

Clinical evaluation and reproducibility of the Pastorex *Aspergillus* antigen latex agglutination test for diagnosing invasive aspergillosis

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Abstract

Aims—The performance of the Pastorex *Aspergillus* antigen latex agglutination test for the detection of galactomannan in sera of patients at risk for invasive aspergillosis was evaluated, and the impact of storage on the reproducibility of the antigen titre was tested.

Methods—During a one year period, 392 serum samples were obtained from 46 patients at risk for invasive aspergillosis and tested for the presence of galactomannan using an *Aspergillus* latex agglutination test (Pastorex). Twenty three positive serum samples which had been stored at -20°C for 2–16 months were retrospectively retested. Furthermore, two positive serum samples were stored at -20°C and -70°C and prospectively tested at three month intervals for a period of 15 months.

Results—The Pastorex *Aspergillus* test was positive in eight patients with microbiological, radiological, or histological evidence for invasive aspergillosis, but was negative in the initial serum sample from five of these patients. In two patients with histological evidence for invasive aspergillosis no positive reaction was found in six samples. Six of 13 (45%) serum samples which had been stored at -20°C for longer than six months had lost reactivity, while one of 10 (10%) samples had lost reactivity when stored up to six months. Two serum samples which had been stored at -20°C and -70°C and prospectively retested at three month intervals for 15 months, maintained stable antigen titres.

Conclusions—The Pastorex *Aspergillus* test is too insensitive to diagnose invasive aspergillosis in an early stage, but may contribute to the diagnosis when cultures remain negative and serial samples are obtained. To maintain a good reproducibility, serum samples should be stored at -70°C when the period of storage exceeds six months.

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treatment is associated with a reduced mortality rate,¹ reliable diagnostic tests capable of detecting invasive aspergillosis in an early stage are of great importance. Early diagnosis is, however, extremely difficult. Therefore tests have been developed to detect *Aspergillus* antigens in body fluids.² Several serological tests to detect antigenaemia have been used, including an enzyme linked immunoassay and a radioimmunoassay using purified galactomannan.³⁻⁵ Monoclonal antibodies raised against galactomannan⁶ are the basis of a rapid commercial latex agglutination test (Pastorex *Aspergillus*, Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France) on serum for detection of circulating antigen. This test, which has been applied in many hospitals, yielded sensitivities up to 95% for patients with proven invasive aspergillosis,^{7,8} but has been reported to be of limited value because results became negative when testing was repeated after storage.^{9,10} Here, we report our experience with the Pastorex *Aspergillus* test in patients at risk for invasive aspergillosis, and the results of a study we conducted to determine the impact of the duration of storage and repeated freezing and thawing on the antigen titre.

Methods

During a one year period, 392 serum samples were obtained from 46 patients at risk for invasive aspergillosis. At least two serum samples per patient were collected and processed within 24 hours of collection, and frozen at -20°C until retesting. Testing of all samples was performed by one technician and according to the manufacturer's instructions. Titration of positive samples was performed by successive twofold dilutions (1:2 to 1:32) in glycine dilution to buffer. To determine the impact of storage on the antigen titre, all positive serum samples were retested retrospectively.

In addition, a prospective study was performed with two positive serum samples obtained from one patient with a histologically proven disseminated infection with *A flavus*. The impact of repeated freezing and thawing was studied by storing 6 ml of each serum at -20°C and -70°C , respectively, for a total period of 15 months. At three month intervals the samples were thawed and the agglutination reaction was performed. Positive agglutination reactions were titrated. The impact of storage on the antigen titre was studied by dividing the remainder of the two serum samples into 20

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Invasive aspergillosis is a serious opportunistic infection with a high mortality rate in patients with compromised host defences. Since early

Table 1 Clinical evaluation and reproducibility of Pastorex *Aspergillus* latex agglutination test in patients at risk for invasive aspergillosis

Patient No	Age/sex	Underlying disease	Postmortem diagnosis	No of samples tested/ No of positive samples	Titre	No of samples retested/ No of positive samples	Titre	Time between first/second test (months)
1	16/F	NHL, BMT	<i>Aspergillus</i> in bronchi, not invasive	6/1	1:4	1/0	neg	16
2	58/M	Hepatocellular carcinoma	Mycotic necrotising bronchopneumonia	3/2	1:4	2/2	1:2	15
3	36/F	AML	ND	1/1	1:8	1/1	1:4	14
4	45/F	Kahler, BMT	Survived	18/2	1:8	1/1	1:4	11
5	30/M	AML	Cavitating bronchopneumonia invasive in lung tissue	22/8	1:4	8/3	1:1	9
					1:1		1:1	
					1:4		1:1	
					1:1		neg	
					1:4		neg	
					1:1		neg	
					1:4		neg	
					1:1		neg	
6	44/F	AML, BMT	Mycotic necrotising bronchopneumonia	8/3	1:2	3/3	1:2	6
					1:2		1:2	
					1:2		1:2	
7	53/M	CML, BMT	ND	9/4	1:1	4/3	1:1	2
					1:1		1:1	
					1:2		1:1	
					1:1		neg	
8	37/M	AIDS	ND	3/3	1:1	3/3	1:1	2
					1:1		1:1	
					1:4		1:2	

NHL=non-Hodgkin's lymphoma; BMT=bone marrow transplantation; AML=acute myeloid leukaemia; CML=chronic myeloid leukaemia; AIDS=acquired immunodeficiency syndrome; ND=not done.

