Eur. J. Clin. Microbiol. Infect. Dis., 1996, 15: 139–145

Comparison of an Enzyme Immunoassay and Latex Agglutination Test for Detection of Galactomannan in the Diagnosis of Invasive Aspergillosis

A. Sulahian[1*], M. Tabouret[2], P. Ribaud[3], J. Sarfati[4], E. Gluckman[3], J.P. Latgé[4], F. Derouin[1]

Aspergillus antigenemia was followed up in 215 consecutively observed bone marrow transplant (BMT) patients over a period of two years, using both a latex agglutination test and a sandwich immunocapture enzyme immunoassay (EIA) with a rat anti-galactomannan monoclonal antibody as capture and detector antibody. For each patient, sequential sera (3 to 20) were obtained before and after BMT. No positivity was observed before BMT. After BMT, the EIA and latex agglutination test were positive in 19 and 4 patients respectively of 25 patients with confirmed aspergillosis and 14 and 7 of 15 patients with probable aspergillosis. In 19 of 25 patients with confirmed aspergillosis and 9 of 15 patients with probable aspergillosis, the EIA was more sensitive and detected infection earlier than the latex test. In all positive cases, antigenemia rapidly increased in sequential samples and remained strongly positive. In 31 of 169 (19%) BMT patients without clinical signs of aspergillosis, the EIA was occasionally positive in samples taken within the first month after BMT, giving a specificity of 81% in these patients. In non-BMT patients suffering from other diseases (n = 77), the specificity was 98.7%. The overall positive and negative predictive values for the EIA were 54% and 95% respectively. These results favour the use of EIA for early diagnosis and monitoring of aspergillosis in BMT patients, although the predictive value of transient positivity remains to be ascertained.

Invasive aspergillosis is a major problem in patients undergoing bone marrow transplantation (BMT). This mycosis is usually severe, rapidly progressive, and difficult to diagnose (1, 2), with a fatality rate approaching 100% despite treatment (3).

The mortality rate can be substantially reduced if an early diagnosis is made and the proper therapy given. Antibody detection is unsatisfactory in invasive aspergillosis of the immunocompromised host probably due to profound immunodeficiency (2, 4). The most promising diagnostic approach is the detection of *Aspergillus* antigens in the serum or urine of patients, and more specifically galactomannan, a cell wall component of *Aspergillus* that appears to be a specific indicator of invasive disease (5, 6). Since the pioneering study of Reiss and Lehmann (7), various techniques have been used to detect *Aspergillus* galactomannan, such as enzyme immunoassays (EIAs) (8–11), radioimmunoassays (12, 13), and latex agglutination tests (14–16). However, serious controversy exists regarding the use and utility of these immunological tests, mainly because the number of patients and sera tested has been too limited.

In this study the detection of *Aspergillus* galactomannan in sequential sera of 215 BMT patients by a new sandwich immunocapture EIA was evaluated, and the sensitivity and specificity of the EIA compared to that of the only commercially available latex agglutination kit. Both tests use the

[1] Laboratoire de Parasitologie-Mycologie, Hôpital Saint-Louis, 1 Avenue Claude Vellefaux, 75010 Paris, France.
[2] Sanofi Diagnostics Pasteur, Steenvoorde, France.
[3] Unité de Greffe de Moelle Osseuse, Hôpital Saint-Louis, Paris, France.
[4] Unité de Mycologie Médicale, Institut Pasteur, Paris, France.

Table 1: Comparison of the EIA and latex agglutination test for detection of galactomannan in sera from patients with confirmed or probable aspergillosis, or with an indeterminate diagnosis.

	EIA		Latex agglutination	
	Positive	Negative	Positive*	Negative
Group I				
Confirmed aspergillosis	19	6	4	21
< 30 days before aspergillosis	7		1	
> 30 days before aspergillosis	8		3	
After onset of symptoms	4		–	
Group II				
Probable aspergillosis	14	1	7	8
< 30 days before aspergillosis	4		6	
> 30 days before aspergillosis	5		1	
After onset of symptoms	5		–	
Group III				
Indeterminate	8	0	0	8
< 30 days before aspergillosis	2			
> 30 days before aspergillosis	4			
After onset of symptoms	2			

* No positive latex test was observed in EIA-negative sera.

same monoclonal anti-galactomannan antibody (MAB) EB-A2 which recognizes the (1– > 5)b-D-galactofuranoside side chains of the *Aspergillus* galactomannan (17).

