Comparative Analysis of Prognostic Indicators of Aspergillosis in Haematological Malignancies and HIV Infection


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The objective of this study was to identify the prognostic factors influencing the outcome of aspergillosis in two models of immunodeficiency, namely haematological malignancies and HIV infection. The study is based on a 5 year prospective logistic regression analysis of risk factors, clinical features, radiological findings and therapy affecting the prognosis of aspergillosis in 43 patients, i.e. 27 haematological neoplastic patients (group A) and 16 HIV infected patients (group B). Univariate analysis indicated that neutropenia (P = 0.02), haemoptysis (P = 0.03) and concomitant AIDS (P = 0.02) negatively influenced the prognosis of aspergillosis. Comparing the two groups of patients, significant differences emerged in the prognostic indicators. In particular respiratory failure (P = 0.02) and radiological bilateral involvement of the lungs were associated with a poor prognosis in group A (P = 0.04) and low (<100/mm³) T CD4+ cell count in group B (P = 0.02). At variance, a better prognosis was documented in patients treated with sequential therapy (amphotericin B and itraconazole) only within the group of haematological patients (P = 0.003). On multivariate analysis sequential therapy (P = 0.01) and AIDS (P = 0.03) were independent prognostic indicators of aspergillosis.

In conclusion, our prospective study indicates that aspergillosis, although an uncommon event in patients with HIV infection, has a more severe prognosis in comparison to haematological patients. Future prospective clinical trials are necessary to confirm the real importance of the sequential therapy, with amphotericin B and itraconazole, in patients with aspergillosis.

Introduction

Aspergillosis represents an important cause of morbidity and mortality in immunocompromised hosts. Patients with severe immunodeficiency, organ transplant recipients, patients under cytotoxic chemotherapies or high dose corticosteroids as well as patients with chronic granulomatous disease are at high risk for developing aspergillosis. In particular, aspergillosis is one of the most common fungal infections in haematological neoplastic patients (14–20% of cases) and it has been associated with auto-immune disorders, chemotherapy and neutropenia. Surprisingly, patients with HIV infection are at low risk for aspergillosis (0.9–8.6% in AIDS patients) and this finding contrasts with the presence of major deficits of T-cells, neutrophils and macrophages in these patients. The relationship between aspergillosis and HIV infection has not been fully elucidated, since invasive aspergillosis, initially included among the AIDS defining conditions by the Centers for Disease Control (CDC), was later removed as non-predictive of cellular immunodeficiency.

Prognostic indicators of aspergillosis in immunocompromised patients are still not clearly defined. Disseminated infection and bilateral pulmonary disease have been previously reported as indicators of a low probability of surviving from aspergillosis in patients with haematological malignancies. At present, no previous study has prospectively investigated the factors which influence the prognosis in an unselected sample of patients with aspergillosis, using a multivariate statistical approach.

The objective of this study was to identify the prognostic factors which affect the outcome in patients with Aspergillosis infection prospectively analysing the characteristics of the disease in two models of immunodeficiency, i.e. haematological malignancies and HIV infection through univariate and multivariate statistical analysis.

Patients and Methods

All episodes of aspergillosis, observed in the wards of Haematology and Infectious Diseases of the Catholic
University, Rome, Italy, were intentionally investigated through a case finding technique which included a systematic revision of all clinical and microbiological records as well as direct contacts with the attending physicians of each ward. During the period of the study (January 1990–December 1994) all adult patients in whom an episode of aspergillosis was diagnosed were considered and they were prospectively studied in follow-up. For the purpose of the study we divided the patients with aspergillosis into two groups: group A, haematological patients, i.e. acute myelogenous leukaemia (AML), acute lymphoblastic leukaemia (ALL) and non-Hodgkin’s lymphoma (NHL) and group B, HIV infected patients. These latter patients were classified on the basis of 1992 CDC revised definition of AIDS18 according to the clinical characteristics (A, B or C) and degree of immunodeficiency (1, 2 or 3). This classification was retrospectively used also for patients diagnosed before 1992. For each patient with aspergillosis the following parameters: age, sex, concomitant diseases, antifungal prophylaxis, risk factors for Aspergillus infection,1,14,19 clinical and radiological findings of aspergillosis, microbiologic data and therapy were considered and their role in affecting the outcome of aspergillosis was evaluated. In group B, in addition to the above mentioned parameters, HIV risk behaviour, T CD4 + cell count and AIDS related diseases were evaluated. A patient was considered under prophylaxis for aspergillosis if he/she had received itraconazole or amphotericin B for at least 1 month prior to the development of aspergillosis. Neutropenia was defined as a number of circulating neutrophils <500 mm$. The onset of aspergillosis was identified with the onset of clinical symptoms and radiological pictures. Diagnosis of respiratory failure in the course of pulmonary aspergillosis was done when arterial oxygen tension (PaO$_2$) was less than 60 mmHg and/or PaCO$_2$ was equal or higher than 50 mmHg, while breathing room air.

