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Current Strategies in the Treatment of Invasive *Aspergillus* Infections in Immunocompromised Patients

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Contents

Abstract

Aspergillus infections have a very high mortality rate. Their incidence is growing because of the increasing number of immunocompromised patients. Treatment of *Aspergillus* infection is difficult, and the agents used have numerous adverse effects and toxicities. Recently, new and less nephrotoxic formulations of amphotericin B have come onto the market and other new drugs, such as voriconazole and terbinafine, are under evaluation for this infection.

Restoration of host immune defences by tapering of immunosuppressive therapy in transplant patients or correction of granulocytopenia in haematological disease is the cornerstone of modern treatment of aspergillosis in immunocompromised patients. In patients with invasive aspergillosis it is very important to achieve therapeutic concentrations of antimycotic drugs as quickly as possible. Patients at high risk of developing aspergillosis (e.g. those with granulocytopenia) should be treated on the basis of clinical or radiological criteria alone if microbiological or histological diagnosis would significantly delay treatment.

Conventional amphotericin B is still the first-line treatment for patients with invasive aspergillosis. In transplant patients receiving other nephrotoxic drugs, particularly cyclosporin, first-line therapy with one of the new amphotericin B formulations should be considered. If the emergence of renal toxicity in any

patient precludes aggressive treatment, the patient should be switched to one of the new formulations of amphotericin B. For patients cured with amphotericin B, secondary prophylaxis is needed at the end of the intravenous therapy. Amphotericin B by aerosol or itraconazole are possible solutions.

In non-invasive forms of aspergillosis, such as suppurative bronchitis, patients could be treated either with amphotericin B or itraconazole as first-line therapy.

Amphotericin B has been the most widely used and consistently effective antifungal agent for *Aspergillus* infections over the past 40 years. However, new drugs, such as itraconazole, and new amphotericin B formulations, such as liposomal encapsulation, have more recently entered the market. The aim of this review is to discuss the different pharmacological strategies available today for *Aspergillus* infections (table I), with particular emphasis on invasive infections in immunocompromised patients (fig. 1).

The outcome of invasive aspergillosis is closely related to early diagnosis and aggressive treatment, and the degree and duration of immunosuppression. Tapering of immunosuppressive therapy in transplant patients and the correction of granulocytopenia in haematological diseases are the cornerstone of modern treatment. The principal purpose of antifungal therapy is often simply to keep the patient alive until host immunity is restored.[1] Although amphotericin B is the most widely used antifungal agent for *Aspergillus* infections, the overall response rate with this agent is probably only 30 to 35%.[1] However, in patients who receive amphotericin B for 14 days, the response rate increases to 55%.[2,3] Recently, new delivery forms of amphotericin B and new oral drugs have attracted attention.

Table I. Most common clinical forms of aspergillosis in humans

Hypersensitivity syndromes
Allergic bronchopulmonary aspergillosis
Extrinsic allergic alveolitis
Mycotoxicoses
Toxic effects of fungal products
Local infection
Aspergilloma
Otomycosis
Keratitis
Endophthalmitis
Orbital
Paranasal sinusitis
Gingival
Subcutaneous mycetoma
Noninvasive bronchitis
Invasive infection
Pulmonary
invasive pulmonary aspergillosis
chronic necrotising aspergillosis
invasive bronchitis
Disseminated (heart, central nervous system, bone)

1. Amphotericin B

The drug of choice for the treatment of aspergillosis is amphotericin B. This drug exerts its effect by binding to ergosterol in fungal cell membranes, thereby increasing membrane permeability and adversely influencing cell function and survival. Amphotericin B administered orally is poorly absorbed and, consequently, it must be given by intravenous infusion. Drug resistance has never been described in aspergillosis. In immunosuppressed patients it is important to achieve therapeutic concentrations as quickly as possible, but in the clinical setting high dosages cannot be administered because of the adverse effects and toxicity of the drug.

