

## Sandwich Enzyme-Linked Immunosorbent Assay Compared with Pastorex Latex Agglutination Test for Diagnosing Invasive Aspergillosis in Immunocompromised Patients

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**The performance of a direct sandwich enzyme-linked immunosorbent assay (ELISA) for detecting *Aspergillus galactomannan* was compared with that of the Pastorex *Aspergillus* antigen latex agglutination (LA) test by using 532 serum samples from 61 patients at risk for invasive aspergillosis. The ELISA gave positive results earlier in the course of infection than did the LA test. A sensitivity of 70% and a specificity of 86% were obtained for the LA test and corresponding values of 90 and 84% were obtained for the ELISA when a series of serum samples was employed.**

*Aspergillus fumigatus* is a ubiquitous fungus capable of causing life-threatening opportunistic infections in immunocompromised patients (3). Inhalation of airborne *Aspergillus* conidia and germination of spores in the alveoli primarily result in pulmonary infection, although dissemination to other organs may occur. The successful diagnosis of invasive aspergillosis (IA) is frustrated by the difficulty in obtaining specimens which demonstrate the organism directly. Serologic techniques have been used in an attempt to establish diagnosis in early stages of infection but have proven to be unsuccessful for immunocompromised patients because of the patients' impaired ability to produce humoral responses. The detection of circulating *Aspergillus* antigens in body fluids, e.g., in serum and urine, is promising, but despite the development of several methods for such detection (1, 8), none has gained widespread acceptance. Recently, a direct double sandwich enzyme-linked immunosorbent assay (ELISA) has been developed; this assay employs the rat monoclonal antibody EB-A2 to detect galactomannan (GM), a cell wall component of *Aspergillus* spp. (15, 16). The EB-A2 antibody is also used in the commercially available Pastorex *Aspergillus* latex agglutination (LA) test (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France) (16), but the detection limit can be lowered 10-fold by employing the antibody as a captor and a detector (15), which may allow IA to be diagnosed earlier. We investigated this possibility by testing the ELISA with a series of 532 serum samples which had been previously analyzed by LA (18) to attempt to diagnose IA in 61 immunocompromised patients at high risk for the disease.

A total of 532 serum samples from 61 patients were collected during chemotherapy-induced neutropenia and were stored at  $-80^{\circ}\text{C}$ . At least two serum samples were available from each patient. Before testing, all serum samples were treated as described previously for *Candida* mannan (13) to dissociate immune complexes. All LA tests were performed by the same technician exactly as described by the manufacturer. For the ELISA, the supernatants of the serum samples were obtained

by centrifuging the samples at  $15,000 \times g$  for 10 min. The ELISA was performed as described previously (15). Briefly, 50  $\mu\text{l}$  of the treated sample and 50  $\mu\text{l}$  of horseradish peroxidase-conjugated EB-A2 were placed in the wells of a microtiter plate coated with monoclonal antibody EB-A2, and the plates were incubated at  $37^{\circ}\text{C}$  for 90 min. After the plates were washed, 100  $\mu\text{l}$  of buffer containing *ortho*-phenylenediamine dihydrochloride was added to the wells, and the plates were incubated for 30 min. The reaction was stopped with 50  $\mu\text{l}$  of 4 M sulfuric acid, and the optical density was read at 492 nm. The optical density of antigen-negative serum spiked with 1 ng of GM per ml provided the cutoff value (15), and a positive and a negative control were included with each test.

The results for the 532 serum samples tested by LA and ELISA are shown in Table 1. A total of 325 serum samples were obtained from 33 patients who had a low likelihood of IA, i.e., patients for whom there was no clinical, microbiological, histological, or radiological evidence of infection. Three samples were positive in both tests, 2 samples (1.5%) were positive in the LA test alone, and 22 samples (7.7%) were positive only in the ELISA. There was clinical evidence of IA in the other 28 patients, who had all developed pulmonary infiltrates and had been febrile (temperatures of  $>38^{\circ}\text{C}$ ) for 3 days despite broad-spectrum antimicrobial therapy. For 18 of these patients, the courses of infection and the results of cultures suggested that IA was unlikely. With the 120 serum samples from these 18 patients, positive results were obtained for 3 samples (2.5%) in both tests and 10 samples (8.3%) were positive by ELISA (Table 1). The clinical characteristics of the remaining 10 patients with possible IA (patients showing clinical and radiological evidence for IA but having negative culture results) or proven IA (patients showing histopathological evidence of tissue invasion at autopsy and having positive *Aspergillus* culture results) are shown in Table 2, together with the results of the analyses of 87 serum samples from these patients. GM was not detected by either test in any of the serum from patient 1, who had a proven infection. Very low levels of GM in serum, due to limited fungal invasion of the pulmonary blood vessels or the release of low levels of GM by the fungus into the body fluids, may have contributed to the false-negative result for this patient. For the entire patient population, the sensitivity and specificity of the LA test were 70 and 86%, respectively, and

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TABLE 1. Performance of the double-sandwich ELISA and the Pastorex LA test for diagnosis of IA

Test (result)	Patient group					
	Low index of suspicion for IA <sup>a</sup>		IA suspected but unlikely <sup>b</sup>		High index of suspicion for IA <sup>c</sup>	
	No. of patients	No. of positive samples/no. of samples analyzed	No. of patients	No. of positive samples/no. of samples analyzed	No. of patients	No. of positive samples/no. of samples analyzed
LA (+)	4	5/35	3	3/27	7	16/52
LA (-)	29	0/290	15	0/93	3	0/35
ELISA <sup>d</sup> (+)	10	25/88	5	10/41	9	58/85
ELISA (-)	23	0/237	13	0/79	1	0/2
ELISA <sup>e</sup> (+)	5	20/52	3	8/33	9	58/85
ELISA (-)	28	5/273	15	2/87	1	0/2

<sup>a</sup> Patients in this group showed no clinical, microbiological, histological, or radiological evidence of infection. *n* = 33.