Patients with aspergillosis were divided into the following two groups: pulmonary aspergillosis (PA) and disseminated aspergillosis (DA). It is of note that all patients had radiological abnormalities consistent with invasive aspergillosis. PA was diagnosed on the basis of the following criteria: (a) histology and/or culture of bronchoscopically obtained pulmonary bronchoalveolar lavage (BAL) fluid positive for Aspergillus species (b) multiple sputum cultures positive for Aspergillus and/or (c) histologic demonstration of lung invasion by branched septate hyphae of Aspergillus of biopptic samples or autopsy tissues. An infection was considered disseminated (DA) when a patient with pulmonary aspergillosis had also the presence (culture and/or histology) of Aspergillus in another site (central nervous system, ear, skin). In each case the diagnosis of aspergillosis was established only if other more common aetiologies of pulmonary infections, such as Mycobacteria, Pneumocystis carinii and bacteria were excluded, as active, at the time of the diagnosis of aspergillosis.

Statistical analysis

The differences between mean values were assessed using Student’s two tailed $t$-test and Mann-Whitney rank sum test. Differences between proportions in the two groups were assessed using Fisher’s exact test or the Chi-squared test. Confidence intervals (C.I.) were used as an estimate of the scatter of values. The relationship of mortality to all above considered factors was evaluated by univariate analysis. The dependent variable was mortality. The limits for entering and removing terms were 0.15 for both. To facilitate the statistical analysis of the outcome, the fatalities directly or indirectly related to aspergillosis, were included into a single category, when they occurred before the episode of aspergillosis was considered resolved.

To determine the relative independent prognostic importance of factors to mortality, a multivariate analysis of factors related to outcome was calculated. All variables were entered in the logistic regression as categorical variables with two categories (0 = absent or normal, 1 = present or abnormal). For those variables with more than two categories, a cut-off point was selected according to the results of the univariate analysis. For the statistical analysis of the long-term survival we randomly selected, in the same wards of the cases and in the period of the study, two control groups: 50 patients with haematological malignancies without aspergillosis (group C) and 50 patients with AIDS and without aspergillosis (group D). The survival analysis was performed using the Kaplan-Meier method. The starting date of the study was the time of the diagnosis of AIDS for HIV infected patients and the time of the diagnosis of the neoplastic disease for haematological patients. The end-point of the analysis was death; the follow-up of the patients still alive ended on March 31, 1995. The differences in survival among the groups were assessed by the log-rank test. Univariate analysis to compare survival of patients stratified according to age and sex was performed with Mantel-Cox test. Two tailed tests of significance at the $P$<0.05 level were used to determine statistical significance.

Results

In the period of the study we observed a total number of 43 patients with aspergillosis. The mean age of the patients was 44.9±16.3 years; 30 of them were male and 13 female. The overall mortality was 65%.
Prognostic Indicators of Aspergillosis

Table I. Univariate analysis of prognostic indicators of outcome in 43 patients with aspergillosis.

<table>
<thead>
<tr>
<th>Prognostic indicators</th>
<th>Patients</th>
<th>Death</th>
<th>P</th>
<th>R.R.</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients (n=43)</td>
<td>23 (53)</td>
<td>19/23 (82)</td>
<td>0.02</td>
<td>5.80</td>
<td>1.21-30.15</td>
</tr>
<tr>
<td>Neutropenia*</td>
<td>13 (30)</td>
<td>12/13 (92)</td>
<td>0.03</td>
<td>10.50</td>
<td>1.12-69.74</td>
</tr>
<tr>
<td>AIDS (C3 class of HIV infection)</td>
<td>14 (32)</td>
<td>13/14 (93)</td>
<td>0.02</td>
<td>12.13</td>
<td>1.30-78.45</td>
</tr>
</tbody>
</table>

Group A (n=27)

| Sequential therapy† | 8 (30) | 7/8 (87) | 0.04 | 12.00| 1.02-94.45 |
| Bilateral involvement (chest X-ray) | 6 (22)  | 6/6 (100) | 0.02 | N.D. | N.D. |

Group B (n=16)

| Low number of TCD4 + cells‡ | 13 (81)  | 13/13 (100) | 0.02 | N.D. | N.D. |

* PMN cells <500 mm$^3$ for more than 7 days.
† Amphotericin B (0.7-1.5 mg/kg/daily IV) for at least 4 weeks and then itraconazole (400 mg/daily p.o.) for further 4-12 weeks.
‡ TCD4 + cells <100/mm$^3$.

