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Liposomal amphotericin B compared with amphotericin B deoxycholate in the treatment of documented and suspected neutropenia-associated invasive fungal infections

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Summary. It has been suggested that a better outcome of neutropenia-associated invasive fungal infections can be achieved when high doses of lipid formulations of amphotericin B are used. We now report a randomized multicentre study comparing liposomal amphotericin B (AmBisome, 5 mg/kg/d) to amphotericin B deoxycholate (AmB, 1 mg/kg/d) in the treatment of these infections. Of 106 possible patients, 66 were enrolled and analysed for efficacy: nine had documented fungaemia, 17 had other invasive mould infections and 40 had suspected pulmonary aspergillosis. After completion of the course medication, in the AmBisome group ($n = 32$) 14 patients had achieved complete response, seven a partial response and 11 were failures as compared to 6, 13 and 15 patients ($n = 34$) treated with AmB ($P = 0{\cdot}09$); $P = 0{\cdot}03$ for complete responders. A favourable trend for AmBisome was found at day 14, in patients with documented infections and in patients with pulmonary aspergillosis ($P = 0{\cdot}05$ and $P = 0{\cdot}096$ respectively). Mortality rates were lower in patients treated with AmBisome (adjusted for malignancy status, $P = 0{\cdot}03$). More patients on AmB had a >100% increase of their baseline serum creatinine ($P < 0{\cdot}001$).

The results indicate that, in neutropenic patients with documented or suspected invasive fungal infections AmBisome 5 mg/kg/d was superior to AmB 1 mg/kg/d with respect to efficacy and safety.

Keywords: liposomal amphotericin, AmBisome, aspergillosis, neutropenia, randomized trial.

Invasive fungal infections in haemato-oncologic patients with neutropenia continue to result in high morbidity and mortality rates (Denning & Stevens, 1990; Meunier *et al*, 1992; Wingard, 1994, 1995; Denning, 1996a). Some reports have suggested a better outcome if higher doses (up to 1·5 mg/kg/d) of amphotericin B deoxycholate (AmB) were used (Denning & Stevens, 1990; Ruchlemer *et al*, 1996). However, such regimens are associated with high toxicity rates. Lipid formulations of AmB, which have shown to be less nephrotoxic, have recently been developed (de Marie *et al*, 1994; Leenders & de Marie, 1996; Bratjburg & Bolard, 1996). AmBisome (NeXstar Pharmaceuticals Inc., San Dimas, California, U.S.A.) is a liposomal formulation of AmB and has demonstrated promising results in both animal models of invasive fungal infections and in clinical studies in neutropenic patients with fever of unknown origin (FUO) (Leenders & de Marie, 1996; Prentice *et al*, 1997). A more rapid mycological response to AmBisome as compared to AmB was seen in a study including HIV-infected patients with cryptococcal meningitis (Leenders *et al*, 1997).

We report the results of an open randomized comparative multicentre trial of AmBisome 5 mg/kg/d versus AmB 1 mg/kg/d in the treatment of neutropenia-associated invasive fungal infections.

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METHODS

Study population. Hospitalized patients ⩾16 years of age who were severely neutropenic (neutrophilic granulocytes

$<0{\cdot}5\times10^9$/l) or who presented within 14 d of recovery from severe neutropenia, with untreated documented or highly suspected invasive fungal infection, were eligible for preliminary enrolment following informed consent. Preliminary enrolment was possible either on the basis of (1) positive fungal culture or histology from blood or deep tissue specimen, or (2) positive fungal culture or microscopy showing fungal hyphae from sputum or broncho-alveolar lavage (BAL) fluid together with chest X-ray abnormalities compatible with pulmonary infection, or (3) the presence of fever not responding to broad-spectrum antibiotics plus chest X-ray abnormalities suggestive of pulmonary aspergillosis. In every patient suspected of pulmonary aspergillosis, a BAL was required before start of treatment to confirm fungal infection and exclude other aetiology. During the first 18 months of the study only patients with fungal infection that was documented within 7 d after enrolment were definitely enrolled. After this time, patients with suspected pulmonary aspergillosis were also eligible for definite enrolment provided no other aetiology was established. The protocol required that preliminary-enrolled patients who did not fulfil the criteria for definite enrolment were replaced by new patients. All replaced patients were included in the toxicity evaluation.