An initial test dose of amphotericin B 1mg in 100ml of 5% dextrose in water should be administered over 30 minutes. If no anaphylactic or other serious adverse effects are experienced, an infusion of amphotericin B 0.25 mg/kg in 500ml of 5% dextrose in water may be started 2 hours later and administered over 6 hours. The daily dosage of amphotericin B should be progressively increased with the aim of achieving a dosage of 1 mg/kg/day.

Unfortunately, this goal is rarely accomplished because of adverse effects and toxicity. Although

Fig. 1. Necroscopic specimen of widespread invasive pulmonary aspergillosis in a patient with AIDS.

adverse effects such as fever, thrombophlebitis, headache, nausea and chills can usually be treated by administration of antipyretic or antiemetic drugs, heparin or corticosteroids, major nephrotoxicity nearly always appears, and in almost all cases leads to a reduction in the daily dose of amphotericin B. Renal toxicity is characterised by hypokalaemia, azotaemia and, very occasionally, tubular acidosis; renal failure can appear and lead to a requirement for dialysis. Renal toxicity is dose-related and may regress some months after the end of amphotericin B treatment, but is usually irreversible when therapy is prolonged.[4] In some cases renal toxicity can be reduced by saline loading and/or by alternate-day treatment with a consequent extension of the treatment period. If the plasma creatinine level rises to >200 mmol/L the drug should be interrupted for 48 hours and then restarted at a dosage approximately 20% lower, according to the patient's condition and the clinical setting. Patients developing renal impairment while receiving the conventional formulation of amphotericin B had an improved or stable renal status when liposomal amphotericin was substituted, even when the dosage was increased.[5]

Long-term therapy with amphotericin B is frequently associated with anaemia secondary to bone marrow suppression. Less common toxicities include leucopenia, thrombocytopenia and hepatic dysfunction.[6] Pulmonary reactions with acute dyspnoea, hypoxaemia and interstitial infiltrates can occur when treatment with amphotericin B is combined with granulocyte transfusion. Amphotericin B can enhance the effects of other nephrotoxic drugs, such as aminoglycosides and antineoplastic drugs. Corticosteroids can increase amphotericin B-induced potassium loss and the resulting hypokalaemia.[6]

The optimal duration and dosage of therapy with amphotericin B is not known. It is common practice to measure amphotericin B therapy in terms of total dose administered; in patients with invasive disease a dose ranging from 1 to 1.5g is usually recommended. In all patients, the overall response rate with amphotericin B is probably 30 to 35%.[1]

2. Rifampicin (Rifampin) and Amphotericin B

Data from *in vitro* animal models suggest that rifampicin and amphotericin B are synergistic or additive when used together. It is apparent that the demonstration of synergy is highly strain-dependent. The use of rifampicin poses problems for some patients because of its potent enzyme-inducing properties. This is particularly true in transplant patients receiving treatment with cyclosporin. Use of rifampicin may also preclude the subsequent use of itraconazole because of enzyme induction. Clinical reports on the use of this combination are anecdotal, with varying accounts of success.[7]

3. Flucytosine Alone or in Combination

Denning and Stevens in 1990 reviewed 2121 cases of invasive aspergillosis published in the literature.[1] Only 12 patients were treated with flucytosine alone, and 10 of 12 responded. Six of these 10 patients were described in 1 report;[8] 1 patient with meningitis and 2 with pleural disease were among these. One patient relapsed, possibly as a result of resistance, in this series.

A total of 63 patients were treated with a combination of amphotericin B and flucytosine. Two-thirds of these patients responded to the combination, including 11 (58%) of 19 patients with acute leukaemia.

However, no conclusive prospective randomised study has answered the question of whether this combination is more useful than therapy with amphotericin B alone; the utility of the combination has to be balanced against the potential dose-related myelotoxicity of flucytosine.

To our knowledge, no *in vivo* data are available concerning the usefulness of the combination of flucytosine with the new delivery forms of amphotericin B, such as the liposomal formulation, or with itraconazole.

