Pediatric Pulmonology 48:789–796 (2013)

Diagnosis of Invasive Pulmonary Aspergillosis in Children with Bronchoalveolar Lavage Galactomannan

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Summary. Background: Invasive pulmonary aspergillosis (IPA) is a life-threatening complication in immunocompromised patients. Early diagnosis and therapy improves outcome. Assessment of galactomannan (GM) in bronchoalveolar lavage (BAL) fluid is a proposed tool to diagnose IPA. Little is known about the diagnostic value of BAL GM in children. Materials and Methods: Retrospectively, 72 bronchoscopies were analyzed for GM in patients fulfilling the host factor criteria as defined by the EORTC/MSG. A cut-off index value GM of $\geq$0.5 was used. Clinical data, results of chest CT-scans and BAL cultures were collected. Results: Sensitivity, specificity, PPV, and NPV of BAL GM for a diagnosis of proven and probable IPA (n = 41) were 82.4%, 87.5%, 82.4%, and 87.5% respectively. A significant relation was found for BAL GM and abnormal chest CT ($P = 0.01$). No significant relationship was observed between BAL *Aspergillus* sp. culture and chest CT (n = 47). BAL GM and serum GM correlated significantly. In 9 out of 12 patients classified as possible IPA, antifungal therapy was continued or started, despite a negative BAL GM. Conclusions: BAL GM test had good diagnostic value in children suspected of IPA. However, the decision to continue or start antifungal therapy was mainly determined by the clinical suspicion of IPA based on chest CT-outcome, serum GM index values and failure of antibiotic therapy. **Pediatr Pulmonol. 2013; 48:789–796.**

Key words: galactomannan; aspergillosis; bronchoalveolar lavage; children.

Funding source: none reported.

INTRODUCTION

Invasive Pulmonary Aspergillosis (IPA) is a frequent and increasing cause of morbidity and mortality in immunocompromised patients. To improve the outcome of these often fatal infections, early diagnosis of IPA is of utmost importance.[1]

To diagnose IPA, clinical signs and symptoms and the results of various direct and indirect diagnostic tests, including chest CT, antigen testing and cultures from respiratory specimens, are combined to improve the likelihood of IPA. However, it remains difficult to establish a diagnosis of IPA with certainty. Clinical signs, cultures, and direct microscopy of sputum or

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Conflict of interest: None.

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Received 7 April 2012; Accepted 12 July 2012.

DOI 10.1002/ppul.22670
Published online 04 September 2012 in Wiley Online Library (wileyonlinelibrary.com).

bronchoalveolar lavage (BAL) fluid lack specificity and sensitivity.[2,3] The characteristic air crescent and halo sign, which are frequently observed on thoracic CT of haematological adult IPA patients,[4,5] appear far less common in children.[6–8]

A lung biopsy showing invasive hyphal growth of *Aspergillus* sp. is considered the gold standard for the diagnosis of IPA, but this procedure comes with considerable risks in critically ill patients. Indirect diagnostic tests have facilitated the diagnosis of IPA at an early stage of disease. One of these is the detection of galactomannan (GM) in serum by the Platelia assay (Bio-Rad, Marnes-la-Coquette, France), which has been accepted by the EORTC/MSG as part of their revised classification criteria.[7] In immunocompromised pediatric patients, three important studies[9–11] recognized the important role of this GM assay in serum in children.

Several studies[12–15] have evaluated the Platelia assay in detecting BAL GM in adult patient populations and reported sensitivities ranging from 60% to 100% and specificities from 87.8% to 100% using a cut-off index of $\geq$1.0. Bergeron et al.[16] reported a sensitivity and a specificity of 57.6% and 95.6% respectively when using a cut-off index of BALF GM $\geq$0.5.

Unfortunately, evidence is lacking whether these results can be extrapolated to pediatric patients. It has been suggested that in serum[9] there may be differences in the performance of the Platelia assay, especially regarding false-positives, between adults and children.[17,18] How these differences in test characteristics apply to the detection of GM in BAL fluid in children remains uncertain.

