Author’s response to reviews

Title: Incidence and characteristics of invasive fungal diseases in allogeneic hematopoietic stem cell transplant recipients: a retrospective cohort study

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Author’s response to reviews:

Dear Editor,

We are submitting the revision of our paper “Incidence and characteristics of invasive fungal diseases in allogeneic hematopoietic stem cell transplant recipients: a retrospective cohort study“ by Nicole Harrison, Margit Mitterbauer, Selma Tobudic, Peter Kalhs, Werner Rabitsch, Hildegard Greinix, Heinz Burgmann, Birgit Willinger, Elisabeth Presterl, and Christina Forstner as original article to the Journal “BMC Infectious Diseases”.

We confirm that we have read the instructions for authors and accept the conditions therein. All the named authors have seen and agreed to the submitted version of the manuscript and contributed significantly to the work. On behalf of my co-authors we certify that this article is original. It is not under consideration by another journal and has not been previously published. Correspondence concerning the disposition of the manuscript should be directed to the corresponding author.

Comments and suggestions of both reviewers have been very helpful to improve the quality of our manuscript. Point to point answers to the reviewer comments are given below.

REVIEWER 1:

a- Please explain how the fungi were identified.

ANSWER:

We agree with the reviewers that additional information on the identification of fungi is needed. Therefore we have included a whole paragraph in the methods section detailing microbiological identification of fungi and we added Prof. Dr. Birgit Willinger from the department of laboratory medicine, division of clinical microbiology as additional co-author.

CHANGES (Line 118-136):

Identification of fungal pathogens

All microbiological specimens were analysed for fungal pathogens by the department of clinical microbiology of the Medical University of Vienna. Blood cultures were incubated in the BacTAlert® (BioMerieux, France) for fourteen days and if a positive signal was obtained, a gram stain and a subculture on Sabouraud Glucose Agar (SAB) and Chromagar Candida® (Becton Dickinson, Heidelberg, Germany) were performed. Further identification of yeasts was done using either the Vitek® system or ATB Candida ID32C® (both BioMerieux, France). Also, rice-extract agar was inoculated and incubated at 28°C for the formation and examination of micromorphology. Other specimens such as sputum or bronchoalveolar lavage fluid were inoculated on Sabouraud Glucose Agar (SAB) and Chromagar Candida® and incubated at 35°C and 21°C for seven days. In addition a SAB-broth was inoculated for enrichment. Identification of moulds was performed by examining macro- and micromorphology. In case identification was impossible using conventional methods, sequence analysis was suspended. In addition, the
multiplex PCR Septifast (Roche, USA) was used for more rapid detection of certain Candida species and Aspergillus fumigatus. Aspergillus antigen was determined by using the serum galactomannan ELISA assay (Bio-Rad, Hercules, CA, USA) with a cut-off value for the optical density index of ≥0.5 for serum and ≥0.8 for BAL. For the identification of Pneumocystis jirovecii real-time PCR from BAL was performed. In some cases fungi were identified during autopsy or histological examination of tissues by direct microscopy by the department of pathology.

b- Candida species and Aspergillus species should be replaced by the complete identification.

ANSWER:

Although we agree with the reviewer that a complete identification of the fungal pathogen is preferable, we cannot provide a species in four cases of IFD (one invasive candidiasis, and three cases of invasive aspergillosis). The reason for this is that the fungi were identified by direct microscopy of histological samples by the department of pathology. A further identification of the species was not performed. However, according to the revised definitions for invasive fungal disease by the EORTC Consensus Group, direct microscopy is sufficient for a proven IFD (De Pauw et al, 2008). We referred to the histological examination of fungi in the methods section.

c- Candida glabrata is a species complex. If the identification was performed by phenotypic method it should be named as C. glabrata sensu lato or C. glabrata complex. If molecular identification was performed, it should be named C. glabrata sensu stricto (or C. nivariensis/C. bracarensis).

d- Aspergillus fumigatus and A. niger are species of the Aspergillus section fumigati and A. section nigri, respectively. As for C. glabrata, it is important to molecularly differentiate the species.

