Cryptococcosis in patients with hematologic malignancies. A report from GIMEMA-infection program

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A B S T R A C T

Background and Objectives. Cryptococcosis is an important cause of morbidity and death in immunocompromised patients. The aim of this study was to evaluate clinical and laboratory characteristics, and outcome of patients with cryptococcosis complicating hematologic diseases.

Design and Methods. This was a retrospective study, conducted over a ten-year period (1993-2002) in 21 hematology divisions, in tertiary care or university hospitals.

Results. This study evaluated 17 patients with hematologic diseases who developed cryptococcosis. Possible risk factors recognized before the onset of the infection were: administration of steroids for the underlying malignancy (6 patients), diabetes mellitus (4 patients), cutaneous lesions (2 patients) and autoimmune disease, hepatic cirrhosis, chronic renal failure and exposure to pigeons (1 patient each). Five patients received prophylaxis, consisting of fluconazole in 2 cases. Fever, neurological and respiratory signs developed according to the primary sites of infection (5 blood, 5 central nervous system, 4 lung, and 1 each in gut, skin and mouth). Diagnosis was made by positive microbiological culture, antigen detection in serum or cerebrospinal fluid, or polymerase chain reaction. All patients started specific treatment (fluconazole, 7 patients; amphotericin-B deoxycolate or liposomal amphotericin-B, 10 patients). Two patients died from cryptococcosis within 30 days after diagnosis.

Interpretation and Conclusions. Cryptococcosis in patients with hematologic malignancies is a rare complication. In neutropenic patients, it is less fatal than other fungal infections (i.e. aspergillosis or candidemia). Specific treatment, started promptly, positively influences the outcome.

Key words: *Cryptococccus spp.*, hematologic malignancies.

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Cryptococcosis is an important cause of morbidity and death in immunocompromised patients.[1,2] Although the incidence of this infection among patients with AIDS has decreased in the past years as a consequence of the introduction of highly active antiretroviral therapy (HAART), it still remains a major cause of morbidity and mortality.[3] Currently, AIDS is the predisposing factor in approximately 90% of cryptococcal infections, but other patients with defects of T-cell-mediated immunity are at increased risk of cryptococcosis.[4-5] There are some reports on patients with hematologic malignancies; patients with Hodgkin's disease are those at highest risk of cryptococcosis.[6-15]

There are several reasons for the rarity of cryptococcosis in hematologic patients: the widespread prophylactic use of fluconazole,[16] and the fact that the infection is often asymptomatic or characterized by symptoms undistinguishable from those of the underlying disease.

In this retrospective analysis, we reviewed the features of 17 patients with hematologic malignancies who developed a cryptococcal infection, in order to evaluate the clinical spectrum of the disease, the diagnostic tools, the antifungal approach, and to identify the factors influencing the outcome in these patients.

Design and Methods

We retrospectively collected all cases of microbiologically and/or histologically documented cryptococcosis in adults (>12 years of age) with hematologic malignancies, observed between January 1993 and December 2002 in 21 GIMEMA (Gruppo Italiano Malattie EMatologiche dell'Adulto)

centers. Hospital records of 17 patients with cryptococcosis were reviewed to collect demographic data (age, sex), type and stage of underlying hematologic disease. The following risk factors for cryptococcosis were analyzed: steroid therapy (doses administered according to the therapeutic trial for the hematologic malignancy), bone marrow transplantation, use of tacrolimus or fludarabine, underlying diabetes mellitus, chronic renal failure, cirrhosis, local injury, contact with pigeons or other birds. Patients with an HIV-related hematologic malignancy were excluded from the study. Furthermore, date of diagnosis, clinical presentation, imaging pattern, antigen detection in serum and/or in cerebrospinal fluid (CSF), CSF India ink, culture or microscopic evaluation of CSF, blood, bronchoalveolar lavage (BAL)/sputum, urine, skin, or other samples, were reviewed. Data on type of drugs employed, total doses and duration of treatment for cryptococcosis were also collected. Patients had a follow-up of 100 days after the diagnosis of cryptococcosis. Mortality was judged as due to cryptococcosis when it happened within 30 days of the diagnosis. A post-mortem was carried out on all patients who died. The clinical records and charts were examined by the authors and reviewed by 2 physicians skilled in hematology and/or infectious diseases.

