PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Monitoring of clinical strains and environmental fungal aerocontamination to prevent invasive Aspergillosis infections in hospital during large deconstruction work: a protocol study
AUTHORS	Loeffert, Sophie; Melloul, Elise; Dananché, Cédric; Hénaff, Laetitia; Bénet, Thomas; Cassier, Pierre; Dupont, Damien; Guillot, Jacques; Botterel, Françoise; Wallon, Martine; Gustin, Marie-Paule; Vanhems, Philippe

VERSION 1 – REVIEW

REVIEWER	Astrid Mayr Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria
REVIEW RETURNED	28-Jun-2017

GENERAL COMMENTS	Thank you for the opportunity to review this manuscript. The authors address an important and interesting issue in infection prevention. The manuscript is clear and well written, quantity of data and methods representative. However, there are some aspects of interests for the reader: 1) Had there been a problem/rise in the hospital setting concerning IA before construction. How is the epidemiological situation in this setting-is Aspergillus of concern or other fungi? 2) What kind of preventive measurements/precautions are performed when deconstruction/construction is conducted (e.g. dust protection, education of staff,...)?

REVIEWER	Alanio Alexandre Saint-Louis Hospital, Paris France Already published about invasive aspergillosis and genotyping.
REVIEW RETURNED	12-Jul-2017

GENERAL COMMENTS	The authors describe here a protocol study without giving any results. Major comments: There is well described literature (not cited in the present references) explaining that environmental investigation of Aspergillus fumigatus from the environment and trying to correlate with the appearance of invasive aspergillosis is very difficult if not impossible due to the limitations of sampling in the environment and lack of reliable genotyping tools (microsatellites and VNTR are convenient but not accurate enough due to sex and rebombination of A. fumigatus, see. Klaassen CHW, Gibbons JG, Fedorova ND, Meis JF, Rokas A. Evidence for genetic differentiation and variable recombination rates among Dutch populations of the opportunistic human pathogen Aspergillus fumigatus. Mol Ecol. Blackwell Publishing Ltd; 2012 Jan;21(1):57–70.). See reviews on the subject in literature. In fact, Aspergillus is a mold that produce conidia that are able to disseminate in the environment through air. The diversity is known to be very high in the environment, especially if a lot of dust is generated. By definition, environmental sampling is always limited. And it is known for a long time that fumigatus comes from the environment. Rather than genotyping the authors should focus on other metrics from the environment that would correlate better with the appearance for invasive aspergillosis even if A. fumigatus is not found in culture. Genotypic concordance between environmental and clinical isolates are not observed in some reports and sometimes described but in rare cases. - Chazalet V, Debeaupuis JP, Sarfati J, Lortholary J, Ribaud P, Shah P, et al. Molecular typing of environmental and patient isolates of Aspergillus fumigatus from various hospital settings. J Clin Microbiol. 1998 Jun 1;36(6):1494–500. - Debeaupuis JP, Sarfati J, Chazalet V, Latgé JP. Genetic diversity among clinical and environmental isolates of Aspergillus fumigatus. Infect Immun. 1997 Aug;65(8):3080–5. - Bart-Delabesse E, Cordonnier C, Bretagne S. Usefulness of genotyping with microsatellite markers to investigate hospital-acquired invasive aspergillosis. J Hosp Infect. 1999 Aug 1;42(4):321–7. - Menotti J, Waller J, Meunier O, Letscher-Bru V, Herbrecht R, Candolfi E. Epidemiological study of invasive pulmonary aspergillosis in a haematology unit by molecular typing of environmental and patient isolates of Aspergillus fumigatus. J Hosp Infect. 2005 May;60(1):61–8.

