Chapter 4

Diagnostic performance of galactomannan antigen testing in cerebrospinal fluid

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ABSTRACT

Introduction

Testing cerebrospinal fluid (CSF) for the presence of galactomannan (GM) antigen may help in diagnosing cerebral aspergillosis (CA). However, the use of CSF GM as a diagnostic test never been validated. We evaluated its diagnostic performance by comparing the CSF GM levels at different cut-offs in patients with probable and proven CA to those without CA.

Methods

Patients from 2 tertiary referral hospitals with suspected CA between 2004-2014 and in whom CSF GM had been determined, were selected. EORTC/MSG definitions of invasive aspergillosis and CA were used but excluding the to-be-validated-test (=CSF GM) as a microbiological EORTC/MSG criterion.

Results

The study population consisted of 44 patients (4 proven CA, 13 probable CA and 27 no CA). Of the 17 patients with CA, 15 had a CSF GM of ≥2.0. In patients without CA, 26 of the 27 had a CSF GM of <0.5 and 1 had a CSF GM of 8.2. When a GM CSF cut-off level of 1.0 was used, the sensitivity, specificity, positive and negative predictive values were 88.2%, 96.3%, 93.8% and 92.9%, respectively. The same results were found when using a CSF GM cut-off of 0.5 or 2.0.

Conclusion

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.

INTRODUCTION

Cerebral aspergillosis (CA) is a rare and often fatal invasive fungal disease (IFD) [1,2]. The diagnosis is challenging as radiological findings are non-specific and cerebrospinal fluid (CSF) cultures are only positive in less than one-third of the 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 of 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 little studied. To date, only 3 studies totalling 25 patients have described the value of CSF GM, only 2 of which in patients with suspected CA [3,6,7]. In these 2 studies, CSF GM levels were higher in patients with than without CA, and sensitivity and specificity were 80% and 100% [6,7]. Based on these limited data, GM antigen test in CSF seems a promising test, even though no positive and negative predictive values (PPV, NPV) were determined, and no formal cut-off was established. This study evaluated the diagnostic performance of the Platelia™ GM antigen test (Bio-Rad, Marnes-la-Cocquette) in CSF of patients with suspected CA.

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 had been performed, were considered to be "suspected CA" and were selected. In the Erasmus MC, internal medicine, haematology and paediatric patients were selected from January 2004 to March 2015. In the UZ Leuven, internal medicine and haematology patients from May 2007 to December 2014 were retained. Data on age, sex, underlying disease, microbiology and radiology results were collected. Patients were excluded if no pulmonary or cerebral radiology was present, or no serum and BAL GM had been performed.

The diagnostic performance of the GM antigen test in CSF was evaluated by comparing the GM antigen level 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 defined or ruled out according to the revised EORTC/MSG criteria [5]. To avoid inclusion of the test that we wanted to validate (CSF GM) into 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 ≥ 0.5 and a focal cerebral lesion (e.g. cerebral infarction) but without any other evidence of IA elsewhere would fit the probable CA definition.

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, meningeal enhancement) were present on top of a proven or probable IA elsewhere in the body. Proven CA was diagnosed when cerebral pathological evidence of IA or a positive CSF *Aspergillus* culture was present on top of the IA criteria. Patients with proven or probable IA who had non-specific radiological cerebral signs (no focal lesion, 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 only included 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 at diagnosis was included.

The diagnostic performance of GM antigen in CSF were evaluated by comparing the GM antigen level at different cut-offs in proven and probable CA cases to those without CA. CSF GM levels were correlated with serum levels 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, CSF GM cut-off of 1.0 was used.

RESULTS

GM was determined in 205 CSFs 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 (figure 1). Therefore, the evaluable study population consisted of 44 patients (4 proven CA, 13 probable CA and 27 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 ≥ 2.0. In the patients without CA, 26 had CSF GM <0.5 and 1 had CSF GM of 8.2. When a GM CSF cut-off level 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 cut-off of 0.5 or 2.0 was used. With increasing cut-off values of 3.0 / 4.0 / 5.0, the sensitivity decreased to 76.5 / 70.6 / 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 cut-off of 1.0; this decreased the sensitivity from 88.2 to 76.0% (table 4).