According to the criteria of European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG),[17] the diagnosis of cryptococcosis was made following isolation of the fungus from samples obtained by a sterile procedure from a normally sterile site, and/or the presence of soluble cryptococcal antigen in serum and/or CSF and/or histologic evidence of the fungus in tissue samples.

Results

Patients' clinical and laboratory features

Type and status of hematologic disease, and clinical characteristics of the patients are summarized in Table 1. Since there is not a national register for hematologic diseases, we did not attempt to calculate the real incidence of this complication in hematologic malignancies. Ten patients (59%) had been treated with aggressive chemotherapy. The median time between chemotherapy and onset of infection was 14 days (range 0-37). None of the patients had previously received purine analogs. Four patients (24%) were given high-dose therapy [allogeneic bone marrow transplantation (allo BMT, 2 patients), autologous bone marrow transplantation (auto BMT, 1 patient) and peripheral blood stem cell transplantation (PBSCT, 1 patient)]. The median time between transplantation and cryptococcosis was 635 days (200-1245).

Six patients received 6-methyl-prednisolone as part

Table 1. Clinical characteristics and risk factors in 17 patients with cryptococcosis, observed between 1993 and 2002.

N. of patients	*17*
Median age (years; range)	61 (17-78)
Gender (male/female)	12/5
Underlying hematologic diseases	
Acute myeloid leukemia	6
Non-Hodgkin's lymphoma	3
Acute lymphocytic leukemia	2
Myelodysplastic syndrome	2
Chronic lymphocytic leukemia	1
Chronic myeloid leukemia	
Hodgkin's disease	1
Multiple myeloma	1
Previous chemotherapy (%)	
Yes	10
No	7
Status of underlying hematologic disease at the onset of cryptococcosis	
Diagnosis	2
Complete remission	4
Partial remission	1
Resistance	3
Relapse	7
Steroid treatment (%)	6
Median total dose of Methyl-prednisolone (mg; range)	475 (75-9000)
Median daily dose (range)	32 (15-210)
Median days of treatment (range)	12 (1-360)
Transplantation	4
Allogeneic BMT	2
Peripheral blood stem cell transplant	1
Autologous BMT	1
Prior neutropenia before onset of *cryptococcosis*	
ANC <0.1×10^9/L (%)	4/17
ANC 0.1- 0.5×10^9/L (%)	2 /17
ANC 0.5 - 1.0×10^9/L (%)	none
Median duration of neutropenia (days)	5 (0-18)
Median lymphocyte count at infection (range)	1.07×10^9/L (0-60)
Possible predisposing factors	
Autoimmune disease	1
Diabetes	4
Chronic renal failure	1
Liver cirrhosis	1
Cutaneous wounds	2
Exposures to pigeons	1
Primary site of infection	
Blood	5
Central nervous system	5
Lung	4
Gut	1
Skin	1
Mouth	1
Etiological agent	
Cryptococcus neoformans	15
Cryptococcus laurentii	2
Prophylaxis	5/17
Fluconazole	2
Oral Nystatin	2
Oral AmB	1
Primary antifungal therapy	17/17
Fluconazole	7
Amphotericin B	6
Liposomal-amphotericin B	4
Outcome of infection (at 30 days after diagnosis)	
Recovery	15
Death from infection	2

of their treatment of the hematologic disease. Other concomitant diseases, as possible risk factors for cryptococcosis, were present in 11 patients: diabetes mellitus (4 patients), cutaneous wounds (2 patients), autoimmune disease, liver cirrhosis, chronic renal failure and occupational exposure to pigeons (1 patient each). At the onset of infection, 6 patients (35%) were profoundly neutropenic with neutrophil counts lower than 0.5×10^9/L. The overall median neutrophil count was 1.4×10^9/L (range 0-9.9×10^9/L). All neutropenic patients recovered from neutropenia (neutrophil counts >1×10^9/L) in a median of 5 days from the diagnosis of the infection (range 1-18); none of them received granulocyte-colony stimulating factor (rhG-CSF). Eight patients (47%) had lymphocyte counts lower than 1.0×10^9/L. the overall median value was 1.07×10^9/L (range 0-60×10^9/L)]. The $CD4^+/CD8^+$ ratio was not evaluated in any of the patients. During neutropenia, 5 patients (29%) received oral antifungal prophylaxis, which in 2 patients consisted of fluconazole, for a median of 11 days (range 4-16). Eight febrile patients received empirical treatment with broad-spectrum antibiotics (beta lactam plus aminoglycoside with or without a glycopeptide).