	- Araujo R, Amorim A, Gusmão L. Genetic diversity of Aspergillus fumigatus in indoor hospital environments. Med Mycol. 2010 Sep;48(6):832–8. - Araujo R, Pina-Vaz C, Rodrigues AG, Amorim A, Gusmão L. Simple and highly discriminatory microsatellite-based multiplex PCR for Aspergillus fumigatus strain typing. Clin Microbiol Infect. 2009 Mar;15(3):260–6. The authors did not discuss at all prophylaxis strategies which may prevent the development of IA and so limiting the number of IA even if the inoculum indoor and outdoor is important. This point should be anticipated in the statistics and for the duration of the study to reach the ideal/sufficient number of cases. The authors did not explained why they chose to investigate 100L outdoor and 250 L indoor ? Why these discrepancies? The authors should incubate culture at 45°C-50° rather that 37°C because it will be more selective for A. fumigatus. The authors did not explain why identification of the fungi recovered in culture are important for environmental evaluation. Do identification at the species level is important, at the genus level? Do the number of mold CFU is enough to evaluate environmental contamination? The authors should explain that only molds and not fungi in genral including yeasts have been investigated. • The authors should provide details about what means « mycological results positive for Aspergillus spp. » Positive culture ? Detection of GM or BDG?□□□□□□□□□□□□□□□□□□□□□ ? • Usually, IA cases in prospective studies are investigated by clinician specialized in infections diseases, pneumologists, radiologists and microbiologists. The cases should be reviewed by such committee. • Community-acquired cases should be better defined especially the delay between admission and symptoms, appearance of the nodule and positive GM or culture. • Practically speaking, patients are frequently hospitalize before invasive aspergillosis to perform chemotherapy and sometimes patients stays at the hospital several days and the come back home before coming back to the hospital with invasive aspergillosis. Do these IA really community acquired. These cases are clearly undertermined. Minor comments: Table II should be provided as a editable table and not a .pdf file Reference 6 is not useful since the authors are dealing with invasive aspergillosis

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Thank you for the opportunity to review this manuscript.
The authors address an important and interesting issue in infection prevention. The manuscript is clear and well written, quantity of data and methods representative.
However, there are some aspects of interests for the reader:

1) Had there been a problem/rise in the hospital setting concerning IA before construction.

Response: Thank you for your comment. Indeed, several years before the actual construction works, a 30 months retrospective study was conducted in a hematological unit of our hospital which was undergoing renovation. In 1987, a total of 22 cases of invasive aspergillosis (IA) were reported including 18 deaths for which the link between IA and death were possible*. Similarly, a quasi-experimental study, including a control group and an intervention group was conducted in the 3 adult hematological intensive care units between 2005 and 2006**. This study evaluated the incidence of IA due to the delocalization of hematological intensive care units. In total, 21 cases of IA were included. 18 were nosocomial, and 3 were of undetermined origin. In the relocated unit, the incidence of IA decreased from 13.2% (9 patients) before relocation, to 1.6% (1 patient) after relocation (P=.018). Those studies highlighted the importance of monitoring environmental factors to prevent IA especially during construction works. However, the hematology department was relocated in another hospital several years ago. In 2013, a cluster of 5 IA cases was identified in immunocompromised non-hematological patients. This cluster was attributed to an increased number of small building works in the hospital. Then, clinicians and infection control unit were aware of this risk before the major demolition works in 2015.
*Perraud P, Piens MA, Nicoloyannis N, et al. Invasive nosocomial pulmonary aspergillosis: risk factors and hospital building works. Epidem Infec. 1987; 99: 407-12.

**Bénet T, Nicolle MC, Thiebaut A, et al. Reduction of invasive aspergillosis incidence among immunocompromised patients after control of environmental exposure. Clin Infect Dis. 2007; 45(6): 682-6.

2) How is the epidemiological situation in this setting-is Aspergillus of concern or other fungi?

Response: Among all fungal species, Aspergillus fumigatus remains the major concern in our hospital. This fungus was the one for which air samples were performed. Aspergillus spp. was identified in 41.1% of the air samples, and A. fumigatus was the most common species retrieved (66.7%).We also found other environmental fungi as other Aspergillus, Penicillium sp, Mucorales without precise research question for these agents.

3) What kind of preventive measurements/precautions are performed when deconstruction/construction is conducted (e.g. dust protection, education of staff,...)?

