Chapter 3

Aerosolized liposomal amphotericin-B to prevent aspergillosis in acute myeloid leukemia: Efficacy and cost-effectiveness in real-life

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Int J Antimicrob Agents 2015;46:82-87

ABSTRACT

Background

Chemotherapy-induced neutropenia can be complicated by invasive pulmonary aspergillosis (IPA). In 2008, liposomal amphotericin-B (L-AmB) inhalation was shown to prevent IPA in a placebo-controlled trial. Patients with acute myeloid leukemia (AML) are the subset of hematology patients at high risk for IPA. In 2008, L-AmB inhalation prophylaxis became standard of care for all AML patients in Erasmus University Medical Center. In this study, the efficacy and cost-effectiveness of L-AmB inhalation were evaluated in a prospective cohort of AML patients.

Methods

In total, 127 consecutive AML patients received chemotherapy and prophylactically inhaled L-AmB during their 1st and 2nd chemotherapy cycles; 108 patients treated for AML at the same sites from 2005 to 2008 served as controls. A standardized diagnostic protocol was used and probable/proven IPA served as the primary endpoint. Diagnostic and therapeutic costs were also comprehensively analyzed and compared.

Results

A significant decrease in probable/proven IPA in the L-AmB inhalation group was observed (L-AmB 9.5%, control 23.4%, p=0.0064). Systemic antifungal therapy given at any time during the entire AML therapy decreased from 52.8% to 29.9%. Per patient equipment and drug costs for the L-AmB inhalation (1292 euro/patient) were more than compensated by a decrease in costs for diagnostics and therapeutic voriconazole use (minus 1816 euro/patient). No L-AmB inhalation related serious adverse events were observed.

Conclusion

In an unselected AML patient group, L-AmB inhalation resulted in a significant and substantial decrease in IPA and was cost saving. Now that azole resistant becomes more frequent, non-azole based prophylaxis may become an attractive strategy.

INTRODUCTION

Patients with acute myeloid leukemia (AML) treated with high-dose chemotherapy are at high risk for invasive pulmonary aspergillosis (IPA), which is associated with an increased morbidity and mortality [1,2]. Moreover, IPA increases the medical cost substantially: the mean additional per-patients costs are 8360 euros for possible IPA and 15280 euros for probable/proven IPA [3]. Given these observations and the fact that hospital resources are finite, preventing IPA is an attractive strategy to reduce mortality and IPA-related costs.

Administration of aerosolized liposomal amphotericin-B (L-AmB) is a promising candidate in preventing IPA. *Aspergillus fumigatus* conidia are inhaled and germinate in the lungs; therefore delivering aerosolized L-AmB directly to this site of infection may prevent IPA. A significant decrease in IPA was shown when inhaling L-AmB compared to placebo in a randomized controlled trial (RCT) [4]. No impaired lung function measurements or systemic side effects due to L-AmB inhalations were observed [5].

In 2008, prophylactic L-AmB inhalation was implemented as standard of care for AML patients of the Erasmus University Medical Center (Rotterdam, the Netherlands). In this prospective cohort study, the efficacy and cost-effectiveness of L-AmB inhalations on the incidence of IPA were evaluated in an unselected real-life AML population.

METHODS

Patient population

This study was performed at the Erasmus University Medical Center, a university referral hospital in Rotterdam, the Netherlands. We studied hospitalized patients with newly diagnosed or relapsed AML, myelodysplastic syndrome (MDS) with excess of blasts or chronic myeloid leukemia (CML) in blast crisis, who were aged ≥18 years and were treated during the period of September 2008 through August 2012. Patients were included after providing written informed consent. All patients were treated with remission induction and consolidation chemotherapy according to the Hemato-Oncology voor Volwassenen Nederland (HOVON) guidelines [6,7]. The following information was obtained: age, sex, number of chemotherapy cycles, type of hematopoietic stem cell transplantation (HSCT), duration of neutropenia, mortality and invasive fungal disease (IFD). Patients were excluded if they had proven, probable or possible IPA before start of the prophylactic inhalation therapy. The control group consisted of 108 historical patients with AML, MDS or CML treated with high-dose chemotherapy during the period of April 2005 through April 2008. Control patients did not receive prophylactic inhalation therapy and were not included in the L-AmB inhalation group if hematologic disease

