Original Articles

Outcome of Antifungal Combination Therapy for Invasive Mold Infections in Hematological Patients is Independent of the Chosen Combination

Rafael Rojas¹, José R. Molina¹, Isidro Jarque², Carmen Montes³, Josefina Serrano¹, Jaime Sanz², Juan Besalduch⁴, Enric Carreras⁵, José F. Tomas⁶, Luis Madero⁷, Daniel Rubio⁸, Eulogio Conde³, Miguel A. Sanz² and Antonio Torres¹.

The Departments of Hematology and Stem Cell Transplantation Units of:
1 University Hospital Reina Sofia, Cordoba. Spain.
2 University Hospital La Fe, Valencia. Spain.
3 University Hospital Marqués de Valdecilla, Santander. Spain.
4 University Hospital Son Dureta, Palma de Mallorca. Spain.
5 University Hospital Clinic, Barcelona. Spain.
6 University Hospital MD Anderson, Madrid. Spain.
7 University Hospital Niño Jesús, Madrid. Spain.
8 University Hospital Miguel Servet, Zaragoza. Spain.

Correspondence to: Miguel A. Sanz, MD, PhD. Hematology Department, University Hospital La Fe, Bulevar Sur s/n, 46026 Valencia. Spain. Tel/Fax number: 34-961 245 875. E-mail: msanz@uv.es

Competing interests: The authors have declared that no competing interests exist.

Abstract. Invasive mold infection (IMI) remains a major cause of mortality in high-risk hematological patients. The aim of this multicenter retrospective, observational study was to evaluate antifungal combination therapy (ACT) for proven and probable IMI in hematological patients. We analyzed 61 consecutive cases of proven (n=25) and probable (n=36) IMI treated with ACT collected from eight Spanish hospitals from January 2005 to December 2009. Causal pathogens were: Aspergillus spp (n=49), Zygomycetes (n=6), Fusarium spp (n=3), and Scedosporium spp (n=3). Patients were classified in three groups according to the antifungal combination employed: Group A, liposomal amphotericin B (L-AmB) plus caspofungin (n=20); Group B, L-AmB plus a triazole (n=20), and Group C, voriconazole plus a candin (n=21). ACT was well tolerated with minimal adverse effects. Thirty-eight patients (62%) achieved a favorable response (35 complete). End of treatment and 12-week survival rates were 62% and 57% respectively, without statistical differences among groups. Granulocyte recovery was significantly related to favorable response and survival (p<0.001) in multivariate analysis. Our results suggest that comparable outcomes can be achieved with ACT in high risk hematological patients with proven or probable IMI, whatever the combination of antifungal agents used.
Introduction. Severe neutropenia and immunosuppression resulting from the use of high-dose chemotherapy in hematological diseases followed, in selected cases, by allogeneic stem cell transplantation (allo-SCT), increase the susceptibility of patients to invasive fungal disease (IFD). The growing use of these aggressive therapies has led to a remarkable increase in the incidence of IFD. Although fluconazole prophylaxis has reduced yeast infections in these vulnerable populations, invasive mold infections (IMI), particularly those caused by Aspergillus spp, have steadily increased to a 10-20% incidence rate. Furthermore, a high mortality rate for aspergillosis, the most common IMI, has been described, especially among allo-SCT recipients. In addition, zygomycosis can reach attributable mortality rates of 91% in these patients.

Many attempts have been made to decrease the incidence and mortality of IFD in patients with hematological malignancies. Strategies including empiric therapy, pre-emptive treatment and improved prophylactic regimens with new azoles have been proposed. Monotherapy with new antifungal agents such as candins, triazoles and liposomal amphotericin B (L-AmB) has also been used as treatment for proven or probable IFD. However, though improved survival rates have been documented with the use of these individual agents, overall response and treatment outcome remain suboptimal in cases of IMI with high mortality rates.

Recently, several retrospective or uncontrolled clinical reports and, as far as we know, only one prospective randomized study have addressed the potential benefit of using combinations of new antifungal agents in the treatment of IFD. This issue is conceptually promising, because an additive activity or even synergy of antifungal drugs might be expected. Although these studies provide evidence supporting the use of this approach, the advantages of combined therapy have not yet been clearly demonstrated. Therefore, it is still unclear if antifungal combined therapy (ACT) is superior to monotherapy in severely neutropenic and/or immunocompromised patients with life-threatening IFD.

In this retrospective, observational, multicenter study, we describe the results of ACT in 61 proven or probable cases of IMI occurring in high-risk hematological patients treated with intensive chemotherapy or allo-SCT.

Patients and Methods. We have retrospectively analyzed all consecutive cases of high-risk hematological patients with proven or probable IMI treated with ACT in eight tertiary university hospitals in Spain over a 5-year period (January 2005 - December 2009). For inclusion in the analysis, patients must have received at least seven days of ACT. Antifungal agents used as primary prophylaxis, empiric treatments or in ACT depended on the specific policies of participating hospitals.