Response: We added this paragraph in the manuscript (pages 6-7): ”Several preventive measures were performed (i) doors and windows in front of the deconstruction site were maintained closed during the day and allowed for opening at night, (ii) patient and medical staff movements were limited and special traffic patterns were designed, (iii) masks were requested for hospitalized or non-hospitalized immunocompromised patients outside wards to limit fungal exposure, (iv) adhesive decontamination carpets were installed at the entry of wards, (v) visitors, patients and medical staff were alerted about fungal exposure due to the deconstruction site. In case of high fungal exposure, after results of environmental survey, intensive bio-cleaning was performed.

Preventive measures were also implemented outdoor, at the deconstruction site to limit fungal dispersion. Construction site teams received information and education about IA risks. Humid environment was mandatory for all works completed by regular humidification. Furthermore, ruined buildings rubbles are humidified and covered for evacuation. Circulation pattern for rubbles evacuation is also designed. Damp cleaning of deconstruction site roads are frequently realized with, in addition, cleaning of truck wheels."
We added a link of a short video showing this huge demolition project (page 5): link: https://www.youtube.com/watch?v=Oa7xRufAnhQ

Reviewer: 2

1) The authors describe here a protocol study without giving any results.

Response: We agree with the reviewer but according to the scope of the journal, this article is about objectives and methodology. The results will be the subjects of another article submitted in the next months.

Major comments:
2) There is well described literature (not cited in the present references) explaining that environmental investigation of Aspergillus fumigatus from the environment and trying to correlate with the appearance of invasive aspergillosis is very difficult if not impossible due to the limitations of sampling in the environment and lack of reliable genotyping tools (microsatellites and VNTR are convenient but not accurate enough due to sex and rebombination of A. fumigatus, see. Klaassen CHW, Gibbons JG, Fedorova ND, Meis JF, Rokas A. Evidence for genetic differentiation and variable recombination rates among Dutch populations of the opportunistic human pathogen Aspergillus fumigatus. Mol Ecol. Blackwell Publishing Ltd; 2012 Jan;21(1):57–70.).See reviews on the subject in literature.

Response: We do agree with the reviewer: environmental sampling and genotyping have both limitations and the correlation between environmental contamination and the occurrence of IA is hardly ever established. However, as shown by few studies (Guinea et al., 2011; Bart-Delabesse et al., 1999; Chazalet et al., 1998), genotyping of environmental and/or clinical A. fumigatus isolates may help to better understand the local disease epidemiology, and to identify the source and the routes of transmission. As mentioned in a study from the group of the reviewer (Alanio et al., 2017), "there is no consensus on a universal typing system". We think that the question remains still open. We choose MLVA typing method; which showed a high discriminatory power with a Simpson's diversity index of 0.9994, good reproducibility for analysis of epidemiological relationships between large amounts of A. fumigatus isolates over a long period of time in hospitals. (Thierry et al., 2010). Of course, as mentioned, the genetic differentiation and variable recombination rates in A. fumigatus could prevent correct analysis of genotyping result.

We added a sentence in the discussion to describe these limits:
-Page (17):"Correlation between environmental contamination and the occurrence of IA is hardly ever established due to limitations of both environmental sampling and genotyping."
- Page 17: "Principally two methods of genotyping have emerged for study of medically important fungi: multilocus sequence typing (MLST) and method bases on short tandem repeats"
- Page 18: "Klassen et al. have underlined the existence of possible genetic differentiation and variable recombination rates of A. fumigatus which could prevent correct analysis of genotyping result"