Eppendorf tubes (300 µl). Ten tubes were stored at -20°C and 10 at -70°C for a total period of 15 months. At three month intervals two tubes stored at -20°C and two stored at -70°C were thawed. The first of each pair of samples was used to perform the agglutination reaction. When positive, the second sample was used for titration.

The Mann-Whitney test was used to determine statistical significance.

Results

A total of 392 serum samples was tested from 46 patients suspected of invasive aspergillosis. Seventy serum samples were collected from eight patients with microbiological, radiological, or histological evidence of invasive aspergillosis (table 1). Of these, 24 samples were found to be positive (titres ranged from 1:1 to 1:8). In all cases the Pastorex *Aspergillus* test results were interpreted in the whole clinical context of the patient. Antifungal therapy was not given in two cases (table 1, patients Nos 2 and 4). In five cases the antigen test was

negative at the time antifungal therapy was started, but became positive during treatment (table 1, patients Nos 1, 3, 5, 7, and 8). In one case (table 1, patient No. 6) antifungal therapy was initiated when the Pastorex *Aspergillus* test was found positive after five successive samples had been negative. Culture of a broncho-alveolar lavage performed the same day yielded *A fumigatus*. Two patients (acute lymphoblastic leukaemia and myelodysplastic syndrome) with histological evidence of invasive aspergillosis showed no positive reaction in six samples. We found only one false positive agglutination in a sample which arrived at the laboratory more than 48 hours after collection. Eight other samples from this patient with chronic granulomatous disease, processed within six hours of collection, were negative. A necropsy performed on this patient, who died of cardiac failure, revealed no evidence of invasive aspergillosis.

Twenty three positive samples, which had been stored at -20°C for 2-16 months, were available for retesting. The results of retesting are shown in table 1. Nine out of 10 (90%) positive serum samples, which had been stored up to six months at -20°C, showed a positive agglutination reaction when retested retrospectively, while seven of 13 (54%) samples were found positive when stored at -20°C for longer than six months (table 1; p=0.067).

The effect of storage at -20°C and -70°C and of repetitive freezing and thawing of serum on the galactomannan antigen titre is shown in table 2.

Discussion

Since serial antigen determination has been shown to be of great importance,⁵ we obtained

Table 2 Effect of storage on *Aspergillus* antigen titre. A: Storage at -20°C and -70°C. Serum samples were thawed before every agglutination test. B: Storage at -20°C and -70°C. Serum samples were stored until antigen titre was determined. Serum 1 (S1) and 2 (S2) were taken 3 days and 1 day, respectively, before death due to invasive aspergillosis

Time between tests (months)	Storage			
	A		B	
	S1 (-20°C)	S2 (-70°C)	S1 (-20°C)	S2 (-70°C)
0	1:8	1:32	1:8	1:32
3	1:8	1:16	1:8	1:16
6	1:8	1:16	1:8	1:16
9	1:8	1:16	1:8	1:16
12	1:8	1:16	1:8	1:16
15	1:8	1:16	1:8	1:16

as many serum samples as possible from patients at risk for invasive aspergillosis. Several investigators have reported the use of a number of methods to detect circulating *Aspergillus* antigen in body fluids.^{2-5,11} Although antigen detection appears to be highly specific, the sensitivity of the tests used until now has been quite low. The Pastorex *Aspergillus* test is the only antigen test which is commercially available, but its use to diagnose invasive aspergillosis in an early stage is controversial. Several investigators have reported a high sensitivity of this test, up to 95%,^{7,8} and found that the Pastorex test allowed diagnosis of invasive aspergillosis to be made earlier in 68% of cases when compared to conventional methods.⁸ However, other studies have shown that the Pastorex *Aspergillus* test had a low sensitivity¹² and a poor positive predictive value^{8,13} in necropsy proven cases of invasive aspergillosis. Our results also indicate that the Pastorex *Aspergillus* test is too insensitive to diagnose invasive aspergillosis in an early stage, but may contribute in the diagnosis if cultures remain negative and serial serum samples are obtained.

Poor reproducibility of the Pastorex *Aspergillus* test has been reported,^{9,10} which can make the interpretation of positive agglutination results difficult. Warnock *et al*⁹ retested 10 positive serum samples which had been stored for three days to 10 months at -30°C , and found that all samples had become negative, and Knight *et al*¹⁰ retested 32 positive samples with a different batch and found loss of reactivity in 14 samples. However, these reactions could have been false positive as the manufacturer's instructions were not followed.¹⁴ In the present study, serum samples which had been stored at -20°C for more than six months tended to lose reactivity. Although, because of the limited number of positive samples available, the difference was not statistically significant in comparison to serum samples stored for less than six months, it shows a trend towards loss of reactivity when serum samples are stored at -20°C for long periods of time. Also, the titre found at retesting tended to be lower when the period of storage became longer.

