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mycoses
Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Original article

High diversity of non-sporulating moulds in respiratory specimens of immunocompromised patients: should all the species be reported when diagnosing invasive aspergillosis?

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Summary

Non-sporulating moulds (NSMs) isolated from respiratory specimens are usually discarded without further testing although they may have pathogenic effects in immunocompromised patients. The objective of this study was to determine the identity and frequency of NSMs in patients with haematological malignancies. We analysed the mycological results of 251 consecutive respiratory samples from 104 haematology patients. Yeast and sporulating moulds were identified at the genus/species level according to their phenotypic features. NSMs were identified by internal transcribed spacer (ITS) sequencing. We detected 179 positive samples, of which 10.1% (18/179) were mixtures of moulds and 26.3% (47/179) were mixtures of moulds and yeast. We identified 142 moulds belonging to 11 different genera/species or groups, with *Aspergillus fumigatus* (n = 50), *Penicillium* spp. (n = 31) and NSM (n = 24) being the most frequently isolated species. Twenty-two NSMs were successfully sequenced: 18 were basidiomycetes and six were ascomycetes, corresponding to 16 different genera/species. NSMs were isolated with *A. fumigatus* in the same sample or in a subsequent sample in five patients with probable invasive aspergillosis. The conclusion is that the respiratory specimens of immunocompromised patients frequently contain very diverse mould species that may increase the virulence of pathogenic species. Reporting all mould species isolated when diagnosing invasive fungal infection could test this hypothesis.

Key words: Respiratory specimens, non-sporulating mould, immunocompromised patient, invasive aspergillosis.

Introduction

Invasive mould diseases (IMD) are the main cause of fungal disease in immunocompromised patients with haematological disorders. In epidemiological and therapeutic studies, IMD are diagnosed according to consensus criteria defined by the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG).[1] These criteria include the recovery of a mould in cultures of non-sterile specimens such as sputum, broncho-alveolar lavage fluid, bronchial brush or sinus aspirates. Such samples are easily obtained and can lead to the diagnosis of probable IMD.[1] The EORTC/MSG criteria do not grade the identified mould according to whether it was isolated as a pure colony and/or from repeat samples.

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Furthermore, the mould species included in the EORTC/MSG criteria are not clearly defined, although it is usually accepted that only species with known pathogenic potential should be considered when analysing respiratory specimens. This includes *Aspergillus, Fusarium,* Mucorales and *Scedosporium* spp., with *Aspergillus fumigatus* being by far the main species responsible for invasive aspergillosis (IA).[2–4] Apart from these well-+known pathogenic moulds, other moulds isolated from respiratory specimens are usually deemed at best colonising moulds or irrelevant environmental contaminants. This prevents all further analysis and even the reporting of such moulds.

To challenge this attitude, we analysed the results of mycological cultures of respiratory specimens obtained from haematology patients during routine practice in our laboratory. We identified non-sporulating moulds (NSMs) by sequencing the nuclear ribosomal internal transcribed spacer (ITS) region as consensually proposed.[5] Sequencing can identify putative emergent pathogenic species without *a priori*.[6] On the other hand, pathogenic moulds without reproductive structures, such as non-sporulating *A. fumigatus*,[7] may be falsely discarded as contaminants. The goal of this study was to describe the diversity of fungi recovered from cultured respiratory specimens from haematology patients, focusing on non-sporulating moulds. In particular, we sought to determine how often such moulds are isolated pure or in association with other fungi.

Materials and methods

Respiratory specimens

We performed a prospective single-centre hospital-based study including every sputum and broncho-tracheal aspirate received in our laboratory from June 15, 2010 to December 31, 2011 as part of routine laboratory testing for suspected IMD in the context of pneumonia and/or unexplained fever in patients with haematological malignancies. Most broncho-tracheal aspirates were obtained by bronchoalveolar lavage. Patients were definitively classified into probable IMD or invasive aspergillosis (IA) according to the 2008 EORTC/MSG criteria[1] every 2 months during a multidisciplinary medical meeting. The clinicians were aware of the presence of NSM, but not of the molecular analysis; therefore, our findings did not influence any medical decisions. According to French Public Health Law (CSP Art L1121-1.1), our study protocol required neither the approval of an ethics committee nor informed consent for the use of patient samples.