Definitions. Diagnosis of IMI was established according to the revised EORTC/MSG criteria. Responses to antifungal therapy were defined according to the MSG/EORTC consensus criteria as either favorable response (complete or partial) or failure (stable disease, progression or death from any cause).

Prophylaxis was defined as primary when patients did not have a previous history of IFD, while it was defined as secondary in patients with a previous IFD history but no signs or symptoms of fungal infection when the new hematological treatment was initiated.

ACT was started when the diagnosis of proven or probable IFD was made. The following doses were used in the treatment of adult patients: voriconazole, loading dose of 6 mg/kg/12 hours x 2 doses followed by 4 mg/kg/12 hours; posaconazole, 400 mg/12 hours; caspofungin, 70 mg on day 1 and 50 mg/day starting from day 2; anidulafungin, 200 mg on day 1 and 100 mg/day after and L-AmB, 3 mg/kg/day. The doses used in the treatment of children were: voriconazole, 7 mg/kg/12 hours; caspofungin, 70 mg/m² (maximum 70 mg) on the first day and 50 mg/m² (maximum 50 mg) each subsequent day and L-AmB, 3 mg/kg/day. All drugs were given intravenously but posaconazole was administered orally. Azole plasma levels were not monitored.

De novo ACT was defined as the combination of two antifungal drugs not used before for prophylaxis or empiric treatment. Sequential ACT occurred when an antifungal drug was added to another already being used for prophylaxis or empiric treatment.

Statistical Analysis. Data were collected in a SPSS database and all statistical results were performed using SPSS version 17. Either the chi-square or the Fisher’s exact test were used to compare data. Overall survival was analyzed using temporal series Kaplan-Meier analysis and comparisons between different treatments groups were performed using the log-rank test. For multivariate analysis, logistic regression was used. A p value of less than 0.05 was defined as statistically significant.

Results. 

Patients. Sixty-one patients were included in the study. The mean age was 43 years (range, 3-73) with 7 patients younger than 18 years. Thirty-four patients had received intensive chemotherapy for induction (n=28)...
Table 1. Patient characteristics

| Characteristics | All patients n (%)=61 (100) | Group A n (%)=20 (33) | Group B n (%)=20 (33) | Group C n (%)=21 (34) |
|----------------|----------------------------|------------------------|------------------------|------------------------|
| Age mean (range) | 43 (3-73) | 44 (8-64) | 48 (21-73) | 39 (3-72) |
| Sex (M/F) | 34 (56)/27 (44) | 11 (55)/ 9 (45) | 14 (70)/ 6 (30) | 9 (43)/12 (57) |
| Underlying disease | | | | |
| AML | 27 (44) | 11 (55) | 7 (35) | 9 (43) |
| ALL | 19 (31) | 3 (15) | 9 (45) | 7 (33) |
| SAA | 2 (3) | 1 (5) | 1 (5) | 0 |
| MDS | 8 (13) | 3 (15) | 2 (10) | 3 (14) |
| NHL | 3 (5) | 1 (5) | 1 (5) | 1 (5) |
| MM | 1 (2) | 1 (5) | 0 | 0 |
| BPL | 1 (2) | 0 | 0 | 1 (5) |
| Allo-SCT | 25 (41) | 8 (40) | 8 (40) | 9 (43) |
| HLA-identical sibling | 10 (16) | 2 (10) | 2 (10) | 6 (29) |
| Adult unrelated donor | 4 (6) | 1 (5) | 2 (10) | 1 (5) |
| Cord blood | 11 (18) | 5 (25) | 4 (20) | 2 (10) |
| High risk condition | | | | |
| SN | 45 (74) | 17 (85) | 13 (65) | 15 (71) |
| Acute GvHD + SN | 9 (15) | 1 (5) | 3 (15) | 5 (24) |
| Acute GvHD | 3 (5) | 1 (5) | 2 (10) | 0 |
| Chronic GvHD | 3 (5) | 1 (5) | 1 (5) | 1 (5) |
| Severe IS | 1 (2) | 0 | 1 (5) | 0 |
| Acute GvHD | Grade 0 | 13 (21) | 6 (30) | 3 (15) | 4 (19) |
| Grade I-II | 9 (15) | 2 (10) | 3 (15) | 4 (19) |
| Grade III-IV | 3 (5) | 0 | 2 (10) | 1 (5) |
| IFD diagnosis (EORTC/MSG) | | | | |
| Proven | 25 (41) | 8 (40) | 10 (50) | 7 (33) |
| Probable | 36 (59) | 12 (60) | 10 (50) | 14 (67) |
| Sites of Infection | | | | |
| Pulmonary | 51 (84) | 19 (95) | 13 (65) | 19 (90) |
| Disseminated | 8 (13) | 1 (5) | 5 (25) | 2 (10) |
| Paranasal sinuses | 2 (3) | 0 | 2 (10) | 0 |
| Fungal Pathogen | | | | |
| Aspergillus spp | 49 (67) | 18 (90) | 14 (70) | 17 (81) |
| Zygomycetes | 6 (10) | 0 | 3 (15) | 3 (14) |
| Scedosporium spp | 3 (5) | 1 (5) | 1 (5) | 1 (5) |
| Fusarium spp | 3 (5) | 1 (5) | 2 (10) | 0 |