relapsed on a later point in time. Both groups received prophylaxis with oral fluconazole 400 milligrams daily (or intravenous when oral intake was impossible). The institutional review board approved the study. According to the diagnostic protocol for evaluation of neutropenic fever, patients underwent high-resolution computed tomography (HRCT) at day 5 of unexplained fever despite treatment with antibiotics. HRCT was repeated 5-7 days later if fever persisted. Patients with intrapulmonary lesions underwent bronchoscopically-guided bronchoalveolar lavage (BAL) of the most representative lung lesion. BAL fluid was cultured for bacteria, mycobacteria and fungi, as well as measurement of galactomannan antigen levels. A galactomannan level of ≥0.5 in BAL fluid was considered positive. If bronchoscopy was impossible to perform because of the location or small size of the lung lesion and serum galactomannan was negative (<0.5), a biopsy of the lung lesions was performed if feasible. Voriconazole was the first-line therapy for IPA.

L-AmB inhalation

Prophylactic inhalation of 12.5 milligrams L-AmB (AmBisome; Gilead Sciences Europe Ltd., Uxbridge, UK) was used twice a week and was initiated at the start of the 1st remission induction chemotherapy cycle. Nebulization of L-AmB was performed with an adaptive aerosol delivery (AAD) system (Prodose® AAD nebuliser from 2008 to 2011, Akita® AAD nebuliser from 2011; Romedic, Meerssen, the Netherlands). Both are advanced nebulizer systems that adapt to individual breathing patterns, nebulizing only during inspiration and therefore the intrapulmonary delivery of the prescribed dose was guaranteed. They generate particles with a mean diameter of 1.9 µm; optimal deposition in the peripheral lung regions is therefore ensured. Inhalation was continued until neutrophil recovery, which was defined as two consecutive neutrophil counts ≥0.2 x10^9/l or one ≥0.5 x10^9/l, after which patients were discharged from the hospital. It was re-initiated during the next chemotherapy cycle until neutrophil recovery. Inhalation therapy was not continued during autologous or allogeneic HSCT following chemotherapy.

IPA classification

IPA was categorized according to the updated criteria of the European Organization for Research and Treatment of Cancer Mycosis Study Group (EORTC/MSG) [8]. Neutropenic patients are considered to have possible IPA if a new and otherwise unexplained well-defined intrapulmonary nodule (with or without halo sign), an air-crescent sign, or a cavity within an area of consolidations is radiologically documented. Probable IPA is diagnosed when on top of these radiological findings microbiological proof of *Aspergillus fumigatus* infection is documented by galactomannan antigen detection or cultures of *Aspergillus fumigatus*. Proven IPA is defined as histopathologic evidence of invasive mold infection and microbiological proof of *Aspergillus fumigatus* infection. Patients with more than 1 episode of IPA during hematological treatment were classified accord-

ing to the highest diagnostic IPA category (e.g., a patient with possible IPA during the 1st cycle of chemotherapy but probable IPA at a later point in time was categorized as having probable IPA). In addition, patients who could not be categorized into possible, probable or proven IPA but were treated with antifungal therapy were subdivided into the following categories: patients with nonspecific intrapulmonary abnormalities with positive galactomannan antigen detection, patients with nonspecific intrapulmonary abnormalities with negative galactomannan antigen detection, and patients with normal CT and negative galactomannan antigen detection. IFD was defined as a culture or biopsy proven infection with a yeast or non-*Aspergillus* mold.

Primary endpoint

The primary endpoint was the incidence of proven or probable IPA during 1st and 2nd cycles of chemotherapy until 28 days after neutrophil recovery.