ANSWER:

We are grateful to the reviewer for pointing this out. Considering that identification was mostly carried out by phenotypic methods, as detailed in the methods section, we changed the taxonomy to Candida glabrata complex, Aspergillus section fumigati and Aspergillus section nigri.
Among the proven IFDs the following fungal species were identified: Candida albicans (n=1), Candida glabrata complex (n=2), Candida krusei (n=1), Candida species (n=1), Aspergillus section fumigati (n=1) and Aspergillus species (n=2). Pathogens of probable IFDs included Candida albicans (n=2), Pneumocystis jirovecii (n=3), Aspergillus section fumigati (n=1), Aspergillus section nigri (n=1), Aspergillus species (n=1) and 12 cases of invasive aspergillosis diagnosed on basis of a suspicious CT-scan and positive test for galactomannan antigen.

Figure 1: The diagnosis of IFD after allogeneic HSCT was very quick. In other reports (Pappas CID 2010, Omer et al Biol Blood Marrow Transplant 2013, Sun et al Clinical Microbiology and Infection 2013, etc) the median was around +120-180 days for Aspergillus spp. and +50 for Candida spp. (depending on the type of transplant). This point should be discussed.

ANSWER:

We agree with the reviewer that the early onset of IFDs at our centre is an interesting point to discuss. Therefore we added a new paragraph about this topic to our discussion. We also added the above-mentioned references except for Pappas et al. 2010 (the reference Pappas et al. was replaced by Kontoyiannis et al. because Pappas et al. included only data on fungal diseases after solid organ transplantation, whereas Kontoyiannis published data on fungal diseases after HSCT, both being part of the TRANSNET group).

Compared to other studies, we observed that IFDs occurred quite early after HSCT at our centre. The median time to IFD was only 36 days (8 days for candidiasis, 36 days for aspergillosis) while other studies reported a median time to IFD of 139 days [13] or 174 days [24] and the TRANSNET study observed that candidiasis and aspergillosis occurred after at a median of 61 and 99 days, respectively [23]. In our cohort 15 IFDs occurred very early after HSCT (median of 9 days, range 1; 41), while all other IFDs occurred more than 150 days after HSCT. In the early IFD group only 33.3% received prophylaxis during aplasia (fluconazole, n=2; posaconazole, n=3). In other centres all patients [13, 24] or the majority of patients [11] received at least fluconazole prophylaxis during aplasia. Limited use of fluconazole prophylaxis at our centre might explain the early onset of candidiasis [8], but not of invasive aspergillosis. Other factors like time to engraftment, conditioning regimen and donor grafts did not provide an explanation for the early incidence of IFDs in our cohort compared to other centres. Interestingly, Sun et al described that matched sibling donors had a much later onset of IFDs (median of 142 days) than haploidentical donors (median of 23 days) [11]. Similarly, we observed that the median time to
IFD was much earlier for mismatched unrelated donors (median of 14 days) and matched unrelated donors (median of 41 days) than for matched sibling donors (median of 156 days).

REVIEWER 2:

The laboratory procedures for the diagnosis of fungal infection must be clearly stated in the paper: who were screened for invasive fungal disease and which methods were applied in the detection and speciation of fungi. The method to diagnose Pneumocystis jirovecii was not included in reference 9 and must be stated.

ANSWER:

We are very grateful to the reviewer for these comments. We added a new paragraph on the identification of fungi including Pneumocystis jirovecii to the methods section (see answer a for reviewer 1) and we provided the necessary references. Furthermore, we added a new paragraph about the screening and the diagnostic procedures concerning IFDs to the methods section.

CHANGES (Line 104-116):

Screening and diagnostic procedures for IFDs

All patients were screened for invasive aspergillosis using serum galactomannan before HSCT and twice weekly after HSCT until they were discharged. During the follow-up patients were screened with serum galactomannan every two weeks until day +100. In case of fever the standard procedures included blood cultures at two different time points and a multiplex PCR which is capable to detect five different Candida species and Aspergillus fumigatus. Patients presenting with respiratory symptoms were first examined by chest X-ray and in case of infiltrates or worsening gas exchange followed-up with a computed tomography (CT) scan of the lungs. If the CT scan showed pulmonary infiltrates, bronchoscopy was performed and fungal culture, galactomannan and PCR for fungal pathogens including Pneumocystis jirovecii were conducted from the bronchoalveolar lavage fluid [19]. Transbronchial biopsy during bronchoscopy or CT-guided biopsy of suspected liver lesions was performed in some cases. Patients with neurological symptoms received magnetic resonance imaging (MRI) of the brain and lumbar puncture including culture and PCR for different fungal pathogens.

Thank you for considering our paper.
Sincerely yours,

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