The serum samples which were stored at -20°C and -70°C and prospectively retested at three month intervals were all found to be positive with stable antigen titres. Repeated freezing and thawing of these serum samples (table 2, A) did not appear to have an effect on the reproducibility. However, to maintain a good reproducibility in the Pastorex *Aspergillus* test, we recommend that serum samples should be stored at -70°C when the period of storage exceeds six months.

In the absence of better commercial antigen detection, the Pastorex *Aspergillus* test remains

useful, although a better test needs to be developed. Other techniques, including DNA based assays, may contribute in the diagnosis of invasive aspergillosis in an early stage. Several investigators have reported the use of the polymerase chain reaction (PCR) for the detection of *Aspergillus* DNA in BAL fluid.¹⁵⁻¹⁸ The PCR showed a very high sensitivity and specificity in patients at high risk for invasive aspergillosis. However, the value of the PCR in the diagnosis of this condition remains to be established and prospective evaluations are now being performed. The diagnosis of invasive aspergillosis is difficult and demands a multidisciplinary approach. The implementation and combination of novel or improved methods, for example PCR and antigen detection, to diagnose invasive aspergillosis in an early stage or in guiding treatment may improve the clinical outcome of this disease.

- 1 Aisner J, Schimpff S, Wiernik PH. Treatment of invasive aspergillosis: relation of early diagnosis and treatment to response. *Ann Intern Med* 1977;86:539-43.
- 2 Andriole VT. Infections with *Aspergillus* species. *Clin Infect Dis* 1993 (suppl 2);17:481-6.
- 3 Sabetta JR, Minter P, Andriole VT. The diagnosis of invasive aspergillosis by an enzyme-linked immunosorbent assay for circulating antigen. *J Infect Dis* 1985;152:946-53.
- 4 Talbot GH, Weiner MH, Gerson SL, Provencher M, Hurwitz S. Serodiagnosis of invasive aspergillosis in patients with hematologic malignancy: validation of the *Aspergillus fumigatus* antigen radioimmunoassay. *J Infect Dis* 1987;155:12-27.
- 5 Weiner MH, Talbot GH, Gerson SL, Provencher M, Hurwitz S. Galactomannan antigenemia and antigenuria in aspergillosis: utility in controlled, blinded trials. *Ann Intern Med* 1987;99:777-82.
- 6 Stynen D, Sarfati J, Goris A, *et al*. Rat monoclonal antibodies against *Aspergillus* galactomannan. *Infect Immun* 1992;60:2237-45.
- 7 Dupont B, Improvisi L, Provost F. Détection de galactomannane dans les aspergilloses invasives humaines et animales avec un test au latex. *Bull Soc Fr Mycol Med* 1990;19:35-41.
- 8 Haynes K, Rogers TR. Retrospective evaluation of a latex agglutination test for diagnosis of invasive aspergillosis in immunocompromised patients. *Eur J Clin Microbiol Infect Dis* 1994;13:670-4.
- 9 Warnock DW, Foot ABM, Johnson EM, Mitchell SB, Cornish JM, Oakhill A. *Aspergillus* antigen latex test for diagnosis of invasive aspergillosis. *Lancet* 1991;338:1023-4.
- 10 Knight F, Mackenzie DWR. *Aspergillus* antigen latex test for diagnosis of invasive aspergillosis. *Lancet* 1992;339:188.
- 11 Kurup VP, Kumar A. Immunodiagnosis of aspergillosis. *Clin Microbiol Rev* 1991;4:439-56.
- 12 Burnie JP, Matthews RC. Recent laboratory observations in the diagnosis of systemic fungal infection: *Candida* and *Aspergillus*. In: Holmberg K, Meyer R, eds. *Diagnosis and therapy of systemic fungal infections*. New York: Raven, 1989: 101.
- 13 Ansorg R, Heintschel von Heinegg E, Rath PM. *Aspergillus* antigenuria compared to antigenemia in bone marrow transplant recipients. *Eur J Clin Microbiol Infect Dis* 1994;13:582-9.
- 14 Stynen D, Meulemans L, Garrigues ML. *Aspergillus* antigen latex test for diagnosis of invasive aspergillosis. *Lancet* 1992;339:188.
- 15 Reddy LV, Kumar A, Kurup VP. Specific amplification of *Aspergillus fumigatus* DNA by polymerase chain reaction. *Mol Cell Probes* 1993;7:121-6.
- 16 Spreadbury C, Holden D, Aufauvre-Brown A, Bainbridge B, Cohen J. Detection of *Aspergillus fumigatus* by polymerase chain reaction. *J Clin Microbiol* 1993;31:615-21.
- 17 Tang CM, Holden DW, Aufauvre-Brown A, Cohen J. The detection of *Aspergillus* spp. by polymerase chain reaction and its evaluation in bronchoalveolar lavage fluid. *Am Rev Resp Dis* 1993;148:1313-7.
- 18 Melchers WJG, Verweij PE, Van den Hurk P, *et al*. General primer-mediated PCR for detection of *Aspergillus* species. *J Clin Microbiol* 1994;32:1710-7.