4. New Formulations of Amphotericin B

Although the sodium deoxycholate complex of amphotericin B remains the treatment of choice for *Aspergillus* infections, its use is limited by the high frequency of toxic events, as described in section 1. When amphotericin B is encapsulated into liposomes or bound to other lipid carriers, toxicity is greatly reduced. This reduction in toxicity may be the result of different affinities for the cholesterol in the human cell membrane (lowest affinity), for the lipids of the lipid carrier and for the ergosterol in the fungal membrane (highest affinity).[9] This may lead to a selective transfer of amphotericin B from the lipid carrier to the fungus, thus minimising any interaction with the human cell membrane.[10] In spite of a lower antifungal activity, dependent on the particular liposome or lipid carrier, these formulations display a markedly increased therapeutic index compared with the conventional form of amphotericin B.

4.1 Amphotericin B Lipid Complex

The lipid composition of this formulation is similar to that of liposomes, but this formulation exists as sheets and not vesicles. Because of its large size, amphotericin B lipid complex is rapidly entrapped by the mononuclear phagocyte system (MPS), resulting in blood concentrations that are considerably lower than those obtained with conventional amphotericin B.[11,12] This compound is usually less nephrotoxic than amphotericin B and therefore higher doses can be delivered for a longer period of time.

4.2 Amphotericin B Colloid Dispersion

Amphotericin B colloid dispersion ('ABCD') is a new formulation of amphotericin B and sodium cholesteryl sulphate in a ratio of approximately 1 : 1, forming very small, uniform, disc-shaped particles of about 115nm in diameter.[13,14] Because of the rapid uptake of ABCD by the liver, the plasma pharmacokinetics and tissue disposition of amphotericin B differ in several respects after the administration of ABCD and the conventional form of the drug.[14,15] Studies in animal models have shown that ABCD produces adverse effects similar to those produced by amphotericin B but at dose levels 4 to 5 times higher. The low toxicity of ABCD is probably due to its plasma stability and the fact that it is rapidly and almost entirely adsorbed by the liver. The rapid hepatic uptake results in a markedly decreased peak plasma concentration of amphotericin B in ABCD-treated animals and a significantly reduced distribution of the drug in the kidney. *In vitro*, ABCD is as effective as amphotericin B against *Aspergillus* spp. In a rabbit model of invasive pulmonary aspergillosis, ABCD was found to be less potent than amphotericin B with dose-dependent therapeutic efficacy and nephrotoxicity.[14]

Oppenheim et al.[13] carried out a non-blind trial in 168 patients with documented or presumed systemic mycoses, using ABCD at a dosage as high as

6 mg/kg/day. All patients had had an incomplete response to at least 7 days of conventional amphotericin B treatment, had experienced amphotericin B-induced nephrotoxic effects, had pre-existing renal impairment or had experienced other amphotericin B-related, treatment-limiting, toxic effects. 97 patients could be evaluated for response. Complete clinical response or improvement was registered in 48 (49%) patients after a treatment duration of 18.5 days; for patients with aspergillosis, 11 of 32 (34%) were judged to have responded. The all-cause mortality rate within 4 weeks of the last dose of ABCD was 52% (88 of 168 patients died). All patients that entered the study could be evaluated for toxicity. Only 14 (8%) of the 168 patients enrolled in the study were withdrawn from the trial because of adverse events. In 11 of 14, the adverse events leading to withdrawal included chills, fever and hypotension. Infusion-related chills were experienced by 64 patients (38%). ABCD had little renal toxicity: the mean change in serum creatinine from baseline to final value was –0.02 mg/dl. Hypokalaemia was recorded in 8 patients (5%).