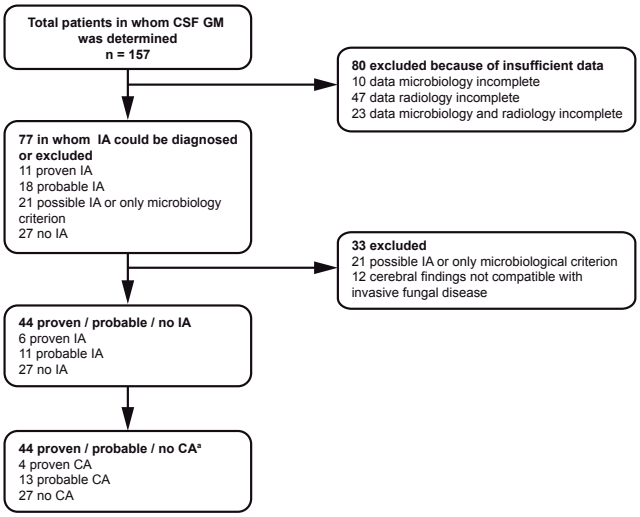

Figure 1. Flowchart of the 44 patients with suspected cerebral aspergillosis (CA).
Two patients with proven IA had histopathological evidence of IA outside the cerebrum and therefore were classified as probable CA cases. CSF, cerebrospinal fluid; GM, galactomannan; IA, invasive aspergillosis.

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

Serum GM was available in 16/17 patients with CA. CSF GM was higher than 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-value = 0.27). In the patients without CA, serum GM was available in 25/27 patients. CSF GM was higher than serum GM in 4 patients, lower in 4 patients, and equal in the remaining 17 patients. The median GM was 0.1 in CSF and 0.1 in serum (p-value = 0.77).

Table 1. Clinical characteristics, radiological and microbiological findings for the patients with probable or proven cerebral aspergillosis (CA).

Patient no.	Age (y)/ sex[a]	IA[b]	CA	Underlying disease / host factor(s)[c]	Radiological findings[d]		Galactomannan level[e]			Culture[f]		Pathology
					Cerebral	Lungs	CSF	BAL	Serum	CSF / cerebral biopsy	BAL	
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	*A. fumigatus*	NA
3	58/M	Proven	Proven	No host factors	Multiple abscesses	Non-specific	2.3	0.1	0.2	*A. fumigatus*	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	*A. fumigatus*	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	*A. fumigatus*	NA	NA
9	69/M	Proven	Proven	Peripheral arterial disease	Meningitis with infarct	Normal	4.3	NA	NA	*A. fumigatus*	NA	Neg, cerebral autopsy
10	54/M	Probable	Probable	AML, allogeneic HSCT	Multiple lesions	Specific	5.0	0.5	0.1	Neg	*A. fumigatus*	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	*A. fumigatus*	Pos, lung autopsy
15	67/M	Probable	Probable	AML	Semi-recent ischemia	Specific	0.1	NA	1.5	Neg	Neg	Neg, CSF cytology

Table 1. Clinical characteristics, radiological and microbiological findings for the patients with probable or proven cerebral aspergillosis (CA) (*continued*)

Patient no.	Age (y)/ sex[a]	IA[b]	CA	Underlying disease / host factor(s)[c]	Radiological findings[d]		Galactomannan level[e]			Culture[f]		Pathology
					Cerebral	Lungs	CSF	BAL	Serum	CSF / cerebral biopsy	BAL	
16	66/F	Proven	Probable	AML, allogeneic HSCT	Hypodense lesion	Non-specific	0.2	5.1	0.1	NA	*A. fumigatus*	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 leukaemia. HSCT, hematopoietic stem cell transplantation. AML, acute myeloid leukaemia. NHL, non-Hodgkin lymphoma. [d] NA, not available. [e] CSF, cerebrospinal fluid. [f] Neg, negative. Pos, positive.

Table 2. Cerebrospinal fluid (CSF) galactomannan (GM) related to patients with suspected cerebral aspergillosis (CA).

	Patients with CA	Patients without CA	Total
CSF with positive GM (≥ 0.5 / 1.0 / 2.0)	15	1	16
CSF with negative GM (<0.5 / 1.0 / 2.0)	2	26	28
Total	17	27	44

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 galactomannan (GM) in cerebrospinal fluid (CSF) according to different cut-offs.

