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Recent Developments in Prophylaxis and Therapy of Invasive Fungal Infections in Granulocytopenic Cancer Patients

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INFECTION is a frequent complication of granulocytopenia which results from chemotherapy of neoplastic disease [1–3]. Although Gram-negative bacillary bacteremia is responsible for the early morbidity and mortality of febrile granulocytopenic cancer patients (FGCP), it has been known, for some time, that fungal infections were also important in these patients, as evidenced by the frequent demonstration of invasive candidiasis and aspergillosis, among others, at autopsy. De Gregorio *et al.* [4] found a 51% (27/52) incidence of fungal infections in autopsied patients with acute leukemia; *Candida* sp. were found in 12, *Aspergillus* sp. in seven and both pathogens in seven. Lungs, for both pathogens, were frequently involved, but a substantial number of these patients had disseminated candidiasis in the liver, spleen, kidneys and heart.

Besides candidiasis and aspergillosis, many other fungal pathogens can be involved in clinical diseases in neutropenic patients; they are much less frequent than the two former organisms, but their frequency is significant (Table 1).

Table 1. *Classical pathogens*

Candida species
Aspergillus species
Mucorales
Torulopsis glabrata
Cryptococcus neoformans
Trichosporon species
Geotrichum species
P. boydii
Histoplasma capsulatum
Coccidioides immitis

Infection results from both exogenous and endogenous sources; air, food, i.v. products and i.v. catheters serve as important portals of entry for acquired fungal pathogens while skin, oral mucous membranes and the gastrointestinal tract represent the major endogenous reservoir for candidiasis. Overall, the role of surveillance cultures performed in high risk patients remains controversial as well as their impact on clinical management. However, there is good evidence in *Aspergillus* sp. and some *Candida* sp. strains (such as *C. tropicalis*) that heavy and persistent colonization of the upper respiratory or the digestive tracts, respectively, often precedes the onset of severe local and/or systemic infection [5–7]. These considerations also support the value of surveillance cultures to identify the patients who are at risk of developing fungal infections; however, for ubiquitous potential pathogens such as *C. albicans*, the role of such a surveillance is understandably limited. In a review of 110 patients with fungemia, more than 30% of them did not show any positive culture for the pathogenic yeasts prior to fungemia from any sites and despite the high rate of disseminated infection among these patients [8]. Non-microbiological considerations are equally important for the prediction of invasive fungal disease in FGCP; among them the duration of severe granulocytopenia is of extreme importance: it has been shown that the incidence of aspergillosis markedly increases when neutropenia has been present for more than 20 days [7]. Severe immunodepression is also a major predisposing condition, as illustrated by the frequency of candidiasis and

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Table 2. General measures to prevent the acquisition of potential fungal pathogens

Education of the staff, the patient and the family
personal hygiene
hand washing
training of house-keepers
Cooked-food diet
Control the quality of the environment such as surveillance of the air, climination of plants, isolation in LAF rooms
Well-trained i.v. team (particularly for TPN)

Table 3. Oral antifungal prophylaxis: prevention of candidiasis

Agents
Polyenes (not absorbed):
Nystatin
Amphotericin B
Imidazoles:
Miconazole
Ketoconazole
Others: new triazoles:
itraconazole
fluconazole (well absorbed)

cryptococcosis in AIDS patients. The proven relationship between the high dosage of corticosteroids and the incidence of invasive aspergillosis has also been reported in renal transplant recipients [9]. Finally, breaks in skin or mucous membranes are a likely focus for entry of fungal pathogens, and the frequently used i.v. catheters, which are maintained for prolonged periods of time, have also been recognized as major sources for candidemia caused by *C. parapilosis* [8, 10].

Accurate diagnosis of candidiasis or aspergillosis is often difficult to demonstrate on a clinical basis and only histological proof of fungal pathogens is accepted as a definite diagnosis. Invasive diagnostic procedures are generally withheld in patients with abnormal coagulation tests or very low platelet counts. Serological means, including detection of antibodies and antigen or metabolites, show false positive as well as false negative results and so far remain research tools without evidence of significant benefit at the bedside.

In the neutropenic patient with persistent fever despite broad spectrum antibiotics, invasive fungal infections are often suspected and empiric antifungal therapy initiated. However, numerous side-effects are observed with amphotericin B therapy and in such circumstances both the physicians and the patient may be reluctant to employ this empiric approach.