Material and Methods

Patients. From August 1992 to October 1994, 211 consecutively observed patients (128 males and 83 females) with various hematological malignancies were followed up before and after allogeneic BMT. The BMT procedure and conditioning therapy have been described previously (18). The standard procedure for prophylaxis of infection was as follows: after BMT patients were maintained in a laminar airflow room for at least 30 days and received nonabsorbable antibiotics for gut decontamination, fluconazole (100 mg/day) and ketoconazole (400 mg/day) for prevention of fungal infections, and acyclovir for prevention of herpes virus infection. Patients were divided into four groups according to the criteria presently advocated for diagnosis of aspergillosis. Group I consisted of 25 patients with confirmed aspergillosis. The diagnosis was based on the isolation of *Aspergillus* from clinical specimens, and/or histological detection of mycelial filaments in biopsy or autopsy specimens, and presence of characteristic lesions on CT scans; the species isolated were *Aspergillus fumigatus* (n = 25), *Aspergillus flavus* (n = 2), and *Aspergillus nidulans* (n = 1). Group II consisted of 15 patients with probable aspergillosis; this diagnosis was based on the presence of characteristic features on CT scans, pulmonary symptoms, persistent fever ≥ 38°C unresponsive to broad-spectrum antimicrobial therapy, but no mycological evidence of aspergillosis. Group III consisted of eight patients in whom the diagnosis of aspergillosis was considered indeterminate on the basis of pulmonary symptoms (cough, chest pain), persistent fever not responding to antimicrobial therapy, and nonspecific radiographic findings. Group IV (control group) consisted of 163 patients with no evidence of aspergillosis or any other fungal infection.

Sera and Other Samples. A total of 2,161 serial serum samples (3 to 20 sera per patient, mean 10 sera) were obtained 15 or 7 days before BMT (n = 426), weekly the first month after BMT, and then monthly (n = 1,735). Two hundred ninety-five samples were tested in the first group, 165 in the second group, 69 in the third group, and 1,632 in the control group. The mean follow-up period was 90 days, with a range of 1 to 150 days. Whenever aspergillosis was suspected clinically or radiologically, sera were obtained daily and tested. Sera were kept at 4°C until testing (within 48 hours), then stored at −20°C.

The specificity of the EIA was evaluated by testing 77 sera from patients with candidiasis (n = 18), cryptococcosis (n = 19), protozoal infections (n = 10), bacterial infections (n = 13), or dysproteinemia (n = 17). Other samples tested to assess the specificity of the EIA comprised rabbit and horse anti-lymphocytic sera (n = 6), alone or with cyclosporin A added, and 12 samples of blood transfusion products which had been filtered on cellulose.

Antigen Detection Techniques. Circulating *Aspergillus* galactomannan was detected using both a latex agglutination test (Pastorex *Aspergillus,* Sanofi Diagnostics Pasteur, France) and a sandwich immunocapture EIA (Sanofi Diagnostics Pasteur, France) with a rat-galactomannan MAB Eb-A2 as the capture and detector antibody (10). For both techniques, immune complexes were disrupted by adding 100 µl 4% w/v EDTA to 300 µl of undiluted serum in a tightly closed Eppendorf tube which was heated to 100°C for 3 min, then centrifuged at 14,000 x *g* for 10 min. The supernatant was used for both tests.

For the latex test, 40 µl of the supernatant were transferred onto disposable plastic cards. Ten µl of the latex reagent was added and mixed with the supernatant using mixing sticks. The results were recorded after 5 min of rotation at 170 rpm. A galactomannan control of 16.5 ng/ml was included with the kit and tested with each batch of serum. Whenever positive results were obtained, twofold dilutions were used. The limit of sensitivity of the latex agglutination test, as determined by the manufacturer, was 15 ng/ml of purified galactomannan.

For the EIA technique, all reagents were provided by the manufacturer. The sensitized microtiter plates were washed once with a wash solution, and then each well was filled with 50 µl of horseradish peroxidase MAB Eb-A2 in conjugate buffer followed by 50 µl of the supernatant to be tested.