At entry the following characteristics were recorded: medical history, underlying malignancy, previous anticancer therapy and malignancy disease status at entry (partial remission, complete remission, stable disease or progressive disease). Patients with progressive disease either received their first induction therapy for acute leukaemia, had relapsing progressive leukaemia, or had refractory haematological malignancy.

The study protocol was reviewed and approved by the ethics committees of all participating Dutch centres and, according to French regulations, by the ethics committee of one French centre.

Antifungal therapy. After preliminary enrolment, patients were randomized 1:1 to receive either AmBisome 5 mg/kg/d i.v. or AmB 1 mg/kg/d i.v. Randomization was performed centrally by one Dutch and one French centre stratified by participating institution. After 2 weeks of full dose the AmBisome and AmB doses were reduced to 3 mg/kg/d and 0·7 mg/kg/d respectively, provided the neutrophil count was $>0{\cdot}5\times10^9$/l, otherwise full doses were continued for as long as the neutrophil counts remained below that level.

AmBisome was administered immediately at full dose, infused over 45 min; no central intravenous catheter was required. The dose of AmB was escalated to a full dose over a 24 h period and was infused over 6 h; the use of a central intravenous catheter was recommended. Sodium supplementation prior to AmB infusion to prevent nephrotoxicity and medication to prevent acute reactions was allowed.

Dose adjustments were required when serum creatinine rose from normal to $\geqslant$300% or from an already elevated baseline value to $\geqslant$200%. Study medication was then to be discontinued for 2 d and subsequently to be reinstituted at half dose, when creatinine levels had fallen. If a rise in serum creatinine did not occur, doses were again escalated to full; if serum creatinine remained at the above high levels after 5 d of reduced dose, the study medication was discontinued.

Clinical evaluation and outcome assessment: efficacy. During the first 4 weeks on study, patients were evaluated daily for vital signs, temperature, specific signs of fungal infection and side-effects. Physical examination was performed frequently and, in case of pulmonary infection, chest X-rays at least once a week. During follow-up patients were examined and a chest X-ray was taken every 2 weeks. Chest X-rays of patients with documented or suspected pulmonary aspergillosis were re-evaluated by a pulmonologist with respect to localization, extension and nature of lesions at the outset, changes during treatment and time to improvement.

The following criteria were used for clinical evaluation. (1) Complete response: normalization of all pre-treatment signs and symptoms together with, if applicable, progressive improvement of chest X-rays. (2) Partial response: decrease of pretreatment signs and symptoms and a stable or improved chest X-ray. (3) Failure: unchanged or progressive pretreatment signs and symptoms. (4) Relapse: recurrence of any sign or symptom of fungal infection during follow-up after an initial response. After discontinuation of study drugs patients were followed for 4 weeks to document relapses. Mortality was evaluated during treatment and follow-up.

Toxicity. Blood urea nitrogen (BUN), serum creatinine and potassium were determined daily and additional chemistry (including liver enzymes) and haematological parameters weekly.

Discontinuation of therapy. Study drug treatment was prematurely discontinued in case of a sustained rise in serum creatinine levels, other serious adverse events judged to be study drug related by the treating physician, or if requested by the patient or physician.

Serum concentrations of amphotericin B. Trough-and-peak serum samples were obtained after 3 and 8 d of therapy. Levels of amphotericin B were all measured by high-performance liquid chromatography at the Erasmus University Medical Centre Rotterdam, as previously described (van Etten *et al*, 1993).

