Universal antifungal therapy is not needed in persistent febrile neutropenia: a tailored diagnostic and therapeutic approach

by Manuela Aguilar-Guisado, Almudena Martin-Pena, Ildefonso Espigado, Maite Ruiz Perez de Pipaon, Jose' Falantes, Fatima de la Cruz, and Jose' Miguel Cisneros

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Universal antifungal therapy is not needed in persistent febrile neutropenia: a tailored diagnostic and therapeutic approach

Manuela Aguilar-Guisado¹,², Almudena Martín-Peña¹,², Ildefonso Espigado¹,³,
Maite Ruiz Pérez de Pipaon², José Falantes³, Fátima de la Cruz,³
and José M. Cisneros¹,²

Spanish Network for Research in Infectious Disease¹; Service of Infectious Diseases, Clinical Microbiology and Preventive Medicine², University Hospital Virgen del Rocío, Sevilla, Spain; Hematology Service³, University Hospital Virgen del Rocío, Sevilla, Spain, University Hospital Virgen del Rocío/ Instituto de Biomedicina de Sevilla (IBIS), 41013, Sevilla, Spain

MAG and AMP contributed equally to this manuscript.

Correspondence
Almudena Martín-Peña, Service of Infectious Diseases, Clinical Microbiology and Preventive Medicine University Hospital Virgen del Rocío, Ave. Manuel Siurot s/n. 41013 Sevilla, Spain. Phone: international + 0034.95.5012185.
E-mail: almudena.martin.pena@gmail.com

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Abstract

Background. Antifungal therapy exclusively in selected patients with persistent febrile neutropenia may avoid over-treatment without increasing mortality. The aim of this study was to validate an innovative diagnostic and therapeutic approach based on risk profile and clinical criteria for selecting patients to receive antifungal therapy and to compare its efficacy with universal empirical antifungal therapy.

Design and methods. This was a prospective study which included all consecutive hematological adult patients with neutropenia and fever refractory to 5 days of empiric antibacterial therapy admitted to a teaching hospital in Spain over a 2-year period. A diagnostic and therapeutic approach based on clinical criteria and risk profile was applied in order to select patients for antifungal therapy. Sensitivity, specificity and negative predictive value of this approach and also overall successful response according to the same criteria of efficacy described in classical clinical trials were analyzed.

Results. Eighty-five episodes were included, 35 of them (41.2%) in invasive fungal infection high-risk patients. Antifungal therapy was not indicated in 33 episodes (38.8%). The overall incidence of proven and probable invasive fungal infection was 14.1%, all of them in patients who had received empirical antifungal therapy. The 30 day-crude mortality was 15.3% and invasive fungal infection-related mortality was 2.8% (2/72). The overall successful response following the diagnostic and therapeutic approach was 36.5% compared with 33.9% and 33.7% obtained in the trial of Walsh et al. Sensitivity, specificity and negative predictive value of the study approach were 100%, 52.4% and 100%, respectively.

Conclusions. Based on the high negative predictive value of this diagnostic and therapeutic approach, in persistent febrile neutropenic patients with hematological malignancies or hematopoietic stem cell transplant recipients, it is useful for identifying patients who are not likely to develop invasive fungal infection, therefore do not require antifungal therapy, and with an effectiveness similar to that reported in controlled trials which indicate empirical antifungal therapy universally.
Introduction

Invasive fungal infection (IFI) is a major cause of mortality among patients with hematological malignancies and hematopoietic stem cell transplant (HSCT) recipients.\(^1\) Its incidence has increased over the past years,\(^2\)\(^-\)\(^4\) often resulting in limitations to chemotherapy administration and worsening the prognosis of hematological disease. Since the main risk factor for IFI in these patients is profound and prolonged neutropenia, universal empirical antifungal therapy in neutropenic patients after 5-7 days of persistent fever has been recommended by the Infectious Diseases Society of America (IDSA)\(^5\) over the last two decades. Although this standard of care is supported by weak scientific evidence\(^6\) and it has important drawbacks such as toxicity, increased cost and risk of antifungal resistance.\(^7\)\(^-\)\(^9\) In recent years, some authors have suggested that indicating antifungal therapy in selected patients may avoid over-treatment without increasing IFI-related mortality,\(^10\)\(^-\)\(^14\) but it would be desirable to design a feasible, effective and safe approach for selecting patients for antifungal therapy indication. Moreover, this approach has not been evaluated in patients with the highest-risk of IFI such as allogeneic HSCT recipients.

Cisneros et al.\(^12\) have proposed an approach for selecting patients for antifungal therapy indication based on risk profile and driven by clinical criteria,\(^12\) whose efficacy and safety were established in a pilot study conducted in our center.\(^10\) Those results were limited by the non-routine determination of serum galactomannan antigen (GM) and the high proportion of patients in whom antifungal therapy indication was based on an individualized decision.

The aim of this prospective study was to establish the sensitivity, specificity and negative predictive value of this innovative diagnostic and therapeutic approach based on risk profile and driven by clinical criteria to select patients with persistent febrile neutropenia for antifungal therapy indication and to compare its efficacy with universal empirical antifungal therapy.
Design and Methods

Ethics statement

The study was approved by the Ethics Committee and the Infections Committee (PI0068/2009) of the University Hospital Virgen del Rocío, Sevilla (Spain) and was performed in accordance with the Declaration of Helsinki. Written consent was not required.