One recent study focused on the detection of GM in the BAL fluid and reported an overall sensitivity of 78% and specificity of 92% at a cut-off index of 0.98 in children.[19] However, they included also immunocompetent patients, whereas we aim to study the diagnostic value of BAL GM in a specific group of immunocompromised patients.

We conducted a retrospective study of the BAL GM test in children classified as immunocompromised according to the EORTC/MSG criteria. In addition, we analyzed the relation between positive BAL culture for *Aspergillus* sp., positive BAL GM and chest CT findings. Furthermore, the added value of BAL GM compared to serum GM and influence on antifungal therapy prescription was analyzed.

MATERIALS AND METHODS

Study Design and Study Population

Retrospectively, BAL fluid obtained from 456 bronchoscopies between July 2002 and June 2008 were evaluated. Pediatric patients fulfilling the host factor criteria as defined by the EORTC/MSG[8] were included. Patients suffering from cystic fibrosis, allergic bronchopulmonary aspergillosis, and other primary lung diseases were excluded. During the study period, a diagnostic protocol was used in febrile neutropenic children, due to chemotherapy for an underlying malignancy. GM in serum was analyzed twice weekly during neutropenia in high risk patients. If patients were persistently febrile during neutropenia while on broad-spectrum antibiotics, a high resolution CT of the chest was performed. A bronchoscopy and BAL was indicated if abnormalities were detected on the chest CT.

Data Collection and Patient Classification

Clinical information was collected from the medical records and the electronic patient information system. Data included underlying disease, age, sex, reports of CT-scans, cultures from sputum, and BAL, and GM in serum and BAL. Antibiotic use was recorded if started at least 1 day before bronchoscopy. The use of antifungal prophylaxis and therapy was recorded. Patients were regarded as neutropenic if neutrophil count was $<0.5 \times 10^9$/L. Children were classified as proven, probable, possible IPA according to the EORTC/MSG criteria.[8] Children with a CT scan not indicative for IPA were regarded as having no IPA. According to Dutch legislation, no approval from an IRB was required for this retrospective study. There was signed informed consent from parents and if applicable, patients for anonymous use of data.

Diagnostic Tests

The BAL was performed in the most affected lobe on the chest CT. Three aliquots of 1 ml/kg (max 20 ml) saline were rinsed in and suctioned out of the lobe. The aliquots were pooled in one sample. The pooled sample was split in a sample for microbiology and for virology for processing. The Platelia ELISA (Biorad Laboratories, France) was used to measure the levels of GM in serum and BAL according to the instructions of the manufacturer. An optical density index of $\geq$0.5 was considered positive in serum[20] and in BAL fluid.[16] BAL samples were centrifuged for 10 min at 3,000 rpm, and a total of 300 μl of supernatant was used for measurement. The assay was run three times weekly in the routine microbiology laboratory. Positive, negative, and cut-off controls were incorporated in each assay. Pretesting samples were stored at 2–8°C for a maximum of 2 days. If samples could not be processed within this timeframe, they were stored at −80°C. All tests were performed by technicians who were unaware of the clinical condition of the patient. Serum GM samples were taken twice weekly in pediatric patients considered to be at high risk (e.g., relapsed leukemia, acute myeloid leukemia, severe aplastic anemia, myelodysplastic

syndrome, and post-HSCT) for invasive aspergillosis. Sputum cultures as well as GM detection in serum had to be performed in a window of 1 week before or after bronchoscopy. Chest CT-scans were analyzed by trained and experienced pediatric radiologists. Using the EORTC/MSG criteria,[7] the chest CT was interpreted indicative or not indicative for IPA.

Data Analyses

To analyze the diagnostic value of the BAL, sensitivity, specificity, and positive and negative predictive value (PPV, NPV) were calculated for BAL GM index on a per patient basis. An optimal cut-off for the BAL GM index was determined by receiver operating characteristic analysis. Patients classified as proven or probable IPA were regarded as cases. Specificity was calculated with the patients classified as having no or possible IPA. We compared BAL GM detection and chest CT-scan abnormalities with the Mann–Whitney *U*-test. BAL culture and CT scan results were analyzed with Fisher's exact test. A correlation between BAL GM and serum GM was calculated according to Spearman's method. We analyzed the association between GM and *Aspergillus* sp. culture from BAL and therapy prescription using descriptive statistics (SPSS software version 15.0).