Secondary endpoints

Secondary endpoints were (1) the incidence of proven or probable IPA 12 weeks after the end of all hematological treatment (including HSCT) (2) the incidence of proven, probable or possible IPA 12 weeks after at the end of all hematological treatment (including HSCT) (3) the incidence of proven, probable or possible IPA during the 1st and 2nd cycles of chemotherapy (4) overall and IPA-related (=attributable) mortality 12 weeks after recovery from neutropenia from the last chemotherapy cycle.

Cost calculations

All costs were studied from a hospital perspective. Costs taken into account were the total hospital-based costs per patient. We distinguished diagnostic costs, costs for medical treatment, costs for hospital stay, and costs for the transfusion of blood products. Diagnostic costs taken into account were radiological imaging, microbiological investigations (blood cultures, staining and cultures of BAL fluid specimens, and galactomannan antigen measurement in serum or BAL fluid specimen) and other diagnostics, e.g. bronchoscopy, pathology, colonoscopy, gastroscopy, echocardiogram. Costs of the medical treatment included costs for antifungal and antibiotic treatment, and costs for L-AmB inhalations. Costs of transfusions included costs for erythrocytes, thrombocytes, and/or fresh frozen plasma. Included extramural costs were all costs mentioned above when they occurred *between* the 1st and 2nd cycle of chemotherapy. The extramural costs *after* the 2nd chemotherapy cycle were limited to antifungal costs only and no longer than 4 weeks after discharge of the 2nd chemotherapy cycle. This was done so to avoid bias as patients that subsequently undergo a 3rd chemotherapy cycle will get this 3rd cycle (and its accompanying diagnostics and transfusion costs) within 1 or 2 weeks after the 2nd chemotherapy cycle, while an allogeneic transplantation will generally take place

later in time. The evaluation was patient based and started from day 1 of hospitalization of the 1st cycle of high dose chemotherapy for newly diagnosed or relapsed AML, MDS with excess of blasts or CML in blast crisis, which means that for each patient the costs of the 2 consecutive treatment episodes were totaled.

In the Netherlands, a detailed fee-for-service system is used for the remuneration of the medical interventions and diagnostic procedures, enabling the calculation of the costs. Therefore, medical costs were calculated by multiplying the volumes of health care use per patient with the corresponding official Euro unit prices for each diagnostic or therapeutic procedure. For drugs, the actual number of milligram administered was multiplied by the costs per milligram, as charged by the hospital pharmacy. The costs for inpatient days were calculated by multiplying the number of days with the unit price as charged for a nursing ward or intensive care unit (ICU), counting the hotel costs only.

To be able to compare the costs between groups in an unbiased way, the cost calculations for the primary analysis were limited to the first two chemotherapy cycles. The reasons that we could not include the AML therapy that followed (e.g. 3rd chemotherapy cycle, HSCT) are threefold. Firstly, over the 7 years of the study period the risk classification of AML changed substantially which resulted in a shift of patients from the intermediate to the high risk AML category. The consequence is that more patients in the L-AmB group underwent an allogeneic HSCT (36% versus 26%). Secondly, if L-AmB inhalation is indeed effective and therefore reduces the incidences of IPA, it will also have an impact on the AML therapy given after the 1st and/or 2nd chemotherapy cycle because patients that have an IPA during the 1st or 2nd chemotherapy cycle will be less likely to undergo an allogeneic HSCT. Thirdly, prophylaxis with L-AmB inhalations was only given to patients admitted to the hospital during AML therapy. During the 1st and 2nd chemotherapy cycles all patients stayed in the hospital until neutrophil recovery. In contrast, only part of the patients stayed in the hospital for the 3rd chemotherapy cycle.

