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New developments in the diagnosis and management of invasive fungal infections

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ABSTRACT

Invasive fungal infections in cancer patients are on the increase. Candidemia is now the fourth leading cause of bloodstream infections in many intensive care units (ICUs). Although a number of risk factors have been identified, antifungal therapy should not be started in non-neutropenic patients until a diagnosis of invasive candidiasis or candidemia is made or presumed in order to avoid the development of resistance. Even a single positive blood culture should be treated, and requires removal of intravascular lines. Fluconazole is the first line agent for treatment candidemia other than that caused by *Candida glabrata* or *C. krusei*. High-resolution CT scan pictures showing a halo sign or crescent air sign are helpful for establishing the diagnosis of invasive aspergillosis. Sandwich ELISA can be used to detect circulating galactomannan in serial serum samples. Polymerase chain reaction (PCR) of blood samples may also be used. There are only a few randomized studies of newly developed antifungal drugs compared to conventional amphotericin B (AmB). So far, both AmB colloidal dispersion and AmB lipid complex have failed to show more favorable efficacy or lesser toxicity rates, except for nephrotoxicity. Liposomal AmB, used during febrile neutropenia, did have a significantly lower toxicity rate. In neutropenic patients with invasive fungal infections liposomal AmB proved to be better than conventional AmB in terms of clinical efficacy, mortality and nephrotoxicity rates. The use of tests to achieve an earlier diagnosis combined with more potent treatment formulations such as liposomal AmB may be significant steps towards successful management of invasive fungal infections.

Key words: mycoses, candida, candidiasis, aspergillus, aspergillosis, antifungal agents, liposome, amphotericin, neutropenia, intensive care

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Invasive fungal infections have been becoming increasingly common in cancer patients in recent years. The most striking examples are candidemia and invasive candidiasis in intensive care unit (ICU) patients and invasive aspergillosis in neutropenic patients and bone marrow transplant recipients. These infections are still associated with high morbidity and unacceptably high mortality rates. This review focuses on new developments in diagnosis and treatment of invasive fungal infections.

Hematogenous and invasive candidiasis in ICU patients

In the USA candidemia cases almost doubled from 2.0 per 1000 discharges in 1980 to 3.8 in 1990.[1,2] This trend was seen in all kinds of ICUs. In many centers *Candida* is now the fourth leading cause of bloodstream infections.[3] Together with an increase in overall incidence there is a shift towards infections by non-*albicans* species.[4,5] In our University Hospital in Rotterdam *Candida albicans* caused over 90% of all candidemias in the late eighties but fewer than 50% in recent years.

Incidence and risk factors

In view of the serious nature of these infections, with an attributable mortality of over 30% it is important to recognize the presence of risk factors for acquisition of candidemia and invasive disease. In a large prospective survey in the USA presented recently, the following factors were associated with a significantly increased risk ratio in multivariate analysis: central line, surgical procedure, acute renal failure, disseminated intravascular coagulopathy and parenteral nutrition.[6] In other studies the use of broad-spectrum antibiotics appeared to be an independent risk factor.

To address the question of whether surveillance cultures could predict invasive fungal infection in ICU patients, Feltz *et al.* performed twice weekly cultures from the respiratory and GI tracts and urine of patients admitted to an ICU.[7] When using the criterion of at least 2 sites giving a positive culture they found a sensitivity of 92% and an odds ratio of 8.7. However, the specificity was only 42%, the positive predictive value 12%, but the negative predictive value 98%. So, the conclusion from this study was that negative *Candida* cultures almost exclude the possibility of invasive disease, but that the predictive value of positive cultures alone is low. Other studies

have shown that extensive colonization together with deterioration of the patient's condition was strongly associated with the development of invasive or hematogenous candidiasis.[8]

In a study performed by Nassoura *et al.,* 17 of a total of 27 retrospectively studied patients who were treated with local bladder installation of AmB solution, appeared to have invasive candidiasis; they showed a significantly higher APACHE II score and had a much higher mortality than the 10 patients with candiduria without invasive candidiasis.[9] When, subsequently, 20 patients with candiduria were treated with fluconazole instead of bladder instillation of AmB all but one survived. This suggests that candiduria could be an indication for systemic antifungal therapy in critically ill patients.