According to standard practice in our laboratory, following their arrival, respiratory samples were centrifuged and seeded onto (i) one plate containing chromogenic medium (Candiselect 2 BioMérieux, Marcy l'Etoile, France) incubated at 37 °C for 1 week, and (ii) two Sabouraud agar slants supplemented with chloramphenicol and gentamicin (Bio-Rad, Marnes la Coquette, France) incubated at 37 °C and at 30 °C (one at each temperature) for 3 weeks. Cultures were checked daily and each colony was identified using ID32C (BioMérieux) for yeast, and macro and microscopic phenotypic features for moulds according to standard practices.[8] If several colonies were observed, each colony was seeded onto a new plate to obtain pure colonies. In the absence of fructification suggestive of a species or a genus, the colony was categorised as NSM.

DNA extraction, amplification and sequencing

The NSM colony was scraped from the agar slant, transferred to 1 ml of PBS, vortexed for 60 s and then heated for 15 min at 100 °C. The suspension was vortexed briefly and then pelleted for 5 min at a maximum speed of 16 000 ***g*** in a microcentrifuge. DNA was extracted from the supernatant using the Qiagen DNA Blood kit (QIAgen, Courtaboeuf, France) according to the manufacturer's recommendations. DNA was finally eluted in 50 µl of the elution buffer and quantified using a NanoDrop DN-1000 Spectrophotometer (Fisher Scientific, Illkirch, France).

A total of 10 ng of DNA was amplified for both the ITS1 and ITS2 regions in two independent reactions in a final volume of 50 µl. An M13 tail was added to the ITS primers[9] to standardise the amplification and sequencing parameters (ITS5-M13F: 5′-GTAGCGCAG CGGCCAGTGGAAGTAAAAGTCGTAACAAGG-3′; ITS2-M13R: 5′-CAGGGCGCAGCGATGACGCTGCGTTCTTCA TCGATGC-3′; ITS3-M13F: 5′-GTAGCGCAGCGGCCAGT GCATCGATGAAGAACGCAGC-3′; ITS4-M13R: 5-CA GGGCGCAGCGATGACTCCTCCGCTTATTGATATGC-3′). The amplification reaction mixture consisted of 1X Fast Start PCR Buffer, 3 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} of each dNTP, 0.2 µmol L^{-1} of each primer and 1.25 U of Fast Start Taq DNA polymerase (Roche Diagnostics, Meylan, France). After an initial denaturation step at 95 °C for 8 min, samples were amplified for 35 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. PCR products were visualised on a QIAxcel

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instrument (QIAgen) and then enzymatically cleaned with shrimp alkaline phosphatase (SAP) and exonuclease I (Exo) to remove un-incorporated dNTPs and primers, respectively. A total of 3 µl of corresponding PCR reaction was incubated with 2 µl of ExoSAP-IT (USB Europe, Staufen, Germany) for 30 min at 37 °C followed by 15 min at 80 °C to inactivate the enzyme, and was then sequenced bidirectionally using the Dideoxy Terminator Cycle Sequencing Kit v1.1 protocol (Applied Biosystems, Courtaboeuf, France). The reaction products were purified by gel filtration using Sephadex G-50 (Amersham, Courtaboeuf, France), run on an ABI PRISM 3130 Genetic Analyser and analysed with the SEQSCAPE v2.6 software (Applied Biosystems, Courtaboeuf, France).

The obtained sequences were subjected to pairwise alignments against curated fungal reference databases available at the on-line MycoBank database (http://www.mycobank.org/). In addition, a BLAST search against known sequences in NCBI Genbank was performed. Sequence identities with a cut off ≥ 97% over 450 nucleotides in length were considered significant. The best BLAST hits were those showing the highest percentage similarity for a query coverage of more than 90%. Sequences were aligned with the CLUSTALX (version 1.83) computer program.[10] A distance tree was obtained using MEGA v.5.1 software with the neighbour-joining algorithm and the Kimura two-parameter model with a gamma distribution. *Rhizopus arrhizus* (CNRMA8.507) and *Rhizopus microsporus* (UMIP1124.75) were chosen as out-groups, and the robustness of the branches was evaluated by 1000 bootstrap replications.

