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# Value of Antigen and Antibody Detection in the Serological Diagnosis of Invasive Aspergillosis in Patients with Hematological Malignancies

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**In a study to determine the value of antigen and antibody detection in the serological diagnosis of invasive aspergillosis in patients with hematological malignancies, 436 sequential serum samples from 79 neutropenic patients were tested. Circulating galactomannan antigen was detected with a latex agglutination method (Pastorex Aspergillus) and antibody to *Aspergillus fumigatus* with an immunodiffusion test on agar gel. Among the 79 patients 18 cases of invasive aspergillosis were detected (4 proven, 6 highly probable, 3 probable, 5 suspected). Antigen was detected in 7 of these 18 patients. The sensitivity of the antigen test was 38.8 % and the specificity 95 %. The antibody test showed 55.5 % sensitivity and 100 % specificity. In this study the antigen test was less sensitive than previously reported but highly specific and might serve as a supplementary diagnostic test.**

Invasive aspergillosis is one of the major causes of morbidity and mortality in patients with acute leukemia, lymphoma or organ transplants. The mortality due to invasive aspergillosis is very high and can be substantially reduced if an early diagnosis is made and the proper therapy given. This

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is difficult, however, because the clinical signs and symptoms are usually non-specific and there is frequently failure to isolate the causative fungus. The most common clinical manifestations are unremitting fever and pulmonary infiltrates despite broad-spectrum antibiotic therapy. The definitive diagnosis requires histopathological detection of the characteristic hyphae in tissue sections and a positive culture, but invasive procedures have a high complication rate in such patients. Recent approaches in an effort to establish a rapid diagnosis include the detection of circulating antigens (1–3). To establish the value of serological tests in the diagnosis of invasive aspergillosis, we conducted a prospective evaluation of *Aspergillus* antigen and antibody detection in serum of neutropenic patients.

***Patients and Methods.*** From June 1991 to June 1993, 436 sequential serum samples were collected from 79 adult neutropenic patients (45 male, 34 female) undergoing remission induction therapy for hematological malignancies at the Clinical Hematological Unit, Hospital of Torrette, Ancona, Italy. Serum was collected on admission and once or twice a week during hospitalization. Samples were maintained at 4°C until testing within 48 hours, and then stored at -20°C. The number of sera studied per patient ranged from 2 to 17 (mean 4).

The likelihood of a diagnosis of invasive aspergillosis on admission was categorized as follows: A) proven infection: histological detection in biopsy or autopsy material of tissue invasion by septate, acutely branching hyphae and positive culture; B) highly probable infection: documented site of infection, and positive culture of bronchoalveolar lavage fluid; C) probable infection: typical clinical and radiographic findings such as pulmonary cavitation and/or air formation, but negative cultures; D) suspected infection: temperature > 38°C for more than 5 days, no response to antibacterial agents, and one or more of the following signs and symptoms: pleural chest pain, dyspnea, aspecific radiographic findings (such as pulmonary density or pleural effusion), nasal swab positive for *Aspergillus* but no other documented pathogens.

Data recorded included the number of positive sera out of the total number of sera tested, the first date of detection of serum antigen in relation to day 0 (defined as the first day with symptoms of aspergillosis) and in relation to the date of beginning antifungal therapy, the date of death and the date of seroconversion (if it occurred) after day 0.

Circulating *Aspergillus* galactomannan was detected with a latex agglutination test (Pastorex Aspergillus, Sanofi Diagnostics Pasteur, France). The latex used in this test is sensitized with a rat IgM monoclonal antibody and can detect galactomannan at very low concentrations (15 ng/ml). The immune complexes were disrupted by adding 100 µl EDTA to 300 µl serum in a tightly-closed Eppendorf tube which was heated to 100°C for 3 min, then centrifuged at 10,000 x *g* for 10 min. Forty µl of supernatant were mixed with 10 µl of sensitized latex on an agglutination card and agitated at room temperature for 10 min, then the result was read. The control provided (*Aspergillus fumigatus* galactomannan antigen, 75 ng/ml) was included in all test sets and gave positive results throughout. Initially we did not retest positive samples immediately, however later we did so at the manufacturer's recommendation (4).

Precipitins were detected by an immunodiffusion test on agar gel according to the technique of Ouchterlony (5) using two types of antigens (somatic and metabolic) obtained from *Aspergillus fumigatus* cultures (Sanofi Diagnostics Pasteur). Control sera (known positive antisera) were run in wells adjacent to test sera (patient sera). The specificity of the precipitin band was determined by the formation of identical bands in patient and control sera.