Prevention of acquisition of fungal pathogens from exogenous sources would thus presumably help in the prevention of opportunistic fungal infections in neutropenic patients. Although no controlled studies in that respect have been made so far, measures as simple as hand-washing and a series of other general hygienic measures, if strictly enforced, would probably reduce the acquisition of fungal pathogens and the subsequent infections (Table 2).

So far, chemoprophylaxis of fungal infections has been attempted with only a limited number of drugs (Table 3), most of which have been poorly or not absorbed when given by the oral route. This might be an advantage for those situations (candidiasis) where the GI tract serves as the portal of entry. The concept of chemoprophylaxis of candidiasis by the administration of non-absorbable antifungal agents stems from the observed efficacy of non-absorbable antibiotics in the prevention of Gram-negative bacillary bacteremia in neutropenic patients [11].

As far as aspergillosis is concerned, adequate measures for prevention of colonization and/or early infection require the use of drugs that should be applied ideally to the upper respiratory tract since aspergillosis is an airborne infection. The role of systemic antifungal agents for prevention of fungal infections is mostly unknown; it has been shown, however, that low doses of amphotericin B are effective for the control of superficial candidiasis such as that limited to mucous membranes (esophagitis). Whether minimal systemic absorption of poorly absorbable antifungal drugs given orally or other topical routes might be important remains an open question. However, failures have been reported using systemic prophylaxis with i.v. miconazole [12].

More recently, ketoconazole, the reabsorbtion of which is variable, has been widely investigated at various dosages. In one study performed at the Institut J. Bordet, neither miconazole nor ketoconazole (200 mg daily) prevented aspergillosis [13]. The recent development of new imidazoles (such as itraconazole and fluconazole) may represent significant progress for the prophylactic approach of invasive fungal infections; itraconazole seems to be more effective on *Aspergillus* sp. and fluconazole (which is mainly active on *Candida* sp.) has an excellent pharmacokinetic profile, indicating predictable and reproducible serum levels.

PREVENTION OF CANDIDIASIS

Several limited studies have suggested that various antifungal agents (amphotericin B, ketoconazole), when administered orally, might prevent the occurrence of local or systemic *Candida* sp. infection; some studies suggest that ketoconazole might be superior, in that respect, to nystatin [14]. Most of the studies available have recently been extensively reviewed [15]. However, placebo-controlled studies

on the efficacy of nystatin are extremely limited, making it difficult to appreciate the intrinsic role of that agent. In a large study performed at the Institut J. Bordet and comparing the respective efficacy of amphotericin B (500 mg q8 h), ketoconazole (200 mg q8 h) and a placebo, we found that true colonization of the GI tract by yeasts could be significantly reduced by amphotericin B as compared to the placebo, but not by ketoconazole. As also reported by others, *T. glabrata* predominated in the stools obtained from ketoconazole-treated patients and *C. albicans* in those from the patients who received the placebo. However, the incidence of documented candidiasis was reduced in the ketoconazole group as compared to that of patients receiving amphotericin B or the placebo, even though these differences were not statistically significant. Moreover, no systemic candidiasis was seen among the patients receiving ketoconazole. The incidence of aspergillosis was similar in all three groups (10%). These results suggested that ketoconazole might be an effective chemoprophylaxis for candidiasis, perhaps due to the moderate systemic absorption of that drug. On the other hand, no beneficial effect has been found for the prevention of aspergillosis (ketoconazole is ineffective on *Aspergillus* sp. and amphotericin B is minimally absorbed and probably inactive against aspergillosis of which the initial site of entry is outside the GI tract). Whether fluconazole will decrease the incidence of candidiasis remains to be studied but encouraging data are already available with this compound for the therapy of oropharyngeal candidiasis [16].

PREVENTION OF ASPERGILLOSIS

As mentioned above, aspergillosis occurs via the airborne routes and colonization with *Aspergillus* sp. of the upper respiratory tract (nose, sinuses) often precedes pulmonary and/or systemic infection, which is usually fatal in neutropenic patients, unless therapy is undertaken very soon after the onset of the symptoms [17]. These may be quite non-specific in patients with cancer and neutropenia who usually suffer from multiple complications related to their underlying neoplastic condition and its therapy. Moreover, the microbiological diagnosis of invasive aspergillosis is difficult; bronchial washings or biopsies, obtained by fiberoptic bronchoscopy, reveal the pathogen in 25–40% of cases, in the best series [18]. These difficulties in clinical and microbiological diagnosis of aspergillosis are a good rationale for the prevention of this highly lethal complication of granulocytopenia.