M: male, F: female, HLA: human leukocyte antigen. AML: acute myeloid leukemia. ALL: acute lymphoblastic leukemia. SAA: severe aplastic anemia. MDS: myelodysplastic syndrome. NHL: non-Hodgkin lymphoma. MM: multiple myeloma. BPL: biphenotypic leukemia. SN: severe neutropenia. GvHD: Graft versus Host Disease. Group A: L-amB plus caspofungin. Group B: L-amB plus triazole (16 voriconazole and 4 posaconazole). Group C: Voriconazole plus candid (20 caspofungin and 1 anidulafungin).

or consolidation (n=6). Twenty-five patients were recipients of allo-SCT and one patient received an autologous SCT. The main demographic and clinical data are shown in Table 1.

Fifty-four patients (89%) were severely neutropenic (absolute neutrophil count <500/µL for at least 10 consecutive days) at the onset of IFD; of these, 9 had at the same time acute graft versus host disease (GvHD). Only 7 patients developed an IFD without severe neutropenia: 3 patients had acute GvHD, 3 patients chronic GvHD and 1 patient severe aplastic anemia under immunosuppressive treatment.

Prior antifungal prophylaxis was administered to 60 patients (23 voriconazole, 18 fluconazole, 18 itraconazol and 1 L-AmB). In 35 patients, prophylaxis was stopped when empirical antifungal treatment was initiated (9 patients were given voriconazole, 16 caspofungin and 10 L-AmB). The patient not receiving prophylaxis started empirical treatment with voriconazole. Only 3 patients with prior history of IFD (2 candidemias and 1 probable pulmonary aspergillosis) received secondary prophylaxis (1 fluconazole, 2 voriconazole).

_De novo_ ACT was started in 26 patients. In 23 other patients, combined treatment was initiated by adding a new antifungal agent to the empirical treatment. In the remaining 12 patients, a second antifungal drug was added to the prophylaxis regimen.

The diagnosis of proven IFD was confirmed in 25 patients, while the remaining 36 patients met the criteria for probable IFD. The molds isolated in proven IFD cases were as follows: _Aspergillus fumigatus_ (n=6), _A. flavus_ (n=3), _A. terreus_ (n=1), _Aspergillus_ spp. (n=3), _Mucor_ spp. (n=6), _Fusarium oxysporum_
The therapeutic results of different ACT regimens in the global series (n=61) and the patients diagnosed with proven or probable invasive aspergillosis (n=49) are summarized in Tables 2 and 3, respectively.

**Table 2. Therapeutic results of different ACT regimens in the global series (n=61)**

| Response | Group A (n=20) | Group B (n=20) | Group C (n=21) |
|----------|----------------|----------------|----------------|
|          | Alive at the end of treatment | Alive at 12 weeks | Alive at the end of treatment | Alive at 12 weeks |
| Favorable | 13 (65) | 12 (60) | 13 (62) |
| Complete  | 11 (55) | 11 (55) | 13 (62) |
| Partial  | 2 (10) | 1 (5) | 0 (0) |
| Failure  | 7 (35) | 8 (40) | 8 (38) |
| Death related to IFD | 6 (30) | 8 (40) | 8 (38) |

**Table 3. Therapeutic results of different ACT regimens in patients diagnosed with proven or probable invasive aspergillosis (n=49)**

| Response | Group A (n=18) | Group B (n=14) | Group C (n=17) |
|----------|----------------|----------------|----------------|
|          | Alive at the end of treatment | Alive at 12 weeks | Alive at the end of treatment | Alive at 12 weeks |
| Favorable | 13 (72) | 8 (57) | 11 (65) |
| Complete  | 11 (61) | 8 (57) | 11 (65) |
| Partial  | 2 (11) | 0 (0) | 0 (0) |
| Failure  | 5 (28) | 6 (43) | 6 (35) |
| Death related to IFD | 5 (28) | 5 (36) | 6 (35) |

IFD indicates invasive fungal disease. Group A: L-amB plus caspofungin. Group B: L-amB plus triazole (16 voriconazole and 4 posaconazole). Group C: Voriconazole plus candin (20 caspofungin, 1 anidulafungin). (n=1), Fusarium spp. (n=2), Scedosporium apiospermum (n=2) and S. prolificans (n=1). All cases of probable IFD were aspergillosis. All patients with mucormycosis were treated with ACT and surgery, with the exception of one patient with pulmonary mucormycosis diagnosed at postmortem examination.