Diagnosis

The diagnosis of invasive candidiasis is still complicated. Blood cultures become positive in a minority of deep-seated infections. Positive cultures from other sites may represent colonization rather than invasive disease. A documented infection is defined by either 1) a positive blood culture; 2) positive cultures from a normally sterile organ, body cavity or fluid; or 3) clinical evidence of disseminated disease such as characteristic ophthalmoscopic abnormalities or bulls' eye lesions on ultrasound or CT imaging of liver and spleen.[10] The contributions from serologic antibody or antigen tests remain unsatisfactory; although some studies have shown promising PCR results especially in neutropenic patients; the predictive value of this test needs to be confirmed in prospective studies.[11,12]

The study by Lecciones *et al.* in cancer patients with candidemia is important for interpreting the results of blood cultures.[13] Patients with positive cultures from a catheter but negative peripheral blood cultures had the same rates of mortality and documented dissemination as patients with both positive catheter and peripheral cultures. Moreover, 58% of all these patients had only one positive culture. This indicates that even a single positive blood culture even from just the catheter should be considered evidence for candidemia and requires prompt treatment.

Another issue is whether the results of *in vitro* susceptibility tests have clinical significance.

In a recent study, the failure of candidemia to respond to fluconazole therapy (defined as persistence of positive blood cultures for more than 5 days) appeared to be related to the *in vitro* minimal inhibitory concentration (MIC) to fluconazole when using cut-offs for sensitivity (MIC < 8 mg/L), intermediate sensitivity (8-32 mg/L), or resistance (64 mg/L or higher).[14] Fluconazole therapy failed in almost half of the patient with sensitive organisms in contrast to 100% with resistant *Candida*. As a comparison *Candida* isolates from the oropharynx of HIV-infected patients with thrush were studied. The results showed the same pattern with even more significance: no failure in case of sensitivity compared to 100% failure in case of resistance, and intermediate figures for intermediate sensitivity.

AmB susceptibility tests may also predict therapy response. Nguyen *et al.* showed that *Candida* strains with a MIC or minimum lethal concentration (MLC) < 1 mg/L were predominantly isolated from patients successfully treated with AmB therapy, while strains with a higher MIC or MLC were predominantly isolated from patients in whom treatment had failed.[15]

Treatment

Although we know that antifungal treatment can convert blood cultures in responders, does that mean that antifungal treatment will reduce mortality? In a retrospective study by Nguyen *et al.* mortality in treated patients was found to be much lower than in untreated ones, both at day 14 (27% vs 74%) and day 30 (37% vs 76%); stratification into critically ill and not critically ill patients showed the same patterns with most benefit from therapy in the critically ill.[16]

Rex *et al.* showed that clinical response and mortality rates in 103 patients treated with fluconazole were comparable to those in 103 patients treated with AmB.[17] Based on this and other comparative trials fluconazole is now the drug of choice for the treatment of candidemia.[18,19]

That catheter removal alone is not enough in mild cases of candidemia was demonstrated in a study performed by Lecciones *et al.* In 48 patients, whose only management was catheter removal, evidence of dissemination appeared in 35%.[13] On the other hand, antifungal therapy without catheter removal resulted in prolonged duration of positive blood cultures after initiation of therapy, as shown by Rex *et al*.[20]

In the therapeutic approach to the patient with hematogenous or invasive fungal infection different strategies can be distinguished.

First, overall antifungal prophylaxis, whose efficacy is established for neutropenic patients, is controversial in other cancer patients. Because of the risks of development of resistance, prophylaxis is not recommended in the ICU, although a recent randomized study showed a reduction in both colonization and invasive candidiasis after abdominal surgery.[21]

Pre-emptive therapy is therapy on the basis of additional risk factors as mentioned above. No prospective data are available to support this approach at the moment, although some authors do recommend this strategy, especially in heavily colonized patients.

Empirical therapy is therapy based on the assumption that fungal disease already exists in critically ill patients. Given the serious nature of the infection this approach is recommended as is, of course, every kind of directed therapy in documented cases.

One major drawback of antifungal therapy is the development of resistance. Nguyen demonstrated that resistance to fluconazole was significantly associated with prior fluconazole use: 72% vs 12% with a relative risk of 6.0.[5] The same study suggested a relation between the duration of therapy with AmB and the development of decreased susceptibility to this agent.

Another result of fluconazole use is the shift towards infections caused by non-*albicans* species. These species show different sensitivities to antifungal agents. *C. glabrata* and *C. krusei* are less sensitive or resistant to fluconazole. *C. lusitaneae* is generally less sensitive to AmB. So, the choice of agents should not only be dictated by the clinical diagnosis, but also by the prior use of a specific antifungal agent.