RESULTS

Patients

From July 2002 to June 2008, a total of 456 bronchoscopies were performed. Selection and classification according to the EORTC/MSG host factor criteria resulted in 72 bronchoscopies (15.8% of total) performed in immunocompromised pediatric patients. Eleven bronchoscopies were excluded because of multiple bronchoscopies within one patient. If more than one bronchoscopy was performed within a patient, the bronchoscopy done for diagnosing IPA was taken. Fourteen of the remaining 61 patients were excluded since the chest CT-scan was not related to the time of bronchoscopy (Fig. 1). In 6 of the 47 remaining patients a bronchoscopy was performed before the CT-scan was made. Indications for these bronchoscopies were new infiltrates on conventional chest X-ray and/or no improvement of pulmonary symptoms despite therapy. Of the 47 patients, 2 were classified as proven IPA, 17 as probable, 12 as possible, and 16 as having no IPA. The two cases of proven IPA were diagnosed by culture of lung tissue obtained at either autopsy or lung resection. The median age of the 47 children was 9.8 years (range 1.1–18.2). Most were diagnosed with a hematologic disease (n = 31) of whom two received a HSCT. At

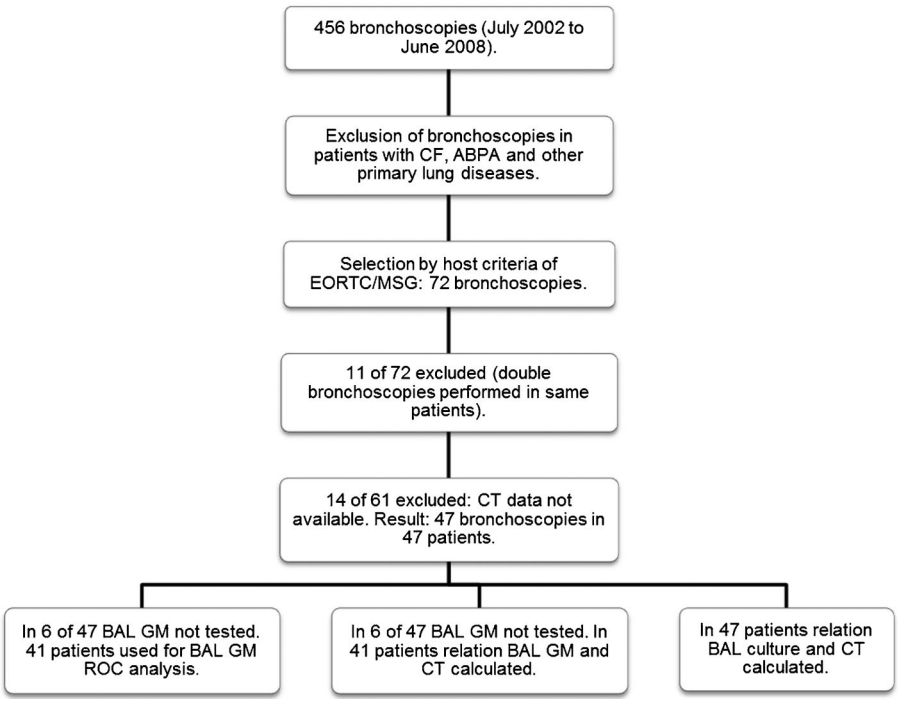

Fig. 1. Selection and classification process of bronchoscopies according to the EORTC/MSG host factor criteria.

least 23 patients were neutropenic at the time of bronchoscopy. Other diagnoses included kidney transplant (n = 3), solid tumours (n = 12), and chronic granulomatous disease (n 1). Of all included patients, 87% used antibiotics > 1 day, 8 used antifungal prophylaxis and 45% antifungals > 2 days at the time of bronchoscopy (Table 1).

