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Detection of Circulating *Aspergillus fumigatus* Galactomannan: Value and Limits of the Platelia Test for Diagnosing Invasive Aspergillosis

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The effectiveness of galactomannan detection with the Platelia test was evaluated in a prospective study of 3,327 sera from 807 patients. The specificity was 99.6% (748 of 751 cases). For the groups of patients with proven and probable invasive aspergillosis, the sensitivity was 50.0% (17 of 34 cases). The disappointing sensitivity associated with the presence of rare false-positive cases underlines the limits of this test.

The incidence of invasive aspergillosis (IA) has significantly increased in neutropenic patients, matched unrelated donor transplant recipients, and patients with aggressive immunosuppressive regimens that impair macrophage functions (steroids, chemotherapy, etc.) (1, 15). This severe opportunistic fungal infection is characterized by a high mortality rate in these at-risk patients (5). An antifungal therapy instituted early should improve the patients' prognosis. However, IA is always extremely difficult to diagnose. The clinical symptoms are not specific; blood cultures and specific antibody detection are seldom positive. The major criteria for IA suspicion are essentially based on a set of arguments such as abnormal chest computed tomography scans (11), a positive fungal culture, and the detection of circulating galactomannan. Proven IA diagnosis based on *Aspergillus* isolation in deep tissues (3) is rarely established since these samples remain questionable in highly neutropenic patients (9). To detect circulating *Aspergillus* galactomannan, the commercialized enzyme-linked immunosorbent assay method (Platelia test) now commonly used is far more sensitive than the previous latex agglutination test with the same monoclonal antibody (10, 12). However, high variations in sensitivity and specificity have been reported (10, 18). These variations were mainly related to the patient populations examined, the test conditions, and the frequency of the serological survey.

Our study evaluated the efficiency for IA diagnosis of the Platelia *Aspergillus* test routinely used in a prospective serological survey of at-risk patients from January 1998 to January 2001.

During this 3-year period, 3,327 serum samples from 807 patients from Grenoble University Hospital that were at risk for IA were tested in routine screening programs, including one or two blood samples obtained per week. Patients were from hematological departments and intensive care units. The patients were classified according to IA diagnosis criteria of European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) (3) with three levels of certainty: proven, probable, and possible. In our study, IA was rarely proven, deep tissue samples were not systematically taken, and autopsy was never performed because of the nonacceptance of the families. The detection of galactomannan by the Platelia *Aspergillus* test (Bio-Rad, Marnes-la-Coquette, France) was carried out exactly according to the manufacturer's instructions. However, an index of 1.0 or more was considered positive instead of 1.5 recommended by the manufacturer. All of the positive samples were retested with a new sample obtained from the patient because of false-positive results due to sample contamination or lack of reproducibility. Two consecutive positive patient samples were necessary to suspect IA. The sensitivity of the test was calculated from the results obtained in the groups of proven and probable IA. The specificity was calculated from the results obtained in immunocompromised patients without sufficient criteria to retain IA.

As indicated in Table 1, the galactomannan antigenemia was positive in 106 sera (34 patients) and negative in 3,221 sera (773 patients). The specificity of this test was 99.6% (748 of 751). The predictive positive and negative values were 85.0 and 96.8%, respectively. Of the 748 patients without IA, 721 presented with negative antigenemia, and 27 patients presented with only one positive antigenemia result. For 15 of these 27 patients, the results were linked to a lack of technique reproducibility because the repeated test on the same sample was

TABLE 1. Results of antigenemia survey of patients with proven, probable, or possible IA or without a retained diagnosis of IA

Patient case category	No. of patients with:		Total no. of patients
	Positive Platelia result[a]	Negative Platelia result	
Proven IA	0	3	3
Probable IA	17	14	31
Possible IA	14	8	22
IA not retained	3	748	751
Total	34	773	807

[a] Patients with at least two positive antigenemia results were included.

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TABLE 2. Characteristics of patients with proven and probable IA.