References:
• Guinea J, García de Viedma D, Peláez T, Escribano P, Muñoz P, Meis JF, Klaassen CH, Bouza E. Molecular epidemiology of Aspergillus fumigatus: an in-depth genotypic analysis of isolates involved in an outbreak of invasive aspergillosis. J Clin Microbiol 2011;49(10):3498-503.
• Chazalet V, Debeaupuis JP, Sarfati J, Lortholary J, Ribaud P, Shah P, et al. Molecular typing of environmental and patient isolates of Aspergillus fumigatus from various hospital settings. J Clin Microbiol. 1998 Jun 1;36(6):1494–500.
• Bart-Delabesse E, Cordonnier C, Bretagne S. Usefulness of genotyping with microsatellite markers to investigate hospital-acquired invasive aspergillosis. J Hosp Infect. 1999 Aug 1;42(4):321–7.
• Alanio A, Desnos-Ollivier M, Garcia-Hermoso D, Bretagne S. Investigating Clinical Issues by Genotyping of Medically Important Fungi: Why and How? Clin Microbiol Rev. 2017;30(3):671-707.
• Thierry S, Wang D, Arné P, Deville M, De Bruin B, Nieguitsila A, Pourcel C, Laroucau K, Chermette R, Huang W, Botterel F, Guillot J. Multiple-locus variable-number tandem repeat analysis for molecular typing of Aspergillus fumigatus. BMC Microbiol 2010;10:315.
• Klaassen CHW, Gibbons JG, Fedorova ND, Meis JF, Rokas A. Evidence for genetic differentiation and variable recombination rates among Dutch populations of the opportunistic human pathogen Aspergillus fumigatus. Mol Ecol. Blackwell Publishing Ltd; 2012;21(1):57–70.

3) In fact, Aspergillus is a mold that produce conidia that are able to disseminate in the environment through air. The diversity is known to be very high in the environment, especially if a lot of dust is generated. By definition, environmental sampling is always limited.

Response: Yes indeed, environmental sampling is always limited if we consider the large number of potential sources of Aspergillus development and dispersal. When there is demolition works, a lot of conidia are disseminated and an increase of outdoor contamination could happen. The increase of the number of Aspergillus conidia outdoor is known to impact the level of indoor contamination. Outdoor warning system could help to prevent indoor Aspergillus contamination. In the present study we tried to evaluate the efficacy of a non-cultivable method (Hirst spore –traps sampler), which continuously monitor Aspergillaceae conidia. Large-scale surveillance systems are needed to detect outdoor fungal spores and alert hospitals to quickly implement indoor control measures.

4) And it is known for a long time that fumigatus comes from the environment.

Response: We do agree with the reviewer. However, we considered that the period of major deconstruction work was an opportunity to describe and analyse the increase of A. fumigatus concentration in the environment. Most study failed to correlate the presence of construction works with higher contamination of A. fumigatus in the hospital (Hansen D et al 2008; Berthelot P et al 2006; Fournel et al., 2010; Reboux et al.,2014).

References:
Hansen D, Blahout B, Benner D, Popp W. Environmental sampling of particulate matter and fungal spores during demolition of a building on a hospital area. J Hosp Infect. 2008;70(3):259-64. doi: 10.1016/j.jhin.2008.07.010.

Berthelot P, Loulergue P, Raberin H, et al. Efficacy of environmental measures to decrease the risk of hospital-acquired aspergillosis in patientshospitalised in haematology wards. Clin microbiol Infect. 2006;12 : 738-44.

Reboux G, Gbaguidi-Haore H, Bellanger AP, et al. A 10-year survey of fungal aerocontamination in hospital corridors: a reliable sentinel to predict fungal exposure risk? J Hosp Infect 2014;87:34-40.
Fournel I, Sautour M, Lafon I, et al. Airborne Aspergillus contamination during hospital construction works: efficacy of protective measures. Am J Infect Control 2010;38(3):189-94.

4) Rather than genotyping the authors should focus on other metrics from the environment that would correlate better with the appearance for invasive aspergillosis even if A. fumigatus is not found in culture.

Response: Thank you for your comment. We hope that “other metrics” suggested by the reviewer are those related with meteorological conditions.
In this study we collected meteorological parameters (rainfall, relative humidity, temperature, wind speed and direction) every 2 hours during one year. As found by previous study (Brenier-Pinchart et al., 2009; Cavallo et al., 2013) analysis of meteorological conditions could explain some variation in indoor Aspergillus contamination, even if some other internal factors have to be collected for a precise analysis. Then, we aimed to analyze the relationship between these climatic conditions and A. fumigatus concentration in the environment (outside and inside hospital blocks).