**Antifungal Combination Therapy.** The mean duration of ACT was 31 days (range, 8-127). Three antifungal combination groups were identified: group A, L-AmB plus caspofungin (20 patients); group B, L-AmB plus triazole (voriconazole 16 and posaconazole 4) and group C, voriconazole plus candin (20 caspofungin and 1 anidulafungin).

**Toxicity.** ACT was well tolerated except for a mild increase in liver enzymes in patients receiving voriconazole and a mild increase in serum creatinine levels in two patients receiving L-AmB. In one case, L-AmB treatment was discontinued for two days returning the serum creatinine level to normal following a dose reduction from 3 to 1.5 mg/kg/day for two additional days. Hypokalemia was observed in patients receiving L-AmB, but they responded well to potassium supplements.

**Outcome.** Thirty-eight patients had a favorable response to ACT [35 complete response (CR) and 3 partial responses (PR)]. The survival rate at the end of treatment was 62% for the whole series and the 12-week survival rate after initiation of ACT was 57%.

In group A, 13 (65%) patients had favorable response (11 CR, 2 PR), in group B, 12 (60%) patients (11 CR, 1 PR response) and in group C, 13 (62%) patients (13 CR). We found no statistical differences in terms of clinically favorable responses among groups A, B and C (Table 2).

Survival rates at the end of treatment for groups A, B and C were 65%, 60% and 62%, respectively. The probability of survival at 12 weeks for groups A, B, and C were 55%, 55% and 62%, respectively (Figure 1A). We found no significant differences in the probability of survival at 12 weeks between patients treated with de novo ACT compared with those treated with sequential ACT through addition of another antifungal agent to previous prophylaxis or empiric therapy (Figure 1B).

A total of 20 out of 27 patients (74%) receiving induction or consolidation chemotherapy for AML achieved a favorable response to ACT while a favorable response occurred in only 14 out of 25 recipients of allo-SCT (56%). Survival at 12 weeks was higher in AML (64%) compared to allo-SCT (52%). However these differences were not statistically significant.

Twenty-three patients died. Deaths were related to IFD alone (n=8), IFD in the setting of progressive underlying disease (n=14), and cytomegalovirus infection plus acute GvHD (n=1). From the end of the combined treatment to 12 weeks after the initiation of ACT, 3 additional patients died due to chronic GvHD (n=1) and leukemia relapse (n=2). IFD-related mortality at 12 weeks was 39%.

**Antifungal Combination Therapy in Aspergillosis.** Invasive aspergillosis (IA) was diagnosed in 49 patients (13 proven and 36 probable). A total of 32 (65%) patients responded favorably to treatment (30 CR and 2 PR), and the end of treatment and 12-week survival rates were 65% and 61%, respectively.

In group A, 13 of 18 patients (72%) responded (11 CR and 2 PR), in group B, 8 of 14 patients (57%, 8 CR) and in group C, 11 of 17 patients (65%, 11 CR). We found no statistical difference between response rates among groups A, B and C (Table 3). Furthermore, there were no significant differences in survival at 12 weeks among groups A, B and C (Figure 2A). Moreover, no significant differences were observed between patients treated with de novo ACT and those with sequential ACT (Figure 2B). The probability of death at 12 weeks attributable to IA was found to be 34%.
Analysis of Prognostic Factors. Univariate analysis revealed that the use of non-active mold prophylaxis (fluconazole or no prophylaxis) influenced unfavorably the ACT response (p=0.03) and mortality (p=0.03), while granulocyte recovery and complete remission of the underlying malignancy improved the response to ACT (p<0.001 and p=0.009, respectively) and decreased mortality (p<0.001 and p=0.002 respectively). Other clinical variables such as age, stem cell transplantation, de novo ACT and IA did not show significant influence on response or mortality. Logistic regression multivariate analysis revealed that granulocytic recovery was the only statistically significant variable (p<0.001) (Table 4).

When we analyzed these variables separately in groups A, B and C only granulocyte recovery was statistically significant for response (p=0.004 and p=0.02 for group A and B, respectively) and survival at 12 weeks (p=0.02 and p=0.007 for group A and B, respectively). In group C all patients with no granulocyte recovery died.

