

Use of the Pastorex aspergillus antigen latex agglutination test for the diagnosis of invasive aspergillosis

V Hopwood, E M Johnson, J M Cornish, A B M Foot, E G V Evans, D W Warnock

Abstract

Aims—To evaluate the Pastorex aspergillus antigen latex agglutination test for the diagnosis of invasive aspergillosis in patients undergoing liver or bone marrow transplantation.

Methods—Serum samples were taken at least twice weekly post-transplant and tested for *Aspergillus* antigen. Latex agglutination test results were compared with microbiological examination of respiratory, urine and bile specimens. Serum samples from liver transplant patients were also tested for antibodies to *Aspergillus fumigatus* by counter immunoelectrophoresis.

Results—Eight of the 91 patients studied developed invasive aspergillosis. Positive latex agglutination tests were obtained in eight of 187 (4.3%) serum samples from four of these eight patients. The other four patients with invasive aspergillosis gave consistently negative latex agglutination tests. A positive latex agglutination test was the first indication of invasive aspergillosis in two patients; these patients were already on amphotericin B. Positive latex agglutination tests were the only evidence of invasive aspergillosis in one patient who subsequently died of the infection. False positive latex agglutination tests were obtained in five of 83 (6%) patients with no evidence of invasive aspergillosis and misleading positive cultures seen in nine of 83 (10.8%). No antibodies were detected in three of four liver transplant patients with invasive aspergillosis. Conversely, antibodies were detected in 63 of 262 (24%) serum samples from 43 liver transplant patients with no evidence of invasive aspergillosis; one of these patients had an antibody titre of 1:2 on four separate occasions.

Conclusions—The Pastorex aspergillus antigen latex agglutination test, when used alone, lacks sensitivity and specificity for the early diagnosis of invasive aspergillosis. A diagnosis was made in all patients with invasive aspergillosis when both culture and antigen tests were performed although using these criteria a false positive diagnosis would have been made in 13 of 83 (15.6%) patients. Microbiological and serial serological investigations for antigen should both be performed and the results considered in

conjunction with radiological and clinical evidence.

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Invasive aspergillosis is a major cause of morbidity and mortality in immunocompromised patients undergoing intensive chemotherapy for cancer or organ transplantation. The response of immunosuppressed patients with invasive aspergillosis to specific antifungal therapy is poor, with an overall mortality rate of 45% and a mortality rate in bone marrow transplant recipients of 94%.¹ The major problem associated with invasive aspergillosis is the difficulty in diagnosing this infection early enough to be of value in patient management. Cultures of blood or respiratory specimens are seldom positive especially during the early stages of the disease. Aisner *et al*² demonstrated mortality of 83% in six patients where there was early evidence of pulmonary aspergillosis whereas mortality was 100% in patients whose infection was diagnosed late.

At present, reliable antemortem diagnosis of invasive aspergillosis requires invasive procedures to obtain biopsy material for histological and microbiological examination. However, clinicians are often reluctant to perform these procedures because of the associated morbidity and mortality.

Serological procedures are used as an adjunct to microbiological methods for the diagnosis of invasive aspergillosis but current tests lack sensitivity. Antibody detection is often negative because of the fulminant nature of the disease or the poor immunological status of the host, or both.³ Antigen detection tests have been developed^{3,4} but most have only been evaluated in individual laboratories. Recently, however, a monoclonal antibody based latex particle agglutination test for the detection of *Aspergillus* galactomannan in serum has become available commercially. We report our experience with this test, over two years, for the routine surveillance of patients undergoing liver and bone marrow transplantation.

Methods

MYCOLOGICAL SURVEILLANCE

Forty seven liver transplant recipients were screened for fungal infection over two years.

PHLS Mycology
Reference Laboratory,
Department of
Microbiology,
General Infirmary,
University of Leeds,
Leeds LS2 9JT
V Hopwood
E G V Evans

PHLS Mycology
Reference Laboratory,
Bristol Public
Health Laboratory,
Bristol BS2 8EL
E M Johnson
D W Warnock

Bone Marrow
Transplant Unit,
Royal Hospital
for Sick Children,
Bristol BS2 8BU
J M Cornish
A B M Foot

Correspondence to:
Dr V Hopwood.