5) Genotypic concordance between environmental and clinical isolates are not observed in some reports and sometimes described but in rare cases.

- Chazalet V, Debeaupuis JP, Sarfati J, Lortholary J, Ribaud P, Shah P, et al. Molecular typing of environmental and patient isolates of Aspergillus fumigatus from various hospital settings. J Clin Microbiol. 1998 Jun 1;36(6):1494–500.
- Debeaupuis JP, Sarfati J, Chazalet V, Latgé JP. Genetic diversity among clinical and environmental isolates of Aspergillus fumigatus. Infect Immun. 1997 Aug;65(8):3080–5.
- Bart-Delabesse E, Cordonnier C, Bretagne S. Usefulness of genotyping with microsatellite markers to investigate hospital-acquired invasive aspergillosis. J Hosp Infect. 1999 Aug 1;42(4):321–7.
- Menotti J, Waller J, Meunier O, Letscher-Bru V, Herbrecht R, Candolfi E. Epidemiological study of invasive pulmonary aspergillosis in a haematology unit by molecular typing of environmental and patient isolates of Aspergillus fumigatus. J Hosp Infect. 2005 May;60(1):61–8.
- Araujo R, Amorim A, Gusmão L. Genetic diversity of Aspergillus fumigatus in indoor hospital environments. Med Mycol. 2010 Sep;48(6):832–8.
- Araujo R, Pina-Vaz C, Rodrigues AG, Amorim A, Gusmão L. Simple and highly discriminatory microsatellite-based multiplex PCR for Aspergillus fumigatus strain typing. Clin Microbiol Infect. 2009 Mar;15(3):260–6.

Response: Some of these references are now included in the discussion of the manuscript (page, 17 references 39,40). We did not include the studies from Araujo et al., (2009 and 2010) because environmental (2010) or clinical isolates (2009) were analysed independently. The other studies (Chazalet et al., 1998; Debeaupuis et al., 1997; Bart-Delabesse et al., 1999; Menotti et al., 2005) genotyped isolates coming from the environment and from clinical samples. Debeaupuis et al 1997; Bart-Delabesse et al 1999 and Menotti et al 2005 made only indoors environmental sampling. Chazalet et al (1998) performed both indoor and outdoor sampling but every two weeks in 4 different hospitals, while we performed 28 air samples (indoor: 16, outdoor: 12) per days during 11-months. Previous investigations found concordance between environmental and clinical isolates. In the present study, we were able to perform a very large environmental investigation (112 air samples were made every week). We collected 600 environmental A. fumigatus isolates in a 11-month period. We would like to genotype at least 400 environmental isolates coming from indoor and outdoor sites completed by clinical samples.

6) The authors did not discuss at all prophylaxis strategies which may prevent the development of IA and so limiting the number of IA even if the inoculum indoor and outdoor is important.

Response: Thank you for your comment. As we have no more hematological department in our hospital, the high risk populations susceptible to received prophylaxis strategies are more limited. Before deconstruction works, a multidisciplinary meeting including the president of the medical committee, the president of the hospital infection control committee and 2 hospital practitioners decided not to extend indications of prophylaxis to immunocompromised patients without acute leukemia. In case of occurrence of increased incidence of IA, this possibility would be discussed. We added information in the manuscript (page16): “No specific prophylaxis strategy was implemented at our hospital for immunocompromised patients. Only patients with acute leukemia were susceptible to received prophylaxis therapy according to their condition”.

7) This point should be anticipated in the statistics and for the duration of the study to reach the ideal/sufficient number of cases.

Response: The present study was not performed to estimate the impact of prophylaxis in patient at risk. We agree that prophylaxis might be discussed but to confirm similarities between environmental and clinical isolates, no statistic test was planned. Sample size calculation was not necessary to be planned. The objective of this study was essentially to describe potential relationship between environmental and clinical isolates coming from potential IA clinical cases only. Because no comparison between groups was planned, the sample size calculation was not done. Some cases might be missed but well documented cases, with isolates found both in the patient and the environment will be an interesting finding.