IA group and patient no.	Sex[a]	Underlying disorder[b]	Neutropenia during the diagnosis[c]	Steroids >14 days[c]	Computed tomography scan result	Positive samples/total samples	Site of fungal culture[d]	Direct results[e]	Culture result	Treatment	Outcome
Proven IA											
1	F	AML	+	+	Halo sign	0/9	Lung biopsy	Hyphae	*A. fumigatus*	L AmB	Survival
2	F	AML	+	−	Sinusitis plus osteolysis	0/10	Sinus (bone)	Hyphae	*A. terreus*	L AmB plus Itra (S)	Survival
3	M	NHL	−	−	Sinusitis plus osteolysis	0/3	Sinus (bone)	Hyphae	*A. fumigatus*	Itra (S)	Death
Probable IA[g]											
4	M	CLL	+	+	Halo sign	2/2	BAL	Hyphae	*A. fumigatus*	AmB	Death
5	M	AL	+	−	Halo plus necrosis	2/5	BA	Hyphae	Negative	AmB	Death
6	M	NHL (2)	+	+	Sinusitis	4/10	Nostril	Hyphae	*A. flavus*	AmB	Death
7	F	AML	+	−	Pulmonary infiltrate	4/4	BA	Hyphae	*A. fumigatus*	AmB	Death
8	M	CLL	−	−	Halo sign	5/5	Trachea	Hyphae	*A. fumigatus*	L AmB	Death
9	M	Kidney Tx	−	+	Bilateral infiltrate	2/3	Sputum, BA	Hyphae	*A. fumigatus*	L AmB	Death
10	F	AML (1)	+	−	Halo sign	7/13	Sputum, BA	No hyphae	Negative	L AmB	Survival
11	M	AML	+	−	Halo sign	3/3	Sputum	Hyphae	*A. fumigatus*	L AmB	Survival
12	F	AML (2)	+	−	Pulmonary necrosis	3/6	Sputum, BA	No hyphae	Negative	Itra	Death
13	F	NHL	+	+	Cavitation	2/3	Sputum, BA	Hyphae	*A. fumigatus*	AmB	Death
14	M	CML (1)	+	−	Pulmonary infiltrate	6/10	Sputum	No hyphae	Negative	AmB	Survival
15	M	AML	+	−	Pulmonary infiltrate	3/9	BAL, BA	Hyphae	*A. fumigatus*	AmB plus Itra	Survival
16	F	NHL	+	+	Halo sign	3/3	BA, trachea	Hyphae	*A. fumigatus*	AmB	Death
17	F	AML (2)	−	+	Pulmonary nodules	4/7	BAL, BA	Hyphae	Negative	AmB	Death
18	F	CLL (1)	+	+	Bilateral infiltrate	2/2	BAL, BA	Hyphae	*A. fumigatus*	Itra	Survival
19	M	CML	+	−	Pulmonary nodules	6/6	Sputum	Hyphae	Negative	AmB	Survival
20	M	NHL	−	−	Pulmonary infiltrate plus meningitis	2/3	CSF	No hyphae	Negative	AmB plus Itra	Survival
Probable IA[i]											
21	M	NHL	+	−	Halo sign	0/5	BA	Hyphae	*A. fumigatus*	AmB	Death
22	F	AML	+	+	Infiltrate plus nodules	0/4	Sputum	Hyphae	*A. fumigatus*	AmB	Death
23	M	NHL	+	+	Halo sign	0/2	Sputum	Hyphae	*A. fumigatus*	AmB	Death
24	F	AML	+	−	Pulmonary nodules	0/6	Sputum	Hyphae	*A. flavus*	AmB	Death
25	M	MDS	−	−	Halo sign	0/4	BA	Hyphae	*A. fumigatus*	AmB	Death
26	M	NHL	+	+	Pulmonary nodules	0/6	BAL, BA	Hyphae	*A. fumigatus*	AmB	Death
27	M	AML (2)	−	+	Pulmonary nodules	0/3	BAL, BA	Hyphae	*A. fumigatus*	AmB	Death
28	M	Liver Tx	+	−	Cavitation	0/5	Sputum, BA	Hyphae	*A. fumigatus*	L AmB plus Itra	Survival
29	M	CLL	−	+	Halo sign	0/3	BAL, BA	Hyphae	*A. fumigatus*	AmB	Survival
30	F	NHL (2)	+	+	Halo sign	0/3	BAL	Hyphae	NI[h]	AmB	Survival
31	M	Severe CBPO	−	+	Cavitation	0/3	Trachea	Hyphae	*A. fumigatus*	AmB	Death
32	M	Pulmonary Tx	+	−	Pulmonary infiltrate	0/3	BAL, BA	Hyphae	*A. fumigatus*	Itra	Survival
33	M	Severe CBPO	−	+	Pulmonary nodules	0/2	BA	Hyphae	*A. fumigatus*	Itra	Survival
34	F	AML	+	−	Cavitation	0/2	BA	Hyphae	*A. flavus*	AmB	Survival

[a] M, male, F, female.
[b] AML, acute myeloid leukemia; NHL, non-Hodgkin's lymphoma; CLL, chronic lymphocytic leukemia; AL, acute leukemia; MDS, myelodysplastic syndrome; Tx, transplantation; CBPO, chronic brondhopulmonary obstruction; (1), autologous bone marrow transplantation; (2), allogeneic bone marrow transplantation.
[c] +, Present; −, absent.
[d] BAL, bronchoalveolar lavage; BA, bronchoaspiration; CSF, cerebral spinal fluid.
[e] Presence of separate hyphae on direct microscopic observations.
[f] AmB, amphotericin B; L AmB, liposomial amphotericin B; Itra, itraconazole; (S), surgical resection.
[g] With positive antigenemia.
[h] NI, *Aspergillus* species not identified.
[i] With negative antigenemia.