<sup>b</sup> Patients in this group showed fever and pulmonary infiltrates, but culture results and courses of infection suggested that IA was unlikely. *n* = 18.

<sup>c</sup> Patients in this group had histopathological, radiological, or microbiological evidence of IA. *n* = 10.

<sup>d</sup> A patient was considered positive when one or more serum samples was positive by ELISA.

<sup>e</sup> A patient was considered positive when two or more consecutive serum samples were positive by ELISA.

the sensitivity and specificity of the ELISA were 90 and 71%, respectively. When a true-positive result for the ELISA was defined as two consecutive positive serum samples, the sensitivity was similar (90%) but the specificity increased to 84%.

Diagnosing IA at an early stage of infection is difficult. The culture of *Aspergillus* spp. from the sputa or bronchoalveolar lavage fluids of patients at high risk for IA is highly indicative of infection, but the diagnostic reliability of culture is low (11). Therefore, more-sensitive methods, such as PCR (10, 12, 14, 17) and *Aspergillus* antigen detection (2, 4-9, 15, 18, 19), are under investigation. The detection of GM by the Pastorex *Aspergillus* LA test has been evaluated at several institutes (2, 4-7, 9, 18, 19), and the test showed sensitivities of up to 95% with serum samples from patients with a high index of suspicion for IA (4, 5). The LA test was also found to yield positive results earlier than conventional tests for 68% of patients with proven IA (5). However, these observations were not con-

firmed by others (2, 6, 7, 9, 18, 19), and a sensitivity of only 38% has been reported (9). In our study, the LA test yielded positive results only during advanced stages of infection in most patients with suspected IA and did not contribute to an earlier diagnosis (18). The present study showed that the ELISA detected GM in serum up to 5 days earlier than the LA test did. Although both LA and ELISA failed to detect one proven infection, the ELISA detected GM in two additional patients for whom the LA test continued to yield negative results. Moreover, GM was detected in more serum samples by ELISA than by LA. This suggests that monitoring sequential serum samples from high-risk patients during neutropenia may allow the diagnosis of IA to be made at an earlier stage of infection.

The increased sensitivity of the ELISA, however, was associated with an increase in false-positive results. A rate of 8% false positives by ELISA has been found by others (15). There-

TABLE 2. Clinical characteristics and serum analyses of 10 neutropenic patients with proven or possible IA

Patient no.	Sex <sup>a</sup> /age (yr)	Underlying condition(s) <sup>b</sup>	<i>Aspergillus</i> infection <sup>c</sup>	No. of serum samples tested	No. positive by LA	No. positive by ELISA	Time (days) between fever and positive test by <sup>d</sup> :	
							LA	ELISA
1	F/33	AML	Proven, bronchopneumonia	2	0	0		
2	M/30	AML	Proven, cavitating bronchopneumonia	19	5	17	+10	+7
3	F/44	AML, BMT	Proven, bronchopneumonia	5	3	5	+12	+9
4	F/60	Kidney transplant	Proven, disseminated	2	1	2	+14	+9
5	F/51	MDS, BMT	Proven, disseminated	12	1	7	+10	+7
6	M/58	Hepatocellular carcinoma	Proven, bronchopneumonia	2	2	2	+4	+4
7	M/53	CML, BMT	Possible	9	1	8	+16	+13
8	F/50	CML, BMT	Possible	15	0	6		-5
9	M/38	CML, BMT	Possible	18	0	8		-4
10	M/37	AIDS	Possible	3	3	3	+90	+90
Total				87	16	58		

<sup>a</sup> F, female; M, male.

<sup>b</sup> AML, acute myeloid leukemia; BMT, bone marrow transplantation; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia. All patients died.

<sup>c</sup> "Proven" refers to patients with histopathological evidence of tissue invasion and positive *Aspergillus* cultures. "Possible" indicates that cultures from sputa or bronchoalveolar lavage fluids were negative for *Aspergillus* species but clinical and radiological evidence for IA was present.

<sup>d</sup> Values are numbers of days elapsed between the first day of fever (temperature of >38.5°C) and a positive result by either test. Patient 9 developed severe graft-versus-host disease (III) and was treated with high doses of corticosteroids. With patient 10, a fever of unknown origin was present for 3 months, but 3 days before the first serum sample was obtained, respiratory failure developed and infiltrates were demonstrated by chest roentgenogram.

fore, in order to identify a genuine elevation of the GM level in serum, positive ELISA results should be found for at least two consecutive serum samples.

Our results suggest that antigen detection at regular intervals by the double sandwich ELISA may allow the early diagnosis of IA in immunocompromised patients. Twice-weekly collection and testing of serum samples from a patient during the period of neutropenia should be sufficient to detect an increase in the GM level in serum early in the course of infection. In the case of a positive ELISA result, the collection and testing of serum samples should be continued in order to exclude the possibility of a false-positive result. Furthermore, confirmation of suspected IA should be obtained by chest roentgenogram, computer tomography, or bronchoalveolar lavage. Prospective studies following these guidelines are needed to establish the clinical value of this new ELISA.

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