Diagnostic Value of GM Assay in BAL

In 41 of the 47 patients, results of GM in BAL were available. Therefore, sensitivity/specificity calculations were performed using the BAL GM index of 41 patients. We reclassified these 41 patients disregarding the BAL GM results to prevent bias. This reclassified two patients from ''probable'' IPA to ''possible'' IPA, in whom the only mycological criterium was a BAL GM $\geq$ 0.5. Of these 41 patients, 13 were classified as having no IPA, 11 as possible, 15 as probable, and 2 as proven IPA. Mycological results for the 41 patients are summarized in Tables 2 and 3.

In one patient classified as having no IPA, the BAL GM index was 3.3, and *Aspergillus* sp. was cultured from the BAL fluid. Based on this result, the patient would be classified as ''probable'' IPA. However the CT-scan was not indicative for a diagnosis of IPA, resulting in a classification as having no IPA.

At a cut-off index value of $\geq$0.5, GM detection in BALF had a sensitivity of 82.4% and specificity of 87.5%. PPV and NPV were 82.4% and 87.5% (Fig. 2). When applying the cut-off of $\geq$0.87 as proposed by Desai and colleagues a sensitivity, specificity, PPV and NPV of respectively 64.7%, 91.7%, 84.6% and 78.6% was obtained.[16] Applying a cut-off index of $\geq$1.0 as is proposed in adults resulted in the same sensitivity, specificity, PPV, and NVP as when using a cut-off index of $\geq$0.87.[12–15]

Added Value of BAL GM Compared With Serum GM

To determine a possible additional value of BAL GM over serum GM we compared the results of these tests and examined if a correlation existed between these two variables. Among the 13 patients with a positive serum GM, 11 had a positive BAL GM. In 11 of the 15 patients with a positive BAL GM, and in whom serum GM was measured, serum GM was positive. Two of the four patients with negative serum GM were categorized as possible IPA. These two cases were regarded as false-positives in our study since in one a *Bacillus cereus* was cultured and in the other patient all tests, except CT and BAL GM, were negative. Of the 17 cases with IPA, two had a positive serum GM, whereas BAL GM was negative. We observed a significant correlation between BAL and serum GM (Spearman's correlation coefficient of 0.719 (two-tailed; $P = <0.01$). In Figure 3 the relation between BAL GM and serum GM is demonstrated.

Relation BAL GM and Chest CT-Scan

The same 41 patients were selected to compare BAL GM detection and chest CT-scan. We divided the 41 patients into two groups, one with a CT-scan indicative of IPA (n = 31) and one with aspecific abnormalities (n = 10). A significant difference was found for the distribution of BAL GM indices between the two groups ($P = 0.012$). High BAL GM index values were associated with an indicative chest CT scan for IPA, whereas low index values were related to a CT scan not indicative for IPA.

Relation BAL Culture and Chest CT-Scan

Of 34 patients with an indicative CT scan, an *Aspergillus* sp. was cultured in six. In only one of the 13

TABLE 1— Characteristics of Patients

	All patients (n = 47)	Proven[1] (n = 2)	Probable[1] (n = 17)	Possible (n = 12)	No (n = 16)
Gender					
Male	27	0	10	3	14
Female	20	2	7	9	2
Median age, years (range)	9.8 (1.1–18.2)	9.1 (8.5–9.7)	9.6 (1.1–18.2)	10.3 (1.5–17.3)	9.6 (2.0–16.6)
Underlying diseases					
Hematologic disease	31	2	15	6	8
Solid organ transplantation	3	0	0	1	2
Solid tumor	12	0	2	5	5
Other diagnoses	1	0	0	0	1
Antibiotics	41	2	15	10	14
β-lactam		2	14	9	12
Non-β-lactam		2	15	10	14
Antifungal prophylaxis	8	1	1	2	4

[1]For patients with proven, and probable invasive pulmonary aspergillosis, bronchoalveolar lavage galactomannan results were included as part of the classification criteria.

