mycoses
Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Original article

Multicentre surveillance study on feasibility, safety and efficacy of antifungal combination therapy for proven or probable invasive fungal diseases in haematological patients: the SEIFEM real-life combo study

A. Candoni,[1] M. Caira,[2] S. Cesaro,[3,a] A. Busca,[3,a] M. Giacchino,[3,a] R. Fanci,[3,a] M. Delia,[3,a] A. Nosari,[3,a] A. Bonini,[3,a] C. Cattaneo,[3,a] L. Melillo,[3,a] C. Caramatti,[3,a] G. Milone,[3,a] R. Scime',[3,a] M. Picardi,[3,a] R. Fanin,[1] L. Pagano[2] on behalf of the SEIFEM GROUP (Sorveglianza Epidemiologica Infezioni Fungine nelle Emopatie Maligne)

[1]*Division of Hematology, University of Udine, Italy,* [2]*Division of Hematology, UCSC, Rome, Italy and* [3]*SEIFEM Group, Italy*

Summary

This multicentre observational study evaluated the feasibility, efficacy and toxicity of antifungal combination therapy (combo) as treatment of proven or probable invasive fungal diseases (IFDs) in patients with haematological malignancies. Between January 2005 and January 2010, 84 cases of IFDs (39 proven and 45 probable) treated with combo were collected in 20 Hematological Italian Centres, in patients who underwent chemotherapy or allogeneic haematopoietic stem cell transplantation for haematological diseases. Median age of patients was 34 years (range 1–73) and 37% had less than 18 years. Acute leukaemia was the most common underlying haematological disease (68/84; 81%). The phase of treatment was as follows: first induction in 21/84 (25%), consolidation phase in 18/84 (21%) and reinduction/salvage in 45/84 (54%). The main site of infection was lung with or without other sites. The principal fungal pathogens were as follows: *Aspergillus* sp. 68 cases (81%), *Candida* sp. six cases (8%), *Zygomycetes* four cases (5%) and *Fusarium* sp. four cases (5%). The most used combo was caspofungin+voriconazole 35/84 (42%), caspofungin + liposomal amphotericin B (L-AmB) 20/84 (24%) and L-AmB+voriconazole 15/84 (18%). The median duration of combo was 19 days (range 3–180). The overall response rate (ORR) was 73% (61/84 responders) without significant differences between the combo regimens. The most important factor that significantly influenced the response was granulocyte (PMN) recovery (*P* 0.009). Only one patient discontinued therapy (voriconazole-related neurotoxicity) and 22% experienced mild and reversible adverse events (hypokalaemia, ALT/AST increase and creatinine increase). The IFDs-attributable mortality was 17%. This study indicates that combo was both well tolerated and effective in haematological patients. The most used combo regimens were caspofungin + voriconazole (ORR 80%) and caspofungin + L-AmB (ORR 70%). The ORR was 73% and the mortality IFD related was 17%. PMN recovery

Correspondence: Anna Candoni, MD, Division of Hematology and BMT, University of Udine, Italy.
Tel.:+39 432 559662. Fax:+39 432 559661.
E-mail: candoni.anna@aoud.sanita.fvg.it

Note. A part of this study was presented (oral communication) at 21st European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Milan, 2011.

[a]See appendix for authors' affiliation.

Submitted for publication 27 August 2013
Revised 14 October 2013
Accepted for publication 9 November 2013

during combo predicts a favourable outcome. Clinical Trials Registration: NCT00906633.

Key words: Combined antifungal therapy, invasive fungal disease, caspofungin, amphothericin, voriconazole, posaconazole.

Introduction

Recent data from haematological series suggest that mortality attributable to invasive fungal infections (IFDs) has decreased, probably due to the application of a correct and timely diagnostic evaluation and to the availability of newer, well tolerated and more efficacious drugs (echinocandins, extended spectrum triazoles, lipid formulations of Amphotericin B).[1–4] However, the efficacy of current antifungal therapies is still suboptimal, particularly in some categories of patients (allogenic HSCT recipients) or in some rare fungal infections (zygomycosis, fusariosis).[2,3] The expansion of the antifungal armamentarium led to the opportunity to revise the traditional approaches to treat IFDs and to the chance to further improve the outcome.

Combination antifungal therapy (combo) is not a new concept, as its role has been well established for various infectious diseases, such as cryptococcal meningitis.[5,6] Unfortunately, whether combo may affect the outcome of IFDs is still controversial, since only limited data are available. This is even more unclear for the issue of rare non-*Aspergillus* moulds. A large number of studies performed *in vitro* or in animal models have examined the effects of combo suggesting potential benefits for difficult-to-treat mycosis.[7–13] Unfortunately, results of preclinical studies cannot be translated into clinical decisions and many unanswered questions do persist. Efficacy and safety still need to be proved, particularly in the setting of a prospective clinical trial.

The aim of this multicentre observational study was to collect data on the use of this off-label treatment for proven and probable IFDs among Italian Hematological Centres and to assess the feasibility, toxicity and efficacy of antifungal combo strategy in patients with haematological malignancies.