Patients and methods

This is a prospective study of consecutive persistent febrile neutropenia episodes in patients with hematological malignancies or HSCT recipients admitted to the Hematology Service of a tertiary Center from October 2007 to October 2009.

Patients were included in the study if they fulfilled the following criteria: (i) age over 14 years; (ii) neutropenia post-chemotherapy or after HSCT (absolute neutrophil count inferior to 0.5x10^9/L or less than 1.0x10^9/L if rapid decrease was predicted in the following 24-48 hours) and (iii) persistent fever (more than 96 hours of axillary temperature of >38°C recorded twice or >38.3°C recorded once) refractory to empirical antibacterial therapy and without etiological diagnosis. Patients with solid neoplasm, neutropenia secondary to other causes and persistent fever with known etiology were not included.

Demographic data, variables related to the hematological diseases and persistent febrile neutropenia episodes were prospectively recorded.
Definitions

Final diagnosis of persistent febrile neutropenia episode: (i) infectious fever (other than IFI) was considered proven if there was an organ-specific or systemic infection with microbiological isolation and probable when there was no microbiological isolation but there was response to empiric antimicrobial therapy; (ii) tumoral fever when active hematological disease was demonstrated and there was response to non-steroidal anti-inflammatory drugs (NSAIDs) or steroids in absence of proven or probable infection; (iii) drug fever when it was temporarily related to drug administration and disappeared 24-48 hours after withdrawal, in the absence of proven or probable infection. It was necessary to rule out a probable or proven IFI in all cases.

Possible, probable and proven IFI were defined according to EORTC/MSG criteria.15

IFI high-risk patients were allogeneic HSCT recipients and patients with acute myeloblastic leukemia receiving induction or re-induction chemotherapy.

Efficacy assessment: the efficacy end point was an overall successful response of a five component end point, used in previous studies of empirical antifungal therapy,7-9;16;17 were met: successful treatment of any baseline fungal infection, absence of any breakthrough fungal infection during therapy or within seven days after the completion of therapy, survival for seven days after the completion of therapy, no premature discontinuation of antifungal therapy because of drug related toxicity or lack of efficacy, and resolution of fever (temperature below 38°C for at least 48 hours) during neutropenia.

Mortality: 30 day-crude mortality was defined as mortality for any cause 30 days after the onset of febrile neutropenia. IFI-related mortality was defined as death during the treatment of a probable or proven IFI with refractory underlying disease (progression or failure to improve) in the absence of any other condition believed to have caused death.
Severe sepsis and septic shock were defined according to internationally accepted criteria and profound neutropenia was defined as an absolute neutrophil count < 0.1x10^9/L.

**Antimicrobial prophylaxis protocol**

Every patient received prophylaxis with levofloxacin (500 mg/day from the first day of chemotherapy or transplant conditioning until the onset of fever) and trimethoprim/sulfamethoxazole 800/160 mg on alternative days. Antifungal prophylaxis was administered only in allogeneic HSCT recipients, with fluconazole 400 mg/day or, posaconazole 200 mg tid in patients with chronic GVHD.

**Febrile neutropenia management**

Routine management of febrile neutropenia episodes included a complete physical examination, a chest X ray, blood cultures (catheter and peripheral blood samples) and additional samples from infected sites as clinically indicated. After obtaining cultures, empiric antimicrobial therapy was started with an antipseudomonal beta-lactam with or without an aminoglycoside. A glycopeptide was added in patients with severe sepsis or septic shock, suspected catheter infection or severe mucositis, according to IDSA recommendations. Blood cultures were repeated in 72 hours if initial results were negative.

Those patients with neutropenia and fever after 5 days of empiric antibacterial therapy, without etiological diagnosis, were defined as patients with persistent febrile neutropenia and were included in the study. The following diagnostic and therapeutic approach recommended by the Andalusian Society of Infectious Diseases was applied in order to select patients for antifungal therapy indication.

**Diagnostic and therapeutic approach of persistent febrile neutropenia episodes.**

The first step was to evaluate the severity of the episode (severe sepsis or septic shock) and the second step was the identification of any clinical infectious foci of possible fungal etiology.

In patients with neither severe signs nor infectious foci, antifungal therapy was not initially indicated and further diagnostic evaluation was performed, including serial
serum GM tests twice a week (with an index >0.5 considered positive), thoracic thin-section computed tomography (TSCT), abdominal ultrasound (US), repeated blood cultures and other ancillary tests until etiological or syndromic diagnosis was reached or fever disappearance.

Regarding the rest of the patients, antifungal therapy was indicated with the most appropriate antifungal for the most likely etiology of IFI according to the following clinical criteria: (i) in patients with signs of severe sepsis or septic shock, caspofungin (70 mg/day and 50 mg/day on the following days) was indicated as primary therapy or liposomal amphotericin (3-5 mg/kg/day) as alternative therapy; (ii) in patients without severe sepsis or septic shock and with any infectious foci suspected of being of fungal etiology: pulmonary, central nervous system and sinus, voriconazole (6 mg/kg/day and 4 mg/kg/day on the following days) was used as primary therapy and liposomal amphotericin (3-5 mg/kg/day) or caspofungin (70 mg/day and 50 mg/day on the following days) as alternative therapies, while in case of abdominal or skin focus, caspofungin (70 mg/day and 50 mg/day on the following days) was indicated as primary therapy and liposomal amphotericin (3-5 mg/kg/day) or fluconazole (50-800 mg/day) as alternative therapies; (iii) in patients with GM detected in serum (index > 0.5), voriconazole (6 mg/kg/day and 4 mg/kg/day on the following day) was initiated.