Results

During the study period, we received 251 respiratory samples (median of two samples/patient; range 1–19) from 104 patients (mean age: 56 ± 14 years; sex ratio M/F: 1.3). Among these 251 respiratory samples, 129 were sputa and 122 were broncho-tracheal aspirates. Fungal species were found more frequently in cultures of sputa [76.7% (99/129)] than in cultures of broncho-tracheal aspirates [65.6% (80/122)] ($P = 0.053$). Among the 179 positive samples, 82 (45.8%) contained mixtures of different fungal species with 33.5% (60/179) containing two species and 12.3% (22/179) containing >2 fungal species. The mixtures were composed of yeast and moulds (26.3%; 47/179), and different moulds (10.1%; 18/179).

From the 179 positive samples, we classified 287 fungal colonies (142 moulds and 145 yeasts) based on routine examination (Table 1). We identified 17 yeast genera/species, with *Candida albicans* and *Candida glabrata* being the two main species. Based on microscopic features, we identified 11 different genera/species of moulds, with *A. fumigatus*, *Penicillium* spp., and NSM being the main species (Table 1).

We isolated 24 NSMs in total from 19 patients (Table 2), 22 of which were identified by ITS sequencing. For the remaining two isolates, we failed to obtain pure colonies and therefore to obtain an analyzable chromatogram (Patients 18 and 19, Table 2). Eighteen isolates belonged to the *Basidiomycota* and six to the *Ascomycota* (Fig. 1). All sequences were assigned to the species level except for two basidiomycetes (NSMs 2 and 10), which were only identified to the

Table 1 Fungal identification obtained for the 287 isolates upon routine identification.

Mould	Aspiration $n = 60$	Sputum $n = 83$	n (%) $n = 142$	Yeast	Aspiration $n = 65$	Sputum $n = 80$	n (%) $n = 145$
Aspergillus fumigatus	28	22	50 (35.2)	*Candida albicans*	31	33	64 (44.1)
Penicillium sp.	9	22	31 (21.8)	*C. glabrata*	16	13	29 (20.0)
NSM	15	9	24 (16.9)	*Geotrichum* sp.	2	13	15 (10.3)
A. niger	5	8	13 (9.2)	*C. kefyr*	4	7	11 (7.6)
Fusarium sp.	0	5	5 (3.5)	*C. lusitaniae*	2	3	5 (3.4)
Trichoderma sp.	1	4	5 (3.5)	*C. krusei*	2	1	3 (2.1)
A. flavus	0	4	4 (2.8)	*Candida* sp.	2	1	3 (2.1)
A. versicolor	0	3	3 (2.1)	*C. tropicalis*	1	1	2 (1.4)
Aspergillus sp.	1	1	2 (1.4)	*C. inconspicua*	2	0	2 (1.4)
Alternaria sp.	0	2	2 (1.4)	*C. famata*	1	1	2 (1.4)
Cladosporium sp.	1	1	2 (1.4)	*Saccharomyces cerevisiae*	0	2	2 (1.4)
Mucorales	0	1	1 (0.7)	Others[1]	2	5	7 (4.8)

[1] *C. cantenulata*, *C. colliculosa*, *C. guilliermondii*, *C. norvegensis*, *C. peliculosa*, *C. pulcherrima*, and *Candida* sp. one isolate each. Yeast name as provided by API 32C, Biomérieux.