Patients and methods

Patients with any type and stage of haematological malignancy and patients undergoing autologous or allogeneic HSCT were considered eligible for this survey. All participating centres had received a specific Case Report Form to retrospectively register all cases treated with combo. Patient data were queried for haematological underling disease, medical history, predisposing factors to IFDs, IFD sites and aetiology, IFD-related therapy and outcome. The presence of neutropenia was assessed at baseline and at the end of combo for all cases. The Platelia Aspergillus enzyme-linked immunoassay (Bio-Rad, Redmont, WA) was used to detect galactomannan and index of 0,5 or greater in two consecutive samples was considered as positive.[4,14] All reported cases were reviewed by two independent physicians (AC and LP) and only those identified as proven or probable IFDs (according to EORTC criteria) were included in the survey.[14]

Efficacy of combo was based on the investigator assessment at the end of treatment (considering clinical, radiographic and microbiological response). In line with current standard definitions, both complete (CR) and partial responses (PR) were considered as success; all other responses were classified as failure.[15] In particular, patients were considered refractory to the treatment if clinical and radiological signs indicated a progressive infection after at least 7 days of antifungal combo therapy with adequate drug doses. Toxicity was evaluated and graded in accordance with WHO criteria.

Overall mortality was defined as any death within 12 weeks from the diagnosis of proven or probable IFD. Mortality was considered attributable to the IFD (IFD-attributable mortality) when patients died within 12 weeks from the onset of a fever with microbiological, histological, or clinical evidence of an active IFD and if other potential causes of death could be excluded by the responsible physician.

Statistical analysis

Univariate analysis was performed using the *chi-squared test* with the following independent variables: age (cut-off 50 years), paediatric or adult patient (cut-off 18 years), certainty of diagnosis (proven vs. probable), status of haematological malignancies

(active or in remission), days of therapy (using cut-off 14 days), neutrophil count (PMN) at the end of therapy (cut-off 500/mmc), type of combo treatment (sequential vs. *ex novo*). Multivariate analysis was performed using a logistic regression model. Adjusted HRs and 95%-CIs were calculated. Statistical significance was set at P-value < 0.05. Endpoint for univariate and multivariate analysis was response to combo antifungal treatment (partial and complete responses). Survival curves were generated by the Kaplan–Meier method. Overall survival (OS) was calculated from the data of IFD diagnosis to death or to the last date of follow-up. The impact of clinical variables on survival was evaluated using the Cox hazard regression. The statistical analysis was performed using MedCalc version 12.5.0.0 (MedCalc statistical Software bvba, Belgium).

Results

From January 2005 to January 2010, 84 cases of combo were collected among the 20 participating Italian centres. They were 38 females and 46 males with a median age of 34 years (range 1–73). Thirty-one patients were younger than 18 years (paediatric cases), but only 3/31 were less than 3 years old. All cases had haematological disease, as reported in Table 1, and 35% (29/84) of them had undergone HSCT.

Acute leukaemia was the most common underlying haematological disease (68/84; 81%).

The majority of patients had refractory or relapsing disease (45/84, 54%), whereas the remaining were at the onset of their disease (21/84, 25%) or in remission (18/84, 21%).

Causative agents and infection sites

IFDs were classified as proven in 39/84 cases (46%) and probable in 45/84 cases (54%). The site of IFD was lung in 56 of 84 patients (67%) whereas 25/84 (30%) of cases had disseminated infections with two or more sites involved.

Aspergillus species was identified as causative agent in the majority of cases (68/84, 81%) (Table 1). In detail, the causative agents in proven IFD cases were as follows: *Aspergillus fumigatus* (6), *Aspergillus flavus* (5), *Aspergillus niger* (2), *Aspergillus terreus* (1), *Aspergillus* sp. (9), *Fusarium* sp. (4), *Mucor* sp. (2), *Rhizopus* (2), *Candida not albicans* (6), *Blastoschizomyces* (1) and *Peacillomyces* (1).

Previous therapy

A total of 57/84 (68%) of patients had received previous antifungal prophylaxis with fluconazole (31/57), itraconazole (20/57), posaconazole (3/57) or L-AmB (3/57). Hence, 67/84 (80%) had received a previous antifungal monotherapy (empiric 39/67, preemptive 22/67 or target 6/67), with a median duration of 9 days (range 3–46). Prior antifungal therapies included amphothericin B lipid formulations (45/84, 54%), caspofungin (10/84, 12%), voriconazole (9/84, 11%), posaconazole or itraconazole (3/84, 4%). In addition, 8/84 (10%) patients had previously received more than one line of antifungal monotherapy.

Table 1 Patient and IFD characteristics.