White et al.[16] assessed the efficacy and safety of ABCD in 82 patients with proven or probable aspergillosis; 261 historical cases treated with amphotericin B were used as control. The 2 groups were balanced in terms of underlying disease; ABCD recipients were younger and more likely to have pre-existing renal insufficiency (42.5 *vs* 15.9%, respectively). Response rates (48.8%) and survival rates (50%) among ABCD-treated patients were higher than those among amphotericin B-treated patients (23.4 and 28.4%, respectively; $p < 0.001$ for both comparisons). Renal dysfunction developed less frequently in ABCD recipients than in amphotericin B recipients (8.2 *vs* 43.1%). Multivariate analysis revealed that treatment group was the best predictor of response, mortality and nephrotoxicity.

4.3 Liposomal Amphotericin B

Liposomal amphotericin B consists of small unilamellar vesicles of about 80nm, each containing 10% amphotericin B on a molar basis. Because of its small diameter and rigidity, this liposome is less rapidly cleared by the MPS, resulting in relatively high serum concentrations of amphotericin B.[5] Liposomal amphotericin B has recently been approved by the US Food and Drug Administration (FDA) for the treatment of fungal infections and fever of unknown origin in neutropenic patients. Among the 3 new formulations of amphotericin B discussed here, this is the only one that is truly liposomic.

In various animal models, liposomal amphotericin B was found to be as effective as conventional amphotericin B and more effective in pulmonary aspergillosis. The liposomes of this formulation are captured by the MPS more slowly than the other amphotericin B compounds because of the larger volume of lipid complex or because of the colloidal particles that it contains. Thus, its plasma concentration is higher and it remains in the blood stream longer.[5,17]

In several studies, the nephrotoxicity of liposomal amphotericin B was found to be lower than that reported with conventional amphotericin B; and in some patients with renal failure due to previous treatment with amphotericin B, renal function improved during therapy with liposomal amphotericin B.[5] Adverse effects such as fever, chills and phlebitis are less frequent than usually reported during treatment with conventional amphotericin B; in a study carried out in 187 patients, only 7% reported adverse effects.[18] During infusion of liposomal amphotericin B, some patients report the appearance of back pain which can be reversed by slowing the drug infusion rate; the pathogenesis of this adverse effect remains unclear.

Our (so far unpublished) experience with liposomal amphotericin B is described here. Between January 1991 and March 1997, we have recorded 20 thoracic organ transplant recipients with *Aspergillus* infections (14 lung, 5 heart and 1 heart and lung transplants). Seven patients had pulmonary invasive disease, 1 pleural, 2 disseminated (lung, brain and heart) and 10 non-invasive bronchitis. The first 14 patients were initially treated with con-

ventional amphotericin B and then changed to liposomal amphotericin B after developing renal toxicity. Since then, liposomal amphotericin B has been used as first-line treatment. The dosages of liposomal amphotericin B ranged between 1 and 6 mg/kg/day for a total dose of between 1 and 6g. Three patients died: 2 had disseminated aspergillosis and 1 had lung-invasive relapse. The adverse effects of liposomal amphotericin B were as follows: 4 episodes of cutaneous rash, 4 of back pain, 3 of hypokalaemia, 2 of allergic reactions leading to interruption of the treatment, 4 of myelotoxicity and 2 of abnormal liver enzymes. Renal toxicity was minor and easily managed. No patient needed dialysis or haemofiltration.

The optimal dosage of liposomal amphotericin B is still controversial and recently a prospective randomised clinical trial failed to demonstrate any advantage in using a higher and more expensive dosage.[19]

5. Azole Antifungal Drugs

The azole antifungal agents are from the imidazole and triazole groups. These agents are structurally similar, with a 5-member azole ring containing either 2 or 3 nitrogens. The imidazoles, miconazole and ketoconazole, have a narrower spectrum of activity, more adverse effects and a shorter half-life than the newer triazole agents fluconazole and itraconazole. All azole agents have the same mechanism of action. They act by inhibiting ergosterol synthesis in the cell membrane of yeast and fungi by binding to the cytochrome P450 (CYP)-mediated enzyme 14α-demethylase. This results in the accumulation of 14-methylated ergosterol precursors that destabilise the cell membrane, leading to inhibition of cell growth and ultimately to cell death. There are theoretical considerations for possible antagonism of azoles and polyenes, but the sequential use of the agents did not bear out this concern.[20]

Drugs such as rifampicin, which increase hepatic CYP enzymes, increase the metabolism of azole drugs. Conversely, azole drugs can inhibit the metabolism of other agents. Ketoconazole, for example, inhibits the metabolism of cyclosporin, prolonging its half-life and increasing the risk of nephrotoxicity.