Based on present knowledge the following guidelines can be formulated: fluconazole is the first line agent for candidemia, peritonitis, and urinary tract infection. AmB should be used for fluconazole-resistant *Candida*, or in case of fluconazole failure. The combination AmB with flucytosine is indicated in cases of septic shock, endocarditis, other endovascular infections, severe endophthalmitis or meningitis, or in neonates.[10]

Invasive aspergillosis in neutropenic patients

The crude mortality rate of invasive aspergillosis (IA) in cancer patients is still unacceptably high. Recent figures from the literature give this rate as ranging from over 90% in BMT patients to over 50% in leukemia patients.[22]

Diagnosis

Clinical signs such as pleuritic chest pain and hemoptysis are suggestive of IA but are insensitive during neutropenia; most of these patients show only persistent fever.

The radiologic features include a wide range of abnormalities; the most specific pictures are pleura-based, wedge-shaped, nodular infiltrates, which may become cavitary during bone marrow recovery. Unfortunately these images are relatively late in the course of aspergillosis and are not always that specific.

An important contribution to establishing a diagnosis of IA comes from high resolution computerized tomography (CT). This CT scanning takes ultra-thin slices of 1 mm. The sensitivity and specificity of the pictures are greater than those of conventional chest X-rays; in addition the imaging may be used to guide diagnostic transthoracic biopsies. The so-called *crescent air sign* is highly suggestive, although not completely specific for aspergillosis. This air sign is formed after retraction of a central necrotic mass. While the crescent air sign is seen mostly after recovery of BM, the *halo sign* is very sensitive during severe neutropenia. It is an area of low attenuation and represents hemorrhagic infiltrates surrounding necrotic lung tissue full of fungal hyphae. In one study the implementation of CT led to an significant earlier diagnosis and even lower mortality rates compared to historical controls.[23]

Respiratory tract cultures in high-risk patients are fairly specific (specificity in neutropenic patients >80%) but unfortunately their sensitivity is often less than 30%.[24]

Other promising developments are the detection of circulating antigen and of DNA sequences.

The sandwich ELISA for detecting circulating galactomannan, a component of the fungal cell wall has been shown in some studies to have a sensitivity around 85% and a specificity higher than 80%.[25,26] The specificity is higher when two consecutive samples are required to be positive.

Einsele and colleagues, have recently demonstrated that PCR of blood was sensitive and specific in detecting aspergillosis and other invasive fungal infections; persistingly positive PCR results seemed also to be prognostic of outcome.[11]

Although both PCR and galactomannan tests seem to be promising, the exact clinical impact of testing serial samples on the outcome of febrile neutropenia needs to be established in prospective randomized studies.

In a recent publication by Bretagne, ELISA was shown to be superior to PCR in a total of 22 neutropenic patients with proven or suspected aspergillosis: 82% of the patients were positive by ELISA testing whereas only 54% were by PCR.[27] In most patients ELISA was positive earlier and for a longer period during the neutropenic episode: 44% of all samples were positive with ELISA compared to only 10% with PCR. None of the control samples was PCR positive but 4% were ELISA positive.

Antifungal treatment

Amphotericin B (AmB) is the mainstay of treatment of invasive fungal infections in neutropenic patients. However, it is not only a toxic drug, but one with a variable and often insufficient efficacy. Other less toxic compounds active against *Aspergillus* are available, such as itraconazole, or are being developed, such as voriconazole, pneumocandin and echinocandin. Although these agents hold promise, so far no data from comparative studies of treatment of patients with diagnosed fungal infections are available.

The rationale for using lipid formulations of AmB is that the antifungal activity of conventional AmB is dose-dependent, but toxicity limits the dosage resulting in sub-optimal tissue concentrations which reduces the potential clinical efficacy. Lipid formulations show a slightly reduced activity when compared with AmB, together with considerable reduction of toxicity, especially nephrotoxicity. This allows the use of much higher dosages which should lead to an increased therapeutic index with the potential of higher success rates.

This concept has been validated for the different formulations by some, but not all, animal experimental studies.[28]

At this moment there are 3 commercially developed lipid formulations, all with different conformations and pharmacokinetics.[29,30] ABLC (AbelcetR) is a large lipid-complex of 11,000 nm with the configuration of sheets. Due to its large size ABLC is rapidly removed from the blood by the liver and spleen, which results in low blood concentrations. ABCD (Amphocil®/Amphotec®) is a colloidal dispersion of complex of AmB with cholesterylsulfate, forming very flat discs. This compound gives peak blood concentrations lower than conventional AmB but its half-life is much longer. Liposomal AmB (AmBisome®) is a small unilamellar vesicle (diameter 80 nm) and gives much higher and more sustained blood concentrations because it escapes the macrophage-phagocyte system to some extent.[31]

Only data from non-comparative studies of diagnosed infections are available on the efficacy of ABCD.[32] A recently published comparative study in patients with fever of unknown origin (FUO) showed higher rates of infusion-related toxicity – including dyspnea – for ABCD 4 mg/kg than for conventional AmB 0.8 mg/kg, but a lower rate of nephrotoxicity.[33] Response rates to the two drugs were comparable in this study.