TABLE 2— Mycological Results in Bronchoalveolar Lavage Fluid and Serum in 41 Patients With Possible, Probable, or Proven Invasive Pulmonary Aspergillosis or Without

	Proven[1] (n = 2)	Probable[1] (n = 15)	Possible[1] (n = 11)	No (n = 13)	Total (n = 41)
BAL GM					
Positive ≥0.5 (range)	2 (0.7–8.2)	12 (0.5–17.0)	2 (0.7–4.0)	1 (3.3)	17 (0.5–17.0)
Negative <0.5	0	3	9	12	24
Serum GM					
Positive ≥0.5 (range)	1 (9.8)	12 (0.5–23.3)	0	0	13 (0.5–23.3)
Negative <0.5		2	11	12	25
Not performed	1	1		1	3
BAL microscopy					
Positive direct examination	0/2	1/9	0/7	1/8	2/26
Negative direct examination	2/2	8/9	7/7	7/8	24/26
BAL *Aspergillus* sp.					
Positive culture	0	6	0	1	7
Negative culture	2	9	11	12	34
Tissue diagnosis	2	—	—	—	2

[1]Classification performed disregarding the BAL GM results.

patients with a negative CT scan, *Aspergillus* sp. was cultured (Fisher's exact test, two-tailed $P = 0.655$). Microorganisms other than *Aspergillus* sp. were cultured from BAL in 16 of 47 patients. In 4 of these 16 patients also *Aspergillus* sp. were cultured. Fifteen of these 16 patients showed abnormalities on chest CT suggestive for infection. The chest CT-scans of 12 of these 15 patients (80%) showed abnormalities suggestive for IPA according to the EORTC/MSG criteria,[7] while only four of them actually had a positive *Aspergillus* sp. culture (indicating a relatively poor *Aspergillus* sp. culture sensitivity of 33%).

Clinical Impact of BAL fluid

To evaluate the clinical impact of the BAL GM and cultures we analyzed the outcome of the BAL in relation to antifungal therapy. In 9 out of 12 patients originally classified as having possible IPA antifungal therapy was continued or started despite a negative BAL GM and culture. In BAL fluid of 16 patients with other microorganisms, an *Aspergillus* sp. was also found in four. Antibiotic therapy was continued or started in 14 of the 16 patients. In 10 of these, antifungal therapy was continued or initiated irrespective of BAL findings.

DISCUSSION

Our study suggests that BAL GM is a valuable diagnostic tool for diagnosing IPA in children, with high sensitivity, specificity, PPV, and NPV for the BAL GM index. When there was a strong suspicion based on clinical criteria (i.e., indicative chest CT scan, failure of antibiotic treatment, and positive serum GM) the result of BAL GM had no apparent added value for clinical decision making.

Previous studies investigating BAL GM reported results comparable with ours.[13,14,16,21] However none of these had an exclusively pediatric study population. Until now, only one other study addressed the test characteristics of BAL GM in pediatric patients,[19] and reported a sensitivity of 78% and specificity of 100% for BAL GM at a cut-off index of ≥0.87 in the

TABLE 3— Bronchoalveolar Lavage Culture Results in 41 Patients With Possible, Probable, or Proven Invasive Pulmonary Aspergillosis or Without

	Proven (n = 2)	Probable (n = 15)	Possible (n = 11)	No (n = 13)
Aspergillus sp.	—	*A. fumigatus* (n = 3) *A. flavus* (n = 2) *Aspergillus* sp. (n = 1)	—	*A. fumigatus* (n = 1)
Molds/yeasts	—	*Candida albicans* (n = 1) *Mucor* sp. (n = 1) *Candida* sp. (n = 1)	—	*Nocardia brasiliensis* (n = 1) *Mycobacterium tuberculosis* (n = 1)
Bacteria	—	*Streptococcus* sp. (n = 1) *Absidia corymbifera* (n = 1) *Bacillus* sp. (n = 1) *Pseudomonas* sp. (n = 1)	*Bacillus cereus* (n = 1) *Methylobacterium* sp. (n = 1) *Staphylococcus aureus* (n = 1) *Haemophilus influenzae* (n = 1)	*Candida albicans* (n = 2) *Candida* sp. (n = 1)

