Diagnostic Performance of Galactomannan Antigen Testing in Cerebrospinal Fluid

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Testing cerebrospinal fluid (CSF) for the presence of galactomannan (GM) antigen may help in diagnosing cerebral aspergillosis (CA). However, the use of the CSF GM test as a diagnostic test has been little studied. We evaluated its diagnostic performance by comparing the CSF GM optical density indexes (ODI) at different cutoffs in patients with probable and proven CA to those in patients without CA. Patients from 2 tertiary referral hospitals with suspected CA between 2004 and 2014 and in whom CSF GM ODI had been determined were selected. European Organization for Research and Treatment of Cancer/Invasive Infectious Diseases Study Mycoses Group (EORTC/MSG) definitions of invasive aspergillosis and CA were used, but with the exclusion of the test to be validated (i.e., the CSF GM test) as a microbiological EORTC/MSG criterion. The study population consisted of 44 patients (4 with proven CA, 13 with probable CA, and 27 with no CA). Of the 17 patients with CA, 15 had a CSF GM ODI of ≥ 2.0 . Of 27 patients without CA, 26 had a CSF GM ODI of <0.5 and 1 had a CSF GM ODI of 8.2. When a GM CSF ODI cutoff of 1.0 was used, the sensitivity, specificity, and positive and negative predictive values were 88.2%, 96.3%, 93.8%, and 92.9%, respectively. The same results were found when a CSF GM ODI cutoff of 0.5 or 2.0 was used. Testing GM in CSF has a high diagnostic performance for diagnosing CA and may be useful to diagnose or virtually rule out the infection without the need for a cerebral biopsy.

Cerebral aspergillosis (CA) is a rare and often fatal invasive fungal disease (IFD) (1, 2). The diagnosis is challenging, as radiological findings are nonspecific and cerebrospinal fluid (CSF) cultures are positive only in less than one-third of cases (3, 4). Moreover, obtaining brain tissue for histopathological examination, the diagnostic gold standard, is frequently not feasible. Galactomannan (GM) antigen detection in CSF is one of the microbiological criteria in the revised European Organization for Research and Treatment of Cancer/Invasive Infectious Diseases Study Mycoses Group (EORTC/MSG) criteria (5). However, its diagnostic performance has been studied very little. To date, only 3 studies including a total of 25 patients have described the value of CSF GM, only 2 of which were carried out with patients with suspected CA (3, 6, 7). In these 2 studies, CSF GM optical density indexes (ODI) were higher in patients with CA than in those without CA, and sensitivity and specificity were 80% and 100% (6, 7). Based on these limited data, the GM antigen test in CSF seems promising, even though no positive and negative predictive values (PPV and NPV) were determined and no formal cutoff was established. This study evaluated the diagnostic performance of the Platelia GM antigen test (Bio-Rad, Marnes-la-Coquette) in CSF of patients with suspected CA.

(This information was presented at the 7th Trends in Medical Mycology, Lisbon, Portugal, 9 to 12 October 2015 [8].)

MATERIALS AND METHODS

This retrospective study was performed at the Erasmus University Medical Center (Erasmus MC) in the Netherlands and University Hospitals Leuven (UZ Leuven) in Belgium. Patients in whom a CSF GM test had been performed were considered to have suspected CA and were selected. In the Erasmus MC, internal medicine, hematology, and pediatric patients were selected from January 2004 to March 2015. In the UZ Leuven, internal medicine and hematology patients from May 2007 to December 2014 were chosen. Data on age, sex, underlying disease, and microbiology

and radiology results were collected. Patients were excluded if no pulmonary or cerebral radiology was present or no GM testing in serum and bronchoalveolar lavage (BAL) fluid had been performed.

The diagnostic performance of the GM antigen test in CSF was evaluated by comparing the GM antigen ODI in CSF of patients with proven or probable CA to patients without CA. Patients with proven, probable, or no CA were selected in two steps. First, invasive aspergillosis (IA) was identified or ruled out according to the revised EORTC/MSG criteria (5). To avoid inclusion of the test that we wanted to validate (CSF GM test) in the gold standard, CSF GM was removed from the microbiology criteria. To avoid overlap between the definition of IA and CA, cerebral radiology was also excluded from the clinical EORTC/MSG criteria. This was deemed necessary because otherwise, patients with an isolated serum GM ODI of ≥ 0.5 and a focal cerebral lesion (e.g., cerebral infarction) but without any other evidence of IA elsewhere would fit the definition of probable CA.