Statistical analysis

Baseline characteristics and incidence of IPA and IFD were tested with the independent sample t-test or Fisher's exact test as appropriate. Cost difference between the control group and L-AmB group were analysed using the Mann-Whitney U test. Since cost data per patient (but not per day care) are typically highly skewed, we used nonparametric bootstrap techniques to derive a 95% confidence interval for the differences in distributions of the direct medical costs. For the analysis of IPA incidence during all AML therapy (including 3rd chemotherapy cycle or HSCT), patients were censored 3 months after hospital discharge from the last AML treatment episode. For the primary endpoint a 2-sided P value <0.05 was considered to be statistically significant. Analyses were done with SPSS version 20. GMC, SP and BJAR analyzed the data.

RESULTS

From September 2008 through August 2012, 127 patients in the L-AmB group received high dose chemotherapy or a HSCT during 310 treatment episodes. 226 of these were for the 1st or 2nd cycle of chemotherapy. The control group consisted of 108 historical patients who were treated during the period of April 2005 through April 2008 and had in total 262 treatment episodes. 200 of these were for the 1st or 2nd cycle of chemotherapy. Two patients, 1 of the control group and 1 of the L-AmB group, could not be included in the cost-calculation population as they had an unusual combination of AML therapy during 1 hospital admission (1 patient had the 1st, 3rd chemotherapy cycle and HSCT in 1 treatment episode, and 1 patient had the 1st chemotherapy cycle and HSCT in 1 treatment episode). From 3 patients only the 1st chemotherapy cycle could be included in the cost-calculation population as they had their 2nd chemotherapy cycle combined with an allogeneic HSCT in the same hospital admission (1 L-AmB and 2 controls). Baseline characteristics of the study groups are summarized in table 1.

Table 1. Characteristics of the cohort of 233 patients with acute myeloid leukaemia-myelodysplastic syndrome treated with high dose chemotherapy.

Characteristics	Control group n = 108	L-AmB inhalation group n = 127	p-value
Age, mean years (range)	52.1 (20.2 - 76.9)	55.7 (20.4 - 79.7)	0.051
Male gender, n (%)	61 (57.0%)	67 (53.2%)	0.5983
Diagnosis, n (%)			>0.1
Acute myeloid leukaemia	90 (83.3%)	113 (89.0%)	
Myelodysplastic syndrome with excess of blasts	18 (16.7%)	12 (9.5%)	
Chronic myeloid leukaemia in blast crisis	0	2 (1.6%)	
Mean duration neutropenia per chemotherapy treatment[a], n (%)			0.1
<10 days	0	1 (0.8%)	
≥10 – 20 days	14 (13.2%)	17 (13.6%)	
≥20 days	92 (86.8%)	107 (85.6%)	

[a] Neutrophil count <0.5 x10^9/l or leucocyte count <1.0 x10^9/l.

The incidence of IPA during the 1st and 2nd cycles of chemotherapy is given in table 2. Twenty-five patients (23.4%) in the control group developed probable or proven IPA versus 12 patients (9.5%) in the L-AmB group, a significant decrease in probable or proven IPA (p=0.0064). In a separate analysis, the incidence of probable or proven IPA according to treatment episode was analysed for the entire cohort of 235 patients and included all IPA cases diagnosed until 3 months after all therapy had been completed, including HSCT if applicable (table 3). During the 1st chemotherapy cycle, 16 of 108 patients in the control group developed probable or proven IPA versus 10 of 127 patients in the L-AmB group (p=0.1). In

total, 92 patients in the control group received the 2nd chemotherapy cycle and 99 patients in the L-AmB group. During the 2nd chemotherapy cycle, control patients developed IPA significantly more often than L-AmB patients (p=0.0246). The difference in IPA numbers in table 2 versus table 3 is explained by the fact that 6 patients with IPA were excluded from table 2 (2 patients with unusual combination of chemotherapy in 1 hospital stay as mentioned above, 1 patient receiving the 2nd chemotherapy cycle combined with HSCT in 1 hospital stay, and 3 patients who developed IPA during or shortly after their HSCT).