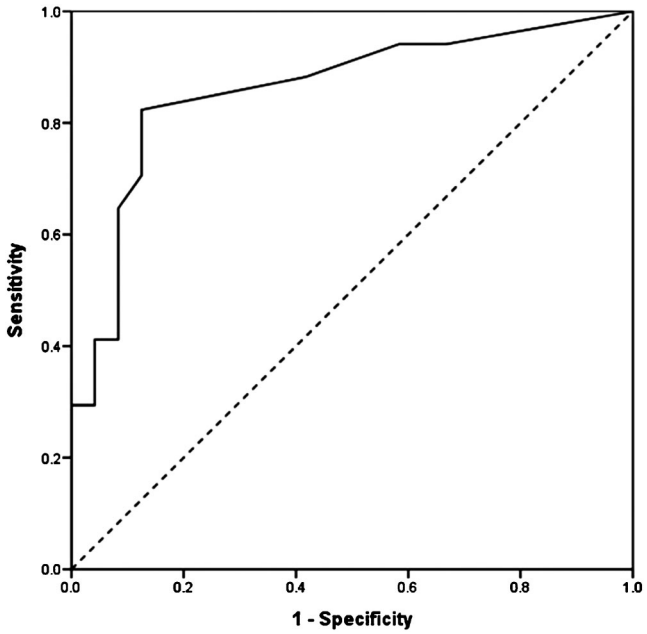

Fig. 2. ROC curve for bronchoalveolar lavage galactomannan detection in 41 patients. Using a cut-off value of ≥0.5 resulted in a sensitivity of 82% and specificity of 87%. Area under the curve (AUC) = 0.86.

subgroup of immunocompromised patients. When we applied this cut-off value, sensitivity dropped considerably to 64.7% whereas specificity increased to 91.7%. Applying a cut-off of ≥1.0, as in previous studies[12–15] did not result in improved characteristics compared with a cut-off of ≥0.87. In immunocompromised pediatric patients suspected of IPA, missing a case of IPA comes with considerable morbidity and even mortality. We therefore, want to emphasize the importance of the NPV. Applying a higher cut-off then 0.5, in our study resulted in a considerable decrease of the NPV indicating an increase in the number of false-negatives and an elevated risk to miss a case of IPA. Based on these results we propose a cut-off of ≥0.5 in immunocompromised pediatric patients for use in future studies.

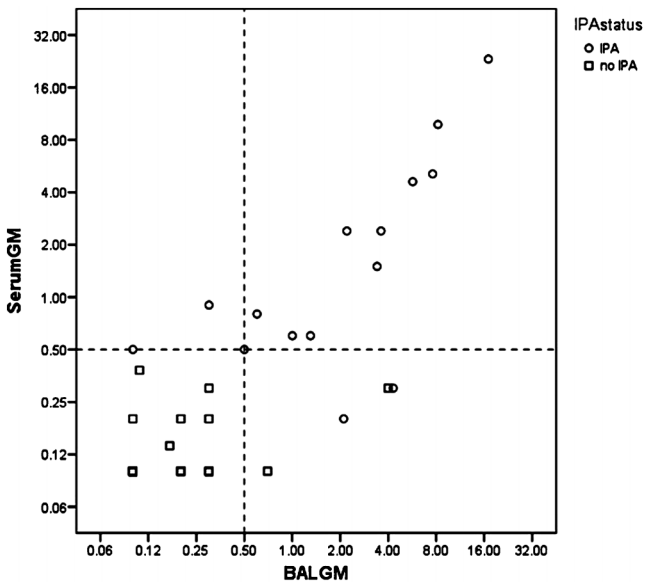

Fig. 3. Scatterplot of bronchoalveolar lavage and serum galactomannan. Spearman correlation coefficient 0.72 ($P < 0.01$). Some datapoints represent several patients. Note the logarithmically scaled axes.

Several diagnostic studies have shown that the detection of BAL GM has better test performance than the detection of GM in serum.[12,13] In our study, most of the patients with a positive serum GM also had a positive BAL GM and vice versa. Moreover a significant positive correlation between BAL GM and serum GM was observed. These results raise questions about the true additional diagnostic value of BAL GM compared with serum GM and should therefore be addressed in future studies in a larger group of immunocompromised patients.