Plates were incubated for 90 min at 37°C. After thorough washings (5 times), the reaction was obtained by 30 min incubation in darkness with 100 µl of buffer containing o-phenylenediamine. The optical density (OD) was read at λ = 492/620 nm. In each set of experiments a negative control and two positive controls containing 1 and 10 ng respectively of galactomannan were included. The threshold of positivity was estimated to be 0.8 from the mean OD values of 100 control sera plus 5 standard deviations; it corresponded to approximately 1 ng/ml of galactomannan. All sera were tested

Table 2: Results of the EIA and latex agglutination test (LA) in 25 BMT patients with proven aspergillosis.

Patient no.	Underlying disease[a]	Site of infection	Onset of symptoms after transplant (days)	First day of positivity after transplant[b]		
				Culture[c]	EIA	LA
1	ALL	disseminated	26	post-mortem (death day 40) lungs, heart	12 (10)	34 (10)
2	ALL	lungs	22	80 nose	– (27)	– (27)
3	CML	lungs	75	79 BAL	76 (13)	– (13)
4	AML	lungs, brain	63	66 BAL	63 (8)	– (8)
5	myeloid splenomegaly	lungs	137	150 BAL	7 (9)	– (9)
6	AML	disseminated	276	276 BAL	276 (5)	– (5)
7	ALL	lungs	127	131 BAL	– (19)	– (19)
8	ALL	disseminated	150	post-mortem (death day 179) BAL	61 (20)	– (20)
9	PNH	disseminated	3	12 BAL	12 (20)	12 (20)
10	congenital dyskeratosis	disseminated	192	204 sputum	5 (8)	– (8)
11	AML	disseminated	97	post-mortem (death day 104) liver	97 (8)	– (8)
12	AML	lungs	100	116 BAL	– (18)	– (18)
13	myeloproliferative syndrome	lungs	92	138 sputum	6 (8)	– (8)
14	AA	lungs, brain	36	8, 95 nose, pleura	6 (17)	– (17)
15	CML	lungs	70	70 BAL	19 (9)	– (9)
16	AML	lungs	227	290 BAL	– (12)	– (12)
17	CML	lungs	237	238 lung	41 (15)	– (15)
18	RAEB	lungs, brain	40	40 BAL	39 (8)	39 (8)
19	CML	lungs	40	102, 110 lung	34 (14)	– (14)
20	ALL	disseminated	48	52 BAL	53 (5)	– (5)
21	AML	sinus	228	270 sinus	– (8)	– (8)
22	AML	lungs	37	37 BAL	– (9)	– (9)
23	CML	lungs	54	55 BAL	6 (17)	– (17)
24	CML	brain, lungs	85	85 sputum	90 (15)	91 (15)
25	CML	brain	21	24 BAL	6 (10)	– (10)

[a]ALL = acute lymphoblastic leukemia; AML = acute myeloid or nonlymphoblastic leukemia; CML = chronic myeloblastic leukemia; AA = aplastic anemia; RAEB = refractory anemia with excess blasts; PNH = paroxystic nocturnal hemoglobinuria.
[b]Number of tests performed is given in brackets.
[c]*Aspergillus fumigatus* was cultured in all cases except patients no. 2 and 13 (*A. flavus*) and no. 17 (*A. nidulans*).

Table 3: Results of the EIA and latex agglutination test (LA) in 15 BMT patients with probable aspergillosis who were culture negative.

Patient no.	Underlying disease[a]	Site of infection	Onset of symptoms after transplant (days)	First day of positivity after transplant[b]	
				EIA	LA
1	CML	disseminated	120	82 (17)	125 (17)
2	myeloid splenomegaly	lungs	78	– (9)	– (9)
3	lymphoma	lungs	240	11 (14)	– (14)
4	lymphoma	lungs	1	25 (10)	– (10)
5	CML	lungs, brain	56	56 (13)	56 (13)
6	CML	disseminated	75	143 (17)	148 (17)
7	Fanconi's syndrome	lungs	74	6 (5)	– (5)
8	AML	lungs, brain	117	6 (11)	– (11)
9	ALL	lungs, brain	112	114 (1)	114 (1)
10	CML	brain	62	60 (12)	64 (12)
11	myeloproliferative syndrome	lungs, brain	105	6 (13)	113 (13)
12	RAEB	disseminated	64	74 (10)	– (10)
13	AA	brain	111	112 (10)	– (10)
14	ALL	brain	81	81 (14)	89 (14)
15	AL	brain, lungs	19	11 (9)	– (9)

[a]ALL = acute lymphoblastic leukemia; AML = acute myeloid or nonlymphoblastic leukemia; CML = chronic myeloblastic leukemia; AA = aplastic anemia; RAEB = refractory anemia with excess blasts.
[b]Number of tests performed is given in brackets.

in duplicate using two different coated plates. Results of the EIA were kept confidential until the end of the study and thus did not interfere with any decisions concerning treatment or prophylaxis.