Statistical analysis. The study was primarily powered to show superiority of AmBisome over AmB in achieving a more rapid clinical response (complete and partial). Assuming a difference in response rates of 40% after 14 d between AmBisome and AmB (primary endpoint), 30 definite enrolled patients in each arm were required (two-sided $P=0{\cdot}05$, power = 90%). Secondary endpoints defined in the protocol were: rates of clinical success and improvement, mortality rate, time to clinical success, mycological eradication, percentage of patients needing dose reduction, percentage of patients with >100% increase of serum creatinine and the average peak increase in serum creatinine. Furthermore, outcome analyses were performed on subgroups of patients based on characteristics which were recorded according to the protocol. Cumulative percentages of patients with clinical response were calculated according to the method of Kaplan and Meier. Comparison of these curves was done with the log-rank test. The relationship between response rates and neutropenic status was determined using Cox regression with time-dependent variables

(Cox, 1972). Graded response outcomes or percentages were compared the Mann-Whitney *U* test or Fisher's exact test, respectively. Approximate normally distributed variables were compared by Student's *t*-test. Comparison of mortality, taking account of malignancy status, was done using exact logistic regression (Mehta & Patel, 1993). The change from baseline of logarithmically transformed creatinine values was compared using repeated measurements analysis of variance (RmANOVA) (Dixon, 1990). A P value $\leqslant 0{\cdot}05$ was considered significant.

All analyses of clinical and mycological efficacy data of definitely included patients were performed on an intention-to-treat basis and were continued until all antifungal treatment was discontinued. For analysis of toxicity, patients were evaluated up to the time of final discontinuation of the study drug.

RESULTS

Study population

Between January 1992 and January 1996, 106 patients with documented or suspected invasive fungal infection were preliminarily enrolled in the study. All were evaluated for toxicity. A total of 40/106 patients were ineligible for definite enrolment. During the first 18 months, 18 patients were withdrawn because no fungi had been cultured from pretreatment materials within 7 d. After protocol revision at 18 months, another 22 patients were not eligible for definite enrolment: pretreatment nonfungal causes were found to explain the symptoms in 15 patients, invasive candidiasis could not be documented in four cases, previous treatment with itraconazole (one case) and lack of pretreatment cultures (one case). Finally, one patient was preliminarily enrolled when *A. fumigatus* was cultured from his sputum but judged ineligible for definite enrolment when it became apparent that this was due to mass laboratory contamination (>100 culture media were contaminated).

Of the 66 definite enrolled patients, 32 had been assigned to AmBisome and 34 to AmB. Demographic characteristics, clinical parameters, duration of neutropenia before enrolment, type and status of underlying malignancy, the number of bone marrow transplantations, and baseline haematological laboratory values did not differ significantly between the groups (Table I). Baseline levels of creatinine, BUN, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase and bilirubin also did not differ significantly. Sites and causal agents of infection among the 66 patients were: nine documented fungaemia (*Candida* spp., $n=7$; *Cryptococcus neoformans*, $n=1$; *Fusarium* spp., $n=1$), 17 other documented invasive mould (all filamentous fungi) infections (15 pulmonary infection, one aspergillosis of a vertebra, one sinusitis caused by *Absidia* species) and 40 suspected pulmonary mould infections (Table II).

The median duration of neutropenia after enrolment was 5·5 d (range 0–98) for patients assigned to AmBisome, versus 6·5 d (range 0–36) for patients assigned to AmB ($P=0{\cdot}30$). Six patients had recovered within 14 d from neutropenia, four assigned to AmB (at 1, 6, 11 and 14 d) and two assigned to AmBisome (1 and 12 d).

Table I. Demographic characteristics, clinical parameters, underlying malignancies and baseline laboratory values of 66 neutropenic patients treated for documented or suspected invasive fungal infection.