Multiple attempts have been made to prevent aspergillosis in neutropenic patients (Table 4), all being directed to the control of acquisition of the pathogen through the air. These measures include air filtration, decontamination of the air through formaldehyde fumigation and isolation of the patients in LAF units. Although no controlled studies of the efficacy of these measures are available, anecdotal data suggest efficacy, especially when the risk of contamination of the air is high, as is the case when nearby construction work takes place. The need for elimination of potential sources within the hospital (e.g. plants) and the role of surveillance cultures of the nasopharynx have already been discussed.

Table 4. Prevention of aspergillosis

Control the air: formaldehyde fumigation HEPA filters
Eliminate plants
Intensive surveillance if construction work
Control sinusal status as well as nasal colonization
Isolation in LAF rooms
Potential role of nasal sprays of amphotericin B administered prophylatically in neutropenic patients

Chemoprophylaxis of aspergillosis necessarily requires the use of the only presently available drugs which act against *Aspergillus* sp.: amphotericin B and itraconazole. In addition, it might be necessary that the drug should be distributed adequately at the site of primary colonization, i.e. the nasopharynx and the sinuses. The role of systemic prophylaxis remains an open question but it may be suspected that such prevention would make it necessary to expose an excessive number of patients to the systemic toxicity of amphotericin B to the benefit of only few of them. Local administration of amphotericin B in patients at risk has been attempted, at the Institut J. Bordet, with intranasal instillations of amphotericin B in high risk patients; administration was three times per day with 3 mg per instillation [19]. Among 71 patients treated with intranasal chemoprophylaxis, we have observed three (4.2%) cases of invasive aspergillosis while the disease occurred in 11/84 (13%) patients previously observed who did not receive amphotericin B intranasally. These results suggest that locally administered chemoprophylaxis (amphotericin B) might be effective in preventing aspergillosis in neutropenic patients. Our current prospective randomized study shows similar results [20]. The value of itraconazole is also presently being evaluated by several investigators.

EMPIRIC THERAPY OF FUNGAL INFECTIONS

We have already alluded to the need of early treatment of disseminated aspergillosis [17]; the same conclusions apply to that of invasive candidiasis.

The mortality secondary to fungemia has always been extremely high [8, 21], and still is in most patients [10]. It has been shown that only patients who can be treated for more than 3 days survive, suggesting that critically ill patients, who presumably had a late diagnosis, do not benefit from therapy [22].

Pizzo *et al.* found that the early administration of amphotericin B to FGCP, 7 days after the onset of an unsuccessful course with antimicrobial agents, was associated with an improved survival and a reduction of documented fungal infections [23]. These were limited, though provocative, observations and have been the incentive to investigate, within our EORTC Antimicrobial Therapy Cooperative Group the value of early empiric therapy with amphotericin B in FGCP who do not respond to broad spectrum antibiotics and may actually present occult fungal infections.

A favorable clinical response was observed in 69% of the patients who received amphotericin B (Fungizone®) and in 53% of the patients who were not receiving empiric amphotericin B [24]. A reduction in the number of documented fungal infections among the patients who were treated empirically with amphotericin B was also observed. However, the empiric treatment with amphotericin B did not influence the overall survival. These results suggest that fungal infections might be a relatively unimportant cause of death in patients, who often had an advanced neoplastic disease; nevertheless, they suggest that empiric therapy of fungal infection is a sound approach in certain patients whose underlying neoplastic disease and other non-infectious complications can be adequately controlled. Subgroups of patients who had not received previous antifungal prophylaxis and who remained severely neutropenic (PMM <100 μl) were those who benefited most from empiric amphotericin B therapy. These observations might serve as possible guidelines for empiric antifungal therapy in FGCP.

ANTIFUNGAL THERAPY: FUTURE PERSPECTIVES

So far it is difficult to establish the level of efficacy of antifungal therapy for demonstrated infections in FGCP. Only retrospective studies are available and they are biased by the heterogeneity of the populations examined, both from the point of view of the underlying neoplastic disease and its related complications, including the duration of neutropenia and the type of infection.