	Cut-off value of GM in CSF					
	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 galactomannan (GM) in cerebrospinal fluid (CSF).

	Cut-off value of GM in CSF	
	GM level < 1.0	**GM level ≥ 1.0**
Proven CA	0	4
Probable CA	7	15
Possible CA	1	2
No CA	44	4
Total	52	25

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 of 77 patients were available to diagnose or exclude cerebral aspergillosis (CA). Patients with probable or proven cerebral aspergillosis (CA) were compared to patients without CA. Patients with possible CA were excluded. When a GM level of 1.0 in CSF was used, the sensitivity was 76.0% (19/25).

DISCUSSION

In this study, the GM antigen test on CSF showed a good performance for diagnosing CA in patients with proven/probable CA when using a cut-off of 0.5 to 2.0. To validate the CSF GM antigen test, we logically had to exclude CSF GM from the EORTC/MSG criterion.

This made it possible to measure the sensitivity of CSF GM to diagnose CA. As such, we found that in patients with a proven/probable CA based on culture, tissue biopsy or GM in serum or BAL in combination with a suspected radiological cerebral finding, 88.2% of the patients had a positive CSF GM, while cultures were only positive in 17.6% of the cases. As explained in the methods, we chose to remove the 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 cut-off 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 [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/probable CA [7]. The median GM level was 10.52 and was significantly higher in patients with CA compared to 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 in patients with proven CA and negative in all patients without CA. Based on the findings of Viscoli et al. and Kami et al., the CSF GM 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 CSF GM 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 CSF GM is a useful test to rule in or rule out CA.

Among the patients without CA in our study, there was 1 patient with positive CSF GM of 8.2. This patient had a cerebral abscess on magnetic resonance imaging. As he did not have any other localisations of IA, and no positive culture or brain biopsy he was classified as having no CA according to our study criteria in which we excluded CSF GM. However, according to the revised EORTC/MSG, this patient had probable IA and 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. The study was performed retrospectively and for logistical reasons, patients could be selected only from the departments where 1 of the co-authors worked. Secondly, we excluded patients who according to EORTC/MSG had a possible IA or had an isolated positive microbiological criterion. Including these uncertain IA cases as suffering from IA (or the opposite) would unavoidably lead to an uncertain number of misclassification. Therefore, to make a validation of GM antigen testing in CSF possible, we could only but exclude them.

In conclusions, GM detection in CSF showed a good diagnostic performance when a cut-off 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.

REFERENCES

1 Jantunen E, Volin L, Salonen O, Piilonen A, Parkkali T, Anttila VJ, et al. Central nervous system aspergillosis in allogeneic stem cell transplant recipients. Bone Marrow Transplant. 2003;31(3):191-196.

2 Lin SJ, Schranz J, Teutsch SM. Aspergillosis case-fatality rate: systematic review of the literature. Clin Infect Dis. 2001;32(3):358-366.

3 Antinori S, Corbellino M, Meroni L, Resta F, Sollima S, Tonolini M, et al. Aspergillus meningitis: a rare clinical manifestation of central nervous system aspergillosis. Case report and review of 92 cases. J Infect. 2013;66(3):218-238.

4 Kourkoumpetis TK, Desalermos A, Muhammed M, Mylonakis E. Central nervous system aspergillosis: a series of 14 cases from a general hospital and review of 123 cases from the literature. Medicine (Baltimore). 2012;91(6):328-336.

5 De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis. 2008;46(12):1813-1821.

6 Kami M, Ogawa S, Kanda Y, Tanaka Y, Machida U, Matsumura T, et al. Early diagnosis of central nervous system aspergillosis using polymerase chain reaction, latex agglutination test, and enzyme-linked immunosorbent assay. Br J Haematol. 1999;106(2):536-537.

7 Viscoli C, Machetti M, Gazzola P, De Maria A, Paola D, Van Lint MT, et al. Aspergillus galactomannan antigen in the cerebrospinal fluid of bone marrow transplant recipients with probable cerebral aspergillosis. J Clin Microbiol. 2002;40(4):1496-1499.