Total cases	84		
Age (years)		Duration of combo therapy	
Mean ± SD	35 ± 21	Mean ± SD	31 ± 33
Median (range)	34 (1–73)	Median (range)	19 (3–180)
		IFD diagnosis (EORTC/MSG)	
Adult patients	53/84	Proven	39/84 (46%)
Paediatric patients	31/84	Probable	45/84 (54%)
Underlying haematological disease		Fungal pathogens	
Acute leukaemia	68/84	*Aspergillus* spp.	68/84
Lymphoma/myeloma	5/84	Zygomycetes	4/84
MDS/SAA	6/84	*Fusarium* spp.	4/84
Chronic leukaemia	5/84	*Candida* spp.	6/84
		Other*	2/84
Status of underling disease		Sites of IFD	
Onset	21/84	Pulmonary	56/84
Relapse/refractory	45/84	Paranasal sinuses only	3/84
Complete remission	18/84	Disseminated (two sites or more)	25/84
Previous stem cell transplant	29/84 (35%)	Surgery	14/84

*1 Blastoschizomyces; 1 Peacillomyces; SAA, severe aplastic anaemia; MDS, myelodysplastic syndromes.

The neutrophil count (PMN) at the start of combo was below 1000/mmc in 75% of cases and 56/84 (67%) patients had PMN less than 500/mmc. Only 21/84 (25%) of patients had more than 1000 PMN/mmc.

Type of combo

Combo antifungal therapy was started *de novo* (with two new antifungal drugs) in 45/84 patients (54%) whereas in 39/84 cases (46%) it was a sequential therapy, adding a new antifungal agent to the prior antifungal monotherapy. The most frequently used combo was as follows: caspofungin + voriconazole (35/84, 42%), caspofungin + liposomal amphotericin B (L-AmB) (20/84, 24%) and L-AmB + voriconazole (15/84, 18%) (Table 2). The median duration of combo was 19 days (range 3–180).

Response to treatment and prognostic factors

The overall response rate (ORR) was 73% (61/84 responders) with 29 (35%) of CR and 32 (38%) of PR, without significant differences between the combo regimens as reported in Table 2.

If we consider the response to combo according to the different causative agents we had: 71% (48/68) of ORR in Aspergillosis, 50% (2/4) of ORR in Zygomycosis, 100% (6/6) in Candidiasis and 75% (3/4) in Fusariosis.

No differences in terms of ORR were recorded between paediatric and adult cases (81% vs. 71% respectively; *P* 0.4). However, paediatric cases (31) compared to adult patients (53) had these significant differences: more frequent use of caspofungin + voriconazole combination (19/31 vs. 15/53; *P* 0.006), longer duration of combo therapy (40 vs. 18 days; *P* 0.005) and more frequent use of surgery (9/31 vs. 5/53, *P* 0.004).

In 14/84 cases of IFDs (nine paediatric and five adult patients) the combo was associated with a surgical approach including: lung lobectomy (9 cases), liver lobectomy (2 cases), tonsillectomy (1 case), vitrectomy (1 case) and excision of mycotic brain abscess (1 case). The ORR of combo plus surgery in these cases was 79% (11/14).

In univariate analysis, the only factor that significantly influenced the response of combo was PMN recovery (PMN greater than 500/mmc) during combo (*P*-value 0.001) and the type of IFD (proven vs. probable IFDs, *P*-value 0.02). Other factors such as patient's age (greater or less than 50 years, paediatric cases vs. adults), status of underlying disease, previous antifungal prophylaxis, strategies for use of antifungal drugs (sequential vs. *de novo* combo), and duration of combo, did not affect the outcome (Table 3). At multivariate analysis, only PMN recovery during combo remained significant (*P*-value 0.009).

Toxicity of combo

Overall, combo was well tolerated. No serious side effects were observed. Only one patient discontinued therapy (voriconazole-related neurotoxicity) and 22% experienced mild (grades I–II WHO) and reversible adverse events (hypokalaemia, liver toxicity and transient creatinine increase) (Table 4).

Follow-up

After combo, 74% (45/61) of responsive patients received a maintenance therapy with oral voriconazole (34) or posaconazole (11) for a median of 62 days (range 14–380).

After a median follow-up of 4 months (range 1–48), 48/84 (57%) of the patients included in this study have died, mainly due to the refractory or progressive underlying haematological disease. In 14 cases, the IFD was the primary cause of death, with an IFDs-attributable mortality rate of 17% (14/84 cases).

At 12- and 24-week probability of survival (OS) of these patients was 62% and 52%, respectively

Table 2 Responses (overall response rate, ORR; complete response, CR; partial response, PR) to combo therapy in proven and probable IFD.

Combo therapy and responses	Total cases	ORR	CR	PR	NR
Caspofungin + Voriconazole	35 (42%)	28 (80%)	14	14	7
L-AMB + Caspofungin	20 (24%)	14 (70%)	10	4	6
L-AMB + Voriconazole	15 (18%)	11 (73%)	2	9	4
L-AMB + Caspo + Vorico	5 (6%)	2 (40%)	1	1	3
L-AMB + Posaconazole	4 (5%)	3 (75%)	1	2	1
Abelcet + Caspo or Vorico	2 (2.5%)	1 (50%)	0	1	1
Caspofungin + Posaconazole	3 (4%)	2 (67%)	1	1	1
Total	84	61 (73%)	29 (35%)	32 (38%)	23 (27%)

Table 3 Factors affecting response to combo antifungal therapy.