Discussion. This study shows that ACT seems to be suitable for the treatment of IFD in severely immunocompromised patients. Our results are in line with those previously reported. It should be noted that the three types of ACT employed in our patients resulted in similar response rates in both the overall series and in patients with IA. These findings do not support the results that in both in vitro and animal models have been reported. These studies have suggested an antagonism with the combination of L-AmB and voriconazole. Probably, as Segal & Steinbach have stated, a major limitation in the antifungal field is the lack of consistent ability to use in vitro models to predict clinical efficacy. In general,
### Table 1: Response and Survival at 12 Weeks

| Variable                                      | n (%)                  | Response n (%)  | p value | Survival at 12 weeks n (%) | p value |
|-----------------------------------------------|------------------------|-----------------|---------|---------------------------|---------|
| Age (adults/children)                         | 54 (89) / 7 (12)       | 35 (65) / 3 (43)| 0.3     | 32 (59) / 3 (43)          | 0.4     |
| Stem cell transplantation (yes/no)           | 26 (43) / 35 (57)      | 15 (58) / 23 (66)| 0.5     | 14 (54) / 21 (60)        | 0.6     |
| Non-active mold prophylaxis (yes/no)         | 19 (31) / 42 (69)      | 8 (42) / 30 (71)| 0.03    | 7 (37) / 28 (67)         | 0.033   |
| ACT “de novo” (yes/no)                       | 26 (43) / 35 (57)      | 15 (58) / 23 (66)| 0.5     | 14 (54) / 21 (60)        | 0.6     |
| Granulocytic recovery* (yes/no)              | 35 (57) / 26 (43)      | 33 (94) / 5 (19) | <0.001  | 31 (89) / 4 (15)         | <0.001  |
| Aspergillosis IFD (yes/no)                   | 49 (80) / 12 (20)      | 32 (65) / 6 (50) | 0.3     | 30 (61) / 5 (42)        | 0.2     |
| Complete remission status (yes/no)           | 44 (72) / 17 (28)      | 32 (73) / 6 (35)| **0.009** | 31 (71) / 4 (24)       | **0.002** |

ACT indicates antifungal combination therapy. IFD: invasive fungal disease.

*Only granulocyte recovery was significantly associated to an improved response and survival in multivariate analysis (p<0.001).

Antagonism has not been apparent in the clinical setting. In fact, a recent study by Cornely et al. suggested that prior azole prophylaxis or therapy did not affect overall response nor mortality in patients who were treated with L-AmB for IMI (49% response rate in patients given previously azoles vs. 46% in those without prior azole exposure). Survival at 12 weeks was also similar (64% vs. 66%).

Although our study is not a prospectively randomized trial, we believe it provides valuable information on the potential impact of ACT in seriously ill patients with hematological diseases. In fact, as far as we know, the only prospective randomized trial reported is a pilot study by Caillot et al. which included a small number of patients (15 patients in each arm). High dose L-AmB (10 mg/kg/day) was compared with a combination of standard dose L-AmB (3 mg/kg/day) plus caspofungin in 30 patients with hematologic malignancies and proven or probable invasive aspergillosis (COMBISTRAT trial). Response rates at the end of treatment were 67% for the combination vs. 27% with monotherapy (p= 0.03). Survival rates at 12 weeks were similar, 100% for the combination and 80% for monotherapy. Nevertheless, this study indicated the superiority of standard L-AmB plus caspofungin over high-dose L-AmB monotherapy, which in addition had a higher toxicity. On the other hand, the consistently better results in most retrospective case studies using ACT in the treatment of probable and proven IFD are similar to those reported in the present study. However, retrospective studies usually suffer from potential bias. For instance, in our study only patients who survived at least 7 days from the start of ACT were considered for analysis, and consequently, patients with very severe infections may have been excluded from the study. This criticism may be applied to most clinical trials that exclude patients with poor performance status (ECOG ≥2), life-expectancy less than one week or severe organ dysfunction.

Regarding prognostic factors, granulocyte recovery resulting from the control of the underlying disease is the most important variable in IFD patients regardless of the ACT used. The use of ACT instead of monotherapy may more efficiently stabilize the IFD, preventing fatal progression while neutrophil counts recover and the immune system is restored. This finding raises again the question about the potential utility of granulocyte transfusions as adjunctive treatment. After years of controversy, the RING (Resolving Infections in Neutropenia with Granulocytes) study has been designed to evaluate the effectiveness of transfusing large numbers of G-CSF/dexamethasone mobilized granulocytes from community donors. When available, the results of this multicenter phase III randomized controlled clinical trial may be of great value to definitely address this unresolved issue.

**Conflict of Interest Disclosures.** R. Rojas has received honoraria for speaking at symposia organized by Gilead Science and Merck Sharp and Dohme (MSD). JR. Molina has received honoraria for speaking at medical education events supported by Gilead Science and Pfizer. I. Jarque has received honoraria for speaking at symposia organized by Pfizer, MSD, and Gilead Science. J. Besalduch receives honoraria for participation as a speaker at
medical education events supported by Gilead Science. 