Characteristic	Amphotericin B deoxycholate ($n=34$)	AmBisome ($n=32$)
Age (yr)		
Median	48	52·5
Range	20–68	18–74
Sex, no. of patients (%)		
Male	10 (29)	6 (19)
Female	24 (71)	26 (81)
W.H.O. status, no. of patients (%)		
Fully active	7 (21)	5 (16)
Restricted activity	6 (18)	8 (25)
Fatigue, full self-care	8 (24)	6 (19)
Limited self-care	10 (29)	12 (32)
Completely disabled	3 (9)	1 (3)
Malignancy, no. of patients (%)		
Acute nonlymphocytic leukaemia/ myelodysplastic syndromes	20 (59)	18 (56)
Acute lymphocytic leukaemia	2 (6)	6 (19)
Chronic leukaemias	3 (9)	2 (6)
Other	9 (26)	6 (19)
Malignancy status, no. of patients (%)		
Complete remission	9 (26)	6 (19)
Partial remission	4 (12)	3 (9)
Stable disease	4 (12)	4 (13)
Progressive disease	16 (47)	19 (59)
Unknown	1 (3)	0
Previous bone marrow transplantation, no. of patients (%)	5 (15)	4 (13)
Previous stem cell transplantation, no. of patients (%)	1 (3)	0
Neutropenic at enrolment, no. of patients (%)	30 (88)	30 (94)
Duration of neutropenia prior to enrolment (days)		
Median	15	19
Range	5–94	3–113
Haemoglobin (g/dl)		
Median	6·15	6·45
Range	4·9–12·0	5·1–9·5
Leucocytes ($\times 10^9$/l)		
Median	0·4	0·3
Range	0·1–19·8	0·0–22·4
Neutrophils ($\times 10^9$/l)		
Median	0·05	0·03
Range	0·0–17·8	0·0–6·8
Creatinine (μmol/l)		
Median	70	83
Range	30–187	21–195

Table II. Causal agents in 66 neutropenic patients treated for documented or suspected invasive fungal infections according to assigned antifungal therapy.

Fungal infection	Amphotericin B deoxycholate ($n=34$*)	AmBisome ($n=32$*)
Documented		
Aspergillus fumigatus/spp.	4	7
Mould not further determined	4	1
Other moulds†	2	0
Candida albicans	1	2
Candida non-albicans	1	3
Yeast not further determined	0	1
Other yeasts‡	1	1
Suspected		
Pulmonary aspergillosis	22	18

*Two patients (one in each arm) had more than one fungal pathogen isolated.

† *Fusarium* spp. and *Absidia* spp.

‡ *Saccharomyces cerevisiae* and *Cryptococcus neoformans.*

Efficacy

Outcome at day 14 and at completion of therapy. The median duration of treatment was 14·5 d (range 2–149) for patients on AmBisome versus 16·5 d (range 4–76) for patients on AmB.

As shown in Table III, at 14 d, five (17%) patients assigned to AmBisome had achieved a complete response, 10 (33%) a partial response and 15 (50%) had failed versus two (6%), six (18%) and 25 (76%) patients assigned to AmB ($P=0·03$), respectively, with overall response (complete or partial) rates of 15/30 versus 8/33 ($P=0·04$). For patients with documented fungal infection, overall response rates were

Table III. Outcome at day 14, at completion of therapy and mortality of 66 neutropenic patients treated for documented or suspected invasive fungal infections with either amphotericin B or AmBisome.

	Amphotericin B deoxycholate ($n=34$)				AmBisome ($n=32$)				
	Complete response	Partial response	Failure	NE*	Complete response	Partial response	Failure	NE*	*P* value
Response at day 14									
All patients	2	6	25	1	5	10	15	2	0·03
With documented infection	0	2	10	0	4	2	6	2	0·05
With pulmonary aspergillosis	2	6	20	1	3	10	12	1	0·096
Response at completion									
All patients	6	13	15	0	14	7	11	0	0·09†
With progressive malignancy	1	4	11	0	6	3	10	0	0·19†
With documented infection	2	3	7	0	9	0	5	0	0·07†
With pulmonary aspergillosis	6	11	12	0	11	7	8	0	0·16†
Mortality (no. of patients)									
All patients	13/34				7/32				0·19‡
With progressive malignancy	12/16				7/19				0·04
With non-progressive malignancy	1/18				0/13				1·00
With documented infection	6/12				4/14				0·42
With pulmonary aspergillosis	11/29				5/26				0·15

*Three patients were not evaluable at day 14 because no chest X-ray was performed ($n=2$) or because no signs and symptoms were noted.