Results

Internal controls for the EIA and latex agglutination test yielded no false-positive or false-negative results. The reproducibility of the EIA estimated from 50 determinations was satisfactory, with a mean OD value (with standard deviation) of 0.70 ± 0.19 (coefficient of variation 28%) for the 1 ng galactomannan/ml positive control and 2.53 ± 0.34 (coefficient of variation 13%) for the 10 ng galactomannan/ml positive control. The specificity of the EIA was 98.7% since only one positive reaction was observed among the 77 sera from patients with various diseases other than aspergillosis; this reaction occurred in a case of candidemia (OD 1.03).

Cumulative results for all patient groups are summarized in Table 1, and individual results for each group are shown in Tables 2 and 3.

In the group of patients with confirmed aspergillosis (Group I, Table 2), the EIA was positive in 19 of 25 patients (Table 1). The EIA was positive less than 30 days before the onset of symptoms in 7 patients and more than 30 days before the onset of symptoms in 8 patients; in 2 patients the test was constantly positive until aspergillosis (Figure 1A); 6 showed transient positivity before aspergillosis (i.e. alternately positive and negative samples) (Figure 1B). Four of 21 patients were EIA positive after onset of symptoms. In six patients, the EIA was constantly negative during follow-up. In the latex agglutination test, 4 of 25 patients were positive (Table 1). One patient was positive less than 30 days before the onset of symptoms and three after the onset of symptoms. No positivity was observed in either the EIA or latex test before BMT.

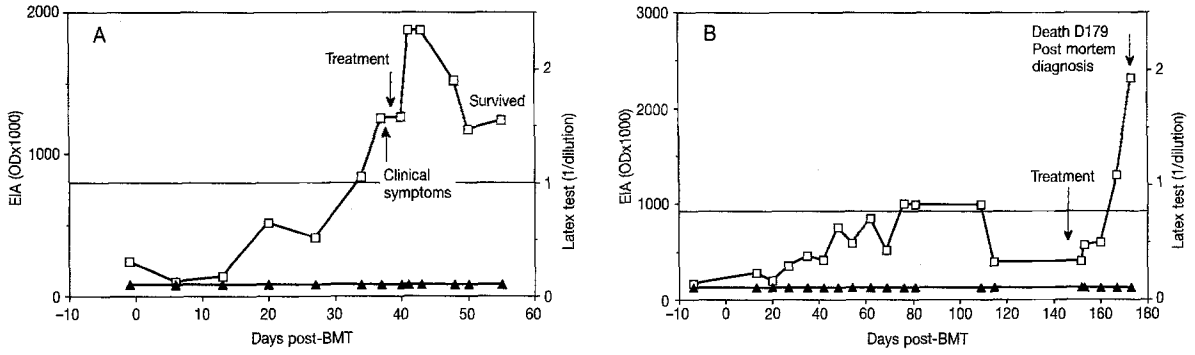

Figure 1: Follow-up of antigenemia by EIA (□□□) and latex agglutination test (▲▲▲) in patients with confirmed aspergillosis. **A:** constantly positive EIA until occurrence of aspergillosis (clinical symptoms on day 40) with constantly negative latex test (Table 2, patient no. 19). **B:** transiently positive EIA with no clinical symptoms, then strongly positive EIA at the time of disseminated aspergillosis (confirmed by isolation of *Aspergillus fumigatus* at autopsy on day 179). Constantly negative latex test (Table 2, patient no. 8). Horizontal line indicates threshold of positivity of EIA (OD 0.8).

In the group of patients with probable aspergillosis (Group II, Table 3), 14 of 15 patients were positive in the EIA and 7 of 15 in the latex test (Table 1). The EIA was positive less than 30 days before the onset of symptoms in 4 patients and more than 30 days before the onset of symptoms in 5 patients. In two patients the test was constantly positive until occurrence of aspergillosis, and three showed transient positivity before aspergillosis. In the latex agglutination test, six patients were positive less than 30 days before the onset of symptoms and one patient was positive after the onset of symptoms. No positivity was observed in either test before BMT.