N.D.: not done; R.R.: relative risk; 95% C.I.: 95% confidence interval.

distribution of the patients within the two groups was as follows: group A included 27 cases of aspergillosis; 17 patients were male and 10 female with a mean age of 50.4±17.2 years (range 19-76 years). The underlying malignancies were AML in 21 patients (78%), ALL in five patients (18%), NHL in one patient (4%). All patients received intensive timed sequential induction therapy for newly diagnosed or relapsed leukaemia and/or intensive therapy while in early first complete remission. Twenty patients (74%) were in the induction or reinduction phase of the treatment, five (18%) in remission and two (8%) were considered resistant to the chemotherapy. In haematological patients with aspergillosis mortality was 52%. Group B included 16 HIV infected patients; 14 of them (87%) were in C3 and two (13%) in B3 class according to CDC classification; 13 patients were male and three female, with a mean age of 35.6±9.4 years (range 24-47 years). In HIV infected patients with aspergillosis mortality was 87%.

Univariate analysis of the prognostic indicators

The significant prognostic indicators of the outcome of aspergillosis, as evaluated by univariate analysis, are listed in Table I. In the following sections we have separately considered all analysed factors.

Underlying diseases. AIDS (i.e. C3 class of HIV infection) is the only underlying disease significantly associated with an unfavourable prognosis of aspergillosis (P=0.02). In group A we did not find any relationship between the type and stage of haematological malignancies and the outcome of aspergillosis. In group B, low number (<100/mm$^3$) of circulating T CD4 + cells was a negative prognostic indicator (P=0.02) while AIDS related diseases were not statistically associated with the evolution of aspergillosis. In both groups, the following concurrent diseases: diabetes mellitus, cirrhosis, hepatic failure and cardiovascular disease were not associated with a higher failure rate of aspergillosis.

Predisposing factors. Among the predisposing factors, only neutropenia was associated with an unfavourable outcome (P=0.02). There was no relationship between the prognosis of aspergillosis and the following risk factors: long term broad-spectrum antibiotic therapy (81% of all patients with aspergillosis), cytotoxic chemotherapeutic agents (63%), corticosteroid therapy (16%), previous (non-active) pulmonary diseases without cavitary lesions (14%), alcoholism (5%) and marijuana smoking (2%).

Diagnosis. Aspergillus was identified by culture or histology in: sputum (67%), BAL (32%), mucosa of the nose (7%), skin lesion (5%), secretion of the ear (5%), transthoracic biopsy tissue (2%) and blood (2%). Five patients (12%) had the diagnosis of aspergillosis confirmed at autopsy, while in three cases (7%) the diagnosis was achieved at autopsy only. Aspergillus fumigatus was identified in 15 patients (35%), A. flavus in 12 (28%), A. fumigatus and A. flavus in six (14%), A. niger in two (5%), A. terreus and A. fumigatus in two (5%), A. versicolor in two (5%) and A. penicilloides in one (2%). In three patients (7%) the Aspergillus species was not further characterized since the diagnosis was done at autopsy. In both groups of patients the outcome was not influenced by the species of isolated Aspergillus.
Haematological parameters. The only haematological parameter associated with an unfavourable prognosis in both groups of patients was neutropenia for more than 7 days \((P = 0.02)\). In HIV infected patients the low number of TCD4 + cells \(< 100/\text{mm}^3\) was also a prognostic indicator \((P = 0.02)\).

Clinical and radiological findings. Thirty-five patients (81\%) were affected by PA and eight (19\%) by DA. The type of infection, localized in the lung or disseminated, was not a negative prognostic factor for aspergillosis. The only symptom associated with an unfavourable prognosis was haemoptysis \((P = 0.03)\). Comparing the two groups of patients, respiratory failure was statistically associated with an unfavourable prognosis in haematological patients only \((P = 0.02)\).