Subsequently, proven or probable CA was determined in patients with proven or probable IA. IA remains uncertain in patients with possible IA or in patients with only an isolated positive microbiological criterion but no clinical or radiological criterion. Therefore, these patients were excluded from the analysis. Probable CA was diagnosed when cerebral radiological signs compatible with IFD (e.g., focal lesions and meningeal enhancement) were present in addition to a proven or probable IA else-

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FIG 1 Flowchart of the 44 patients with suspected cerebral aspergillosis (CA). Two patients with proven IA had histopathological evidence of IA outside the cerebrum and therefore were classified as probable CA cases. CSF, cerebrospinal fluid; GM, galactomannan; IA, invasive aspergillosis.

where in the body. Proven CA was diagnosed when cerebral pathological evidence of IA or a positive CSF *Aspergillus* culture was present in addition to the IA criteria. Patients with proven or probable IA who had nonspecific radiological cerebral signs (no focal lesion and no meningeal enhancement) were excluded from analysis. Patients classified as being without CA had no IA and did not have cerebral abnormalities or had a convincing alternative diagnosis for the cerebral abnormalities. Patients were included only once, and those who had more than one episode of suspected CA were classified according to the highest CA category. Per patient, only the CSF GM ODI at diagnosis was included.

The diagnostic performance of the test for the GM antigen in CSF was evaluated by comparing the GM antigen ODI at different cutoffs in proven and probable CA cases to those without CA. CSF GM ODI were correlated with serum GM ODI and CSF cultures. The independent *t* test or Mann-Whitney U test was used as appropriate to compare the CSF and serum GM values (IBM SPSS Statistics, version 21).

In addition, an extra sensitivity analysis was performed to look at the diagnostic performance of CSF GM when cerebral radiology was not excluded from the clinical EORTC/MSG criteria. For this sensitivity analysis, a CSF GM cutoff of 1.0 was used.

RESULTS

GM was determined in 205 CSF samples of 157 patients. Eighty patients were excluded because of insufficient microbiology ($n = 10$), radiology ($n = 47$), or both ($n = 23$). Further, 12 patients with possible IA, 9 with an isolated microbiology criterion, and 12 with cerebral findings not compatible with IFD were excluded (Fig. 1). Therefore, the evaluable study population consisted of 44 patients (4 with proven CA, 13 with probable CA, and 27 with no CA). Table 1 shows the clinical, radiological, and microbiological findings for those with CA. Fifteen of the 17 patients with CA had CSF

GM ODI of ≥ 2.0 . In the patients without CA, 26 had CSF GM ODI of < 0.5 and 1 had a CSF GM ODI of 8.2. When a GM CSF cutoff of 1.0 was used, the sensitivity, specificity, PPV, and NPV were 88.2%, 96.3%, 93.8%, and 92.9% (Table 2). The same results were found when a cutoff of 0.5 or 2.0 was used. With increasing cutoff values of 3.0, 4.0, and 5.0, the sensitivity decreased to 76.5, 70.6, and 58.8% (Table 3). As a sensitivity analysis, we looked at the impact of including cerebral radiology in the clinical EORTC/MSG criteria using a GM cutoff of 1.0; this decreased the sensitivity from 88.2 to 76.0% (Table 4).

Of the 17 CA patients, 3 were culture positive (2 CSF and 1 biopsy specimen), and all three cultures grew *Aspergillus fumigatus*.

Serum GM was available in 16/17 patients with CA. The ODI of CSF GM was higher than that of serum GM in 10 patients and lower in 1 patient with probable CA (Table 1). The mean GM in CSF was higher than in serum (4.89 versus 3.72; $P = 0.27$). In the patients without CA, the serum GM ODI was available for 25/27 patients. The CSF GM ODI was higher than the serum GM ODI in 4 patients, lower in 4 patients, and equal in the remaining 17 patients. The median GM ODI was 0.1 in CSF and 0.1 in serum ($P = 0.77$).