Table 2. Incidence of IPA and IFI during 1st and 2nd cycles of chemotherapy according to control versus L-AmB inhalation group.

Category	Control group n = 107		L-AmB inhalation group n = 126		p-value
	Count	%	Count	%	
IPA					
Probable or proven IPA	25	23.4%	12	9.5%	0.0064
No IPA and no antifungal therapy	54	50.5%	92	73.0%	0.0004
Possible/probable/proven	34	31.8%	18	14.3%	0.0014
Possible IPA (specific abnormalities on CT but culture plus antigen negative)	9	8.4%	6	4.8%	
Treatment for nonspecific abnormalities on CT, culture or antigen positive	0		3	2.4%	
Treatment for nonspecific abnormalities on CT, culture or antigen negative	10	9.3%	10	7.9%	
Empirical therapy (CT normal, antigen negative)	9	8.4%	3	2.4%	
IFD					
No IFD	101	94.4%	120	95.2%	1.0000
Candida spp.	2	1.9%	3	2.4%	
Mucor spp.	2	1.9%	3	2.4%	
Other IFD[a]	2	1.9%	0		

NOTE. IPA, invasive pulmonary aspergillosis. IFD, invasive fungal disease, excluding *Aspergillus spp*. CT, computed tomography.

[a] Other IFD, including *Cladosporium-speciës*, undefined fungal species.

Table 3. Incidence of proven/probable invasive pulmonary aspergillosis (IPA) according to treatment.

Treatment	Control group n = 108	L-AmB inhalation group n = 127	p-value
Overall	28/108	15/127	0.0066
1st chemotherapy[a]	16/108	10/127	0.0994
2nd chemotherapy[a]	11/92	3/99	0.0246
3rd chemotherapy	0/34	0/38	NA
Allogeneic HSCT	1/28	2/46	1.0000

NOTE. HSCT, hematopoietic stem cell transplantation. All 235 patients were used for this analysis, included patients who underwent HSCT.

[a] Patients who had their 1st or 2nd chemotherapy cycle combined with another chemotherapy cycle or HSCT and developed proven/probable IPA are counted in the initial chemotherapy group.

No difference in the incidence of IFD other than *Aspergillus* species was seen (table 2). During the 1st and 2nd chemotherapy cycle, systemic antifungal therapy decreased from 49.5% to 27.0% (table 2). Systemic antifungal therapy given at any time during the entire AML therapy decreased from 52.8% to 29.9%. We did not observe any major safety issues and no treatment-related serious adverse events were seen. The incidence of ICU admission during the 1st or 2nd chemotherapy cycle was comparable in both groups (20 in controls, 23 in L-AmB). None of the ICU admissions in the L-AmB group could be related to L-AmB inhalation.

A detailed overview of all medical costs is given in table 4. The control group had higher costs related to diagnostics (1101 euro/patient, p<0.0001) and related to voriconazole therapy (715 euro/patient, p=0.0031) in comparison to the L-AmB group. Therefore, the costs per patient for nebulization equipment and L-AmB drug acquisition (1292

Table 4. Mean medical costs (€) per hospital admission and per patient, control versus L-AmB inhalation group.

Cost category	Hospital admission for chemotherapy cycle								p-values
	Control group				L-AmB inhalation group				
	1 n = 94	2 n = 77	1+2[d] n=13	Total mean cost n=107	1 n = 111	2 n = 83	1+2[d] n=15	Total mean cost n = 126	Total mean costs
Diagnostics									
Total	1625	1561	3789	3011	1237	963	1557	1910	0.0000
Radiologic	622	539	1467	1113	502	345	588	739	0.0000
Microbiologic	826	861	1801	1564	580	543	817	966	0.0000
Other[a]	177	161	521	334	155	76	152	205	0.0082
Medication									
Total	2629	4251	7558	6287	4371	6107	4267	7530	0.4325
Voriconazole[b]	1144	1429	4492	2579	815	1469	1497	1864	0.0031
L-AmB inhalation	NA	NA	NA	NA	1292	NA	1292	1292	NA
Other antifungals[c]	951	2370	1874	2769	1709	2955	647	3529	0.5524
Antibiotics	533	453	1192	939	555	389	830	844	0.1553
Hospital stay	26231	28995	45146	49395	26698	26227	41994	45795	0.1046
Transfusions	7276	7071	15873	13409	7716	6383	11645	12388	0.1113
Total costs	37761	41878	72366	72102	40022	38388	59463	67624	0.2806