† $P=0·03$, $P=0·096$, $P=0·02$ and $P=0·14$, respectively, for proportion of patients with complete response.

‡ Adjusted for malignancy status: $P=0·03$.

6/12 (50%) in AmBisome patients versus 2/10 (20%) in AmB patients ($P = 0{\cdot}19$); for patients with suspected or documented pulmonary aspergillosis, overall response rates were 13/25 (52%) in AmBisome patients versus 8/28 (29%) in AmB patients ($P = 0{\cdot}10$).

At completion of therapy, in the AmBisome arm 14 (44%) patients had achieved a complete response versus six (18%) in the AmB arm ($P = 0{\cdot}03$). Among patients with documented infection, nine (64%) AmBisome patients (five aspergillosis, one mould infection and three yeast infections) and two (17%) AmB patients (both with moulds not further determined) had a complete response ($P = 0{\cdot}02$). Within the AmB arm, the patient with *Absidia* sinusitis died, the patient with fusaraemia improved. Among patients with suspected or documented pulmonary aspergillosis, 11 (42%) patients on AmBisome and six (21%) patients on AmB had a complete response ($P = 0{\cdot}14$).

The overall response rates were determined mainly by the malignancy status of the patients, and were 40% in patients with progressive disease versus 83% in patients in remission or with stable disease ($P = 0{\cdot}001$). When the time to response was evaluated, patients who had recovered from neutropenia had a 4·9-fold (95% confidence interval 2·1–11·0) increased response rate as compared to patients who remained neutropenic ($P < 0{\cdot}001$). No differences existed in the proportion of patients who had recovered from neutropenia at day 14 between the treatment arms: 17/32 *v* 20/34. The differences in response rates between patients recovering and not recovering from neutropenia were the same for AmBisome and AmB. The results of patients who had recovered from neutropenia within 14 d prior to enrolment ($n = 6$) were not different from the overall outcome.

Mortality. Overall mortality appeared to be determined considerably by the malignancy status of patients. Mortality rates were 54% among patients with progressive malignancy and 3% in those who were in remission or had stable disease ($P < 0{\cdot}001$). Taking account of the malignancy status using exact logistic regression, the overall mortality rate in patients assigned to AmBisome was significantly lower than in the AmB group: 7/32 (22%) *v* 13/34 (38%) ($P = 0{\cdot}03$). In the AmBisome arm 5/7 and in the AmB arm 10/13 patients were considered to have died due, or at least partly due, to fungal infection. Among patients with progressive malignancy mortality rates were 7/19 (37%) in the AmBisome group versus 12/16 (75%) in the AmB group ($P = 0{\cdot}04$).

Mycological response. In patients with documented fungal infection, at completion of therapy, 7/10 patients assigned to AmBisome showed fungal eradication (4/5 moulds and 3/5 yeasts) versus 2/8 (2/6 moulds and 0/2 yeasts) patients assigned to AmB ($P = 0{\cdot}15$); eight patients were not evaluable for mycological response.

Chest X-rays. Chest X-rays of 47/55 patients with suspected or documented pulmonary aspergillosis were

Table IV. Duration of therapy, cumulative dose and the number of patients with adverse events during treatment with amphotericin B or AmBisome for neutropenia-associated invasive fungal infections.