Clinical presentation

According to the EORTC/MSG criteria, the diagnosis of cryptococcosis was *proven* in all patients. Eleven patients (65%) developed signs and symptoms of cryptococcosis while at home, whereas in 6 patients (35%) the infection was diagnosed during hospitalization. The most frequent sign was fever (71% of cases), with a median temperature of 38.2°C at presentation (range 37.8°-39.7°C); this was the only sign in 4 patients (24%) (Table 2). Other signs and symptoms were related to the primary site of infection: 6 patients had neurological signs, including confusion (5 cases), meningism (4 cases), headache (3 cases), and visual disorders (1 case). Respiratory symptoms were present in 6 patients: cough in 3 cases, dyspnea in 2 cases, and chest pain in 2 cases. Four patients had gastrointestinal manifestations, with abdominal pain and diarrhea. One patient had multiple skin lesions, consisting of 2-5 mm erythematous papules initially resembling petechiae, which progressed to discrete 5-10 mm, dome-shaped, crusted papules with central umbilication.

One patient with NHL in complete remission had thrombocytopenia and the diagnosis of cryptococcosis was made incidentally, with no other signs of cryptococcosis being present. Single or multiple radiological examinations performed in 13 patients (chest X-ray and/or thoracic computed tomography (CT) scans in 10 patients, cerebral CT scans or magnetic resonance imaging in 4 patients), were suggestive of fungal infection in 9 cases (69%).[18] The diagnosis of cryptococcosis was made *in vivo* in all patients and histology was not diagnostic in any of the cases. Microbiology was diagnostic in 9 cases. Nine patients (53%) had a positive culture for cryptococcosis (blood, 3 cases; bronchoalveolar lavage fluid, 2 cases; CSF, stool and skin; 1 case each). *Cryptococcus* was isolated from two sites of infection, blood and mouth, in 1 case. In the 8 patients without culture evidence of cryptococcosis, the diagnosis was made through cryptococcal antigen detection in CSF or serum and India ink staining. Serum antigen detection was positive in all patients in whom it was tested (9/9, 100%). Antigen detection in the CSF using latex agglutination was performed in 6 patients and resulted positive in 4 cases (67%). India ink staining was done in 6 patients, resulting positive in 4 cases (67%). Polymerase chain reaction analysis for *Cryptococcus* was performed in 2 patients and was positive in 1 (50%) (Table 3).

Table 2. Signs and symptoms of fungal infection in the 17 cases studied.

Signs and symptoms	*N. of cases (%)*	*Only sign/ symptom %*
Fever	12 (71)	24
Mental alteration	6 (35)	10
Respiratory symptoms	5 (29)	6
Gastro-intestinal manifestations	4 (24)	–
Meningism	4 (24)	–
Skin lesions	1 (6)	–
Visual disorders	1 (6)	–
Thrombocytopenia	1 (6)	6

Treatment and outcome

Antifungal treatment was administered empirically to all patients after a median of 90 hours of fever unresponsive to broad-spectrum antibiotics.

Seven patients were treated with i.v. fluconazole (liposomal amphotericin-B (L-AmB) was added in 1 patient and AmB in lipid complex was added in another case). The median daily dose of fluconazole was 400 mg (total median dose 3200 mg, range 800-5000) for a median duration of 5 days (range 1-24); it should be noted that one of these patients died after a single day of treatment with fluconazole. Six patients received deoxycolate AmB for a median duration of 26 days (range 12-48) [median daily dose 50 mg (range 40-60), median total dose 1170 mg (range 520-2650)] and 4 patients received L-AmB [median daily dose 150 mg

Table 3. Laboratory diagnostic results in the 17 cases of cryptococcosis studied.

Sample	*Antigen detection pos/tested*[a]	*India ink pos/tested*[b]	*PCR*	*Positive culture*
Serum	9/9	–	1/2	–
CSF	4/6	4/6	–	1
Blood	–	–	–	5
BAL	–	–	–	2
Skin	–	–	–	1
Stool	–	–	–	1

[a]*Range of titer, 1:8 to ≥1: 64,000 ;* [b]*Range of titer, 1:1 to ≥1:32,000.*

(range 100-150), median total dose 2175 mg (range 1400-2700), median duration of treatment 14 days (range 13-17)]. In 7 patients fluconazole was subsequently added as maintenance therapy at a daily dose of 200 mg for a median treatment duration of 62 days (range 19-90).