As regards radiological findings, 20 patients (46\%) had single or multiple cavitary lobe lesions, 11 (26\%) focal alveolar infiltrate, 10 (23\%) bilateral alveolar infiltrates and two (5\%) diffuse interstitial infiltrates. Sixteen patients (37\%) had pleuritic effusion, five (12\%) atelectasia, two (5\%) pulmonary oedema and one (2\%) pneumothorax. We did not find any statistical correlation between the above-mentioned radiological pictures and the outcome. The presence of bilateral disease on chest X-ray was significantly associated \((P = 0.04)\) with a higher mortality in haematological patients only.

Prophylaxis and therapy. Twenty-one patients with haematological diseases (78\%) were under oral antifungal prophylaxis with amphotericin B (80\%) or itraconazole (20\%). None of the HIV infected patients was intentionally on prophylaxis but a significant percentage of them (44\%) had received antifungal therapy with fluconazole (57\%), itraconazole (29\%) or amphotericin B (14\%) for oral or oesophageal candidiasis or cryptococcosis. No correlation was found between the presence or absence of a prophylaxis and the outcome of aspergillosis.

Forty patients were treated with specific antifungal therapy, 18 (45\%) with amphotericin B alone (0.7–1 mg/kg/daily IV) with a mean duration of therapy of 24 ± 9 days \((P = \text{NS}; \ RR = 4.61)\) and eight (20\%) with itraconazole alone (400 mg/daily p.o.) with a mean duration of therapy of 21 ± 10 days \((P = \text{NS}; \ RR = 4.66)\). Fourteen patients (all in group A) treated with amphotericin B (0.7–1.5 mg/kg/daily IV) for at least 4 weeks, and with clinical improvement, received sequential therapy with itraconazole (400 mg/daily p.o.) for a further 4–12 weeks \((P = 0.001; \ RR = 0.04)\). Three patients, in whom the diagnosis was performed at autopsy, did not receive any specific therapy.

The comparative analysis of the two groups indicated

<table>
<thead>
<tr>
<th>Prognostic indicators</th>
<th>(P)</th>
<th>R.R.</th>
<th>95% C.I.</th>
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</thead>
<tbody>
<tr>
<td>Sequential therapy*</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02–0.95</td>
</tr>
<tr>
<td>AIDS (C3 class of HIV infection)</td>
<td>0.03</td>
<td>3.50</td>
<td>1.23–27.6</td>
</tr>
</tbody>
</table>

* Amphotericin B (0.7–1.5 mg/kg/daily IV) for at least 4 weeks and then itraconazole (400 mg/daily p.o.) for further 4–12 weeks.

R.R.: relative risk; 95\% C.I.: 95\% confidence interval.

that in HIV infected patients the prognosis was not influenced by the type of therapy (amphotericin B vs. itraconazole) while, in haematological neoplastic patients the better prognosis was associated with the sequential therapy (amphotericin B and itraconazole) \((P = 0.003; \ RR = 0.04)\).

Multivariate analysis of the prognostic factors

The multivariate analysis showed that sequential therapy \((P = 0.01)\) and AIDS \((P = 0.03)\) were independent prognostic indicators of the outcome. The coefficients of the regression equation are listed in Table II.

Survival analysis

The median follow-up of the patients with aspergillosis was 26 months (range 2–84). Fourteen of 27 patients with aspergillosis and haematological malignancies died with a median interval from the onset of symptoms to death of 2.5 months. The median survival was 300 days in comparison to 653 days for patients without aspergillosis (control group C) \((P = 0.04)\). Fourteen of 16 patients with aspergillosis and HIV infection died with a median interval from onset of symptoms to death of 1.7 months. The median survival of the patients was 124 days in comparison to 438 days for AIDS patients without aspergillosis (control group D) \((P = 0.04)\). The survival curves of the patients stratified according to age and sex did not show any statistically significant difference.