The only drug of this category currently used for aspergillosis is itraconazole, although selected cases cured by ketoconazole have been reported in the literature.

5.1 Itraconazole

Itraconazole is an orally administered triazole antifungal agent active against *Aspergillus* spp. Pharmacokinetic studies demonstrate that itraconazole reaches high tissue concentrations, especially in the skin and liver. Itraconazole has not been detected in the CSF or in urine. One limiting factor of this drug is its unpredictable bioavailability, and an intravenous formulation is currently under investigation. The half-life of itraconazole is longer than that of ketoconazole, and varies from 15 to 24 hours. In patients with renal or hepatic dysfunction, dosage alterations are not necessary.

Minor adverse effects have been reported in 2.4 to 8% of patients receiving itraconazole. Nausea and headache are the most common symptoms reported. Mild reversible elevations in the results of liver function tests have also been registered. Like ketoconazole, itraconazole inhibits the metabolism of cyclosporin and increases its plasma concentrations.

In 1 series of 14 patients with aspergilloma treated with itraconazole, 2 patients were cured and 8 improved, whereas 4 remained unchanged or deteriorated.[3] Of 3 series of immunocompromised patients with invasive aspergillosis, a total of 42 of 54 patients treated with itraconazole 100 to 400 mg/day were considered to be cured of the disease.[21-23] Among 76 patients with invasive aspergillosis treated with itraconazole and evaluable for response,[20] 30 (39%) had a complete or partial response and 3 (4%) had a stable response at the end of treatment. In 20 patients (26%), the protocol therapy was discontinued early because of worsening clinical course or death due to the aspergillosis.

Failure rates with itraconazole varied widely depending on the disease site and the underlying dis-

ease group. Failure rates were 14% for pulmonary and tracheobronchial disease, 50% for sinus disease, 63% for CNS disease and 44% for other sites. In terms of underlying disease, the failure rate was 7% in solid organ and 29% in allogenic bone marrow transplant patients, 14% in those with prolonged granulocytopenia, and 44% in patients with AIDS. The relapse rate among those who completed therapy was 12%.

An oral solution of itraconazole has recently entered the market. The bioavailability of the drug solution compared with tablets seems to be higher. However, clinical experience is still limited.[24]

With the increasing incidence of severe opportunistic fungal infections in immunosuppressed patients and the occurrence of acquired resistance to azoles, new antifungal agents are required to be used either as a monotherapy or in combination with the existing agents. Itraconazole was the primary alternative to amphotericin B in 1 non-blind series of 300 patients[3] and in the non-blind trial including 76 patients discussed previously.[20] Although it is difficult to compare the data in these trials with the results obtained using amphotericin B alone, it appears that the overall response rate with itraconazole in patients with favourable prognostic features is 30 to 40%.