Diagnostic work-up established by the study approach entailed: serum GM test was performed routinely to all patients twice a week and whenever respiratory symptoms or signs appeared; thoracic TSCT in every patient between the fifth and the seventh day of fever and/or if respiratory symptoms or signs developed. Bronchoscopy with bronchoalveolar lavage led by thoracic TSCT was performed when clinically possible in patients with pulmonary infiltrates, for microbiological investigation of bacteria, fungi, Pneumocystis jirovecii and mycobacteria stains and cultures, shell vial and viral culture for cytomegalovirus and rapid test (immunofluorescence) for respiratory viruses (syncytial respiratory virus and influenza virus). In patients with abdominal focus (painful hepatomegaly and/or elevated serum phosphatase alkaline in which hepatoesplenic candidiasis was suspected, or suspicion of necrotizing
enterocolitis without response to support and antibacterial therapy) abdominal US and/or abdominal computer tomography (CT) were performed. Other imaging techniques, invasive procedures such as endoscopy or biopsy and additional cultures of infected sites were performed as clinically indicated (Figure 1).

**Statistical analysis.**

A descriptive analysis of clinical syndrome at presentation, final diagnosis and outcome of every persistent febrile neutropenia episode and the global incidence of proven and probable IFI and its etiology was performed.

A comparative analysis of proven or probable IFI incidence and IFI-related mortality according to the indication or not of antifungal therapy was performed. The number of days between fever onset and the start of antifungal therapy in patients with IFI were also analyzed in order to determine if a delay in antifungal therapy after the 5\textsuperscript{th}-7\textsuperscript{th} day from fever onset (in comparison with standard IDSA approach) could have impacted IFI-related mortality. A sub-analysis of the incidence of proven and probable IFI and IFI-related mortality in IFI high-risk patients was also performed.

Sensitivity, specificity and negative predictive value of the diagnostic and therapeutic approach were analyzed in order to asses its usefulness in the selection of patients for antifungal therapy indication. Possible, probable and proven IFI were considered for their calculation.

The overall successful response according to the same criteria of efficacy as those described in the Walsh et al. clinical trial\textsuperscript{9} was analyzed.

Statistical analysis was performed with software from the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL) version 18.0
Results

Study population

Eighty-five episodes of persistent febrile neutropenia in 72 patients were recorded during the study period. Males represented 48.2% and the median age was 47 years (range: 15-75). The 41.7% of patients were considered IFI high risk (Table 1).

Antifungal therapy

After initial evaluation, in thirty-two episodes (37.6%) antifungal therapy was indicated between the 5th and the 7th day from fever onset, mainly due to pulmonary infiltrates and abdominal focus. In twenty episodes (23.5%), antifungal therapy was indicated after the 7th day mostly due to detection of pulmonary infiltrate in TSCT (10.5%). Overall antifungal therapy indication was established in fifty-two episodes (61.2%) and the median duration in days of antifungal therapy was 11 days (range: 2-164). The main antifungal drugs used were caspofungin and voriconazole in 24 (28.2%) and 22 (25.9%) episodes, respectively, followed by liposomal amphotericin (n=4, 7.7%) and fluconazole (n=2, 3.8%). In the remaining episodes of persistent febrile neutropenia (n=33, 38.8%), and following the diagnostic and therapeutic approach steps, antifungal therapy was not indicated (Figure 2).

Thirty-five persistent febrile neutropenia episodes (41.2%) occurred in patients considered high-risk for IFI and antifungal therapy was indicated in 26 of them (74.3%).

Mean duration of fever was longer in episodes that received antifungal therapy than in episodes that did not (13.9±7.2 vs. 9.91±4.9 days; p= 0.006) whereas there were no differences in mean duration of neutropenia between them (19.8 ± 11.2 vs. 15.8±11.8 days; p= 0.123).

The most frequent final diagnosis of the persistent febrile neutropenia episodes was non-fungal infection (n=46, 54.1%), mostly presenting as non-focused fever or pneumonia without microbiological findings and favorable outcome with antibacterial therapy (Table 2).
Proven and probable invasive fungal infections.

The overall incidence of proven or probable IFI was 14.1% (12 IFI episodes out of 85 persistent febrile neutropenia episodes) with 11 of them diagnosed as baseline IFI and one as breakthrough IFI. There were ten episodes of possible IFI. All IFI episodes appeared in the group of patients who received antifungal therapy.

The proven or probable IFI incidence was 20% (7/35) in high-risk episodes and 10% (5/50) in the rest, p=0.219.

The main etiology of these IFI episodes was moulds (n=8, 66.7%). None of these IFI was a relapse of a previous episode.

Mean days between fever onset and start of antifungal therapy was similar in patients who developed IFI (6.8 ± 2 days) and in patients who did not (7 ± 3.8 days), p=0.85. Every patient who developed IFI and died during the follow up had received antifungal therapy between the fifth and seventh day of fever onset (Table 3).

The values of sensitivity, specificity and negative predictive value of the diagnostic and therapeutic approach for selecting patients who did not need antifungal therapy indication were 100% (CI 95% 85.1-100), 54.2% (CI 95% 40.3-64.2) and 100% (CI 95% 89.6-100), respectively (Table 4).