The antifungal therapy of invasive mycoses has been highly disappointing as confirmed by the frequency of disseminated fungal infections at autopsy [4, 8, 10, 21]. At the present time, the only accepted therapy for disseminated fungal infections in FGCP is amphotericin B; as already mentioned, its efficacy is probably low and it is associated with numerous side-effects (Table 5), many of which might be due to the deoxycholate component. Besides fever, anaphylaxis, hypokaliemia and nephrotoxicity, the use of amphotericin B has been associated with adverse effects on the pulmonary function, especially with concomitant transfusions of leucocytes [25]. At the present time, clinical experience, in neutropenic patients, with ketoconazole and other drugs such as fluconazole or itraconazole for the treatment of invasive fungal infection, is still fragmentary.

Table 5. Amphotericin B—side-effects

Fever
Convulsions
Anaphylaxis
Hypotension
Ventricular fibrillation or cardiac arrest
Phlebitis
Nausea
Vomiting
Anorexia
Metallic taste
Abdominal pain
Nephrotoxicity
Anemia
Hypokaliemia
Hepatotoxicity
Skin rash (very rare)
Myelopathy (after intrathecal administration)

New approaches are thus necessary to improve the present therapy of fungal infections in FGCP and other compromised patients with cancer and/or severe immunodeficiency.

One possible way to improve the 'therapeutic index' of amphotericin B might be the use of liposomes. These lipid structures allow the i.v. injection of water-insoluble drugs such as amphotericin B, and in that case, avoid the concomitant use of deoxycholate, which is probably responsible for some of the toxicity of Fungizone®. Several experimental studies by Lopez-Berestein and others suggest that liposome-encapsulated amphotericin B has an improved efficacy in *C. albicans* infections in mice [26]. Recent limited clinical investigations support its efficacy in severe fungal infections in cancer patients [27, 28].

At the Institut J. Bordet, we have recently investigated the pharmacology and the antifungal efficacy of amphotericin B trapped in sonicated liposomes [29] and we found that the serum levels of amphotericin B were much higher (approx. × 10) and more sustained, for similar doses, when the drug was administered in liposomes. Higher fungistatic and fungicidal activity could also be detected in the

serum of patients treated with amphotericin B in liposomes against test strains of *C. albicans*, *T. glabrata* and *Aspergillus* sp.; significant fungicidal activity could not be detected in the serum of the same patients after the administration of Fungizone®. This serum antifungal activity could be correlated to the levels (measured by HPLC and/or bioassay) of amphotericin B [30]. Very striking also was the almost complete lack of toxicity of amphotericin B in liposomes. Although most of our patients had experienced serious side-effects with Fungizone® (fever, chills, arythmias, dyspnea, bronchospasm, severe hypokaliemia), these adverse reactions almost completely disappeared once these patients were started on amphotericin B in liposomes. Amphotericin B in liposomes had a better 'therapeutic index' than Fungizone® because of better tolerance and also probably a higher antifungal activity as expressed by higher serum concentrations. These results suggest that amphotericin B in liposomes might be clinically effective for the control of systemic fungal diseases and a large randomized study should be performed.

COMMENTS

Invasive fungal infection is a common *post mortem* observation in neutropenic patients; to what extent this is the reflection of an end-stage situation in patients with extensive neoplastic disease, or an intercurrent opportunistic infection, has not been established so far. Probably, both mechanisms are involved. Nevertheless, it appears that prevention and effective therapy of fungal infections are highly desirable in these compromised patients.

These issues have been hampered by difficulties in the microbiological and clinical diagnosis of fungal infections and by the unavailability of safe and effective antifungal drugs.

Our experience indicates that: (1) chemoprophylaxis with orally administered antifungal agents is effective for the prevention of candidiasis in patients at risk; (2) chemoprophylaxis with intranasally administered amphotericin B seems to be useful as a prophylaxis of aspergillosis; (3) empiric therapy with amphotericin B (Fungizone®) is effective in reducing the incidence of systemic fungal infections in severely neutropenic patients who remain febrile despite broad spectrum antimicrobial therapy, particularly if they did not receive previously antifungal prophylaxis and have a clinically documented site of infection; (4) amphotericin B incorporated into liposomes has a better 'therapeutic index' than Fungizone®; and (5) the value of itraconazole and fluconazole as well as their specific indications must be determined in further studies.

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