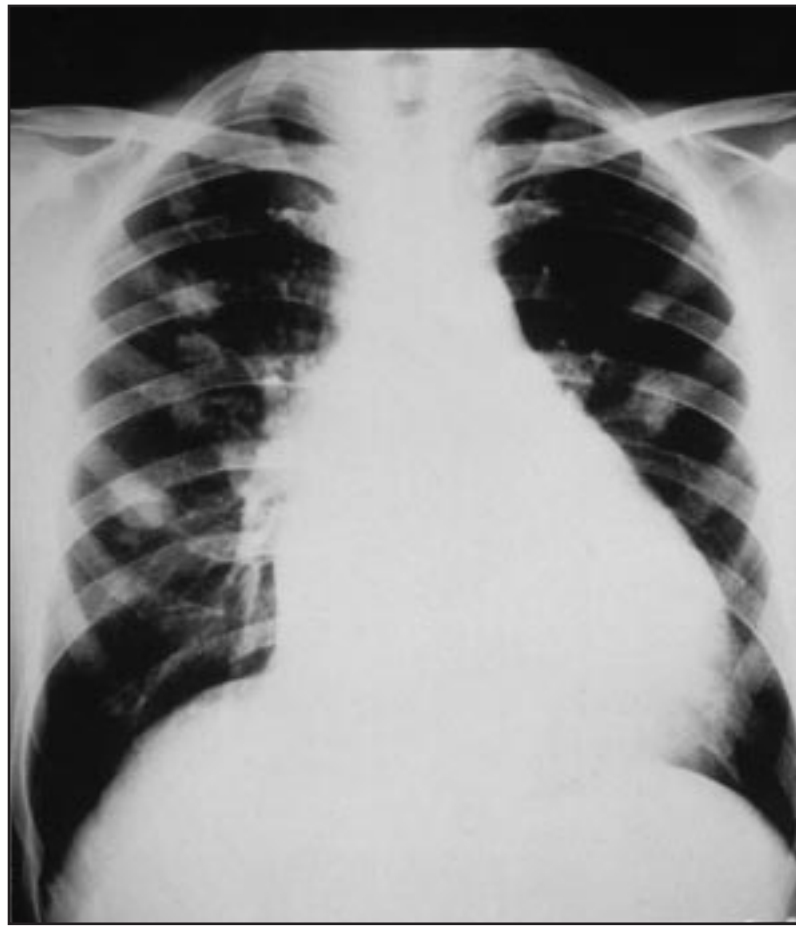

Fig. 2. Chest radiograph of a heart transplant recipient with multiple bilateral nodules caused by *Aspergillus fumigatus*.

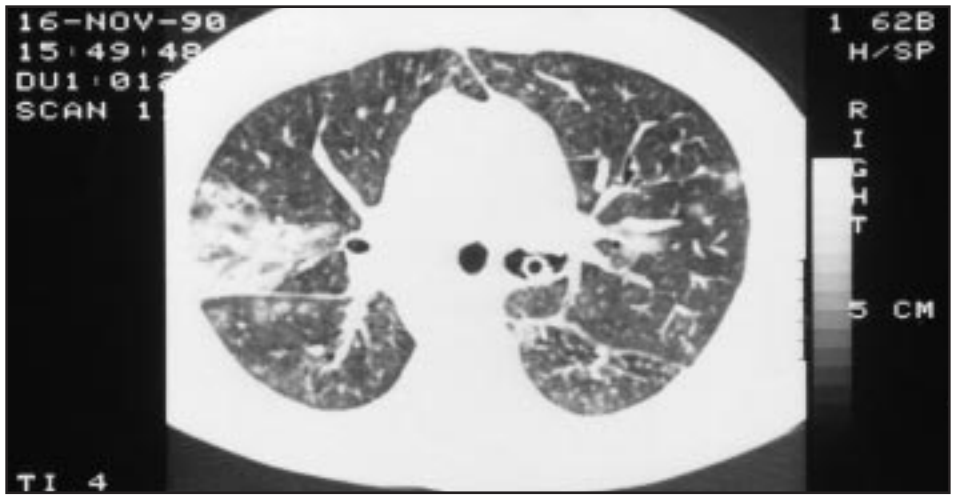

Fig. 3. High resolution computed tomography scan of a double lung transplant recipient: a stent can be seen in the right intermedius bronchus and nodular and alveolar shadows are bilaterally evident. Bronchoalveolar lavage was positive for cytomegalovirus inclusions, *Aspergillus fumigatus* and *Pseudomonas aeruginosa*. The patient made a full recovery after treatment with ganciclovir, ceftazidime and liposomal amphotericin B.