Outcome and patient survival

The overall successful response of persistent febrile neutropenia episodes, following the five-component end-point criteria, after the application of the diagnostic and therapeutic approach was 36.5% versus 33.9% and 33.7% when empirical antifungal therapy is administered in the controlled trial of Walsh et al.9 (Table 5).

The 30 day-crude mortality was 15.3% (11/72) patients, 21.7% (10 out of 46) in the group that received antifungal therapy and 3.8% (1 out of 26) in the group that did not.

IFI-related mortality was 2.8% (2/72), both of them in patients who received antifungal therapy. One patient died after re-induction chemotherapy for relapsed myeloblastic leukemia due to probable invasive pulmonary aspergillosis and the other
one after a second (of a tandem) autologous HSCT for relapsed seminoma due to proven invasive candidiasis (*Candida albicans*).

In the IFI high-risk patient group, 30 day-crude mortality was 10% (3 out of 30) and IFI-related mortality was 3% (1 out of 30). There was no difference in mortality between IFI high-risk and IFI non high-risk patients (*p*=0.754).

**Discussion**

The results of this study demonstrate that the diagnostic and therapeutic approach based on risk profile and driven by clinical criteria have a high sensitivity and negative predictive value for selecting patients who do not need antifungal therapy which represent nearly 40% percent of persistent febrile neutropenia episodes in our series.

These results corroborate the findings of our previous pilot study in which this diagnostic and therapeutic approach was applied in a cohort of 66 consecutive episodes of persistent febrile neutropenia. The main conclusion of this study was that the approach was safe and effective without increasing IFI incidence or IFI related mortality while avoiding over-treatment caused by universal empirical antifungal therapy. However, those results were limited due to the lack of the serum GM test, which could have underestimated the incidence of probable aspergillosis, and a high proportion (34.6%) of antifungal therapy indication were individualized clinical decisions based on risk profile rather than clinical criteria and diagnostic tests. The current study overcomes those limitations, providing improvements in the approach and in its implementation and confirms the effectiveness and safety of this approach.

Recently some authors have proposed other approaches for patient selection guided by clinical criteria and risk profile. Maertens *et al.* designed a preemptive antifungal therapy approach based on serial serum GM test in IFI high-risk hematological patients receiving antifungal prophylaxis against *Candida* spp. In a small subgroup of patients with persistent febrile neutropenia (30 out of 136 patients, 22%)
antifungal therapy was reduced from 35% to 7.7% without an increase in IFI incidence or IFI related mortality. However, the number of patients was relatively small, the study did not include nonmyeloablative allogeneic and autologous HSCT recipients, since this approach is complex for application in daily clinical practice. More recently Cordonnier et al.\textsuperscript{13} published a randomized multicenter study comparing the administration of universal empirical antifungal therapy with pre-emptive therapy (150 patients with hematological malignancies in each arm). The administration of antifungal therapy was 59.8% in the conventional group compared to 1.8% in the pre-emptive therapy group, with similar overall mortality. The results of this study, which excluded allogeneic HSCT recipients, suggest that pre-emptive antifungal therapy is not inferior to universal empirical antifungal therapy, concluding that further studies are needed to investigate the safety and usefulness of this new approach.\textsuperscript{13}

In the present study, we have established the usefulness of a tailored diagnostic and therapeutic approach\textsuperscript{12} based on clinical criteria and patient risk profile in quite a large serie of patients with hematological malignancies. Safety is the main strength of our approach, based on its high negative predictive value. Furthermore, it was applicable and equally safe in patients with very high-risk for developing IFI, including allo-HSCT recipients and relapsed leukemia which represented 41.6% of the study population, and patients with prolonged neutropenia (median of 14 days) or profound neutropenia as suffered by 98.8% of the study population.

The overall successful response of the approach, determined by the five-component end-point, was similar to the results reported by Walsh et al.\textsuperscript{9} in a controlled trial of universal empirical antifungal therapy, but with the main difference that in our study antifungal therapy was indicated only in 60% of episodes.

Unlike other series,\textsuperscript{13,14,20} in this study the choice of antifungal drug was guided by the most probable fungal etiology in each case depending on clinical criteria (i.e septic shock) and/or the diagnostic work-up results (i.e halo sign) following international guidelines for antifungal therapy.\textsuperscript{19,22} The advantage of this individualized antifungal therapy choice is that the patients received the most appropriate antifungal drug early
in the clinical course. This fact should explain the high IFI cure rate obtained in our study 66.7% (8/12). Although voriconazole is not approved for use in persistent febrile neutropenia, it is the drug of choice for invasive aspergillosis and should be used from the beginning if Aspergillus spp. is the most probable etiology. Recent studies have reported a high rate of breakthrough invasive aspergillosis among patients receiving caspofungin for persistent fever. In a study by Lafaurie et al. the suspicion of invasive aspergillosis led to the interruption of caspofungin empirical therapy and switch to voriconazole in all but one case. These supposed breakthrough infections appeared at a median of only 8 days after empirical antifungal therapy initiation, but the authors recognize that, at least in some of these patients, invasive aspergillosis was the probable etiology from the fever onset, but they were overlooked because of the absence of a protocolized chest CT at the start of empirical antifungal therapy.