Response to combo therapy-univariate analysis				Response to combo therapy-multivariate analysis			
Variable	HR	95% CI	*P* Value	Variable	HR	95% CI	*P* Value
Age ≥ 50 vs. <50	0.88	0.33–2.35	0.79				
Adult vs. paediatric patients	1.22	0.46–3.30	0.69				
Proven vs. probable IFD	3.34	1.16–9.62	0.02	Proven vs. probable IFD	2.10	0.65–6.80	0.22
Active haematological disease vs. remission	0.28	1.06–1.31	0.07	Active haematological disease vs. remission	0.76	0.13–0.39	0.76
Days of therapy >14 vs. ≤14	1.02	0.99–1.04	0.1	Days of therapy >14 vs. ≤14	1.30	0.43–3.95	0.64
PMN at stop >500/mmc vs. ≤500/mmc	0.19	0.07–0.53	0.001	PMN at stop >500/mmc vs. ≤500/mmc	0.21	0.07–0.68	0.009
Combo *ex novo* vs. Combo addition	1.94	0.72–5.24	0.19				

HR, hazard ratio; 95% CI, 95% confidential interval.

Table 4 Combo therapy and side effects.

Side effects	Paediatric patients	Adults patients	All cases
Hypokalaemia (I–II WHO)	4/31	4/53	8/84 (10%)
Liver toxicity (I–II WHO)	2/31	2/53	4/84 (5%)
Renal toxicity (I–II WHO)	1/31	2/53	3/84 (4%)
Neurologic toxicity	0/31	2/53	2/84 (3%)
Stop due to toxicity	0/31	1/53	1/84 (1.2%)

(Fig. 1A). The responsive patients to the combo had better OS than non-responder cases and the patients who recovered PMN (>500/mmc) during combo had significantly better survival than neutropenic (PMN < 500/mmc) cases (75% vs. 34% at 12 week, *P* 0.005) (Fig. 1B).

Discussion

The combo antifungal therapy is still an open issue in haematology because the potential advantages compared to monotherapy are not yet demonstrated.[16–20]

Well-designed and randomized trials are required to define the role of this strategy in haematological patients. However, performing a combination trial in the clinical setting of proven/probable IFDs would be expensive and quite challenging.[16–20] In fact, more than 500 cases are required to perform a randomized trial with an adequate power in this context.[16–20]

In vitro and experimental animal models produced very promising results in terms of efficacy and tolerability of this therapeutic approach.[7–13] However, as we do not have any large prospective and randomized clinical trial with an adequate statistical power, combo in IFDs has now a low level of evidence and recommendation in all current international guidelines.[21–24]

In this study, we report the results of a real-life experience in haematological patients.

Despite the limits of our study design (observational, not randomized) we think it may be of help in displaying the current role of combo in daily clinical practice and in analysing its tolerability and feasibility in haematological patients with proven/probable IFDs. Overall, combo antifungal therapy has been rarely used in the 20 participating centres over 2005–2010 (less than one case/year/centre), mostly in patients with acute leukaemia or after allogeneic HSCT. The most common combinations were those including caspofungin (included in 76% of combinations), voriconazole (in 67% of combinations) or L-AmB (in 52% of combinations). The ORR was 73% (with 35% CR and 38% PR) and the only factor significantly associated with response to combo, in multivariate analysis, was the PMN recovery during therapy.

In our experience, combo resulted to be well tolerated, both in children and in adult patients, and only one of them had to withdraw of treatment (1.2%) for voriconazole-related and reversible neurotoxicity. It is worth noticing that the IFDs-related mortality in this study was low (17%, 14/84 cases) and, as expected, that patients responsive to the combo had a significantly better survival than non-responsive cases.

The literature data on combo in haematology focus mainly on invasive aspergillosis (IA) and mucormycosis and seem to confirm the utility of this therapeutic approach. A summary of recent published clinical studies evaluating combination therapy for IA in haematological patients is reported in Table 5.[25–35]

In the context of IA, only two prospective studies on antifungal combo have been published.[25,26] In the first one, 53 patients with prevalence of pulmonary IA (81%) were treated with caspofungin in combination