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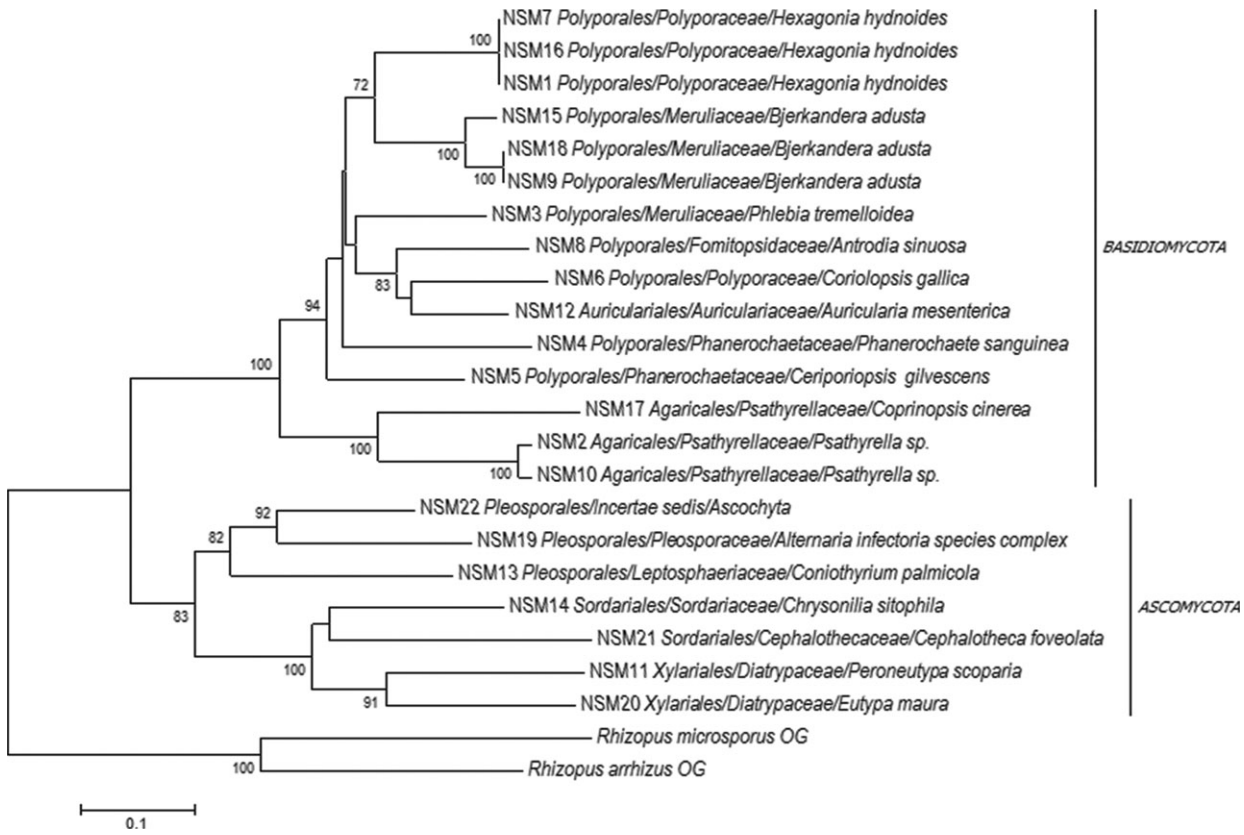

Figure 1 Neighbour-joining tree generated from the internal transcribed spacer (ITS) sequences of the 22 identified non-sporulating moulds listed in Table 2. Bootstrap values are indicated above the branches. Only values above 70% are shown. The bar indicates the number of substitutions per site.

genus level (*Psytharella* spp.). The identity of one ascomycete isolate remained uncertain (NSM 22) because its ITS sequence did not correspond to any known taxa. Different NSM species were observed in one sample from Patient 14 (NSMs 16 and 17) and in serial samples from three other patients (Patients 2: NSMs 2 and 3; Patient 3: NSMs 4 and 5; and Patient 17: NSMs 20, 21 and 22). Globally, NSMs were assigned to 16 different genera/species by sequencing, including three that were detected more than once: *Hexagonia hydnoides* ($n = 3$), *Bjerkandera adusta* ($n = 3$) and *Psathyrella* sp ($n = 2$). Among the *Ascomycota*, no NSM corresponded to non-sporulating *A. fumigatus* or any other species usually involved in IMD.