DISCUSSION

In this study, the GM antigen test on CSF showed good performance for diagnosing CA in patients with proven or probable CA when an ODI cutoff of 0.5 to 2.0 was used. To validate the CSF GM antigen test, we logically had to exclude CSF GM from the EORTC/MSG criteria. This made it possible to measure the sen-

TABLE 1 Clinical characteristics and radiological and microbiological findings for the patients with probable or proven CA

Patient no.	Age (yr)/sex ^a	IA ^b	CA	Underlying disease/host factor(s) ^c	Radiological findings ^d		Galactomannan ODI ^e			Culture ^f		
					Cerebral	Lungs	CSF	BAL	Serum	CSF/cerebral biopsy	BAL	Pathology
1	36/M	Probable	Probable	ALL, allogeneic HSCT	Multiple lesions, sinusitis	Specific	5.5	NA	5.5	Neg	NA	NA
2	67/F	Probable	Probable	AML	Multiple lesions	Specific	6.4	6.3	6.4	Neg	<i>A. fumigatus</i>	NA
3	58/M	Proven	Proven	No host factors	Multiple abscesses	Nonspecific	2.3	0.1	0.2	<i>A. fumigatus</i>	Neg	Pos, cerebral biopsy
4	5/M	Probable	Probable	ALL	Multiple lesions	Specific	7.2	NA	7.2	Neg	NA	NA
5	9/F	Probable	Probable	ALL	Multiple lesions	Specific	9.9	NA	9.9	Neg	NA	NA
6	53/M	Probable	Probable	NHL	Multiple lesions	Specific	5.0	4.2	4.5	Neg	<i>A. fumigatus</i>	NA
7	63/M	Probable	Probable	Cushing's syndrome	Nodular lesion	Specific	3.8	5.6	0.2	Neg	Neg	NA
8	2/F	Proven	Proven	ALL	Multiple lesions	Normal	7.1	NA	2.9	<i>A. fumigatus</i>	NA	NA
9	69/M	Proven	Proven	Peripheral arterial disease	Meningitis with infarct	Normal	4.3	NA	NA	<i>A. fumigatus</i>	NA	Neg, cerebral autopsy
10	54/M	Probable	Probable	AML, allogeneic HSCT	Multiple lesions	Specific	5.0	0.5	0.1	Neg	<i>A. fumigatus</i>	NA
11	59/M	Probable	Probable	AML, allogeneic HSCT	Multiple abscesses	Specific	6.2	NA	6.2	Neg	NA	NA
12	30/M	Probable	Probable	AML, allogeneic HSCT	Multiple lesions	Specific	2.1	NA	2.0	Neg	NA	NA
13	68/F	Proven	Proven	Kidney transplantation	Infarct	NA	7.9	NA	7.4	Neg	NA	Pos, cerebral autopsy
14	66/M	Proven	Probable	NHL	Multiple lesions	NA	5.0	5.3	4.8	Neg	<i>A. fumigatus</i>	Pos, lung autopsy
15	67/M	Probable	Probable	AML	Semi-recent ischemia	Specific	0.1	NA	1.5	Neg	Neg	Neg, CSF cytology
16	66/F	Proven	Probable	AML, allogeneic HSCT	Hypodense lesion	Nonspecific	0.2	5.1	0.1	NA	<i>A. fumigatus</i>	Pos, lung biopsy
17	66/F	Probable	Probable	ALL, allogeneic HSCT	Multiple lesions	Specific	4.5	0.8	0.6	Neg	Neg	NA

^a M, male; F, female.^b IA, invasive aspergillosis.^c ALL, acute lymphatic leukemia; HSCT, hematopoietic stem cell transplantation; AML, acute myeloid leukemia; NHL, non-Hodgkin lymphoma.^d NA, not available.^e ODI, optical density index; CSF, cerebrospinal fluid.^f Neg, negative; Pos, positive.

sitivity of CSF GM for diagnosis of CA. As such, we found that in patients with a proven or probable CA based on culture, tissue biopsy, or the GM test in serum or BAL fluid in combination with a suspected radiological cerebral finding, 88.2% had a positive CSF GM test, while cultures were positive in only 17.6% of the cases. As explained in Materials and Methods, we chose to remove cerebral radiology from the clinical EORTC/MSG criteria. In a sensitivity analysis, we retained the cerebral radiology in the criteria and observed a decrease in sensitivity to 76% (CSF GM ODI cutoff of 1.0). We think that this decrease is rather the result of more misclassifications of patients in the probable-CA group. However, we cannot formally prove this.