***Results and Discussion.*** Among the 79 patients evaluated, 18 cases of invasive aspergillosis (4 proven, 6 highly probable, 3 probable, 5 sus-

**Table 1:** Antigen and antibody test results in 18 neutropenic patients with invasive aspergillosis.

| Aspergillosis category | No. of cases | Antigen positive only | Antibody positive only | Antigen and antibody positive | Negative |
|---|---|---|---|---|---|
| Proven | 4 | 0 | 1 | 1 | 2 |
| Highly probable | 6 | 2 | 3 | 0 | 1 |
| Probable | 3 | 1 | 1 | 0 | 1 |
| Suspected | 5 | 1 | 2 | 2 | 0 |

pected) were detected (Table 1). Galactomannan was detected in seven patients, while seroconversion was observed in ten. The antigen test was also positive in a single serum sample in three patients without any signs of invasive aspergillosis tested in the first months of this study. One of these three samples was positive on retesting one year later, while the other two samples were no longer available.

The sensitivity of the antigen test was 38.8 %, specificity 95 %, positive predictive value 70 % and negative predictive value 84 %. All antigen tests except two were positive only without dilution of the sample. The antibody test showed 55.5 % sensitivity and 100 % specificity.

Data on the patients is reported in Table 2. Of four patients with proven infection, three were observed at autopsy. The antigen test was positive in only one of these patients (no. 1) who had a prolonged clinical course and subsequently showed seroconversion; the other two patients died 6 and 8 days after onset of fever. The fourth patient had pulmonary and cutaneous aspergillosis, and only seroconversion was observed.

Of six patients with highly probable infection, two died. The antigen test was positive in only two of these six patients, in each case in two samples. In patient no. 5, the first sample was taken eight days before administration of amphotericin B, and the second six days after administration of amphotericin B; in patient no. 8 serum was collected two and four days after administration of amphotericin B. Three other patients showed seroconversion.

Of the three patients with probable infection, one patient (no. 12) died of cerebral hemorrhage. In patient no. 13, one serum sample was antigen-positive with a titre of 1:2 (30 ng/ml) after 21 days

**Table 2:** Data on 18 patients with invasive aspergillosis.

| Patient no. | Diagnosis | No. of sera tested | No. of antigen-positive sera | Day of antigen detection | | | Day of sero-conversion after onset of symptoms | Outcome |
|---|---|---|---|---|---|---|---|---|
| | | | | After onset of fever | After initiation of antifungal therapy | Before death | | |
| Proven infection | | | | | | | | |
| 1 | RAEBt | 5 | 1 | 40 | – | 12 | 40 | died |
| 2 | RAEBt | 7 | 0 | – | – | – | – | died |
| 3 | ANLL | 2 | 0 | – | – | – | – | died |
| 4 | ANLL | 9 | 0 | – | – | – | 12 | survived |
| Highly probable infection | | | | | | | | |
| 5 | ALL | 3 | 2 | (-1)[a] 13 | (-8)[a] 6 | 30, 17 | – | died |
| 6 | ANLL | 13 | 0 | – | – | – | – | survived |
| 7 | ANLL | 6 | 0 | – | – | – | 14 | survived |
| 8 | ALL | 10 | 2 | 12, 14 | 2, 4 | – | – | survived |
| 9 | ANLL | 4 | 0 | – | – | – | 16 | survived |
| 10 | ANLL | 4 | 0 | – | – | – | 21 | died |
| Probable infection | | | | | | | | |
| 11 | ANLL | 6 | 0 | – | – | – | 20 | survived |
| 12 | ANLL | 4 | 0 | – | – | – | – | died |
| 13 | ANLL | 11 | 1 | 42 | 21 | – | – | survived |
| Suspected infection | | | | | | | | |
| 14 | ANLL | 3 | 2 | 5, 26 | (-5)[b] 16 | 49, 27 | 20 | died |
| 15 | ANLL | 3 | 1 | 6 | – | 2 | – | died |
| 16 | ANLL | 7 | 0 | – | – | – | 52 | died |
| 17 | ANLL | 2 | 1 | 7 | – | 1 | 7 | died |
| 18 | ALL | 5 | 0 | – | – | – | 8 | died |

[a] First serum sample antigen positive 1 day before onset of fever and 8 days before initiation of antifungal therapy.
[b] First serum sample antigen positive 5 days before initiation of antifungal therapy.
ALL: acute lymphoblastic leukemia; ANLL: acute non-lymphoid leukemia; RAEBt: refractory anemia with excess of blast cells in transformation.

of therapy with itraconazole. In patient no. 11 seroconversion was observed.

All five patients with suspected infection died. Three were antigen-positive, one of whom (no. 14) had two positive serum samples 5 and 16 days after administration of amphotericin B and showed seroconversion. Patient no. 17 was antigen-positive (and positive on retesting one year later) and showed seroconversion in the absence of antifungal therapy. Patient no. 15 was antigen-positive only on the sixth day after onset of fever with a titre of 1:4 (60 ng/ml), and died two days later. Patients no. 16 and 18 showed seroconversion only.

Tests to detect antigen or antibody (seroconversion) in sequential serum samples from neutropenic patients were of value in diagnosing invasive aspergillosis in 12 of 16 patients. The latex antigen test showed lower sensitivity than in previous studies (1, 6). False-positive results in the antigen test have been observed by Warnock et al. (7) who obtained a negative result on retesting. In the course of this study we retested all positive sera with the latex antigen test and considered the results false-positive if samples were negative on retesting. False-positive results have been ascribed to cross-reactivity with several antigens from bacterial and fungal contaminants which may be present in the serum samples (8, 9). False-negative results may be ascribed to variable shedding from the primary focus of infection, to administration of antifungal drugs or to rapid clearance of the circulating antigen.