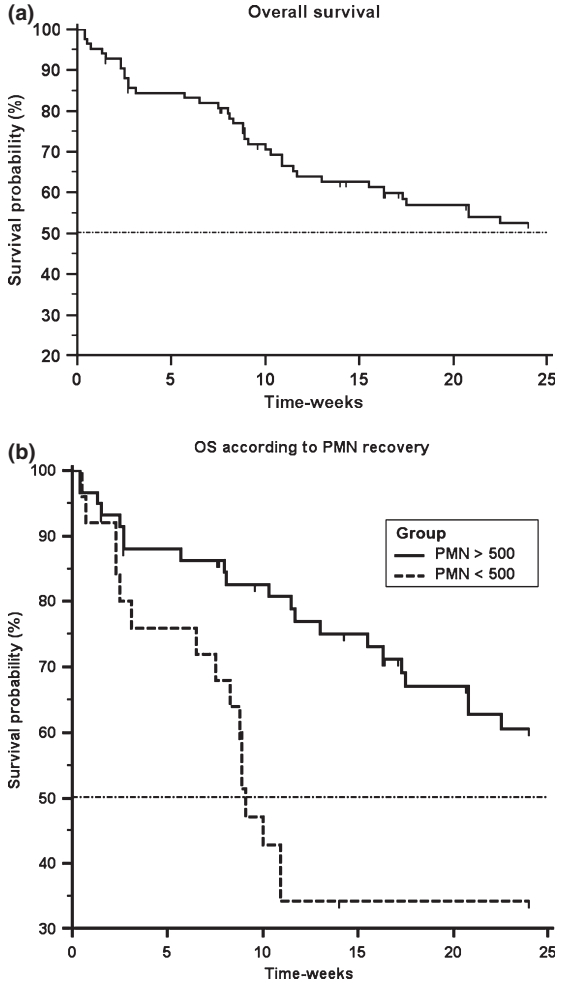

Figure 1 (A) Overall Survival (in weeks) of the whole population (84 cases). (B) Overall Survival according to PMN recovery during combo therapy.

with other antifungals; the most common underlying disease was acute leukaemia (53%) and most patients (87%) were refractory to prior antifungal therapy. The use of combo resulted in an ORR of 55% (29/53). Fifty-seven per cent of patients with neutropenia and 54% who had received an allogeneic HSCT responded favourably with proportion of survivors being 55% by day 84. Combo was well tolerated. Two (4%) serious drug-related adverse events were reported, both attributed to voriconazole. None of the patients discontinued caspofungin due to toxicity. This analysis showed that caspofungin in combination with a triazole or polyene was an effective alternative to salvage monotherapy for patients with IA.[25]

In the second prospective study (Combistrat pilot trial) only 30 patients with haematological malignancies were analysed (15 in each arm). The median duration of treatment was 18 days for the combo group (caspofungin plus standard dose of L-AmB) and 17 days for the high-dose monotherapy group (L-AmB 10 mg Kg^{-1} per day). At the end of treatment, the ORR in the combo arm was significantly superior (67%) compared to monotherapy (27%; *P* 0.028). Survival rates at 12 weeks were 100% and 80% respectively. Infusion-related reactions occurred in three patients in the high-dose monotherapy arm. A twofold increase in serum creatinine occurred in four of 17 patients (23%) who received high-dose monotherapy and one of 15 patients (7%) who received combo.[26]

As for retrospective studies in IA, Kontoyiannis *et al.* [27] analysed 48 cases of documented (23) or possible (25) progressive IA, treated with caspofungin plus L-AmB (65%) as salvage therapy, following 7 or more days of a previous L-AmB monotherapy. The ORR was 42% and no significant toxic effects were seen. In the retrospective analysis by Aliff *et al.* [28], 30 haematological patients were treated with caspofungin plus L-AmB. The median duration of combo was 24 days and the ORR was 60%. In a small, single-centre study, Marr *et al.* [29] evaluated the outcome of 47 patients with IA who experienced failure of first-line therapy with AmB formulations and received either voriconazole (31) or a combination of voriconazole and caspofungin (16) for salvage therapy. The combo of voriconazole plus caspofungin was associated with an improved 3-month survival compared to voriconazole alone.

Another very recent retrospective analysis by Rojas *et al.* [30] reported the outcome of 61 haematological patients treated with different combinations in Spanish Hematologic Centers (L-AmB + caspo, 20; L-AmB + triazole, 20; voriconazole+echinocandin, 21). Combo was well tolerated and 38 (62%) patients achieved a response (with 35 CR), regardless of the combo type.

We have also to underline that Marr *et al.* presented at ECCMID 2012 the final results of the largest (459 patients) prospective, randomized 1:1, phase III trial that compares efficacy and safety of voriconazole plus anidulafungin or placebo in newly diagnosed IA. This study was conducted at 93 sites from 2008 to 2011 and the primary endpoint was 6-week all-cause mortality. Results demonstrated that in the modified intent-to-treat population, 6-week mortality was 19.3% for the patients in voriconazole plus anidulafungin group and 27.5% for the patients in voriconazole plus placebo group (*P* 0.09). This study showed, for the first time, that a combination of two antifungals determines a reduction in early mortality.

Table 5 Summary of recent published studies evaluating combination therapy for invasive aspergillosis in haematological patients.