NOTE. NA, not applicable. Because costs are typically highly skewed, p-values were derived from 1000 non-parametric bootstrap samples drawn with replacement.

[a] Other diagnostic costs (e.g., include bronchoscopy, pathology, colonoscopy, gastroscopy and echocardiogram).

[b] Voriconazole first 8 days intravenous, followed by oral voriconazole if oral intake was possible at that time.

[c] Increased other antifungal costs in L-AmB group were caused by intravenous L-AmB use in 6 patients (median 38 days).

[d] Group of patients who had their 1st and 2nd chemotherapy cycle in one hospital admission.

euro/patient) were more than compensated by the significant decrease in per patient costs of diagnostics and voriconazole therapy. The overall treatment costs per patient were 4478 euro/patient less in the L-AmB group. This was not significantly different from the control group because extreme outliers mainly drove this difference.

IPA-related death, defined as death within 6 weeks after IPA diagnosis was seen in 2 patients (1.9%) in the control group versus 3 (2.4%) in the L-AmB group. During the 1st or 2nd chemotherapy cycle, 14/107 patients (13.1%) died in the control group versus 15/126 (11.9%) in the L-AMB group. The overall mortality 3 months after the end of all therapy was 19 (17.6%) in the control group versus 23 (18.1%) in the L-AmB group.

DISCUSSION

In 2008, the efficacy of inhaled L-AmB for the prevention of IPA was demonstrated in the context of a RCT. In 2010, this resulted in a BI recommendation for the use of this intervention in the European Conference on Infections in Leukaemia (ECIL) guidelines for AML patients during remission induction and consolidation chemotherapy [9]. It is difficult to predict how interventions work in real-life outside the context of a RCT. In this study we established the external validity of the RCT outcomes in a real-life AML patient population. Overall, the incidence of IPA was significantly higher in historical control patients than patients inhaling aerosolized L-AmB (23.4% versus 9.5%). No specific exclusion criteria were used apart from an already established IPA diagnosis at the time of AML diagnosis for which antifungal therapy instead of prophylaxis needed to be initiated. Apart from its efficacy, the use of L-AmB inhalation seems to be cost saving in regard to diagnostics and voriconazole therapy. These cost-savings more than compensated for the costs related to L-AmB inhalation itself.

The difference in IPA incidence was more pronounced during the 2nd than the 1st chemotherapy cycle. This may be the result of undiagnosed IPA at baseline in a small number of patients for which systemic therapy rather than inhalation prophylaxis should have been given. Only a study in which a high-resolution CT is performed in all patients at baseline to exclude patients with pre-existent intrapulmonary lesions could confirm this. One wonders if prophylaxis with a broad spectrum azole during the first 7 to 10 days may further reduce the IPA incidence during the 1st chemotherapy cycle.

We did not observe any major safety issues. The incidence of ICU admissions was comparable in both groups and none of the ICU admissions in the L-AmB group could be related to L-AmB inhalation. Still, an easier and more convenient way to get amphotericin-B inside the lungs would be very welcome. An inhalation amphotericin-B powder was developed several years ago, has favourable pharmacokinetic properties

and seems effective in an animal model [10]. Unfortunately, as far as we know no clinical trials are planned.