Studies of experimental invasive aspergillosis in animal models have shown a significant correlation between galactomannan levels in plasma and the mean number of colony-forming units of *Aspergillus fumigatus* in muscle, kidney, brain, peritoneum, eye and spleen, but not in skin, liver or lung (10). As primary infection in humans is generally pulmonary, the antigen level in sera of patients with invasive aspergillosis may in most cases be below the detection limit of the test.

Antifungal therapy was given to 14 of the 18 patients (10 received amphotericin B and 4 itraconazole), but only 5 of the 18 patients survived. In the 4 patients treated with antifungal agents (3 with amphotericin B, 1 with itraconazole), the antigen test was positive after 4, 6, 16 and 21 days of treatment respectively; all but the first patient died. There is possibly a tendency for levels of circulating galactomannan to increase in non-surviving treated patients, as observed in animals (10).

The values of antibody detection in the diagnosis of invasive aspergillosis is debatable. In our study seroconversion was observed in 10 of 16 patients. Coleman and Kaufman (11) reported precipitins in the sera of 14 of 16 patients, whereas other authors did not find antibodies by this immunodiffusion method (12). The immunodiffusion method has been used successfully to monitor seroconversion in serial serum samples in individual patients (13).

In conclusion, in our study the antigen test to detect invasive aspergillosis was less sensitive than in previous studies but highly specific. In invasive aspergillosis with aspecific signs and symptoms, the antigen test may serve as an additional diagnostic modality. Testing multiple specimens from the same patient greatly increased the rate of detection, so samples should be taken at regular intervals for testing, preferably daily but at least twice a week, during fever in neutropenic patients. Since patients develop antibody only in the terminal stage of the disease, antibody detection is of limited value, but the screening of sequential serum samples can be useful in epidemiological studies and in the management of invasive aspergillosis in patients undergoing a new neutropenic episode.

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# Beta-lactam Resistance in Aerobic Faecal Flora from General Practice Patients in the UK

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**One hundred faecal specimens submitted to a diagnostic laboratory in Edinburgh and found to be negative for gastrointestinal pathogens were examined for the presence of antibiotic-resistant bacteria. The results were compared with findings in the healthy population in the same area. The highest incidence of resistance was observed to cefuroxime (65 %) and ampicillin (60 %). Of the ampicillin-resistant isolates, 62 % could transfer their resistance determinants to a standard *Escherichia coli* host strain. In 100 % of these transconjugants ampicillin resistance was shown to result from the presence of the TEM-1 β-lactamase which was identified in a heterogeneity of plasmid profiles. These plasmids commonly mediated resistance to streptomycin and tetracycline in addition to ampicillin.**

As the prevalence of bacterial resistance to many antimicrobial agents has increased, there has been a simultaneous increase in interest in the identification of the specific reservoirs of antimicrobial resistance genes. The incidence of antibiotic resistance amongst clinical isolates is well documented (1, 2) and reflects the widespread use of antimicrobial agents in the hospital environment. In addition, the commensal flora of the normal healthy population is increasingly well recognised to be a reservoir of resistance genes both in the developed and the developing world (3–7). There has been especial interest in this specific bacterial population given the potential of such bacteria to complicate therapy, either by causing an endogenous infection or by transferring their resistance determinants to invading pathogens (7). It is difficult to encourage participation in surveys which examine faecal flora of the healthy population. It is perhaps for this reason that many of the earlier studies undertaken have looked at specific co-operative populations and their relevance to the general community is therefore questionable. Specific healthy population groups examined for the carriage of antibiotic resistance determinants in faecal flora include children (4) and students (8). In addition, the commensal faecal flora of patients prior to their admission to hospital has been well investigated (9). While this latter population group may not be considered 'healthy', it may provide some indication of the carriage of antibiotic-resistant bacteria in the non-clinical community.

In this study the incidence of antibiotic-resistant bacteria was determined in 100 faecal specimens submitted to a diagnostic laboratory in Edinburgh which were found negative for the presence of recognised gastrointestinal pathogens. Although the donors of the specimens in this group, as with pre-admission patients, may not be considered healthy, no obvious association existed with the clinical community. The results of this survey were compared with those from a survey which examined faecal flora of the healthy population in the same area. In addition, the biochemical and genetic basis for resistance amongst the ampicillin resistant isolates obtained in the current study was examined.

***Materials and Methods.*** Faecal specimens were obtained from the clinical diagnostic laboratories of Edinburgh Royal Infirmary between January and March 1991. The specimens from patients in the Edinburgh region had been submitted by general practitioners and tested negative for the following bacterial pathogens: *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia enterocolitica*, *Clostridium difficile* and *Escherichia coli*

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