On the other hand, the results show that persistent fever is mainly due to nonfungal infection with favorable outcome with antibacterial therapy, whereas probable or proven IFI represents only 14.1% of the episodes. In addition, the incidence of proven or probable IFI in this study that included a high proportion of very high-risk patients was similar to other series as occurred with its IFI related mortality of 2.7%. Our study has some weaknesses. First of all, it is a non-randomized interventional study. Nevertheless, this study included every persistent febrile neutropenia episode occurring over a 2-year period in a tertiary hospital with an active HSCT transplantation program. Also, the specificity of this approach was low in the selection of persistent febrile neutropenia episodes of fungal etiology because, even while avoiding unnecessary antifungal therapy, 76.9% of them that received antifungal therapy did not have proven or probable fungal infection. However, there is still a global reduction of antifungal therapy of 38.8% compared to the standard approach. The development of new diagnostic tests like β-D-glucan and specific fungal PCR would improve the specificity of this approach in the future. Finally, this approach requires intensive clinician involvement and diagnostic work up with the prompt availability of critical diagnostic tests (CT scan, BAL and GM).
In conclusion, based on the high negative predictive value of this diagnostic and therapeutic approach, in persistent febrile neutropenic patients with hematological malignancies or HSCT recipients, it is useful for identifying patients who are not likely to develop invasive fungal infection, therefore do not require antifungal therapy, and with an effectiveness similar to that reported in controlled trials which indicate empirical antifungal therapy universally.

**Authorship and Disclosures**

Information about the contributions of each person named as having participated in the study: Guarantors, i.e., people who are responsible for the integrity of the work as whole: Manuela Aguilar-Guisado, Almudena Martín-Peña and José M. Cisneros. Guarantors of this manuscript confirm that all persons designated as authors qualify for authorship, and that each author has participated sufficiently in the work to take public responsibility for appropriate portions of the content. Authors who participated in the conception of the study: Manuela Aguilar-Guisado, Almudena Martín-Peña, Ildefonso Espigado, Maite Ruiz Pérez de Pipaón, José Falantes, Fátima de la Cruz and José M. Cisneros. Authors who are responsible for the design of the study. The following authors were responsible for specific investigations: Manuela Aguilar-Guisado, Almudena Martín-Peña, Ildefonso Espigado, José M. Cisneros were responsible for design of the clinical study. Manuela Aguilar-Guisado, Ildefonso Espigado, Maite Ruiz Pérez de Pipaón, José Falantes, Fátima de la Cruz and José M. Cisneros were responsible for methods/implementation of the study. Manuela Aguilar-Guisado, Almudena Martín-Peña and José M. Cisneros were responsible for the statistical analysis. Results. The following authors were responsible for specific portions of the results, including figures and tables. Manuela Aguilar-Guisado, Almudena Martín-Peña, Ildefonso Espigado, Maite Ruiz Pérez de Pipaón, José Falantes, Fátima de la Cruz and José M. Cisneros were responsible for evaluation and inclusion of the patients. Manuela Aguilar-Guisado, Almudena Martín-Peña, Ildefonso Espigado and José M. Cisneros were responsible for data analysis and interpretation. Manuela Aguilar-
Guisado, Almudena Martín-Peña and José M. Cisneros were responsible for table 1 to 4 and figure 1 and 2. Almudena Martín-Peña and José M. Cisneros were responsible for table 5. Manuela Aguilar-Guisado and Almudena Martín-Peña were responsible for Online Supplementary Table 1. Writing manuscript. The following authors were responsible for writing the manuscript: Manuela Aguilar-Guisado, Almudena Martín-Peña, Ildefonso Espigado and José M. Cisneros. Critical review of the manuscript and approval of the final version. The following authors were responsible Manuela Aguilar-Guisado, Almudena Martín-Peña, Ildefonso Espigado, Maite Ruiz Pérez de Pipaón, José Falantes, Fátima de la Cruz and José M. Cisneros. The authors reported no potential conflicts of interest.
References


Table 1. Basal characteristics of patients and episodes of persistent febrile neutropenia (PFN).

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Non IFI high-risk</th>
<th>IFI high-risk&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients characteristics</strong></td>
<td>n=42</td>
<td>n=30</td>
<td>n=72</td>
</tr>
<tr>
<td>Underlying hematological disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute myeloblastic leukemia</td>
<td>3</td>
<td>22</td>
<td>25 (34.7)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>17</td>
<td>1</td>
<td>18 (25)</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>11</td>
<td>-</td>
<td>11 (15.3)</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>2</td>
<td>2</td>
<td>4 (5.5)</td>
</tr>
<tr>
<td>Myelodysplastic Syndrome</td>
<td>2</td>
<td>2</td>
<td>4 (5.5)</td>
</tr>
<tr>
<td>Chronic lymphoblastic leukemia</td>
<td>3</td>
<td>-</td>
<td>3 (4.2)</td>
</tr>
<tr>
<td>Chronic myeloblastic leukemia</td>
<td>-</td>
<td>1</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>Others</td>
<td>4</td>
<td>2</td>
<td>6 (8.3)</td>
</tr>
<tr>
<td><strong>PFN episodes&lt;sup&gt;2&lt;/sup&gt;</strong></td>
<td>n=50</td>
<td>n=35</td>
<td>n=85</td>
</tr>
<tr>
<td>Median days of fever, (range)</td>
<td>9 (5-33)</td>
<td>12 (5-37)</td>
<td>10 (5-37)</td>
</tr>
<tr>
<td>Median days of neutropenia, (range)</td>
<td>10 (5-63)</td>
<td>23 (6-56)</td>
<td>14 (5-63)</td>
</tr>
<tr>
<td>Profound neutropenia (ANC&lt;sup&gt;3&lt;/sup&gt; &lt;100µL)</td>
<td>49</td>
<td>35</td>
<td>84 (98.8)</td>
</tr>
<tr>
<td><strong>Risk factors for invasive fungal infection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute myeloblastic leukemia in induction or reinduction phase</td>
<td>-</td>
<td>20</td>
<td>20 (23.5)</td>
</tr>
<tr>
<td>Allogeneic HSCT&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-</td>
<td>15</td>
<td>15 (17.6)</td>
</tr>
<tr>
<td>Prior IFI&lt;sup&gt;5&lt;/sup&gt; episodes</td>
<td>1&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0</td>
<td>1 (1.2)</td>
</tr>
</tbody>
</table>