This study has some limitations. Considering that medicine evolves quickly, a time related bias might occur when controls are not from the same calendar year as cases. However, no considerable change has occurred in the chemotherapeutic agents that were used in the 1st and 2nd chemotherapy cycles during the study period of 2005 till 2012. Secondly, the diagnostic procedures have remained unchanged throughout the study and therefore diagnostic bias is unlikely.

We observed no difference in overall mortality 3 months after all therapy (including HSCT if applicable) between the L-AmB and control group. However, this comparison is likely to be confounded by indication as the chances of getting an allogeneic HSCT is less likely for patients with IPA and higher for patients in more recent years (due to a change in the AML risk classification). We also did not observe a decrease in the mortality attributable to IPA. This is not surprising, given the fact that IPA related mortality has decreased substantially with voriconazole therapy. It was estimated to be as low as 10% in a comparable AML patient population [3]. It is therefore not surprising that, despite the relatively large study population, no significant reduction in overall mortality was demonstrated.

Other prophylactic regimes to prevent IA have been studied in hematological patients with prolonged chemotherapy-induced neutropenia and in allogeneic HSCT patients. Prophylaxis with posaconazole showed a significant decrease in IFD (including IA) in both of these patient groups [11,12]. Voriconazole and itraconazole as prophylaxis have been investigated in allogeneic HSCT patients. Voriconazole showed no difference in the incidence of IFD in comparison to fluconazole in patients undergoing allogeneic HSCT [13]. Itraconazole reduced the incidence of IFD [14] and invasive mold infections [15] in comparison to fluconazole. However, the itraconazole groups in these two studies suffered significantly more gastrointestinal intolerance and toxicity, limiting its success as prophylaxis. Low dose L-AmB at 50 milligrams intravenously every other day reduced the incidence of IFD (including IA) in hematological patients with prolonged chemotherapy-induced neutropenia [16]. However, the control group in this study did not receive fluconazole as anti-yeast prophylaxis. No nephrotoxicity was observed and few patients discontinued L-AmB prophylaxis because of skin rash and infusion-related fever. However, patients with renal insufficiency or liver dysfunction were excluded from participating in this study, whereas in our current study all patients were included except for those who had preexistent IA. Lastly, micafungin as prophylaxis has been studied in allogeneic HSCT patients during their neutropenic period [17]. The study showed that micafungin reduced the IFD incidence, but the reduction in IA was not statistically significant, possibly because micafungin was only administered during the hospital stay

(18 days on average). The cost-effectivity of these intravenously administered antifungals remains to be demonstrated.

Aspergillus fumigatus was cultured in five patients, all in the control group at a time when azole resistance testing was not performed as a routine procedure in our hospital. Azole susceptibility testing was performed in one of the five patients who developed probable pulmonary and cerebral aspergillosis during the 2nd chemotherapy cycle. He was treated with voriconazole and caspofungin. Despite treatment, patient deteriorated clinically and died of progressive IA. The isolate that was cultured from sputum showed resistance to itraconazole (minimum inhibitory concentration (MIC) 16 µg/ml) and voriconazole (MIC 8 µg/ml). In the new millennium, azole resistant *Aspergillus fumigatus* has become a significant problem in the Netherlands [18,19]. More recently, resistance was also observed in several other European countries [20-23]. This is probably the consequence of extensive agricultural azole use [24]. The use of L-AmB inhalation may therefore become an attractive option for the prevention of IPA when prophylactic as well as therapeutic azole use becomes less effective.

With the current study results available and together with the clinical trial data from 2008, we think it is time to move forward and study the use of L-AmB inhalation in other patients at high risk for IPA, in particular in patients with serious graft versus host disease.

Prophylactic L-AmB inhalation administered twice a week to AML or MDS patients undergoing remission induction and consolidation chemotherapy resulted in a substantial decrease in IPA incidence. Furthermore, the costs of inhalation therapy were more than compensated by a decrease diagnostic costs and voriconazole therapy costs, both of which decreased significantly.

FUNDING

This study was supported by a grant from Gilead Sciences to B.J.A. Rijnders.

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