In a large study 5 mg/kg ABLC was compared with 0.6-1 mg/kg AmB in the treatment of invasive candidiasis and candidemia.[34] Only 15% of the 231 patients were neutropenic. Preliminary data from this study showed equal clinical and mycological response rates as well as mortality rates. The rate of nephrotoxicity was lower in the ABCL treated group: 28% percent doubling of baseline serum creatinine concentration compared with 47% of the group treated with conventional AmB. The reduction in nephrotoxicity was confirmed in an open study of 556 cases treated with ABLC.[35]

The safety of liposomal AmB was studied in a double-blind trial in patients with febrile neutropenia by Wals *et al.*[36] They showed that liposomal AmB 3 mg/kg produced lower nephrotoxicity and acute reaction rates than conventional AmB 0.6 mg/kg.

Although the rate of proven breakthrough invasive fungal infections was lower in liposomal AmB treated patients, it is difficult to assess antifungal efficacy in studies of patients with FUO, since neutropenic fever could have a non-fungal origin and improve spontaneously.

Recently we published the results of two randomized studies of treatment of diagnosed fungal infections with liposomal AmB. The first study was performed in AIDS patients with cryptococcal meningitis.[37] We showed that liposomal AmB treatment led to a significantly faster culture conversion of the cerebrospinal fluid. Within only 10 days 50% of the patients thus treated had negative CSF cultures, whereas culture conversion occurred in 10% of patients treated with conventional AmB after a median of more than 21 days. So in this first clinical study, we showed greater mycologic response to liposomal AmB.

We performed another multicenter randomized study in neutropenic patients with either a documented invasive mycosis or a suspected invasive pulmonary aspergillosis.[38]

Patients were treated with 5 mg/kg liposomal AmB or 1 mg/kg conventional AmB. When neutrophil count increased over 500/μL the dosage was reduced after 2 weeks in both arms. Bronchoalveolar lavage was performed in patients with pulmonary infection before enrollment. One hundred and six patients were tentatively included pending culture and other diagnostic results and all were eligible for toxicity studies. Sixty-six patients were definitively included in the efficacy analysis. Two subgroups were analyzed: 26 patients with microbiologically documented infections, and 55 patients with invasive pulmonary aspergillosis, 15 of whom were derived from the other subgroup. The characteristics of the patients at entry in the two arms were similar as far as concerned type of underlying disease, status of malignancy (being in remission or not), the extent of the pulmonary infection, median duration of neutropenia prior to entry as well as after starting treatment. After 2 weeks the failure rate appeared to be significantly different between the two arms: 76% for AmB and only 50% for liposomal AmB. By completion of the treatment, liposomal AmB had more often been successful (44% complete response versus 18%; p=0.03).

The data for the subgroup of patients with documented infections showed the same pattern: significantly better response rates for liposomal AmB after 14 days and after completion of therapy better complete response rates (64 % versus 17%). Overall mortality rates were 38% versus 22%; when adjusted for status of malignancy, this difference was significant. Despite the use of high dosage of liposomal AmB for many days the average increase of serum creatinine concentrations of patients receiving treatment was only 1.5% versus 85%.[38] Although dosages over 5 mg/kg have been administered without an excess of toxicity, the optimal dosage of liposomal AmB is still unknown. Ellis *et al*. studied the efficacy of 4 mg versus 1 mg of this formulation in neutropenic patients with presumed or documented invasive aspergillosis.[39] They did not find a better response rate for the higher dosage. However, the patient groups were not well matched for state of underlying disease, duration of neutropenia after inclusion, of frequency of cerebral involvement. Moreover, there was a better respponse rate in proven infections treated with 4 mg/kg whereas presumed infections responded better to 1 mg/kg. So, this study leaves us with unanswered questions as to the optimal dosage of liposomal AmB.[40]

Two recent studies pointed to lower toxicity for liposomal AmB when compared head to head to ABLC. An *in vitro* study showed a great difference in the rate of potassium leakage from blood cells after exposure to these lipid compounds.[41] Preliminary data from a randomized double-blind trial in neutropenic patients with FUO showed lower rates of acute reactions and nephrotoxicity in patients treated with liposomal AmB compared with those treated with ABLC.[42]

Considering the available results of comparative studies on lipid formulations, we can conclude only for liposomal AmB that higher dosages were superior to conventional AmB in terms of both efficacy and toxicity.

Combining the use of better diagnostic tests to allow earlier initiation of therapy and the use of more potent formulations such as liposomal AmB may result in a significant step forward towards successful management of invasive fungal infections.

Disclosures

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