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Table 1 Antigen test results in patients with invasive aspergillosis

Case No.	No. of blood samples		Other positive samples	Outcome
	Tested	Positive		
1 (LT)	17	0	BAL+TA (weeks 25 and 26)	Died (week 26) ^b
2 (LT)	2	2 (weeks 4 and 5)	Sputum (week 4)	Died (week 5) ^b
3 (LT)	4	0	Bile, urine (weeks 4 and 5)	Died (week 6) ^a
4 (LT)	8	2 (week 6)	Sputum (week 6)	Died (week 6) ^a
5 (BMT)	45	2 (weeks 4 and 20)	Nil	Died (week 22) ^a
6 (BMT)	58	2 (week 22)	BAL (week 9)	Died (week 22) ^a
7 (BMT)	31	0	Nasal swab (week 6)	Died (week 24) ^a
8 (BMT)	22	0	Pus (week 9)	Died (week 11) ^a

^a Invasive aspergillosis confirmed at necropsy.

^b Invasive aspergillosis strongly suspected from radiological and mycological evidence but permission for necropsy refused. LT, liver transplant; BMT, bone marrow transplant; TA, tracheal aspirate.

Clinical specimens (bile, urine, serum) were taken for mycological investigation twice weekly post-transplant until discharge or death. Other specimens (sputum, bronchoalveolar lavage (BAL) fluid, tracheal aspirates) were obtained if there was clinical suspicion of invasive aspergillosis. All specimens were centrifuged and the deposit examined for the presence of fungi by direct microscopy in KOH and plated on to Sabouraud agar containing 0.5% chloramphenicol. Plates were examined for fungal growth after incubation at both 27°C and 37°C for 48 hours (urine and bile) or 96 hours (respiratory specimens).

Serum samples were tested weekly for antibodies to *A. fumigatus* by counterimmunoelectrophoresis⁵ against *A. fumigatus* somatic and culture filtrate antigens (Mercia Diagnostics Ltd., Guildford, UK) at concentrations of 20 mg/ml.

Serum samples were also tested for *Aspergillus* antigen if (i) the patient was pyrexial, (ii) the patient had any signs of chest infection, (iii) an *Aspergillus* species had been isolated from any clinical specimen during routine mycological screening, or (iv) the patient was on the intensive care unit.

Forty four patients undergoing allogeneic bone marrow transplantation were screened for fungal infection over two years. Serum specimens were collected twice weekly post-transplant until discharge or death. Other specimens (nasal swabs, sputum, BAL fluid) were taken at intervals. If invasive aspergillosis was suspected on clinical or mycological grounds, daily testing of serum for *Aspergillus* antigen and empirical treatment with amphotericin B were instituted. Antibody testing was not performed as it was considered that this would not yield useful information.

ASPERGILLUS ANTIGEN TEST

The aspergillus latex agglutination test (Pastorex aspergillus; Sanofi, Diagnostics Pasteur, Paris, France) was carried out according to the manufacturer's instructions with slight modification using half volumes of reagents. Preliminary studies using serial dilutions of positive control antigen and patients' serum samples showed that these modifications did not affect the sensitivity of the test. Briefly, 300 µl of serum was mixed with 100 µl of treatment solution and boiled for three minutes. After centrifugation at 15 000 rpm for 10 minutes the supernatant was tested for galactomannan by

placing 20 µl of specimen and 5 µl of sensitised latex reagent on a clean black slide (manufacturer's instructions: 40 µl specimen and 10 µl latex). After mixing thoroughly with a plastic mixing stick the slide was placed on a rocking table and agglutination recorded after five minutes. Because of previous reports of false positive agglutination tests with these reagents,⁶⁷ all specimens giving positive reactions were retested and were only regarded as true positives if the results were reproducible. Agglutination was scored on a scale of 1+ to 3+.

Results

Over the two years, 279 serum samples from liver transplant recipients were tested for *Aspergillus* antigen; five (1.8%) positive tests were observed.

Two patients (cases 3 and 4) developed invasive aspergillosis, confirmed at necropsy, and two (cases 1 and 2) had presumed invasive aspergillosis although a request for necropsy was refused. *Aspergillus* antigen tests were performed on 31 serum samples from these four patients with four (13%) positive results (table 1). Twenty one serum samples from cases 1 and 3 all gave negative antigen test results. *A. fumigatus* was isolated from BAL fluid and two tracheal aspirate samples from case 1, who died 12 days after the first positive culture. Invasive aspergillosis was confirmed at necropsy in case 3 with widespread involvement of the heart valves, myocardium, kidney, and thyroid.

Two serum samples from each of the other two cases were antigen positive (table 1). Culture of *A. fumigatus* preceded the first positive antigen test by two days in case 2 but occurred four days after the first positive antigen test in case 4. Both patients died within four days of the first positive antigen tests. A necropsy performed on case 4 revealed extensive invasive aspergillosis with involvement of the lungs, brain, kidneys, and thyroid.