Author	Patients number	Type of study	Strategy	Type of combo therapy	Response rate	OS
Maertens *et al.* [25]	53	Prospective	Salvage (proven/probable IA)	Caspo + Itra Caspo + Vorico Caspo + AmB	Overall 55% 43% 60% 50%	55% (12 w)
Caillot *et al.* [26]	30	Prospective	First line (proven/probable IA)	Caspo + L-AmB vs. L-AmB	67% vs. 27%	100% (12 w) vs. 80%(12 w)
Kontoyiannis *et al.* [27]	48	Retrospective	Salvage and first line (proven/probable(23)/ Possible (25) IA)	Caspo + L-AmB	Overall 42% 53% (first line), 35% (salvage)	65% (12 w)
Aliff *et al.* [28]	30	Retrospective	Salvage (proven/probable (10)/ Possible (20) IA)	Caspo + L-AmB	Overall 60%	NR
Marr *et al.* [29]	47	Retrospective	Salvage (proven/probable IA)	Caspo + Vorico (16) vs. Vorico (31)	NR	63% (12 w) vs. 32% (12 w)
Kontoyiannis *et al.* [31]	112	Retrospective	First line (proven/probable IA)	Itraco + L-AmB vs. L-AmB	0% vs. 10%	9% vs. 24%
Rieger *et al.* [32]	56	Retrospective	Salvage [proven (30)/probable (15)/possible (11) IA (62%)]	Caspo + L-AmB (63%) Caspo + Vorico (21%) Other (16%)	Overall 65%	66% (12 w)
Kontoyiannis *et al.* [33]	90	Retrospective	Salvage (85%) first line (15%) (proven/probable IA)	Micafungin + L-AmB	Overall 24%	NR
Mihu *et al.* [34]	159	Retrospective	Salvage (proven/probable IA)	Echino (Caspo > 90%) + L-AmB vs. Caspo or L-AmB	combo 21% vs. 28% 9%	38% (12 w) vs. 39% (12 w) 33% (12 w)
Lellek *et al.* [35]	31	Retrospective	Salvage (proven/probable IA)	Caspo + Vorico	Overall 77%	NR
Rojas *et al.* [30]	61	Retrospective	Salvage [proven/probable IA (80%)]	Caspo + L-AmB (20). Triazole + L-Amb (20). Vorico + Candin (21)	Overall 62%	57% (12w)

NR, not reported.

The evidences on clinical efficacy and tolerability of the combo in Mucormycosis are even more limited, given the rarity of this IFD. The SEIFEM and FUNGISCOPE registries recently published the analysis of 32 cases of proven/probable invasive mucormycosis in haematological patients treated with a combination of L-AmB and posaconazole. Thirteen of them (41%) had also received surgical excision of the infected tissue. After a median follow-up of 3 months the ORR was 78% with 11 CR (34%), 7 PR (22%) and 5 stable diseases (16%). These results suggest a beneficial role of combo plus surgery in invasive mucormycosis.[36]

In summary, all these experiences, although difficult to compare, suggest that combo is well tolerated and it seems to be effective either as first or second line, with an ORR greater than 50% in aspergillosis and mucormycosis.[25–37]

However, the use of combo antifungal therapy may be limited by some drawbacks, like the higher costs when compared to single antifungal agents.

The emergence of resistant strains to antifungal drugs is also strictly related to the issue of combo because a resistant fungal pathogen should be suspected in patients not responsive to first-line antifungal monotherapy.[38,39] This could justify, in selected unresponsive patients the use of combo, especially when *in vitro* tests of resistance are not available.

In conclusion, this multicentre observational study on antifungal combo, as treatment of proven or probable IFDs in haematological patients, confirms the feasibility and good tolerability of combo and supports previous data on its efficacy.

The use of combo may be justified, in patients with responsive haematological cancer and proven or probable IFD, as antifungal bridge until PMN recovery occurs. In clinical practice, the combo therapy can also be used in combination with surgical approach with the aim of eradicating an IFD that could delay the entire therapeutic programme of underling haematological disease.[39] Clearly, a better characterization of patients with a very high risk of IFDs is therefore important for the selection of those who may be candidate to this kind of treatment as first-line approach.[40] Finally, given the high costs of combo treatment, it may also suggest to reserve combo to those patients without refractory or progressive underlying haematological disease for whom we might expect a very good life expectancy if IFD is cured.

In addition, well-controlled studies are still required to adequately determine the most efficacious antifungal combination regimens for specific fungal infections and to evaluate the pharmaco-economic impact of this strategy.

Authors' contributions

AC, MC and LP conceived the study and drafted the manuscript. AC and MC collected the data and performed the statistical analysis. All authors read and approved the final manuscript.

Disclosures

AC has received honoraria from Gilead Sciences, Merck, and Pfizer Pharmaceuticals, she has been speaker for Gilead Sciences, Merck, Pfizer Pharmaceuticals. MC has received honoraria from Gilead Sciences and Merck; she has been speaker for Gilead Sciences, Merck, Pfizer Pharmaceuticals, Schering-Plough, and Astellas Pharma. LP has received honoraria from Gilead Sciences, Schering-Plough, Astellas Pharma, Merck, and Pfizer Pharmaceuticals, he has been speaker for Gilead Sciences, Schering-Plough, Merck, Pfizer Pharmaceuticals, Astellas Pharma.