In both groups I and II, antigenemia was detected earlier with the EIA compared to the latex test; both tests were positive in 11 and 7 cases respectively, but the EIA gave positive results earlier than the latex test by an average of 27 days (range 1–107 days). The galactomannan concentration rapidly increased in sequential samples and remained high (Figure 2A and B). The latex test did not detect any serum that was EIA negative, and overall in groups I and II the sensitivity was 82.5% for the EIA and 27.5% for the latex test.

In the group III patients with an indeterminate diagnosis, 2 were positive in the EIA less than 30 days before onset of symptoms and 4 were positive more than 30 days before; 1 was constantly positive and 3 showed transient positivity before the onset of symptoms; 2 patients became positive after the onset of symptoms. No patient was positive in the latex test.

In the control group IV, 138 of 169 patients (1,449 sera) were negative during follow-up. Thirty-one patients (19%) had at least one positive EIA result (82 of 183 sera), mainly before day 30 (mean day of first positive serum, day 10; range, 5–27 days). In most cases, the OD value exceeded 1.2

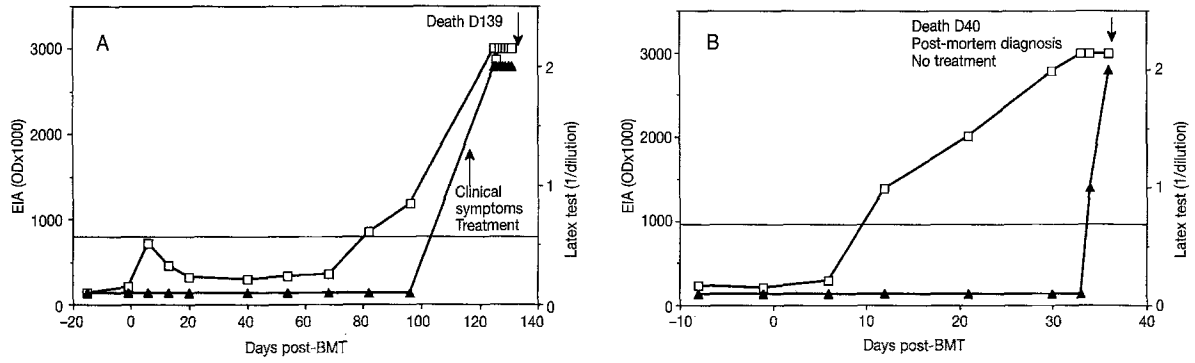

Figure 2: Follow-up of antigenemia by EIA (□□□) and latex agglutination test (▲▲▲) in patients with confirmed or probable aspergillosis. **A:** Probable disseminated aspergillosis on day 120. EIA positive 30 days earlier than latex test (Table 3, patient no. 1). **B:** Disseminated aspergillosis confirmed at autopsy (post-mortem isolation of *Aspergillus fumigatus* on day 40). EIA positive 22 days earlier than latex test (Table 2, patient no. 1). Horizontal line indicates threshold of positivity of EIA (OD 0.8).

and in 12 patients this positivity persisted in at least 3 sequential samples. Review of the clinical records and the results of laboratory tests did not yield any information which would explain this positivity; no patient had received antifungal therapy. Control tests of immune sera (horse and rabbit antilymphocytic sera) alone or with added cyclosporin, as well as of the supernatant of filtered blood products, were all negative. No false-positive results were observed in the latex test.

The predictive values for each test were determined in each group by comparison with the control group without fungal infection. In the groups with confirmed or probable aspergillosis, the positive predictive value was 54% in the EIA and 100% in the latex test, and the negative predictive value was 95% in the EIA and 84.9% in the latex test.

Discussion

In this study two techniques for detection of galactomannan were compared, allowing an evaluation of the potential uses and limitations of these tests in the clinical and laboratory follow-up of BMT patients. Although the tests use the same monoclonal antibody, the results differed markedly in terms of sensitivity and specificity.