negative. The index of these 15 patients was not always near the threshold of the test (for four patients, it ranged between 1.5 and 5.4). For the other 12 of 27 patients, the results of repeat testing were positive but isolated since the successive sera were all negative. Among these 12 patients, 7 were from the pediatric department and 4 were from the geriatric department. Among all of the retested samples, the reproducibility was 85%, and high variations were noted according to the batches. These results underline the necessity to double-check all positive samples because of the possible lack of reproducibility also reported in another study (20). It is also important to consider IA when at least two consecutive positive samples have been obtained, as mentioned by the manufacturer. For 3 patients out of 571, successive samples were highly positive (index of >2). These false-positive cases (Table 1) were from two infants (leukemia and immunoglobulin deficiency) and one elderly patient (76 years old, digestive cancer). However, the diagnosis of IA was totally excluded. It is now well established that this test gives false-positive results, especially in children, due to possible passage through the intestinal mucosa of galactomannan present in milk, rice, or rich protein nutriments (6, 9, 13, 17). Some drugs of fungal origin, such as antibiotics (2) or uricase (C. Pinel, unpublished data), could also cause persistent or transient false-positive results with this sensitive test. This putative process could be involved in a partial explanation of the false-positive results that were obtained with elderly at-risk patients in our study, since some nutriments, highly rich in proteins, have shown high titers in

galactomannan content with the Platelia test. Cross-reactivity with other fungal diseases could also be involved (19).

The sensitivity of this test, calculated on 34 proven and probable IA cases, was low (50.0%) despite the positive cutoff used (Table 1). The Platelia test was disappointing in the three proven IA cases, even though the number of sera was limited for one of the three patients (Table 2, case 3). However, the time of sampling coincided with the acute phase of fever and with the IA episode. Other authors have also noted low efficiency in proven IA cases, especially if at least two positive samples per patient were needed to consider IA (16). The presence of specific antibodies was also another cause of negative antigenemia (8). This is the case for two patients in this group (cases 1 and 2). Furthermore, in animal models, amphotericin B treatment could decrease the galactomannan level. The disappointing sensitivity of the test in our proven IA cases could also be linked to the fungal localization. Cases 2 and 3 corresponded to acute invasive *Aspergillus* sinusitis with extension to the orbit and bony destruction. Moreover, for patient 2 (Table 2), the causative agent was *Aspergillus terreus*. Thus, the galactomannan could be produced in a smaller quantity by some *Aspergillus* species, leading to a weak sensitivity. The test detected only 17 of 31 cases of probable IA. The cutoff index of 1 is a good alternative to increase the sensitivity. In our study, the cutoff of 1 did not modify the specificity. The true false-positive results were higher than 2, and values near the cutoff (index between 1 and 1.5) were related to some probable IA (Table 2, cases 8, 12, and 16).

The Platelia test is able to detect 0.5 to 1 ng of galactomannan per ml. The rapid elimination of this polysaccharide from blood, through a natural process, requires a series of repeated samples for an optimal diagnosis. This is an essential point. Indeed, as shown in Table 2, in the group of probable IA with positive antigenemia, 10 of 17 patients in this group nevertheless showed punctual and negative detection during the survey. The average number of serum samples was lower in the group of probable IA with negative antigenemia than in the group with positive antigenemia (average number of four versus five for the other group [Table 2]). Furthermore, in the course of the disease, the antigenemia level may increase with higher tissue burden. In the group of probable IA, the antigenemia increased in 6 of 10 patients with unfavorable outcome (cases 7, 8, 9, 12, 13, and 17) and remained stable but at a high level for the four other patients. However, the index values decreased for six of seven patients who survived. Thus, the decreasing antigen level often seems associated with a good prognosis (4). In a prospective study, the survey of all at-risk patients should be performed on a regular routine basis to avoid the false-negative results that may be obtained if, during the patient's survey, the antigen detection is not performed on a sufficient number of sera. Moreover, the galactomannan detection remains one of the major criteria, according to the consensus group EORTC/MSG (3), to establish the IA diagnosis even when the mycological detection was negative. Indeed, in our study, four of ten patients were classified in the group of probable IA (Table 2), although the direct-microscopy and the fungal-culture analyses were negative.

Even though this test contributes to improving IA diagnosis, the sensitivity was disappointing in proven and probable IA cases in our study and was lower than previously described (4, 14, 16). Interpretation should be careful during the routine survey because of false-positive results. It would be useful to add other types of specific antigen detection to this reagent (7) or additional methods in order to increase the sensitivity of IA immunodiagnosis.

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