8) The authors did not explained why they chose to investigate 100L outdoor and 250 L indoor ? Why these discrepancies?

Response: We decided to investigate 100L outdoor and 250L indoor according to the guidelines for environmental fungal risk control in French hospitals (Gangneux et al., 2006; Méheust et al., 2013). They recommend a sample volume adapted to the presumed levels of contamination in the environment. Due to the major demolition works ongoing, outdoor air was considered more contaminated by fungi than indoor. So outdoor plates were seeded for only 1 min corresponding to an air volume of 100L to avoid overcrowding on the plates. Indoor samples were supposed to have an intermediate fungal contamination level due to the preventive measures applied to reduce environmental contamination inside units. Therefore, Indoor plates were seeded for 2½ min, resulting in a higher air volume (250L).

We added details in the manuscript (page 9). “Two plates were seeded at each sample site. Air sample volume was chosen according to French guidelines environmental fungal risk control.22,23 They recommend a sample volume adapted to the presumed levels of contamination in the environment. Due to the major demolition works ongoing, outdoor air was considered more contaminated by fungi than indoor. So in order to avoid overcrowding on the plates, outdoor plates were seeded for only 1 min corresponding to an air volume of 100L. Indoor samples were supposed to have an intermediate fungal contamination level due to the preventive measures applied to reduce environmental contamination inside units. Therefore, Indoor plates were seeded for 2½ min, resulting in a higher air volume (250L).”

References:
Méheust D, Gangneux JP, Cann PL. Comparative evaluation of three impactor samplers for measuring airborne bacteria and fungi concentrations. J Occup Environ Hyg. 2013;10(8):455-9.
Gangneux JP, Bousseau A, Cornillet A, Kauffmann-Lacroix C. Control of fungal environmental risk in French Hospitals. Journal de Mycologie Médicale. 2006;16:204–211.

9) The authors should incubate culture at 45°C-50° rather that 37°C because it will be more selective for A. fumigatus.

Response: Thank you for your comment. We selected 37°C as the incubation temperature based on Boff et al* results. In their study, they compared the effect of different incubation temperatures on the recovery of potentially pathogenic fungi particularly Aspergillus species in hospital environment. They showed that an incubation temperature between 35-40°C facilitates the growth of Aspergillus section Fumigati. However, the randomly A. fumigatus colonies choose to be stored for further genotyping analysis were subcultured on Sabouraud dextrose agar and incubated at 45°C in order to select A. fumigatus rather than other related Aspergillus coming from Fumigati section (A. lentulus…).

We added the reference in the methodology and some details in the manuscript (page 11): “During the study, a maximum of 4 A. fumigatus colonies per day among all A. fumigatus environmental cultures incubated at 37°C were arbitrarily isolated, subcultured on Sabouraud dextrose agar and incubated at 45°C in order to select A. fumigatus before being frozen.”

Reference:
*Boff C, Brun CP, Miron D, et al. Technical note: The effect of different incubation temperatures on the recovery of Aspergillus species from hospital air. Am J Infect Control 2012;40:1016-7.

10) The authors did not explain why identification of the fungi recovered in culture are important for environmental evaluation. Do identification at the species level is important, at the genus level? Do the number of mold CFU is enough to evaluate environmental contamination?

Response: Two sampling methods (cultivable and non-cultivable) are included in the protocol of the environmental survey. Non-cultivable methods allow the sampling of numerous spores for large-scale surveillance systems but have limitation in fungal identification which is generally possible only at the genus level. With non-cultivable methods genotyping and evaluation of antifungal susceptibility are not possible. Cultivable methods are time-consuming. Their results depend on the substrate and culture conditions. Cultivable methods generally allow the identification at the species level. Cultivable method is required to perform genotyping and evaluation of antifungal susceptibility of the isolates. In conclusion, both cultivable and non-cultivable methods are useful and complementary for the evaluation of environmental contamination.