Characteristic	Amphotericin B deoxycholate ($n = 54$)	AmBisome ($n = 52$)
Duration of therapy (d), median (range)	13 (3–76)	14 (1–149)
Cumulative dose (mg), median (range)	850 (216–3836)	3865 (360–27000)
Adverse event		
Clinical		
Fever/chills	12*	5*
Nausea	1	0
Exanthema	3	4
Chest pain/tachypnoea	2	3
Other acute reactions†	3	5
Detected in laboratory		
Fall in haemoglobin >2·0 g/dl	3	2
Hypokalaemia (≤2·5 mmol/l)	11	10
Creatinine >2 times baseline	22‡	6‡
Aminotransferase >5 times ULN§	7	7
Alkaline phosphatase >5 times ULN	5	3
Bilirubin >5 times ULN	8	9
None of these adverse events reported	24	23

* $P = 0{\cdot}11$ (Fisher's Exact Test).

† AmB: hypotension, tickling cough and wheezing; AmBisome: hypotension, organic psychosyndrome, headache, oedema legs and acute allergic reaction. See Results section.

‡ $P < 0{\cdot}001$ (both Fisher's Exact Test).

§ ULN = upper limit of normal.

available for the blinded retrospective analysis. For the remaining eight patients the judgement of the local radiologist was accepted. The number of patients with focal lesions was comparable between the treatment arms: 10/26 *v* 11/29. A significantly higher percentage of patients with focal lesions showed complete or partial resolution of X-ray abnormalities, 81% *v* 52% in patients with diffuse lesions ($P = 0{\cdot}04$). Clinical response percentages in these subgroups were 67% *v* 48% ($P = 0{\cdot}24$). No differences were found either in resolution of chest X-ray abnormalities or in clinical response rates between patients with bilateral and unilateral lesions. Analysis showed that the number of patients who had chest X-rays improving was not significantly different between the two treatment arms, 17/26 (65%) for AmBisome versus 16/29 (55%) for AmB ($P = 0{\cdot}58$). Median time to radiological improvement was 3 weeks in the AmBisome group versus 5 weeks in the AmB group ($P = 0{\cdot}71$).

Evaluation of toxicity

The number of patients who received concomitant nephrotoxic medication was high both in patients given AmBisome and AmB: aminoglycosides (23/52 *v* 23/54), glycopeptides (47/52 *v* 40/54) and diuretics (7/52 *v* 11/54). Duration of antifungal treatment and cumulative doses are shown in Table IV. All patients, both in the AmBisome and in the AmB arm, started with full doses. Both treatment regimens were generally well tolerated (Table IV). RmANOVA showed that 14 d after start of treatment the mean change from baseline serum creatinine was 86% (±9%) in the AmB and 1·4% (±5%) in the AmBisome group ($P < 0{\cdot}001$). Significantly more patients treated with AmB had a >100% increase of their baseline serum creatinine; 22/54 (40%) *v* 6/51 (12%) ($P < 0{\cdot}001$). In 18 patients treated with AmB and in two patients treated with AmBisome, medication was temporarily discontinued or lowered in dose due to increase of serum creatinine ($P < 0{\cdot}001$). The number of patients with major changes in biochemical parameters are shown in Table IV. Three patients treated with AmBisome and seven treated with AmB had to stop therapy because of toxicity ($P = 0{\cdot}31$). One AmBisome-treated patient had to stop because of hepatotoxicity, one because infusion-related retrosternal pain and one because of an anaphylactic reaction after infusion, which recurred after switching to AmB. AmB treatment was stopped because of renal toxicity in all cases.

Serum concentrations of amphotericin B

Median serum trough levels (1·80 μg/ml, range 0·34–45·80) and peak levels (19·10 μg/ml, range 5·98–80·00) of amphotericin B in patients treated with AmBisome were higher than those in patients treated with AmB (trough; 0·67 μg/ml, range 0·2–1·32; peak 1·43 μg/ml, range 0·40–2·89; $P = 0{\cdot}022$ for trough and $P < 0{\cdot}0001$ for peak levels). Median serum trough and peak levels at day 3 and day 8 did not differ.