Several NSMs were frequently isolated with other moulds or yeast in the same sample (10/24 for other moulds and 6/24 for yeast; Table 2). Of note, NSMs were isolated in five patients with probable IA either concomitantly with *A. fumigatus* (Patients 15 and 17), or in a subsequent sample (Patients 3, 9 and 17). None of the NSMs were ascribed any specific pathogenic role by clinicians. For the only Mucorales isolate identified (Table 1), the corresponding patient was not diagnosed with IMD and the isolate was not further investigated. This also applied to the five *Fusarium* sp isolates, one of which was obtained from a patient without probable IMD and the other four were isolated along with *A. fumigatus* in patients with probable IA.

Given that no NSM was recovered twice in patients with ≥2 samples, we examined whether this was also true for *A. fumigatus*. Out of 25 patients (16 probable IAs; nine without IA) with ≥2 samples available and at least one *A. fumigatus* isolated, 10 had ≥2 *A. fumigatus* positive samples and six only one (Fisher's exact test, P: 0.097, ns), confirming that *A. fumigatus* more than twice was not taken into account by the clinicians when classifying the patients according to the EORTC/MSG definitions.

Discussion

Little attention has been paid to the details of the mycological results collected for the diagnosis of IMD. Indeed, neither the number of mould-positive cultures

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Table 2 Sequencing results of the 24 non-sporulating moulds of the 19 patients studied with simultaneous isolation of other fungi and the clinical classification of invasive aspergillosis.

NSM number	Patient number Sex/age (yr)	Underlying disease	Allogeneic HSCT (date)	Sampling date	Phylum	Order	Family	Genus/ Species	Identity matches	Match on sequence database	Simultaneous isolation of other fungi	Invasive aspergillosis
1	1 F/26	ALL	No	11/4/2011	*Basidiomycota*	*Polyporales*	*Polyporaceae*	*Hexagonia hydnoides*	629/629	CBS 360.34[1]	*Penicillium* sp.	No
2	2 M/40	AML	No	6/16/2011	*Basidiomycota*	*Agaricales*	*Psathyrellaceae*	*Psathyrella* sp.	693/699	CBS 211.31[1]	*Penicillium* sp.	No
3	2 M/40	AML	No	8/9/2011	*Basidiomycota*	*Polyporales*	*Meruliaceae*	*Phlebia tremelloidea*	573/573	DTO 085 I1[2]	No	No
4	3 M/71	Myeloma	2/3/2010	12/1/2010	*Basidiomycota*	*Polyporales*	*Phanerochaetaceae*	*Phanerochaete sanguinea*	642/642	CBS 251.32[1]	*Penicillium* sp. *Candida glabrata*	Probable[3]
5	3 M/71	Myeloma	2/3/2010	11/17/2010	*Basidiomycota*	*Polyporales*	*Phanerochaetaceae*	*Ceriporiopsis gilvescens*	614/627	DTO 117-F3[2]	No	Probable[3]
6	4 F/61	AML	No	6/14/2010	*Basidiomycota*	*Polyporales*	*Polyporaceae*	*Coriolopsis gallica*	611/611	CBS 428.34[1]	No	No
7	5 F/71	CLL	No	8/11/2011	*Basidiomycota*	*Polyporales*	*Polyporaceae*	*Hexagonia hydnoides*	629/629	CBS 360.34[1]	No	No
8	6 M/37	AML	8/10/2011	12/2/2011	*Basidiomycota*	*Polyporales*	*Fomitopsidaceae*	*Antrodia sinuosa*	653/656	GU991578[4]	No	No
9	7 F/50	Amyloid Light-chain amyloidosis	No	2/17/2011	*Basidiomycota*	*Polyporales*	*Meruliaceae*	*Bjerkandera adusta*	626/626	EF441742[1]	*Candida kefyr*	No
10	8 F/65	AML	3/26/2010	7/28/2010	*Basidiomycota*	*Agaricales*	*Psathyrellaceae*	*Psathyrella* sp.	689/708	CBS 211.31[1]	*Candida albicans*	No
11	9 F/47	NHL	No	7/12/2010	*Ascomycota*	*Xylariales*	*Diatrypaceae*	*Peroneutypa scoparia*	588/594	FJ172283[4]	*Aspergillus niger*	Probable[3]
12	10 F/59	AML	No	12/8/2011	*Basidiomycota*	*Auriculariales*	*Auriculariceae*	*Auricularia mesenterica*	612/612	CBS 119.34[1]	No	No
13	11 M/70	NHL	No	7/8/2011	*Ascomycota*	*Pleosporales*	*Leptosphaeriaceae*	*Coniothyrium palmicola*	569/570	CBS 161.37[1]	No	No
14	12 M/61	AML	No	6/6/2011	*Ascomycota*	*Sordariales*	*Sordariaceae*	*Chrysonilia sitophila*	587/587	CBS 260.47[1]	*Candida albicans*	No
15	13 M/57	NHL	10/9/2008	4/1/2011	*Basidiomycota*	*Polyporales*	*Meruliaceae*	*Bjerkandera adusta*	623/626	EF441742[1]	*Trichoderma* sp.	Probable[3]