Appendix

S. Cesaro: Pediatric Hematology Oncology, AOU Integrata, Verona, It. A. Busca: Stem Cell Transplant Center, Ematologia II, AOU San Giovanni Battista, Turin, It. M. Giacchino: Pediatric Onco-Hematology, SCT and Cellular Therapy Division, Regina Margherita Children's Hospital, Turin, It. Fanci R: Haematology Unit, Careggi Hospital and University of Florence, It. Delia M: Hematology Section, Department of Emergency and Organ Transplantation, University of Bari, It. A. Nosari: Divisione di Ematologia, Niguarda Ca' Granda Hospital, Milano, It. A. Bonini: Transfusion Medicine Unit, IRCCS-Arcispedale Santa Maria Nuova, Reggio Emilia, It. C. Cattaneo: UO Ematologia, Spedali Civili, Brescia, It. L. Melillo: Hematology and SCT Unit, IRCCS, Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, It. C. Caramatti: Ematologia-CTMO AOU Parma, It. G. Milone: Division of Hematology and BMT, Ospedale "Ferrarotto", Catania, It. R. Scimè: Department of Oncology, Hematology Unit, Ospedale "La Maddalena", Palermo, It. M. Picardi: Department of Clinical Medicine and Surgery, Federico II University Medical School, Naples, It.

References

1 Pagano L, Caira M, Picardi M, *et al.* Invasive Aspergillosis in patients with acute leukemia: update on morbidity and mortality–SEIFEM-C report. *Clin Infect Dis* 2007; **44**: 1524–5.

2 Neofytos D, Horn D, Anaissie E, *et al.* Epidemiology and outcome of invasive fungal infection in adult hematopoietic stem cell