In all four patients empirical antifungal therapy with amphotericin B had been instituted several days before confirmation of *Aspergillus* infection by either culture or antigen testing.

Of the 43 liver transplant recipients without invasive aspergillosis, one false positive antigen test (1+ agglutination) was observed out of 248 serum samples tested (0.4%). This occurred in a 13 year old boy (case 9; table 2) who had undergone liver transplantation as a sequel to cystic fibrosis; he was known to be colonised with both *A. fumigatus* and *A. terreus*. Of the

Table 2 Antigen test results in other patients

Case No.	No. of blood samples		Other samples positive	Outcome
	Tested	Positive		
9 (LT)	31	1	Sputum ^f	Now 29/12 post-LT
10 (LT)	4	0	Bile (week 2)	Now 24/12 post-LT
11 (LT)	9	0	Urine (weeks 7 and 8)	Now 11/12 post-LT
12 (LT)	20	0	Sputum (week 1)	Died (week 42)
13 (LT)	7	0	Sputum (weeks 3-5)	Now 12/12 post-LT
14 (LT)	4	0	Bile, urine (weeks 2 and 3)	Now 8/12 post-LT
15 (BMT) ^a	13	1 (week 5)	Nil	Now 27/12 post-BMT ^b
16 (BMT) ^a	46	1 (week 4)	Nil	Died (week 29) ^{b,c}
17 (BMT) ^a	9	1 (week 3)	Nil	Now 11/12 post-BMT ^b
18 (BMT) ^a	17	2 (weeks 4 and 6)	Nil	Now 4/12 post-BMT ^d
19 (BMT)	26	0	BAL (week 15)	Died (week 35) ^{e,f}
20 (BMT)	25	0	Sputum (week 28)	Now 23/12 post-BMT
21 (BMT)	21	0	BAL (week 7)	Now 18/12 post-BMT ^b

^aNo clinical, mycological or radiological evidence of invasive aspergillosis.

^bNo antifungal therapy given.

^cNo evidence of invasive aspergillosis at necropsy.

^dAmphotericin B given on the basis of antigen result.

^ePatient treated with itraconazole on basis of culture result.

^fPatient with cystic fibrosis. Colonised with *A fumigatus* and *A terreus*.

antigen tests performed on 31 serum samples from this patient, only one was positive.

A fumigatus was isolated from five other liver transplant recipients (cases 10 to 14; table 2), four of whom were clinically well two to six months after transplantation without administration of antifungal drugs. One patient (case 12) died 42 weeks after transplantation with no clinical or radiological evidence of invasive aspergillosis.

Of 467 serum samples tested for antibodies to *A fumigatus*, 65 (14%) were positive. Only one of the four patients with invasive aspergillosis (table 1; case 3) had weakly detectable antibodies in two of five serum samples tested; the appearance of antibodies preceded the first positive culture of *A fumigatus* by 14 days. No antibodies were detected in any of the other three liver transplant patients with invasive aspergillosis.

Three of the six patients with no clinical evidence of invasive aspergillosis, but from whom *A fumigatus* was isolated, became weakly antibody positive at some stage (15 of 66 serum samples; cases 9, 10 and 12); the remainder had no detectable antibodies.

Seventeen other patients had positive antibody tests (48 of 196 serum samples, 24%), and one patient had an antibody titre of 1:2 on four occasions despite having no clinical or mycological evidence of invasive aspergillosis.

Serum samples (n=680) from patients undergoing bone marrow transplantation were tested with the latex agglutination reagent. Four patients had invasive aspergillosis confirmed at necropsy (cases 5 to 8; table 1). Four positive aspergillus antigen tests were obtained in 156 serum samples from these patients (2.6%; two were unconfirmed as there was insufficient specimen for repeat testing). All four positive tests were on serum samples from cases 5 and 6, and the aspergillus antigen test was the first indication of fungal infection in case 5, although positive agglutination was not confirmed. *A fumigatus* was isolated from BAL fluid from case 6 at nine weeks post-transplant, at which time antifungal chemotherapy with amphotericin B was instituted. Positive aspergillus antigen tests were not obtained on this patient until 22 weeks post-transplant, the last week of life.

Cases 7 and 8 did not give a positive antigen test despite 53 serum samples being screened. Case 7 had *A fumigatus* isolated from a nasal swab at six weeks post-transplant and died 24 weeks post-transplant. Case 8 developed a cerebral abscess from which *A fumigatus* was isolated at week 9 and died two weeks later.