The CSF GM antigen test is included in the revised EORTC/MSG criteria (5). However, only 2 studies reported on GM antigen testing in CSF in a total of 10 patients with CA. Viscoli et al. measured GM on CSF from 5 patients with proven or probable CA (7). The median GM ODI was 10.52, and GM ODI were significantly higher in patients with CA than in patients without CA. Kami et al. compared different non-culture-based diagnostics on

CSF of 5 patients with proven CA and 11 patients with leukemic, bacterial, viral, or mucor meningitis (6). The GM antigen test was positive in 4 of the 5 CSF samples from patients with proven CA and negative in all samples from patients without CA. Based on the findings of Viscoli et al. and Kami et al., the CSF GM ODI was included in the revised EORTC/MSG definitions. Finally, Antinori et al. reviewed the literature on *Aspergillus* meningitis, which is not the same as CA, and found that the CSF GM test was performed in 15 of the 93 cases (3). The median CSF GM was 6.58, with a range of 2.2 to 578. The sensitivity was 86.7%. Our study, in which 17 patients with CA and 27 without CA were included, confirms that the CSF GM test is useful for ruling in or ruling out CA.

Among the patients without CA in our study, there was 1 patient with a positive CSF GM ODI of 8.2. This patient had a cerebral abscess, as determined by magnetic resonance imaging. As he did not have any other localizations of IA or a positive culture or brain biopsy, he was classified as having no CA according to our study criteria, from which we excluded CSF GM. However, ac-

TABLE 2 Correlation of CSF GM ODI and patient CA status^a

CSF sample type	No. of patients		
	With CA	Without CA	Total
With positive GM ($\geq 0.5/1.0/2.0$)	15	1	16
With negative GM ($< 0.5/1.0/2.0$)	2	26	28
Total	17	27	44

^a Sensitivity, 15/17 (0.8824); specificity, 26/27 (0.9630); positive predictive value, 15/16 (0.9375); negative predictive value, 26/28 (0.9286).**TABLE 3** Diagnostic performance of GM in CSF according to different cut-offs

Parameter	Value (%) with CSF GM ODI cutoff of					
	0.5	1.0	2.0	3.0	4.0	5.0
Sensitivity	88.2	88.2	88.2	76.5	70.6	58.8
Specificity	96.3	96.3	96.3	96.3	96.3	96.3
PPV	93.8	93.8	93.8	92.9	92.3	90.9
NPV	92.9	92.9	92.9	86.7	83.9	78.8

TABLE 4 Sensitivity analysis of the test for GM in CSF^a

Diagnosis	No. of patients with CSF GM cutoff	
	<1.0	≥1.0
Proven CA	0	4
Probable CA	7	15
Possible CA	1	2
No CA	44	4
Total	52	25

^a For the sensitivity analysis, cerebral radiology was included in the clinical criteria of the revised European Organization for Research and Treatment of Cancer/Invasive Infectious Diseases Study Mycoses Group. Sufficient data from 77 patients were available to diagnose or exclude CA. Patients with probable or proven CA were compared to patients without CA. Patients with possible CA were excluded. When a GM ODI of 1.0 in CSF was used, the sensitivity was 76.0% (19/25).

cording to the revised EORTC/MSG criteria, this patient had probable IA, and he was treated with voriconazole. He died 17 days later. Because no autopsy was performed, we cannot exclude that this patient had CA, but this is an intrinsic problem when a new diagnostic test is being validated.

This study has limitations. It was performed retrospectively, and for logistical reasons, patients could be selected only from the departments where one of the coauthors worked. Second, we excluded patients who according to EORTC/MSG criteria had possible IA or had an isolated positive microbiological criterion. Including these uncertain IA cases as cases of IA (or the opposite) would unavoidably lead to an uncertain number of misclassifications. Therefore, to make a validation of GM antigen testing in CSF possible, we could only exclude them.

In conclusion, GM detection in CSF showed a good diagnostic performance when an ODI cutoff of 0.5 to 2.0 was used, and using GM in CSF, CA can be diagnosed or virtually ruled out without the need for cerebral biopsy.

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