5.2 Voriconazole

New triazoles that are highly active *in vitro* against *Aspergillus* spp. are currently under evaluation. In particular, voriconazole is a triazole antifungal agent with a wide spectrum of activity *in vitro* and a fungicidal action against *Aspergillus* spp. Like other azole agents, its primary mode of action is inhibition of fungal CYP-dependent 14α-demethylase.

In a non-blind non-comparative study, voriconazole (6 mg/kg intravenously every 12 hours for 1 day, then 3 mg/kg intravenously every 12 hours for 6 to 27 days, followed by 200mg orally every 12 hours for a total of 24 weeks) was used to treat acute aspergillosis in neutropenic and other immunocompromised patients. Interim analysis of clinical efficacy in 88 patients revealed 17% complete response and 32% partial response. 13 patients died because of aspergillosis or aspergillosis-related events.[25] The most frequent adverse events related to voriconazole administration were visual disturbances, which were reported in 14.5% of patients. Increased liver function tests occurred at an incidence of >10%.

6. Terbinafine

Terbinafine is an allylamine antimycotic agent. The pharmacological basis of the fungicide activity of this compound is related to the inhibition of ergosterol biosynthesis.[26] It inhibits specifically the activity of squalene epoxidase, thus reducing the conversion of squalene to squalene epoxide.[27] This results in a deficiency of membrane sterol production and also in an intracellular accumulation of squalene, which may be toxic to the fungus.

Since the registration of terbinafine for the treatment of onychomycoses, data on adverse event profiles have been obtained on over 2 million patients treated orally. Terbinafine is generally well tolerated with few gastrointestinal adverse events, and rare haematological or biochemical evidence of organ dysfunction.

In a recent report from our group,[28] terbinafine was effective against *Aspergillus* spp. in bronchopulmonary infections unresponsive to the classical treatment. We also reported the successful treatment with this drug of relapsing *Aspergillus* bronchitis in a double lung transplant recipient.[29] These data suggest that terbinafine may be useful in the treatment of aspergillosis and that a study is

required to evaluate the use of this drug in pulmonary fungal infections.

7. Clinical Management of Patients with Aspergillus Infections

Invasive aspergillosis is a disease with a high mortality rate and is difficult to diagnose. Prognostic factors are related to the degree of immunosuppression of the host and to the extent of infection at the time of diagnosis (number of organs involved). It is crucial to diagnose and fight the infection as soon as possible. Patients at high risk of developing aspergillosis (e.g. patients with granulocytopenia) should be treated on the basis of clinical and/or radiological (figs. 2 and 3) criteria alone if microbiological or histological diagnosis is difficult or would significantly delay starting the treatment. Liposomal amphotericin B has recently been approved by the FDA for the treatment of fever of unknown origin in patients with granulocytopenia. It is very important to achieve therapeutic concentrations of antimycotic drugs as quickly as possible.

Conventional amphotericin B is still the first-line treatment for patients with invasive aspergillosis. In transplant patients already undergoing treatment with other nephrotoxic drugs, particularly cyclosporin, the new amphotericin B formulations, such as the liposomal formulation, should be considered as first-line therapy. Solid organ transplant patients may start therapy with amphotericin B and, in the event of renal failure, be switched to one of the new formulations. In these difficult patients, and in patients with granulocytopenia, it is very important to achieve therapeutic concentrations of amphotericin B quickly and to keep the infection under control until the immune defence mechanism of the host has been restored. Thus, if the