Based on studies performed in children investigating the diagnostic value of serum GM, some authors suggested that in pediatric patients the number of false-positives may be increased compared with adults.[10,17,18] Whether these differences in test characteristics also apply to the detection of BAL GM has yet to be clarified. According to our experience, we propose that false-positivity may be explained by BAL GM becoming positive in an earlier phase of the disease compared to serum GM. This would explain why some chest CT's, indicative for IPA, are accompanied by serum GM index values <5. Moreover, in those patients with a chest CT scan negative for IPA, false-positivity could illustrate very early infection, even before abnormalities become visible on chest CT. However, contamination of the sample or colonization of the respiratory tract would also provide an explanation for this. Regarding false-negativity, dilution of the respiratory tract sample has been suggested[16] next to the use of antifungal therapy prior to bronchoscopy.

Both chest CT and BAL cultures are currently the most accurate diagnostic methods to identify IPA, when no tissue diagnosis is available. We assessed whether the outcome of the BAL GM could strengthen the diagnosis on the basis of CT-scan and found that the mean BAL GM value was significantly larger in patients with an indicative chest CT than in those with a CT not suspicious for IPA. However, our study lacked patients in which IPA was identified by the gold standard tissue diagnosis, and therefore we were not able to determine the added value of BAL GM compared to chest CT.

We tried to determine whether BAL GM levels affected medical decisions regarding the prescription of antifungal therapy. In most patients classified as possible IPA, antifungal therapy was continued or started,

despite a negative BAL GM. Furthermore, in 71% of the patients with a negative BAL GM test result, antifungal therapy was given, and stopped in only one. This illustrates that BAL GM had little or no therapeutic consequences in our clinic. This may be due to the fact that the GM test reliability and validity are incompletely known.

Certain limitations of our study deserve to be acknowledged. First, the population size was relatively small, impeding robust conclusions about an optimal cut-off point. Second, we used retrospective data. Third, in several cases test results of the tests mentioned in the EORTC/MSG criteria and used in our study were not available. Still, most of the data were collected according to a standardized protocol, which was introduced before the start of the study. Therefore, the number of missing data was limited. Fourth, classification of patients in our study may be hampered by the fact that classic findings on CT (halo-sign, air-crescent sign) are less common in children. This means that some patients in our study categorized as no IPA, in fact may have had possible IPA. However, it is unlikely that this biased BAL GM test results, since we calculated specificity using the total number of patients with no and possible IPA. At last, we cannot exclude the possibility that our study was biased by disease progression.[15] It is obvious that the timing of BAL has an influence on the BAL GM test result, since performing bronchoscopy too early may result in false-negativity. This may be of considerable importance in patients diagnosed with possible IPA. However, our protocol helped to standardize the correct time of BAL. Moreover, in just 3 of 41 patients the detection of BAL GM resulted in a false-negative test result. We therefore consider this problem of minor importance in our study.

In conclusion, we want to emphasize that diagnosing IPA relies on a combination of diagnostic markers including host criteria, clinical criteria and mycological criteria. Every effort should be undertaken to make the diagnosis with as much certainty as possible. Preferably, a tissue-diagnosis should be made. In our study group, the BAL GM test demonstrated good test characteristics in pediatric patients suspected of IPA. Although the results of the BAL GM did not influence the antifungal therapy, the number of positive BAL GM results was higher when compared to the number of positive serum samples for GM and additional positive culture results for *Aspergillus* sp. in the BAL. Therefore mycological analysis of BAL fluid is relevant in assessing the certainty of the diagnosis. Practically, we would suggest that a negative result gives support to early termination of antifungal therapy, unless there are clinically clear signs to decide otherwise. A positive result should be considered as indicative for proven IPA, and be treated as such. Furthermore, performing a BAL can identify other microorganisms which can lead to a causative diagnosis. Since only two patients with proven IPA were included, it was hard to demonstrate a significant possible added value of BAL GM compared to chest CT.

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