The two fluconazole breakthrough patients received deoxycolate AmB and L-AmB. No patients were given 5-flucytosine. Therapy was successful in 15 patients (88%), while 2 patients (12%) died of cryptococcosis within 30 days after diagnosis. Six further patients (35%), who responded to antifungal therapy, died of hematologic disease progression within the 3 months following the fungal infection without signs or symptoms of infection.

Discussion

Despite the extensive literature accumulated on cryptococcosis in recent years, many aspects of the epidemiological and clinical features of cryptococcosis in HIV-negative patients are poorly documented, particularly in patients with hematologic malignancies. Cryptococcosis in hematologic patients is rarely reported due to the low frequency of this complication or to diagnostic difficulties.[1,6-11] Given the small number of cases reported, risk factors and clinical findings of cryptococcosis in patients with hematological malignancies are poorly documented.[2,7,14,15]

We collected the cases of cryptococcosis observed in Italian Hematology Divisions during the last ten years. At variance from other authors,[5,6] we observed that the patients at highest risk of cryptococcosis are those with acute leukemia (8 patients; 47%), although we did not document any particular risk factors or local clusters of cryptococcosis in these patients. One possible explanation could be that the majority of patients admitted to our Hematology departments had acute leukemia; the other possibility is chance.

Only one of our patients had Hodgkin's disease, suggesting that the incidence of cryptococcosis in this disease may be overestimated. Accordingly, we did not observe an increased cryptococcosis rate in patients treated with purine analogs, which are known to induce marked and prolonged T-lymphocyte depletion. Differently from a French series, in which 4 of 6 patients with cryptococcosis had previously received fludarabine,[12] none of our patients had received purine analogs.

In our study, cryptococcosis mainly caused pneumonia and septicemia. Fungemia due to *Cryptococcus* was one of the most frequent clinical manifestations and extra-hematologic involvement (e.g. skin, gastro-intestinal tract) was consequent to blood dissemination. *Cryptococcus var. laurentii* and *var neoformans* were isolated. The latter is ubiquitous and predominantly affects immunocompromised hosts. In contrast, *Cryptococcus var. gattii* has a limited geographic distribution and predominantly affects immunocompetent male hosts.[19]

It has been reported that disseminated cryptococcosis is invariably fatal if untreated and the prognosis remains poor even when treated.[6,20] In the series from MSKCC, it was reported that patients with cryptococcal meningitis and cancer did significantly worse than patients with HIV infection (improvement or cure in 43% vs. 78% of the patients).[10] However, the mortality rate for cryptococcosis has changed dramatically in the last years. While in the past this rate reached 70-80% in HIV-positive patients and 40-60% in HIV-negative patients, it is now much lower, particularly in HIV-positive patients.[22] It is noteworthy that among our 17 patients, only 2 patients died of complications of their fungal infection (both had cerebral involvement), while all the other patients recovered from the infection and continued treatment for their hematologic malignancy, without any delay.

Although experience in the treatment of cryptococcosis in non-HIV patients is limited, the best results are reported for AmB, followed by a triazole.[6, 21-23] Lipid-compound amphotericin B has been reported to be effective in some cases, in particular for pulmonary and cerebral infections.[24,25] None of our patients received flucytosine, which is usually administered to HIV-positive patients. The possibility of bone marrow depression due to flucytosine is the major limit to using this drug in patients with blood diseases.[26] Since our survey was retrospective, we could not draw any conclusion on the treatment. However, prophylaxis with fluconazole, used to reduce the incidence of candidiasis in hematologic patients, could reduce the incidence of *Cryptococcus* infection, although it did not prevent it in 2 patients. In our patients, both AmB compounds and fluconazole

were effective and resulted in the patients' recovery. Considering that the 2 fatal cases were due to cerebral cryptococcosis, L-AmB, which can cross the blood-brain barrier and results in a high drug concentration in the brain, could be more indicated than conventional AmB.[27]

In conclusion, our study shows that cryptococcosis is a rare complication in patients with hematologic malignancies. Our data confirm the reports in current literature, that the incidence of cryptococcosis has not increased in the last years in this subset of patients. One possible explanation for this is the widespread prophylactic use of fluconazole. Finally, in our experience the mortality rate in cryptococcosis is lower than that observed for other fungal infections in neutropenic patients.

LP co-ordinated the study; LP and LF wrote the paper; all other co-authors collected the clinical data; PM and ADF critically reviewed and approved the final version of the paper.

The authors reported no potential conflicts of interest.

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