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IFI-high-risk patient: allogeneic HSCT recipient and patient with acute myeloblastic leukemia receiving induction or re-induction chemotherapy; PFN episodes persistent febrile neutropenia episodes; ANC: absolute neutrophil count; HSCT: hematopoietic stem cell transplantation; IFI: invasive fungal infection; Hepatosplenic candidiasis during induction chemotherapy in one patient with acute myeloblastic leukemia in consolidation phase at the moment of the study.
Table 2. Final diagnosis of persistent febrile neutropenia (PFN) episodes classified according to the indication or not of antifungal therapy.

<table>
<thead>
<tr>
<th>Final diagnosis of PFN episodes</th>
<th>Antifungal therapy</th>
<th>No antifungal therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Invasive fungal infection</td>
<td>43 (82.7)</td>
<td>25 (75.7)</td>
</tr>
<tr>
<td>Proven IFI¹</td>
<td>22 (42.3)</td>
<td>0</td>
</tr>
<tr>
<td>Probable IFI</td>
<td>9 (17.3)</td>
<td>0</td>
</tr>
<tr>
<td>Possible IFI</td>
<td>10 (19.2)</td>
<td>0</td>
</tr>
<tr>
<td>Non fungal infection</td>
<td>21 (40.4)</td>
<td>25 (75.7)</td>
</tr>
<tr>
<td>Non infection</td>
<td>9 (17.3)</td>
<td>7 (21.2)</td>
</tr>
<tr>
<td>Tumoral fever</td>
<td>5 (9.6)</td>
<td>5 (15.1)</td>
</tr>
<tr>
<td>Drug fever</td>
<td>2 (3.8)</td>
<td>2 (6.1)</td>
</tr>
<tr>
<td>Pulmonary thromboembolism</td>
<td>1² (1.9)</td>
<td>0</td>
</tr>
<tr>
<td>GVHD ²</td>
<td>1 (1.9)</td>
<td>0</td>
</tr>
<tr>
<td>Unknown fever</td>
<td>0</td>
<td>1³ (3)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>52 (100)</td>
<td>33 (100)</td>
</tr>
<tr>
<td>IFI high-risk episodes¹⁴</td>
<td>26 (50)</td>
<td>9 (27.3)</td>
</tr>
<tr>
<td>Non IFI high-risk episodes</td>
<td>26 (50)</td>
<td>24 (72.7)</td>
</tr>
</tbody>
</table>

IFI: invasive fungal infection; GVHD: graft versus host disease; This patient had a lymphoma and during neutropenia post chemotherapy presented with non focused fever, without severity signs, and negative radiological and microbiological tests, that solved without antifungal therapy; IFI high-risk episodes: persistent febrile neutropenia episodes in allogeneic hematopoietic stem cell transplant recipients and in patients with acute myeloblastic leukemia receiving induction or re-induction chemotherapy.
Table 3. Description of proven and probable invasive fungal infections, the positive diagnostic test that established their etiology and clinical outcomes.