E. Carreras receives honoraria for participation as a speaker at medical education events supported by Gilead Science, Pfizer and MSD. JF. Tomas receives honoraria for participation as a speaker at medical education events supported by Gilead Science, MSD and Pfizer. L. Madero receives honoraria for participation as a speaker at medical education events supported by Gilead, Pfizer and MSD. D. Rubio has received honoraria for speaking at symposia organized by Gilead Science, Pfizer and MSD. E. Conde has received honoraria for speaking at symposia organized by Gilead Science and MSD. MA. Sanz has received honoraria for speaking at symposia organized by Pfizer, (MSD), and Gilead Science and has sat on advisory boards on antifungal agents for Pfizer, MSD, and Gilead Science. A. Torres has received honoraria for participation as speaker at medical education and symposia events supported by Gilead, Pfizer and MSD. C. Montes, J. Serrano and J. Sanz declare no competing financial interest. 

References:

1. Marr KA, Carter RA, Boekh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplantation recipients: Changes in epidemiology and risk factors. Blood 2002; 100: 4358-4366. 
2. Sanz MA, Jarque I, Salavert M, Pemán J. Epidemiology of invasive fungal infections due to Aspergillus spp. and Zygomycetes. Clin Microbiol Infect 2006; 12 (Suppl 7): 2-6. 
3. Neofytos D, Horn D, Anaissie E, Steinbach W, Oliyaei A, Fishman J, Pfaffer M, Chang C, Webster K, Marr K. 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. 
4. Lin SJ, Schranz J, Teutsch SM. Aspergillus case-fatality rate: systematic review of the literature. Clin Infect Dis 2001; 32: 358-366. 
5. Upton A, Kirby KA, Carpenter P, Boekh M, Marr KA. Invasive aspergillosis following hematopoietic stem cell transplantation: outcomes and prognostic factors associated with mortality. Clin Infect Dis 2007;44:531-540. 
6. Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisian TA, Schauefeld RJ, Sein M, T, Chiuo CC, Chu JH, Kontoyianannis DP, Walsh TJ. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clin Infect Dis 2005; 41: 634-653. 
7. Walsh TJ, Finberg RW, Arndt C, Hiemenz J, Schwartz C, Bodensteiner D, Pappas P, Seibel N, Greenberg RN, Dummner S, Schuster M, Henning JS, National Institute of Allergy and Infectious Diseases Mycoses Study Group. Liposomal amphotericin B for empirical therapy in patients with persistent fever and neutropenia. N Engl J Med 1999; 340: 764-771. 
8. Walsh TJ, Pappas P, Winston DJ, Petersen F, Raffalli J, Yanovich S, Stiff P, Greenberg R, Donowitz G, Schuster M, Reboli A, Wingard J, Arndt C, Reinhardt J, Hadley S, Finberg R, Lavredièrre M, Perfect J, Garber G, Fioriontani G, Anaissie E, Lee J. Voriconazole compared with liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever. N Engl J Med 2002; 346: 225-234. 
9. Walsh TJ, Teppher H, Donowitz GR, Maertens JA, Baden LR, Dmoszynska A, Cornely OA, Bourque MR, Lupinacci RJ, Sable CA, dePauw BE. Caspofungin versus liposomal amphotericin B for empirical antifungal therapy in patients with persistent fever and neutropenia. N Engl J Med 2004; 351: 1391-1402. 
10. Maertens J, Theunissen K, Verhoel G, Verschakelen J, Lagrou K, Verbeke E, Willner A, Vrehaegge J, Boogaerts M, Van Elsene P, Galactomannan and computer tomography-based preemptive antifungal therapy in neutropenic patients at high risk for invasive fungal infection: A prospective feasibility study. Clin Infect Dis 2005; 41: 1242-1250. 
11. Cordonnier C, Pautas C, Maury S, Vakhoff A, Farhat H, Suarez F, Dhédin N, Isnard F, Aides L, Kuhnowski F, Foulet F, Kuentz M, Ménou P, Bretagne S, Schwarzinger M. Empirical versus preemptive antifungal therapy for high-risk, febrile, neutropenic patients: a randomized, controlled trial. Clin Infect Dis 2009; 48:1042-1051. 
12. Girmenia C, Micoczi A, Gentile G, Santilli S, Arleo E, Cardarelli L, Capria S, Minotti C, Cartoni C, Brocchieri S, Gueavissa V, Meloni G, Foi R, Martino P. Clinically driven diagnostic antifungal approach in neutropenic patients: a prospective feasibility study. J Clin Oncol 2010;28:667-674. 
13. Pagano L, Caira M, Nosari A, Cattaneo C, Fanci R, Bonini A, Vianelli N, Garzia MG, Mancinelli M, Tosti ME, Tumbarello M, Viale P, Aversa F, Rossi G; HEMA e-Chart Group. The use and efficacy of empirical versus pre-emptive therapy in the management of fungal infections: the HEMA e-Chart Project. Haematologica 2011;96:1366-1370. 
14. Winston DJ, Mazziar RT, Chandrasekar PH, Lazarus HM, Goldman M, Blumer JL, Leitz GJ, Territo MC. Intravenous and oral itraconazole versus intravenous and oral fluconazole for long-term antifungal prophylaxis in allogeneic hematopoietic stem-cell transplant recipients: a multicentre, randomized trial. Ann Intern Med 2003; 138: 705-713. 
15. Siwik GT, Pappas MA, Polgreen PM, Cobb S, Hoth P, Magalhaes-Silverman M, Diekema DJ. Incidence of invasive aspergillosis among allogeneic hematopoietic stem cell transplant patients receiving voriconazole prophylaxis. Diagn Microbiol Infect Dis 2006; 55: 209-212. 
16. Wingard JR, Carter SL, Walsh TJ, Kurtzberg J, Small TN, Baden LR, Gersten ID, Mendizabal AM, Leather HL, Confer DL, Mazziar RT, Stadtmaurer EA, Bolaños-Mead J, Brown J, Dipersio JF, Boekh M, Marr KA. Randomized double-blind trial of fluconazole versus voriconazole for prevention of invasive fungal infection after allogeneic hematopoietic cell transplantation. Blood 2010; 116: 5111-5118. 
17. Torres A, Serrano J, Rojas R, Martín V, Martín C, Tabares S, Molina JR, Capote M, Martínez F, Gómez P, Sánchez-Garcia J. Voriconazole as primary antifungal prophylaxis in patients with neutropenia after hematopoietic stem cell transplantation or chemotherapy for acute myeloid leukemia. Eur J Haematol 2009; 84: 271-273. 
18. Cornely OA, Maertens J, Winston DJ, Perfect J, Ullmann AJ, Walsh TJ, Heldring D, Holowiecki J, Stockelberg D, Geh YT, Petrinii M, Hardalo C, Suresh R, Angulo Gonzalez D, Posaconazole vs fluconazole or itraconazole prophylaxis in patients with neutropenia. N Engl J Med 2007; 356: 348-359.
19. Ullmann AJ, Lipton JH, Vesole DH, Chandrasekar P, Langston A, Tarantolo SR, Greinix H, Morais de Azevedo W, Reddy V, Boparai N, Pedicone L, Patino H, Durrant S. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. N Engl J Med 2007; 356: 335-347. (PMID:17251531)

20. Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann JW, Kern WV, Marr KA, Ribaud P, Lortholary O, Sylvester R, Rubin RH, Wingard JR, Stark P, Durand C, Caillot D, Thiel E, Chandrasekar PH, Hogeveen MR, Schlamm HT, Troke P, de Pauw B. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med 2002; 347: 408-415. (PMID:12167683)

21. Van Burik JA, Perfect J, Louie A, Graybill JR, Pedicone L, Raad II. Efficacy of posaconazole (POS) vs standard therapy and safety of POS in hematopoietic stem cell transplant (HSCT) recipients vs other patients with aspergillosis. Biol Blood Marrow Transplant 2006; 12 (Suppl 1): 137. (PMID:16678903)

22. Denning DW, Ribaud P, Milpied N, Caillot D, Herbrecht R, Thiel E, Haas A, Ruhrke M, Loel H. Efficacy and safety of voriconazole in the treatment of acute invasive aspergillosis. Clin Infect Dis 2002; 44: 2-12.

23. Denning DW, Marr KA, Lau WM, Facklam DP, Ratanatharathorn V, Becker C, Ullmann AJ, Soibel NL, Flynn PM, van Burik JA, Baell DN, Patterson TF. Micafungin (FK463), alone or in combination with other systemic antifungal agents, for the treatment of acute invasive aspergillosis. J Infect. 2006; 53: 337-349. (PMID:16678903)

24. Kontoyiannis DP, Hachem R, Lewis RE, Rivero GA, Forrest G, Safdar N, Muñoz P, Pursell K, Kontoyiannis DP, Cordonnier C, Segal BH, Herbrecht R, Stevens DA, Ostrosky-Zeichner L, Sobel KD, Segal DH, Denning DW, Perlman AM, Stevens DA. In vitro susceptibility and synergy studies of Aspergillus species to amphotericin B with azole antifungal drugs: what are we doing? Antimicrob Agents Chemother. 1995; 39: 1907-1912. (PMID:8540690)

25. Singh N, Limaye AP, Forrest G, Saadat N, Muñoz P, Pursell K, Houston S, Rosso F, Montoya JG, Patton P, Del Busto R, Aguado JM, Fisher RA, Klintmalm GB, Miller R, Wagener MM, Lewis RE, Kontoyiannis DP, Husain S. Combination of voriconazole and caspofungin as primary therapy for invasive aspergillosis in solid organ transplant recipients: A prospective multicenter, observational study. Transplantation 2006; 81: 320-326. (PMID:16477215)

26. Marr KA, Bodech M, Carter RA, Kim HW, Corey L. Combination antifungal therapy for invasive aspergillosis. Clin Infect Dis 2004; 39: 797-802. (PMID:15472810)