Discussion

Aspergillosis has been recognized as a significant cause of morbidity and mortality in severely immunocompromised patients. In previous reports, the comparative analysis of clinical and radiological characteristics of aspergillosis in patients with HIV infection and patients with haematological malignancies have not shown any substantial differences. On the contrary, a more severe prognosis of patients with HIV infection in comparison to
haematological neoplastic patients has been recently reported. 19,20 This finding, however, has not been further analysed in terms of the definition of the different indicators of the prognosis nor identified the differences among these patients. Our study substantially confirms a significant difference in the prognosis of aspergillosis between HIV-infected and haematological patients, indicating a better outcome for this latter group. In fact, in line with previous reports, 1,17,19 the overall rate of mortality in our haematological neoplastic patients accounts for 52% of the episodes while the rate of mortality of HIV-infected patients with aspergillosis is 8.7%. No previous report on prognostic factors of aspergillosis has been done in HIV infection and only one study 17 has indicated a better prognosis of aspergillosis in leukaemic patients when in complete leukaemic remission. This observation has not been entirely confirmed in our patients, since the finding of a better prognosis for leukaemic patients responsive to chemotherapy was not supported by a statistically significant result. Our study, through univariate analysis, indicated that neutropenia, haemoptysis and concomitant AIDS negatively influence the prognosis of aspergillosis. There are, however, remarkable differences in the prognostic indicators between the two groups of patients. In haematological patients, radiological bilateral involvement of the lungs and respiratory failure were negative prognostic indicators while sequential therapy (with amphotericin B and itraconazole) was associated with a better prognosis. In HIV-infected patients, a low number of T CD4+ cells was a negative factor for the prognosis of aspergillosis. To further determine the relative independent prognostic importance of the above-mentioned factors we applied the multivariate analysis technique which has proven to be a powerful method of relating covariates, and allows us to assign a relative weight to a large number of cofactors. On the basis of this statistical analysis we were able to establish that the major factor indicating a higher risk of death was concomitant AIDS, while sequential therapy (amphotericin B and itraconazole) was associated with a better outcome.

It is of note that concomitant HIV infection, while it is not per se a risk factor for the onset of aspergillosis, 21,22 is an element which negatively influences the prognosis of the disease. This finding, in our opinion, probably reflects not only the severity of Aspergillus infection in these patients but also the patient's debilitated state and, in some cases, the presence of other opportunistic diseases. Their role in affecting the prognosis is not always easy to evaluate.

Neutropenia was a negative prognostic factor both in haematological neoplastic and HIV-infected patients, usually being the consequence of antineoplastic chemotherapy in the first group of patients and long-term anti-infective therapy (e.g., zidovudine, ganciclovir), prophylaxis (e.g., trimethoprim-sulphamethoxazole) and/or HIV infection itself in the latter group of patients. 23,24 The persistence of neutropenia in affecting an important anti-infective mechanism of defence increases the possibility of the onset of aspergillosis but also of other opportunistic diseases in these patients which negatively influence the prognosis.

Another factor which was significantly associated, in our study, with a poor prognosis was haemoptysis. This symptom, predominantly observed in patients with cavitary upper lobe disease, was often fatal. The acute bleeding is probably secondary to fungal invasion of a large bronchial artery which causes immediate asphyxia and consequent death and it is likely to be due to the peculiar vasculotropic nature of Aspergillus. 5 This dramatic event occurred in five of our patients with pulmonary cavitary disease and it was uniformly observed both in HIV-infected and haematological patients.

The radiological bilateral involvement of the lungs was associated with a poor prognosis in haematological neoplastic patients only and this probably reflects the wide pulmonary and/or systemic diffusion of the infection in these patients. On the contrary, none of the different radiological pictures were associated with poor prognosis in HIV-infected patients. Although it is difficult to comment on this difference between the two groups of patients, it seems reasonable to suggest that the profound anergic "status" induced by HIV infection could have influenced the lesions caused by the fungus in the lungs and the consequent radiological findings. Although the majority of our patients have been treated with amphotericin B, as first choice drug, itraconazole can be considered a suitable alternative, as indicated by the response rates which are comparable to those of amphotericin B. 25-27 It is noteworthy that, in many of our haematological patients, aspergillosis, regardless of the species involved, has been successfully treated with sequential therapy of amphotericin B and itraconazole. The real impact of this therapy in significantly affecting the prognosis, however, remains to be defined and conclusive results will be possible only when controlled clinical studies have been performed. In fact, the selection of our patients, who have been treated with sequential therapy, was done within the group already treated with amphotericin B and with clinical improvement and this, in addition to the limited follow-up, could have biased the results.

From analysis of the survival curves of patients with aspergillosis it emerges that this disease represents a severe clinical condition in terms of reducing the median
survival of patients both with haematological malignancies or HIV infected patients, although with remarkable differences between the two groups. In fact, AIDS patients had a shorter survival in comparison to haematological patients and this probably indicates that aspergillosis can also be the terminal event in the setting of multiple opportunistic diseases in consideration of the poor response to therapy.

In summary, our prospective study indicates that aspergillosis, although an uncommon event in patients with HIV infection, has a more severe prognosis than in haematological patients. Future prospective controlled trials are necessary to confirm the real importance of the sequential therapy (amphotericin B followed by itraconazole) in patients with aspergillosis.

References