<table>
<thead>
<tr>
<th>Etiology diagnostic</th>
<th>Clinical syndrome</th>
<th>Radiological test</th>
<th>Microbiological test</th>
<th>Days fever onsets-AT(^1)</th>
<th>Type of IFI</th>
<th>Antifungal therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proven <em>Aspergillus fumigatus</em></td>
<td>Pulmonary</td>
<td>Thoracic TSCT(^2)</td>
<td>BAL(^3), LB(^4)</td>
<td>+7 Baseline</td>
<td>Voriconazole #</td>
<td>Cure</td>
<td></td>
</tr>
<tr>
<td>Probable <em>Aspergillus</em> spp.</td>
<td>Pulmonary</td>
<td>Thoracic TSCT</td>
<td>BAL</td>
<td>+5 Baseline</td>
<td>Voriconazole #</td>
<td>Cure</td>
<td></td>
</tr>
<tr>
<td>Probable <em>Aspergillus</em> spp.</td>
<td>Individualized clinical decision</td>
<td>Thoracic TSCT</td>
<td>GM(^5)</td>
<td>+8 Break-through</td>
<td>Amphotericin #</td>
<td>Voriconazole ψ</td>
<td>Cure</td>
</tr>
<tr>
<td>Probable <em>Aspergillus</em> spp.</td>
<td>Pulmonary</td>
<td>Thoracic TSCT</td>
<td>GM</td>
<td>+6 Baseline</td>
<td>Voriconazole #</td>
<td>Cure</td>
<td></td>
</tr>
<tr>
<td>Probable <em>Aspergillus</em> spp.</td>
<td>Pulmonary and abdominal</td>
<td>Thoracic TSCT</td>
<td>GM</td>
<td>+7 Baseline</td>
<td>Voriconazole ψ</td>
<td>IPI(^6) related -death</td>
<td></td>
</tr>
<tr>
<td>Probable <em>Aspergillus</em> spp.</td>
<td>Pulmonary</td>
<td>Thoracic TSCT</td>
<td>GM</td>
<td>+10 Baseline</td>
<td>Voriconazole #</td>
<td>Cure</td>
<td></td>
</tr>
<tr>
<td>Probable <em>Aspergillus</em> spp.</td>
<td>Pulmonary</td>
<td>Thoracic TSCT</td>
<td>GM</td>
<td>+5 Baseline</td>
<td>Voriconazole #</td>
<td>Cure</td>
<td></td>
</tr>
<tr>
<td>Probable <em>Aspergillus</em> spp.</td>
<td>Pulmonary</td>
<td>Thoracic TSCT</td>
<td>GM</td>
<td>+11 Baseline</td>
<td>Voriconazole #</td>
<td>Cure</td>
<td></td>
</tr>
<tr>
<td>Probable hepatosplenic candidiasis</td>
<td>Abdominal, ESAF(^7)</td>
<td>Abdominal US(^8)</td>
<td>-</td>
<td>+5 Baseline</td>
<td>Caspofungin #</td>
<td>IFI non related-death</td>
<td></td>
</tr>
<tr>
<td>Proven <em>Candida albicans</em></td>
<td>Severe sepsis/ septic shock</td>
<td>Thoracic TSCT, Abdominal US</td>
<td>BC(^9)</td>
<td>+6 Baseline</td>
<td>Caspofungin #</td>
<td>IFI related-death</td>
<td></td>
</tr>
<tr>
<td>Proven <em>Candida tropicalis</em></td>
<td>Abdominal</td>
<td>-</td>
<td>BC</td>
<td>+5 Baseline</td>
<td>Caspofungin #</td>
<td>IFI non related-death</td>
<td></td>
</tr>
<tr>
<td>Probable candidiasis hepatosplenic</td>
<td>Abdominal, ESAF</td>
<td>Abdominal CT(^10)</td>
<td>-</td>
<td>+7 Baseline</td>
<td>Caspofungin #</td>
<td>Cure</td>
<td></td>
</tr>
</tbody>
</table>
1AT: antifungal therapy; 2Thoracic TSCT: thoracic thin section computed tomography; 3BAL: bronchoalveolar lavage; 4LB: lung biopsy; 5GM: serum galactomannan antigen test; 6IFI: invasive fungal infection; 7Abdominal US: abdominal ultrasound; 8ESAF: elevated serum alkaline phosphatase level (supporting microbiological criteria are not required for probable category); 9BC: blood cultures; 10Abdominal TC: abdominal computed tomography. #Primary antifungal therapy; Ω: Addition of antifungal to primary therapy; ψ: Change of antifungal drug.
Table 4. Sensitivity and specificity of the diagnostic and therapeutic approach for selecting hematological patients with persistent febrile neutropenia for antifungal therapy indication.

<table>
<thead>
<tr>
<th>Invasive fungal infection No.</th>
<th>Non invasive fungal infection No.</th>
<th>Total No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antifungal therapy indication</td>
<td>22(^1)</td>
<td>30</td>
</tr>
<tr>
<td>Non antifungal therapy indication</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>63</td>
</tr>
</tbody>
</table>

95% C. I.

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>Lower limit</th>
<th>Upper limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>100 %</td>
<td>85.1</td>
<td>100</td>
</tr>
<tr>
<td>Specificity</td>
<td>52.4 %</td>
<td>40.3</td>
<td>64.2</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>42.3 %</td>
<td>29.9</td>
<td>55.8</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>100%</td>
<td>89.6</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^1\)22: three proven, nine probable and ten possible invasive fungal infections were considered.
Table 5. Comparison of the overall successful response obtained in the diagnostic and therapeutic approach and in the clinical trial reported by Walsh et al.\textsuperscript{9} indicating universal empirical antifungal therapy.

<table>
<thead>
<tr>
<th>End point</th>
<th>Diagnostic and therapeutic approach (Cisneros et al.\textsuperscript{12}) (No = 85)</th>
<th>Caspofungin (Walsh et al.\textsuperscript{9}) (No = 556)</th>
<th>Liposomal amphotericin B (Walsh et al.\textsuperscript{9}) (No = 539)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall successful response, No. (%)</td>
<td>31 (36.5)</td>
<td>190 (33.9)</td>
<td>181 (33.7)</td>
</tr>
</tbody>
</table>

Components of primary end point

- Successful treatment of baseline proven or probable IFI \textsuperscript{1}  
  9/11 (81.8)  
  14/27 (51.9)  
  7/27 (25.9)

- Absence of proven or probable breakthrough IFI  
  84/85 (98.8)  
  527 (94.8)  
  515 (95.5)

- Survival for ≥ 7 days after completion study therapy  
  74/85 (87)  
  515 (92.6)  
  481 (89.2)

- Resolution of fever for at least 48 hours during neutropenia  
  36/85 (42.3)  
  229 (41.2)  
  223 (41.4)

- No therapy discontinuation prematurely because of toxicity or lack of efficacy  
  80/85 (94.1)  
  449 (89.7)  
  461 (85.5)

\textsuperscript{1} IFI: invasive fungal infection proven and probable were considered.
Figure 1. Diagnostic and therapeutic approach in persistent febrile neutropenic patients with hematological malignancies or hematopoietic stem cell transplant recipients.