Positive aspergillus antigen tests were also obtained on four other patients (cases 15 to 18), although they had no mycological, radiological or clinical evidence of invasive aspergillosis (table 2). Two of these patients remain well, 27 and 11 months after transplantation, despite receiving no antifungal therapy. One patient died but had no evidence of invasive aspergillosis at necropsy and one patient received amphotericin B on the basis of the antigen result and was well at the time of writing.

Thirty six patients (439 serum samples) had negative antigen test results. *A fumigatus* was recovered from specimens from three of these patients (table 2). Case 19 was treated with itraconazole following isolation of *A fumigatus* from BAL fluid 15 weeks post-transplant. Although the patient died 35 weeks post-transplant there was no evidence of invasive aspergillosis at necropsy. The other two patients (cases 20 and 21) remain well 23 and 18 months post-transplant, respectively, although case 20 did receive a four week course of amphotericin B.

Discussion

Reliable diagnosis of invasive aspergillosis is difficult and is often only made at necropsy. *Aspergillus* is only recovered from sputum in 8-34% of patients and sputum samples are often scanty or unavailable.⁸ In some cases recovery of *Aspergillus* from sputum may be because of contamination rather than infection; culture of BAL fluid or tracheal aspirate specimens may give more accurate results. In our series of patients *A fumigatus* was isolated from respiratory specimens (nasal swab, sputum, BAL fluid, tracheal aspirate) from five of the eight (62%) patients with invasive aspergillosis but also from six of 83 (7.2%) patients with no evidence of invasive aspergillosis.

Antibody detection lacked both sensitivity and specificity. Only one of our liver transplant

patients with confirmed invasive aspergillosis had detectable antibodies to *A fumigatus* whereas antibody tests were positive in 20 of 42 patients with no evidence of invasive aspergillosis; one of these latter patients had an antibody titre of 1:2 on at least four occasions. It is unlikely that the presence of antibodies to *Aspergillus* affected antigen detection as heat treatment of serum before testing with the latex agglutination reagent would dissociate any immune complexes.

In this prospective study the latex agglutination aspergillus antigen detection test lacked sufficient sensitivity for the early diagnosis of invasive aspergillosis, which would influence treatment, but was of value in helping to confirm a diagnosis. The antigen test was positive in four of the eight (50%) patients with invasive aspergillosis and was the first indicator of infection in two patients; all of the patients with invasive aspergillosis in this study, however, had been receiving empirical antifungal chemotherapy with amphotericin B for several days before confirmation of infection by either culture or antigen testing. Two positive latex agglutination tests were the only evidence of invasive aspergillosis in a bone marrow transplant recipient who subsequently died of the infection.

Confirmed false positive latex agglutination tests were obtained in five of 83 (6%) cases. The reasons for these false positive antigen tests are not known although it has been suggested that contamination of serum samples with some airborne moulds,⁹ or bacteria,¹⁰ may result in cross-reactivity with the monoclonal antibody used in the kit. Care was taken with other factors which may interfere with the test¹¹; all serum samples were separated from the clot well within 24 hours of sampling, micro-centrifuge tubes were properly sealed during boiling and, as the volumes of reagents were halved, the serum-latex mixtures were covered during mixing to prevent dessication.

Antigen detection tests for the diagnosis of invasive aspergillosis can give more meaningful results by testing sequential rather than single serum samples.⁴ However, in this study, four patients with invasive aspergillosis gave consistently negative antigen test results despite testing multiple samples.

The diagnosis of invasive aspergillosis was enhanced when antigen tests and culture were both performed; a positive culture and/or antigen test occurred in all eight patients with invasive aspergillosis. However, using these criteria a false positive diagnosis would also have been made in 16% (13/83) of patients with no clinical or radiological evidence of invasive aspergillosis.

Clearly, methods for the diagnosis of invasive aspergillosis still need to be improved. Urine¹² and BAL fluid¹³ have both been shown to contain detectable antigen in patients with invasive aspergillosis and these may be better specimens to test than serum. Unfortunately, the latex agglutination reagent evaluated in this study is only suitable for use on serum samples. Until more reliable tests such as the polymerase chain reaction are developed for the diagnosis of invasive aspergillosis, the Pastorex aspergillus antigen latex agglutination test alone should not be relied on to give an early diagnosis of invasive aspergillosis and users of the test should recognise its limitations and interpret its results with care.

Despite the disappointing performance of this latex agglutination test, we still believe that both microbiological and serial serological investigations for aspergillus antigen should be performed to maximise the chances of diagnosing invasive aspergillosis and the results considered in conjunction with radiological and clinical evidence of infection.

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