27. Singh N, Limaye AP, Forrest G, Saadat N, Muñoz P, Pursell K, Houston S, Rosso F, Montoya JG, Patton P, Del Busto R, Aguado JM, Fisher RA, Klintmalm GB, Miller R, Wagener MM, Lewis RE, Kontoyiannis DP, Husain S. Combination of voriconazole and caspofungin as primary therapy for invasive aspergillosis in solid organ transplant recipients: A prospective multicenter, observational study. Transplantation 2006; 81: 320-326. (PMID:16477215)

28. Maertens J, Glasmacher A, Herbrecht R, Thiebaut A, Cordonnier C, Segal BH, Killar J, Taylor A, Kartsounis N, Patterson TF, Aoun M, Caillot D, Sable C. Multicenter, noncomparative study of caspofungin in combination with other antifungal treatment in patients with aspergillosis unresponsive to conventional and new agents. Antimicrob Agents Chemother. 2010; 65: 114-117. (PMID:20462012)

29. Sugar AM. Use of amphotericin B with azole antifungal drugs: what are we doing? Antimicrob Agents Chemother. 1995; 39: 1907-1912. (PMID:8540690)

30. Denning DW, Hanson LH, Perlman AM, Stevens DA. In vitro susceptibility and synergy studies of Aspergillus species to conventional and new agents, order, and timing. Curr Fungal Infect Rep. 2010; 4: 1813-1821. (PMID:20574543)

31. De Pauw B, Walsh TJ, Donnelly IP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kaufman CA, Denning DW, Patterson TF, Maschmeyer G, Bille J, Dismukes WE. Herbrecht R, Hope W, Kibbller C, Kilburg BJ, Marr KA, Muñoz P, Odds FC, Perfect JR, Restrepo A, Ruhrke M, Segal BH, Sobel JD, Sorrell TC, Viscoli C, Wingard JR, Zaatouis T, Bennett JE. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 2008; 46: 1813-1821. (PMID:18637757)

32. Segal BH, Herbrecht R, Stevens DA, Ostrosky-Zeichner L, Sobel J, Viscoli C, Walsh TJ, Maertens J, Patterson TF, Perfect JR, Dupont B, Wingard JR, Calandra T, Kaufman CA, Graybill JR, Baden LR, Pappas PG, Bennett JE, Kontoyiannis DP, Cordonnier C, Viviani MA, Bille J, Almyroudis NG, Wheat LJ, Graninger W, Bow EJ, Holland SM, Kilburg BJ, Dismukes WE, De Pauw BE. 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: 674-683. (PMID:18639566)

33. Criteria. Clin Infect Dis 2008; 47: 674-683. (PMID:18639566)

34. Coryn NY, Oestmann JW, Kern WV, Marr KA, Ribaud P, Lortholary O, Bille J, Dismukes WE. Herbrecht R, Hope W, Kibbller C, Kilburg BJ, Marr KA, Muñoz P, Odds FC, Perfect JR, Restrepo A, Ruhrke M, Segal BH, Sobel JD, Sorrell TC, Viscoli C, Wingard JR, Zaatouis T, Bennett JE. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 2008; 46: 1813-1821. (PMID:18637757)

35. Butterfield J, Reid G, Rice J, Rice P, Leatherman J, Weinberg W, Englund J, Khan A, Patel S, White J. Combination antifungal therapy: Efficacy and toxicity in hematological cancer patients. Ann Hematol 2008; 87: 915-922. (http://dx.doi.org/10.1007/s00277-008-0534-4)

36. Sugar AM. Use of amphotericin B with azole antifungal drugs: what are we doing? Antimicrob Agents Chemother. 1995; 39: 1907-1912. (PMID:8540690)

37. Denning DW, Hanson LH, Perlman AM, Stevens DA. In vitro susceptibility and synergy studies of Aspergillus species to conventional and new agents, order, and timing. Curr Fungal Infect Rep. 2010; 4: 1813-1821. (PMID:20574543)

38. Sugar AM. Use of amphotericin B with azole antifungal drugs: what are we doing? Antimicrob Agents Chemother. 1995; 39: 1907-1912. (PMID:8540690)

39. De Pauw B, Walsh TJ, Donnelly IP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kaufman CA, Denning DW, Patterson TF, Maschmeyer G, Bille J, Dismukes WE. Herbrecht R, Hope W, Kibbller C, Kilburg BJ, Marr KA, Muñoz P, Odds FC, Perfect JR, Restrepo A, Ruhrke M, Segal BH, Sobel JD, Sorrell TC, Viscoli C, Wingard JR, Zaatouis T, Bennett JE. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 2008; 46: 1813-1821. (PMID:18637757)

40. Sugar AM. Use of amphotericin B with azole antifungal drugs: what are we doing? Antimicrob Agents Chemother. 1995; 39: 1907-1912. (PMID:8540690)