PERSISTENT FEBRILE NEUTROPENIA (> OF 4 DAYS)

Severe sepsis/septic shock
Blood cultures

Step one: Clinical evaluation of the severity

No

Step two: Evaluation of the focus of fever

Focused fever

- Caspofungin
- L-Amb

- Pneumonia
  - Thoracic TSCT, BAL, GM

- Rhinosinusitis
  - Sinus CT, rhinoscopy

- CNS Abscess
  - Abscess biopsy

- Abdominal focus
  - Abdominal US/CT

- Skin lesions
  - Skin biopsy

- Voriconazole
- L-Amb*

- Voriconazole
- L-Amb*

- Voriconazole
- L-Amb*

- Caspofungin
- L-Amb

No focused fever

- Thoracic TSCT, GM

- No antifungal therapy

TSCT: thin-section computed tomography; BAL: bronchoalveolar lavage; GM: serum galactomannan antigen test; CNS: central nervous system; US: Ultrasound; L-Amb: liposomal amphotericin; *Liposomal amphotericin will be choice antifungal therapy if mucormycosis is suspected.; **Voriconazole and/or liposomal amphotericin will be choice antifungal therapy if Aspergillus spp., Scedosporium spp. or Fusarium spp. is suspected.
Figure 2. Results of the implementation of the diagnostic and therapeutic approach in persistent febrile neutropenic patients with hematological malignancies or hematopoietic stem cell transplant recipients.

Evaluation between 5th-7th day of fever onset (n = 85 PFN1 episodes)

- Yes AT2
  - 32 (37.6%)
  - AT indications:
    - Pulmonary infiltrate (n=16, 19%)
    - Hepatomegaly/cholestasis (n=6, 7%)
    - Septic shock (n=4, 5%)
    - Rhinosinusitis (n=1, 1%)
    - Necrotizing enterocolitis (n=1, 1%)
    - Individual clinical decision (n=1, 1%)
    - Mucositis (n=1, 1%)
    - Folliculitis (n=1, 1%)

- No AT
  - 53 (62.3%)

Further diagnostic evaluation (after 7th day)

- Yes AT
  - 20 (23.5%) PFN episodes
  - AT indications:
    - Pulmonary infiltrate (n=10, 10.5%)
    - Hepatomegaly/cholestasis (n=6, 7%)
    - Individualized clinical decision (n=3, 3.5%)
    - GM3 positive (n=1, 1%)

- No AT
  - 33 (38.8%) PFN episodes

1PFN: persistent febrile neutropenia; 2AT: antifungal therapy; 3GM: serum galactomannan antigen test.
<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Most probable fungal etiology</th>
<th>Diagnostic tests</th>
<th>Antifungal therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non focused fever</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No severity signs</td>
<td>Non fungal</td>
<td>Serum GM&lt;sup&gt;1&lt;/sup&gt;, blood cultures, abdominal US&lt;sup&gt;2&lt;/sup&gt;</td>
<td>No</td>
</tr>
<tr>
<td>Severe sepsis or septic shock</td>
<td><em>Candida</em> spp.</td>
<td>Blood cultures</td>
<td>Caspofungin&lt;sup&gt;#&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amphotericin&lt;sup&gt;ψ&lt;/sup&gt;</td>
</tr>
<tr>
<td>Focused fever</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary</td>
<td><em>Aspergillus</em> spp.</td>
<td>BAL&lt;sup&gt;3&lt;/sup&gt; Thoracic TSCT&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Voriconazole&lt;sup&gt;#&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amphotericin&lt;sup&gt;ψ&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Caspofungin&lt;sup&gt;ψ&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sinusitis</td>
<td><em>Aspergillus</em> spp.</td>
<td>Rhinoscopy Sinus CT&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Voriconazole&lt;sup&gt;#&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amphotericin&lt;sup&gt;ψ&lt;/sup&gt;</td>
</tr>
<tr>
<td>CNS</td>
<td><em>Aspergillus</em> spp.</td>
<td>CNS&lt;sup&gt;6&lt;/sup&gt; CT</td>
<td>Voriconazole&lt;sup&gt;#&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amphotericin&lt;sup&gt;ψ&lt;/sup&gt;</td>
</tr>
<tr>
<td>Abdominal</td>
<td><em>Candida</em> spp.</td>
<td>Abdominal US or CT Endoscopy</td>
<td>Caspofungin&lt;sup&gt;#&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amphotericin&lt;sup&gt;ψ&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fluconazole&lt;sup&gt;ψ&lt;/sup&gt;</td>
</tr>
<tr>
<td>Skin</td>
<td><em>Candida</em> spp.</td>
<td>Skin biopsy</td>
<td>Caspofungin&lt;sup&gt;#&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amphotericin&lt;sup&gt;ψ&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fluconazole&lt;sup&gt;ψ&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>GM: serum galactomannan antigen test; <sup>2</sup>US: ultrasound; <sup>3</sup>BAL: bronchoalveolar lavage; <sup>4</sup>TSCT: thin-section computed tomography; <sup>5</sup>CT: computed tomography; <sup>6</sup>CNS: central nervous system. *Amphotericin when Mucor spp. infection is suspected; # Primary therapy; ψ Alternative therapy; & Primary therapy except for severely ill patients or with previous azole prophylaxis.