We added sentences in the manuscript (page 16-17):” Non-cultivable methods allow the sampling of numerous spores, useful to carry out surveys but have limitation in fungal spore’s identification only possible at the genus level.35 Cultivable method can identify spores at the species level but is time-consuming and depends on the substrate plated and culture condition applied.36 Cultivable methods generally allow the identification at the species level. Cultivable method is required to perform genotyping and evaluation of antifungal susceptibility of the isolates.”

References

Fernández-Rodríguez S, Tormo-Molina R, Maya-Manzano JM, Silva-Palacios I, Gonzalo-Garijo A. Outdoor airborne fungi captured by viable and nonviable methods. Fungal Ecology 2014;7:16-26

Fernández-Rodríguez S, Molina RT, Palacios IS, Garijo AG. Two sampling methods for the Petri dish detection of airborne fungi. Grana 2011;50:202-7.

11) The authors should explain that only molds and not fungi in general including yeasts have been investigated.

Response: In this study, we tried to correlate the presence of Aspergillus and in particular A. fumigatus in the environment with the occurrence of clinical cases of IA. So, only molds and in particular Aspergillus spp. have been investigated.
We added this precision in the manuscript (page 7): “Only molds, in particular Aspergillus spp. have been investigated in this study”

12) The authors should provide details about what means « mycological results positive for Aspergillus spp. » Positive culture ? Detection of GM or BDG?

Response: Thank you for your comment. We provide more details in the manuscript (page 10): “mycological results positive for Aspergillus spp.. corresponding to cultures showing colony of A. fumigatus were investigated.”

13) Usually, IA cases in prospective studies are investigated by clinician specialized in infections diseases, pneumologists, radiologists and microbiologists. The cases should be reviewed by such committee.

Response: Thank you for pointing this out. First screening of suspected IA cases was realized by 2 infection control practitioners (1 resident and 1 physician) of our hospital. External validation was done in case of uncertain diagnosis by standardized chart, allowing the collection of demographic characteristics, disease history, clinical features, mycological, biological and radiological data, antifungal therapy, and disease outcome. If an increased incidence of IA was detected, infections control specialists and mycologists would be solicited to review IA cases.
We added those precisions in the manuscript (page11):“If an increased incidence of IA was detected, infections control specialists and mycologists would be solicited to review IA cases.”

14) Community-acquired cases should be better defined especially the delay between admission and symptoms, appearance of the nodule and positive GM or culture.

Response: We added details in manuscript (page 10): “Cases were categorized into 3 groups, according to the time between hospital admission and diagnosis: community-acquired, undetermined and nosocomial. Community-acquired cases were defined as incident cases imported from outside the hospital with apportion of clinical symptoms or positive sample in less than 2 days after admission Undetermined cases were defined as incident cases with lag time ranging from 1 to 9 days between admission and the first IA signs without any previous negative sample. Probable nosocomial cases were defined as incident IA with lag time between admission and symptoms onset of at least 10 days or if there is some history of negative sample. Clinical manifestations of IA vary widely and may develop in different clinical scenarios.”

15) Practically speaking, patients are frequently hospitalize before invasive aspergillosis to perform chemotherapy and sometimes patients stays at the hospital several days and the come back home before coming back to the hospital with invasive aspergillosis. Do these IA really community acquired. These cases are clearly undertermined.

Response: We agree with the reviewer. In 2011, Nicolle MC.et al*, from our Infection control unit also reported the complexity to determine the community or nosocomial acquisition of IA. However, genotyping of A. fumigatus isolates have already permitted to help understanding infection routes and possible nosocomial transmission. This is why we would like to genotype clinical and environmental samples, hoping to provide arguments (possible acquisition from hospital environment or not) to help IA cases investigation.

*Nicolle MC, Benet T, Vanhemps P:« Aspergillosis: nosocomial or community-acquired? » Medical Mycology. 2011;49(1):S24-9.

Minor comments:

16) Table II should be provided as a editable table and not a .pdf file

Response: Thank you for pointing this. We changed the table in the manuscript.

17) Reference 6 is not useful since the authors are dealing with invasive aspergillosis

Response: Thank you for your comment. We deleted the reference in the manuscript.