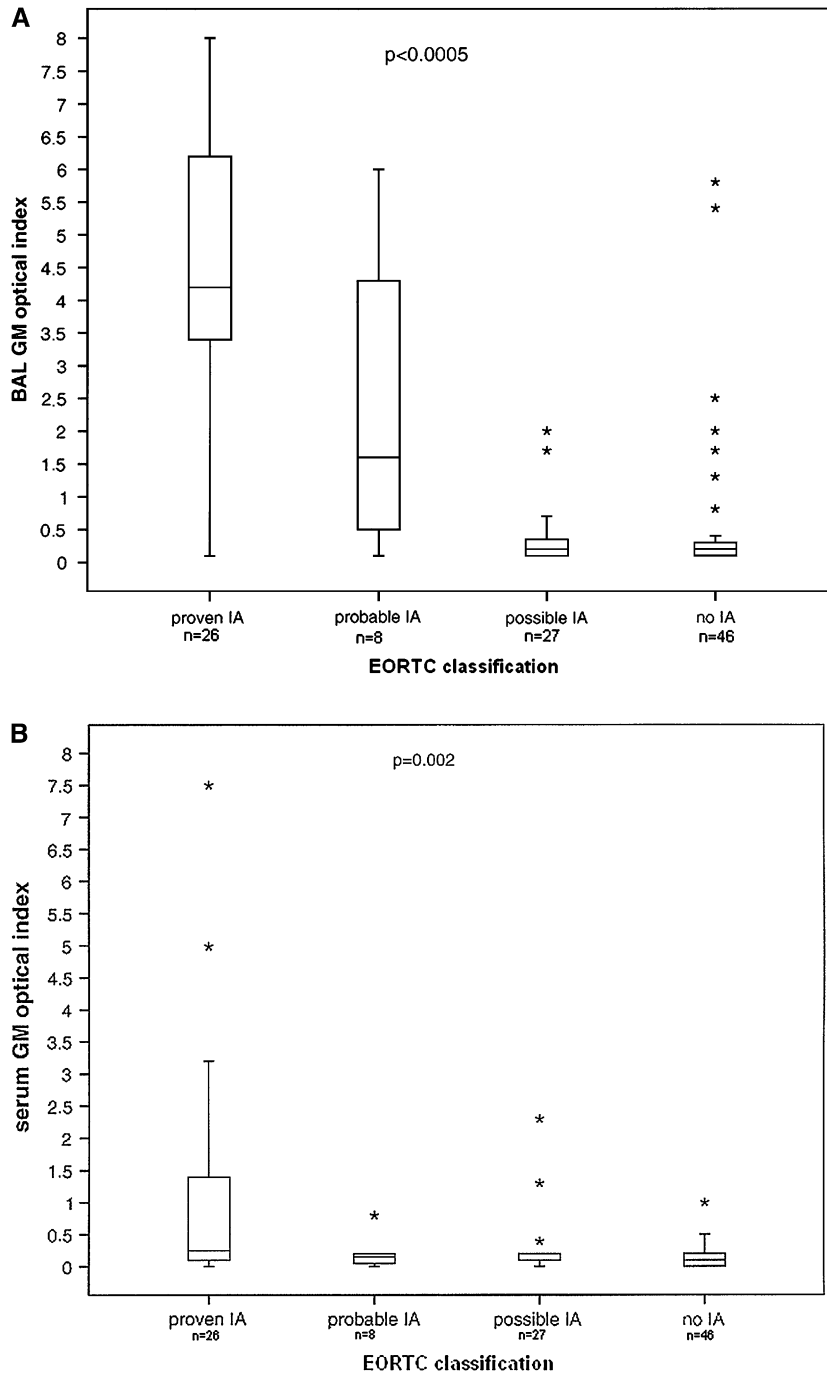
<sup>†</sup> See online supplement for CT and chest X-ray illustrations of some studied patients.

<sup>‡</sup> SAPS II is a score of severity of illness in critically ill patients (range between 12 and 163, with predicted mortalities between 1.3 and 100%, respectively).

<sup>§</sup> Applying an optical density index cut off = 0.5.

## DISCUSSION

To the best of our knowledge, this is the first study that analyzes the diagnostic contribution of GM detection in BAL in ICU. Our findings support the utility of determining GM levels in BAL fluid of critically ill patients at risk for IA. The sensitivity of GM detection was 88% in proven cases when calculated on the first BAL and applying a cutoff value of 0.5. The contribution of serum GM to the diagnosis of IA in our ICU population was much lower (sensitivity, 42%). The specificity of GM detection in serum and BAL was high even in patients treated with piperacillin-tazobactam (96 and 87%, respectively; cutoff, 0.5 ng/ml). Overall, the performance of fungal culture and/or direct examination on BAL was only moderate for the diagnosis of IA (sensitivity, 58% for the proven cases). CT features, such as the halo sign, proved to be of no value in the ICU, even in the neutropenic patients. The histopathologic pattern of IA in autopsy specimens confirmed earlier reports



**Figure 2.** (A) Distribution of bronchoalveolar lavage galactomannan (BAL GM) results on Day 1 of inclusion. (B) Distribution of serum GM results on Day 1 of inclusion. EORTC = European Organization for the Research and Treatment of Cancer; IA = invasive aspergillosis. *P* values by Kruskal-Wallis test. Boxes show interquartile range; whiskers show 95% confidence intervals. EORTC criteria include the "classical" host factors proposed by Asciglu and colleagues (12) and three additional ICU-related host factors (cirrhosis, COPD, and steroids). Asterisks represent outliers.

that steroid patients had more inflammation and less fungal burden in the lungs than neutropenic patients (24).