DISCUSSION

Amphotericin B deoxycholate (AmB) is the drug of choice for the (empirical) treatment of invasive fungal infections in neutropenic patients; however, its efficacy in this setting has been disappointing (Denning & Stevens, 1990; Meunier *et al*, 1992; Wingard, 1994, 1995; Denning, 1996a). Only fluconazole was shown to be more effective than AmB in a subgroup of neutropenic patients with candidiasis in one larger study including only patients with candidial infections (Denning & Stevens, 1990; van't Wout *et al*, 1991; Anaissie *et al*, 1996). However, the lack of activity against moulds prohibits the empirical use of fluconazole in a setting where mould infections have to be considered such as in the case of a febrile neutropenic patient with pulmonary infiltrates. We report an antifungal agent with superior clinical efficacy compared to AmB in the treatment of neutropenia-associated invasive fungal (yeast and mould) infections. AmBisome showed a significantly higher response rate within 14 d and more patients had achieved a complete response at termination of therapy. A complete response to antifungal therapy offers better opportunities for continuing treatment of the underlying malignancy, whereas fungal infections that only partially respond to therapy are more likely to relapse during renewed immunosuppressive therapy. However, patients with a complete response might also relapse from a cryptogenic fungal focus (Karp *et al*, 1988). Although the majority of infections could not be documented, we feel that this does not devalue the results of this study, because in a clinical setting these are exactly the patients who are treated with antifungal therapy. Furthermore, patients with other documented (microbiological) diagnoses in whom antifungal treatment would normally also be discontinued, were not eligible for definite enrolment; these patients were not allowed to receive further free study medication and could therefore not be included in the intention-to-treat analysis.

The favourable trend in response rates to AmBisome seen in patients with documented infection may be influenced by the fact that the two arms were not completely comparable for fungal causes. However, both in patients with fungaemia and in patients with documented aspergillosis or other mould infections, clinical responses among patients treated with AmBisome were better.

Patients with progressive malignancy, including patients who received first induction chemotherapy, responded far less to antifungal therapy. In patients with progressive malignancy, mortality was twice as high among patients on AmB as compared to patients on AmBisome. An analysis taking account of the malignancy status showed that adjusted mortality among all patients was significantly lower in those treated with AmBisome.

The results of patients with documented or suspected pulmonary aspergillosis showed fewer differences between the treatment arms, which may be due to the fact that in the subgroup of patients with suspected aspergillosis, patients without a fungal infection might have been included.

Compared to CT-chest, chest X-rays are known to be extremely heterogenous and less sensitive, particularly in early cases of invasive aspergillosis. With the current knowledge that CT-chest is the most sensitive and therefore preferred radiological method to diagnose invasive aspergillosis, future studies should include regular CTs instead of the

chest X-rays which were carried out in our study (Denning, 1996b). Earlier diagnosis of invasive aspergillosis by using CT may also greatly influence the outcome of therapy.

Resolution of neutropenia is the most important factor influencing the outcome of invasive aspergillosis (Denning & Stevens, 1990; Denning, 1996a). In this study, patients recovering from neutropenia had a 5-fold increased response rate, nevertheless we chose to include patients who had recovered from neutropenia within the preceding 14 d because they probably acquired the infection during the neutropenic period and therefore belong to the same population at risk. Differences of efficacy could not be explained from differences in time to recovery of neutrophils between both arms. Remarkably, in seven patients (four on AmBisome and three on AmB) clinical symptoms had cleared before the neutrophils were restored.