For the latex agglutination test, the specificity was 100%, but the sensitivity was 16% in the patients with confirmed aspergillosis, 47% in the patients with probable infection, and 27.5% in both groups considered together. These results agree with those obtained by Manso et al. (19) who reported a sensitivity of 38.8% and a specificity of 95% in 436 sequential serum samples from 79 neutropenic patients using the same test. However, these results differed from those reported by Haynes and Rogers (20) who did a retrospective study on the detection of circulating *Aspergillus* galactomannan in sera from 121 immunocompromised patients, including 19 with proven invasive aspergillosis; the sensitivity (95%), specificity (90%), and negative predictive values (99%) of the latex test were similar to previously published results obtained with an inhibition EIA (9). However, the positive predictive value of the latex test was only 67% compared to $\geq$ 95% obtained with the inhibition EIA, and the latex test thus had less value in predicting the onset of invasive pulmonary aspergillosis (9, 20). In two other studies (15, 16), the latex test was found to have low sensitivity and specificity in bone marrow and liver transplant patients. These discrepancies in results obtained with the latex test may be due to differences in the selection of patients and/or the test conditions.

EIA antigen detection techniques have previously been shown to have a high sensitivity and specificity (9–11). The presence of multiple epitopes recognized by an MAB on one galactomannan molecule made possible the development of a double-direct sandwich EIA with MAB Eb-A2 as the capture and detector (after conjugation with peroxidase) antibody (21). Using this technique, we obtained a sensitivity of 82.5% in patients with confirmed or probable aspergillosis. In contrast to the generally accepted postulate, antigenemia was found not to be transient in character as the concentration of galactomannan usually increased with progression of the disease.

However, as observed by Verweij et al. (11) using the same EIA, the increase in sensitivity of galactomannan detection was associated with the occurrence of false-positive reactions early after BMT, reducing the specificity to 81% in our patients. These results contrasted with the absence of such false-positive reactions in the 431 sera drawn before BMT and with the high specificity obtained in sera from patients with other infections (98.7%). The false-positive reactions were not associated with any particular disease or with different treatment of samples; most of the false-positive reactions occurred less than 30 days after BMT, a period during which patients receive strong immunosuppressive therapy and frequent transfusions with blood products. Two hypotheses can be advanced to account for these false-positive reactions: the antigenemia may have been transient and induced by the immunosuppressive therapy, or there was cross-reactivity with unidentified serum components. The absence of false-positive reactions with anti-globulin serum or transfused blood products rules out interference of these factors.

Despite these drawbacks, the development of this EIA represents a marked improvement in the serological diagnosis and follow-up of patients at risk for aspergillosis. The EIA detects galactomannan at concentrations less than 1 ng/ml whereas the latex test using the same monoclonal antibody does not detect concentrations less than 15 ng/ml (10). The detection threshold of the EIA is low enough to allow invasive aspergillosis to be suspected prior or concomitant to the onset of early clinical signs. In patients with confirmed or probable aspergillosis, the sensitivity of the EIA was

markedly superior to that of the latex test which was never positive in sera that were negative in the EIA. Our follow-up study indicated that the EIA detected galactomannan earlier than the latex test when the latter was positive. In almost all cases of invasive aspergillosis with detectable antigenemia, the EIA OD values rapidly increased in sequential sera samples and remained strongly positive. In patients undergoing treatment, no sufficiently reliable information could be obtained on the effect of treatment on antigenemia because of the great variety of therapeutic schedules that were administered. However, we can conclude that the EIA is useful for the diagnosis of aspergillosis in BMT patients although the predictive value of transient positivity remains to be ascertained. Additional studies are needed to define the potential value of this test for monitoring therapy.

Acknowledgements

We thank T. Pottet, A. Cousin, C. Chottin, M.C. Serugue, and C. Barnel for their technical assistance and Dr. P.H. David for reviewing the manuscript.