transplant recipients: analysis of Multicenter Prospective Antifungal Therapy (PATH) alliance registry. *Clin Infect Dis* 2009; **48**: 265–73.
3 Pagano L, Valentini CG, Posteraro B, *et al.* Zygomycosis in Italy: a survey of FIMUA-ECMM (Federazione Italiana di Micopatologia Umana ed Animale and European Confederation of Medical Mycology). *J Chemother* 2009; **21**:322–9.
4 Maschmeyer G, Haas A, Cornely OA. Invasive aspergillosis: epidemiology, diagnosis and management in immunocompromised patients. *Drugs* 2007; **67**: 1567–604.
5 Bennett JE, Dismukes WE, Duma RJ, *et al.* A comparison of amphotericin B alone and combined with flucytosine in the treatment of cryptoccal meningitis. *N Engl J Med* 1979; **301**: 126–31.
6 Day JN, Chau IT, Lalloo DG. Combination antifungal therapy for Cryptococcal Meningitis. *N Engl J Med* 2013; **368**: 2522–3.
7 Petraitis V, Petraitiene R, Sarafandi AA, *et al.* Combination therapy in treatment of experimental pulmonary aspergillosis: synergistic interaction between an antifungal triazole and an echinocandin. *J Infect Dis* 2003; **187**: 1834–43.
8 Arikan S, Lozano-Chiu M, Paetznick V, Rex JH. In vitro synergy of caspofungin and amphotericin B against Aspergillus and Fusarium spp.. *Antimicrob Agents Chemother* 2002; **46**: 245–7.
9 Kirkpatrick WR, Coco BJ, Patterson TF. Sequential or combination antifungal therapy with voriconazole and liposomal amphotericin B in a guinea pig model of invasive aspergillosis. *Antimicrob Agents Chemother* 2006; **50**: 1567–9.
10 MacCallum DM, Whyte JA, Odds FC. Efficacy of caspofungin and voriconazole combinations in experimental aspergillosis. *Antimicrob Agents Chemother* 2005; **49**: 3697–701.
11 Petraitis V, Petraitiene R, Hope WW, *et al.* Combination therapy in treatment of experimental pulmonary aspergillosis: in vitro and in vivo correlations of the concentration- and dose- dependent interactions between anidulafungin and voriconazole by Bliss independence drug interaction analysis. *Antimicrob Agents Chemother* 2009; **53**: 2382–91.
12 Ibrahim AS, Gebremariam T, Fu Y, *et al.* Combination echinocandin-polyene treatment of murine mucormycosis. *Antimicrob Agents Chemother* 2008; **52**: 1556–8.
13 Manavathu EK, Alangaden GJ, Chandrasekar PH. Differential activity of triazoles in two-drug combinations with the echinocandin caspofungin against Aspergillus fumigatus. *J Antimicrob Chemother* 2003; **51**: 1423–5.
14 Ascioglu S, Rex JH, de Pauw B, *et al.* Defining opportunistic invasive fungal infections in immune-compromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* 2002; **34**: 7–14.
15 Segal BH, Herbrecht R, Stevens DA, *et al.* Defining responses to therapy and study outcomes in clinical trials of invasive fungal diseases: Mycoses Study Group and European Organization for Research and Treatment of Cancer consensus criteria. *Clin Infect Dis* 2008; **47**: 674–83.
16 Lewis RE. Decision making in antifungal monotherapy versus combination therapy. *Pharmacotherapy* 2006; **26**: 61–67.
17 Lewis RE, Kontoyiannis DP. Rationale for combination antifungal therapy. *Pharmacotherapy* 2001; **21**: 149–64.
18 Kontoyiannis DP, Lewis RE. Toward more effective antifungal therapy: the prospects of combination therapy. *Br J Haematol* 2004; **126**: 165–75.
19 Johnson MD. Perfect JR Combination antifungal therapy: what can and should we expect ? *Bone Marrow Transplant* 2007; **40**: 297–306.
20 Powers JH. Considerations in clinical trials of combination antifungal therapy. *Clin Infect Dis* 2004; **39**(Suppl 4): S228–35.
21 Walsh TJ, Anaissie EJ, Denning DW, *et al.* Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis* 2008; **46**: 327–60.
22 Pappas PG, Kauffman CA, Andes D, *et al.* Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009; **48**: 503–35.
23 Maertens J, Marchetti O, Herbrecht R, *et al.* Third European Conference on Infections in Leukemia. European guidelines for antifungal management in leukemia and hematopoietic stem cell transplant recipients: summary of the ECIL 3–2009 update. *Bone Marrow Transplant* 2011; **46**: 709–18.
24 Böhme A, Ruhnke M, Buchheidt D, *et al.* Treatment of invasive fungal infections in cancer patients–recommendations of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Oncology (DGHO). *Ann Hematol* 2009; **88**: 97–110.
25 Maertens J, Glasmacher A, Herbrecht R, *et al.* Multicenter, noncomparative study of caspofungin in combination with other antifungals as salvage therapy in adults with invasive aspergillosis. *Cancer* 2006; **107**: 2888–97.
26 Caillot D, Thiebaut A, Herbrecht R, *et al.* Liposomal amphotericin B in combination with caspofungin for invasive aspergillosis in patients with hematologic malignancies: a randomized pilot study (Combistrat trial). *Cancer* 2007; **110**: 2740–6.
27 Kontoyiannis DP, Hachem R, Lewis RE, *et al.* Efficacy and toxicity of caspofungin in combination with liposomal amphotericin B as primary or salvage treatment of invasive aspergillosis in patients with hematologic malignancies. *Cancer* 2003; **98**: 292–9.
28 Aliff TB, Maslak PG, Iurcic JC, *et al.* Refractory Aspergillus pneumonia in patients with acute leukemia: successful therapy with combination caspofungin and liposomal amphotericin. *Cancer* 2003; **97**: 1025–32.
29 Marr KA, Boeckh M, Carter RA, *et al.* Combination antifungal therapy for invasive aspergillosis. *Clin Infect Dis* 2004; **39**: 797–802.
30 Rojas R, Molina JR, Jarque I, *et al.* Outcome of antifungal combination therapy for invasive mold infections in hematological patients is indipendent of the chosen combination. *Med J Hem Infect Dis* 2012; **4**: 1–8.
31 Kontoyiannis DP, Boktour M, Hanna H, Torres HA, Hachem R, Raad II. Itraconazole added to a lipid formulation of amphotericin B does not improve outcome of primary treatment of invasive aspergillosis. *Cancer* 2005; **103**: 2334–7.
32 Rieger CT, Ostermann H, Kolb HJ, *et al.* A clinical cohort trial of antifungal combination therapy: efficacy and toxicity in haematological cancer patients. *Ann Hematol* 2008; **87**: 915–22.
33 Kontoyiannis DP, Ratanatharathorn V, Young JA, *et al.* Micafungin alone or in combination with other systemic antifungal therapies in HSCT recipients with invasive aspergillosis. *Transpl Infect Dis* 2009; **11**: 89–93.
34 Mihu CN, Kassis C, Ramos ER, Jiang Y, Hachem RY, Raad II. Does combination of lipid formulation of amphotericin B and echinocandins improve outcome of invasive aspergillosis in hematological malignancy patients ? *Cancer* 2010; **116**: 5290–6.
35 Lellek H, Waldenmaier D, Dahlke J, *et al.* Caspofungin plus Posaconazole as salvage therapy of invasive fungal infections in immunocompromised patients. *Mycoses* 2011; **54**: 39–44.
36 Pagano L, Cornely O, Busca A, *et al.* Combined antifungal approach for the treatment of invasive mucormycosis in patients with hematological diseases: a report from the SEIFEM and FUNGISCOPE registries. *Haematologica* 2013; **98**: 127–30.
37 Marr KA, Schlamm H, Rottinghaus ST, *et al.* A randomised, double-blind study of combination antifungal therapy with voriconazole and anidulafungin versus voriconazole monotherapy for primary treatment of invasive aspergillosis. abstr LB 2812. Abstr. In 22nd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), London, March 2012.
38 Pfaller MA, Messer SA, Woosley LN, Jones RN, Castanheira M. Echinocandin and triazole antifungal susceptibility profiles of opportunistic yeast and mould clinical isolates (2010-2011): application of new CLSI clinical breakpoints and epidemiological cutoff values to characterize geographic and temporal trends of antifungal resistance. *J Clin Microbiol* 2013; **51**: 2571–81.
39 Even C, Bastuji-Garin S, Hicheri Y, *et al.* Impact of invasive fungal disease on the chemotherapy schedule and event-free survival in acute leukemia patients who survived fungal disease: a case-control study. *Haematologica* 2011; **96**: 337–41.
40 Lewis RE, Georgiadou SP, Sampsonas F, Chamilos G, Kontoyiannis DP. Risk factors for early mortality in haematological malignancy patients with pulmonary mucormycosis. *Mycoses* 2013; doi: 10.1111/myc.12101.