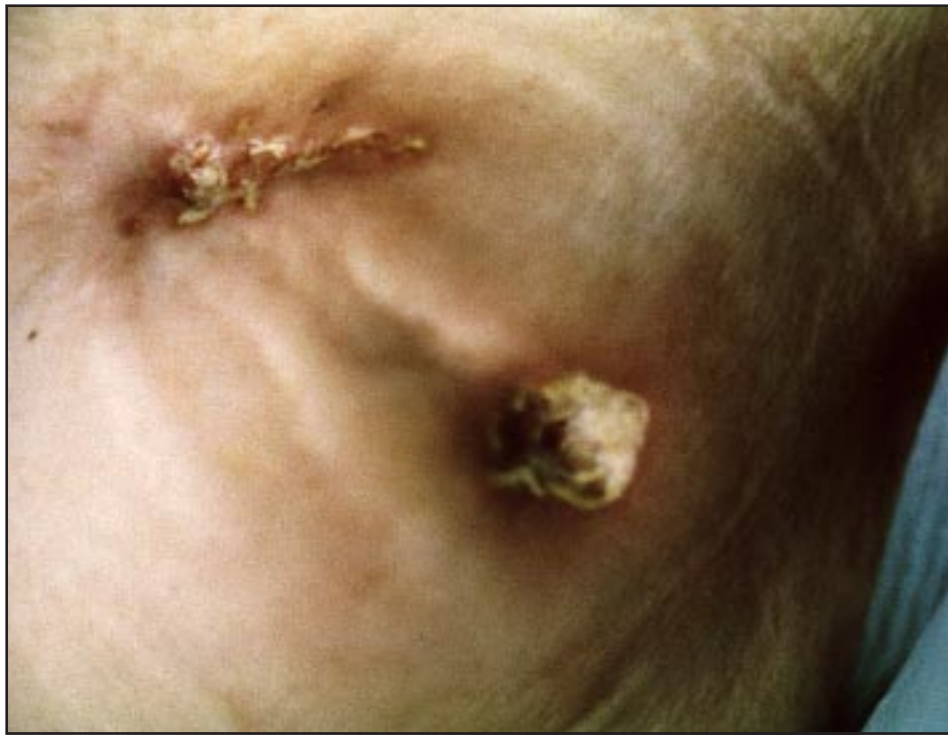

Fig. 4. Subcutaneous abscess of the leg caused by *Aspergillus* in a lung transplant recipient.

emergence of renal toxicity does not allow aggressive and effective treatment, the patient should be switched to one of the new formulations of amphotericin B.

Patients with non-invasive forms of aspergillosis, such as suppurative bronchitis, could be treated either with amphotericin B or itraconazole as first-line therapy. In selected patients, local therapy with amphotericin B can be effective and less toxic than intravenous treatment (e.g. amphotericin B by aerosol for suppurative bronchitis, or bronchial instillation for aspergilloma). Itraconazole can also be used to treat a non-immunocompromised host with *Aspergillus* invasive disease or as an alternative treatment to amphotericin B. For patients cured with amphotericin B, secondary prophylaxis is needed at the end of the intravenous therapy. Amphotericin B by aerosol or itraconazole are possible solutions. In a few patients with treatment failure or adverse effects during itraconazole prophylaxis we have also used terbinafine, but the data are limited and no conclusive results can be drawn.

Cerebral involvement in aspergillosis has a very poor prognosis, and in such patients high doses of liposomal amphotericin B are indicated, as this is the formulation that produces the highest concentration in the CSF.[30] Physicians should also be aware of the possible association between *Aspergillus* infection and viral infection, in particular cytomegalovirus.

Finally, in selected patients localised lesions (e.g. fig. 4) can also be evaluated for surgery.[31,32] For example, a patient with localised lesions and bleeding due to erosion of blood vessels, particularly during neutrophil recovery, should be carefully considered for surgery. Percutaneous computed tomography-guided treatment of inoperable pulmonary aspergillosis has also recently been reported.[33] However, exact indications and interactions with other treatments have yet to be defined.

8. Conclusions

The outcome of *Aspergillus* infection is strictly related to early diagnosis, aggressive treatment, and strict clinical and radiological follow-up. The new formulations of amphotericin B have produced a significant decrease of mortality in this infection and a reduction in adverse effects, particularly in renal toxicity. In addition, the encapsulation in liposomes of other agents such as nystatin is now under investigation.[34] In the future, new compounds such as terbinafine and voriconazole may become valuable alternatives to amphotericin B or be used as second-line treatment for resistant infections.

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