Our study is the first clinical trial to show superior clinical efficacy of a lipid formulation of AmB over the parent drug in the treatment of invasive fungal infections. So far, three lipid products of AmB have been marketed in Europe or the U.S.A.: Abelcet (Amphotericin B Lipid Complex), Amphocil (Amphotericin B Colloidal Dispersion) and AmBisome (de Marie *et al*, 1994; Leenders & de Marie, 1996; Bratjburg *et al*, 1996). The major advantage of these products is the reduction of toxicity which allows the administration of higher doses and should result in an increased therapeutic index. Indeed, this has been reported in several animal models (Leenders & de Marie, 1996). The three formulations differ significantly in pharmacological characteristics and, although they are all less nephrotoxic than AmB, it has been shown that they differ in the rate of other side-effects, especially acute infusion-related reactions (Leenders & de Marie, 1996; Storm & van Etten, 1997; Janknegt *et al*, 1992). Therefore these products need to be compared separately from the parent compound to evaluate their efficacy and safety. The results found in the present study using AmBisome should therefore not be extrapolated to other formulations.

The optimal dose of AmBisome remains to be determined. From the results of studies in patients with FUO it was concluded that lower doses may be effective, although it is very difficult to assess antifungal efficacy in studies with these particular groups of patients (Prentice *et al*, 1997). Results of most animal studies indicate that the highest doses give the best responses (Leenders & de Marie, 1996). However, in an animal model of pulmonary aspergillosis it was shown that lower doses of AmBisome were able to prevent dissemination of infection (Leenders *et al*, 1996). It could therefore be hypothesized that doses as low as 1 mg/kg may well be useful in a prophylactic setting, such as febrile neutropenia. We propose that future clinical studies should address the question whether higher doses (e.g $\geqslant$10 mg/kg/d) of AmBisome can further increase its efficacy in more advanced infections. Toxicity data from the present study clearly indicate that the maximal tolerated doses in patients exceeds 5 mg/kg/d. Due to the high costs of the formulation, the introduction of regimens using AmBisome 5 or 10 mg/kg/d could have a very substantial impact on the costs of the health-care system. The use of this formulation should probably be restricted to certain groups of patients, for example patients with progressive malignancy. Unfortunately, cost–benefit was not an endpoint in our study and we therefore did not collect sufficient data to perform such an analysis. We strongly suggest that future studies comparing this formulation with AmB include a formal cost–benefit analysis, because higher efficacy, less toxicity and easier administration with the use of AmBisome may well lead to a cost-effective regimen.

In this study the response rates of patients with progressive malignant disease to antifungal therapy were much lower. This lower response rate was apparently not caused by a longer duration of neutropenia, which was the same in patients with and without progressive malignancy. It is possible that the functioning of neutrophils in patients with progressive malignancy is less active than when the malignancy is in remission. Because the results of antifungal therapy between these two subgroups is completely different, we recommend that, in future comparative studies, patients are stratified according to their status of malignancy at entry. Differences in the proportion of patients who are in remission can easily confound the observed differences in outcome between treatment arms. If this information is not available it is pointless to compare the results of different studies. The status of malignancy might also be taken into account when antifungal therapy is selected. Therapy with liposomal amphotericin B may be particularly indicated for patients with progressive malignancies and possibly for patients with other factors predictive of a poor outcome such as bone-marrow recipients.

We were surprised to find that serum levels of amphotericin B after administration of AmBisome 5 mg/kg/d were significantly lower than after the administration of AmBisome 4 mg/kg/d in a study we performed simultaneously in patients with AIDS-associated cryptococcal meningitis (Leenders *et al*, 1997). This difference in pharmacokinetics profile points to a higher volume of distribution of AmBisome in the neutropenic patient compared to the HIV-infected patient. Similar observations have been reported for other drugs in these patient groups (Lotholary *et al*, 1996; Prentice *et al*, 1994). The relevance of serum levels as to efficacy, however, is still unclear.

We conclude that in neutropenic patients with documented or suspected invasive fungal infections high-dose ($\geqslant$5 mg/kg) AmBisome was superior to standard AmB with respect to efficacy and safety. AmBisome could be the therapy of choice in the treatment of invasive fungal infections, particularly in patients with progressive malignancy.

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