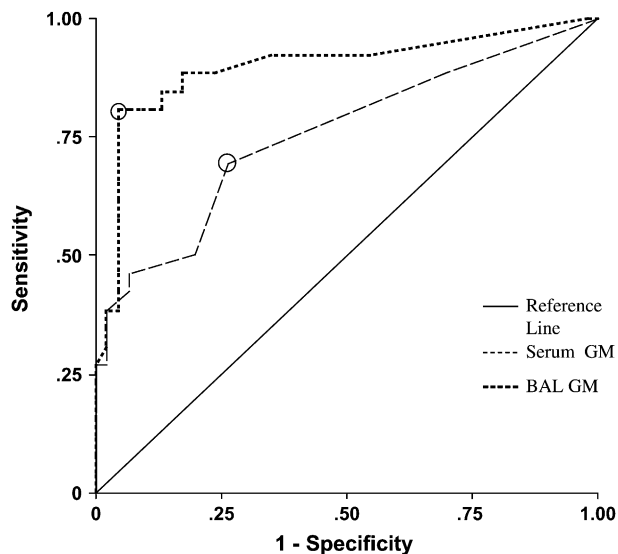
Recent studies indicate that IA may be considered an emerging problem in critically ill patients (4–8). Patients receiving steroids and patients with COPD or liver cirrhosis are especially at risk.

The diagnosis of IA remains difficult. Clinical signs are frequently lacking in mildly immunosuppressed patients. The main problem with the fungal culture as a diagnostic tool is its limited performance and the delay in diagnosis of 48 to 72 hours. Early diagnosis is of great importance because early start of antifungal treatment improves survival (25).

The use of antigen markers have been studied in an effort to improve rapidity and performance of the diagnostic procedure. GM is a polysaccharide cell wall component that is released by

*Aspergillus* during growth. Several large, prospective studies in neutropenic patients reported sensitivities above 90% for GM detection by ELISA in serum (17). However, most ICU patients who are at risk for IA are not neutropenic (78% of the patients in the current study). In addition, it has been hypothesized that neutrophils are capable of clearing GM from the blood by its mannose-binding receptors (17). This might explain the lower sensitivity of serum GM levels (50%) for the diagnosis of IA in our previous study (6). Therefore, we decided to focus on the lungs, reflecting the primary site of entry of *Aspergillus* conidia.

Other studies examining GM values in BAL fluid have mainly been done in liver and lung transplant recipients and showed rather poor sensitivities (30%) (18, 19). A possible explanation is the less severe disease status of the patients included in these studies and the lack of histologic data in most



**Figure 3.** Receiver operating characteristic (ROC) curves for galactomannan (GM) detection in bronchoalveolar lavage (BAL) (vs. serum GM) graphing sensitivity (true positive results) versus 1-specificity (true negative results) using multiple optical density index cutoff values to define positivity. Areas under the ROC curves were 0.898 (95% confidence interval, 0.81–0.96) for a GM BAL cutoff value of 0.5, and 0.755 (95% confidence interval, 0.63–0.82) for a GM serum cutoff value of 0.5.

patients. In patients with hematologic malignancies, better sensitivities have been reported (20, 21).

The excellent sensitivity of GM detection in the BAL fluid might be counterbalanced by a loss of specificity due to a greater occurrence of false-positive results. Although several sources of false positivity are frequently encountered in the ICU (piperacillin–tazobactam or amoxicillin–clavulanate, plasma-lyte infusions) (17), the specificity was still above 85%, both for serum and BAL.

CT scan is an important tool for diagnosis of IA in neutropenic patients (13). Typical lesions, such as the halo sign, were not seen in our study. This observation was confirmed by another study, reflecting the fact that the halo sign is only seen in early IA (4). Moreover, many confounding factors (e.g., pleural effusions and ventilator-associated pneumonia) are present in ICU patients and obscure the presence of characteristic signs.

One of the strengths of our study is the high autopsy rate of 95%. This enabled us to discriminate between invasive disease and colonization. As such, we could demonstrate that in 9 of 26 patients with proven IA who were screened before death, antifungal therapy might have been given if physicians had been aware of the GM value in the BAL fluid.

We do realize that the study design contains limitations. First, the optimal timing for sampling the patient at risk is prone to subjective interpretation and is much more difficult to determine in the broad group of critically ill patients than in previous studies confined to patients with hematologic diseases. Obvious entry criteria, such as 10 days of neutropenia or presence of graft-versus-host disease, used in previous studies cannot be implemented in the broad group of patients admitted to the ICU. Second, because our referral unit cares for patients with severe medical disease only, it is difficult to extrapolate our results to other ICUs. Finally, we cannot provide data on the influence of antifungal therapy on the performance of GM detection in BAL fluid, because the patients did not receive antifungals at the time of the first bronchoscopy.

In summary, IA appears to be an important problem in patients admitted to a medical ICU. The use of GM in BAL fluid as a means of establishing early diagnosis of IA in critically ill patients at risk is very promising. The validity of the data needs to be confirmed in other ICUs. If so, critical care physicians do have a helpful instrument to decide in which circumstances antifungal therapy should be initiated early.

**Conflict of Interest Statement:** W.M. has participated as a speaker for Pfizer and Merck, Sharp and Dohme (MSD). K.L. has participated as a speaker in a scientific meeting organized and financed by Pfizer. J.M. has been a consultant and received lecture fees from AstraZeneca, Schering Plough, MSD, BioRad Laboratories, Pfizer, Johnson and Johnson, and Amgen. A.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. G.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.V. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. I.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. E.V. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. E.V.W. has participated as a speaker and consultant for Pfizer and MSD.

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