References

1. Bodey GP, Vartivarian S: Aspergillosis. European Journal of Clinical Microbiology & Infectious Diseases 1989, 8: 413–437.
2. Kurup VP, Kumar A: Immunodiagnosis of aspergillosis. Clinical Microbiology Reviews 1991, 4: 439–456.
3. Denning DW, Stevens DA: Antifungal and surgical treatment of invasive aspergillosis: a review of 2121 published cases. Reviews of Infectious Diseases 1990, 12: 1147–1201.
4. Barnes RA: *Aspergillus* infection: does serodiagnosis work? Serodiagnosis and Immunotherapy in Infectious Diseases 1993, 5: 135–138.
5. Dupont B, Huber M, Kim SJ, Bennett JE: Galactomannan antigenemia and antigenuria in aspergillosis, studies in patients and experimentally infected rabbits. Journal of Infectious Diseases 1987, 155: 1–11.
6. Fujita SI, Matsubara F, Matsuda T: Demonstration of antigenemia in patients with invasive aspergillosis by biotin-streptavidin enzyme-linked immunosorbent assay. Journal of Laboratory and Clinical Medicine 1988, 112: 464–470.
7. Reiss E, Lehmann PF: Galactomannan antigenemia in invasive aspergillosis. Infection and Immunity 1979, 55: 357–365.
8. Sabetta JR, Miniter P, Andriole VT: The diagnosis of invasive aspergillosis by an enzyme-linked immunosorbent assay for circulating antigen. Journal of Infectious Diseases 1985, 152: 946–953.
9. Rogers TR, Haynes KA, Barnes RA: Value of antigen detection in predicting invasive pulmonary aspergillosis. Lancet 1990, 336: 1210–1213.
10. Stynen D, Goris A, Sarfati J, Latgé JP: A new sensitive sandwich enzyme-linked immunosorbent assay to detect galactofuran in patients with invasive aspergillosis. Journal of Clinical Microbiology 1995, 33: 497–500.
11. Verweij PE, Stynen D, Rijs AJMM, de Pauw BE, Hoogkamp-Korstanje JAA, Meis JFGM: Sandwich enzyme-linked immunosorbent assay compared with Pastorex latex agglutination test for diagnosing invasive aspergillosis in immunocompromised patients. Journal of Clinical Microbiology 1995, 33: 1912–1914.
12. Shaffer PJ, Kobayashi GS, Medoff G: Demonstration of antigenemia in patients with invasive aspergillosis by solid phase (protein A-rich *Staphylococcus aureus*) radioimmunoassay. American Journal of Medicine 1979, 67: 627–630.
13. Talbot JH, Weiner MH, Gerson SL, Provencher M, Hurwitz S: Serodiagnosis of invasive aspergillosis in patients with hematologic malignancy: validation of the *Aspergillus fumigatus* antigen radioimmunoassay. Journal of Infectious Diseases 1987, 155: 12–27.
14. Dupont B, Improvisi L, Prévot F: Détection de galactomannane dans les aspergillosis invasives humaines et animales avec un test au latex. Bulletin de la Société Française de Mycologie Médicale 1990, 21: 35–41.
15. Ansorg R, Heintschel von Heinegg E, Rath PM: *Aspergillus* antigenuria compared to antigenemia in bone marrow transplant recipients. European Journal of Clinical Microbiology & Infectious Diseases 1994, 13: 582–589.
16. Hopwood V, Johnson EM, Cornish JM, Foot ABM, Evans EGV, Warnock DW: Use of the Pastorex aspergillus antigen latex agglutination test for the diagnosis of invasive aspergillosis. Journal of Clinical Pathology 1995, 48: 210–213.
17. Stynen D, Sarfati J, Goris A, Prévost MC, Lesourd M, Kamphuis H, Darras V, Latgé JP: Rat monoclonal antibodies against *Aspergillus* galactomannan. Infection Immunity 1992, 60: 2237–2245.
18. Saugier-Veber P, Devergie A, Sulahian A, Ribaud P, Traoré F, Bourdeau-Esperou H, Gluckman E, Derouin F: Epidemiology and diagnosis of invasive pulmonary aspergillosis in bone marrow transplant patients: results of a 5-year retrospective study. Bone Marrow Transplantation 1993, 12: 121–124.
19. Manso E, Montillo M, De Sio G, D'Amico S, Discepoli G, Leoni P: Value of antigen and antibody detection in the serological diagnosis of invasive aspergillosis in patients with hematological malignancies. European Journal of Clinical Microbiology & Infectious Diseases 1994, 13: 756–760.
20. Haynes K, Rogers TR: Retrospective evaluation of a latex agglutination test for diagnosis of invasive aspergillosis in immunocompromised patients. European Journal of Clinical Microbiology & Infectious Diseases 1994, 13: 670–674.
21. Latgé JP, Kobayashi H, Debeaupuis JP, Diaquin M, Sarfati J, Wieruszeski JM, Parra E, Bouchara JP, Fournet B: Chemical and immunological characterization of the extracellular galactomannan of *Aspergillus fumigatus*. Infection and Immunity 1994, 62: 5424–5433.