(continued)

Table 2 (continued)

NSM number	Patient number Sex/age (yr)	Underlying disease	Allogeneic HSCT (date)	Sampling date	Phylum	Order	Family	Genus/ Species	Identity matches	Match on sequence database	Simultaneous isolation of other fungi	Invasive aspergillosis
16	14 F/27	AML	6/24/2011	8/9/2011	*Basidiomycota*	*Polyporales*	*Polyporaceae*	*Hexagonia hydnoides*	629/629	CBS 360.34[1]	*Coprinopsis cinerea*[5]	No
17	14 F/27	AML	6/24/2011	8/9/2011	*Basidiomycota*	*Agaricales*	*Psathyrellaceae*	*Coprinopsis cinerea*	609/610	CBS 394.65[1]	*Hexagonia hydnoides*	No
18	15 M/39	AML	4/19/2011	9/8/2011	*Basidiomycota*	*Polyporales*	*Meruliaceae*	*Bjerkandera adusta*	623/626	EF441742[1]	*Aspergillus fumigatus*	Probable
19	16 F/59	ALL	6/22/2010	8/1/2010	*Ascomycota*	*Pleosporales*	*Pleosporaceae*	*Alternaria infectoria complex*	497/497	CBS 106.52[1]	No	No
20	17 M/37	AML	9/24/2009	6/29/2010	*Ascomycota*	*Xylariales*	*Diatrypaceae*	*Eutypa maura*	551/553	CBS 219.87[1]	*Aspergillus fumigatus*	Probable
21	17 M/37	AML	9/24/2009	8/4/2010	*Ascomycota*	*Sordariales*	*Cephalothecaceae*	*Cephalotheca foveolata*	566/566	AB278171[4]	No	Probable[3]
22	17 M/37	AML	9/24/2009	9/10/2010	*Ascomycota*	*Pleosporales*	*Incertae sedis*	Genus *Ascochyta*[6]	470/501	CBS 117477[1]	*Aspergillus fumigatus*	Probable
23	18 M/58	AML	No	8/16/2010	Not identifiable NSM						*Candida glabrata*	No
24	19 M/75	AML	No	6/17/2010	Not identifiable NSM						*Candida tropicalis*	No

HSCT, haematopoietic stem cell transplantation; ALL, acute Lymphoid Leukaemia; AML, acute myeloid leukaemia; CLL, chronic lymphoid leukaemia; NHL, non-Hodgkin lymphoma.

[1]Collection strain from FUNCBS database (http://www.cbs.knaw.nl/collections/).

[2]Collection strain from INDOOR database (http://www.cbs.knaw.nl/indoor/).

[3]*Aspergillus fumigatus* isolation on another respiratory sample.

[4]Genbank accession number.

[5]*Syn. Hormographiella aspergillata.*

[6]Uncertain identification since no sequence entry for known taxa was available for these strains.

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nor the presence of a single mould species recovered in culture are considered in the EORTC/MSG definitions of IMD[1] and are therefore not reported.[2,4] By focusing specifically on NSMs, we clearly show that the fungal species isolated from respiratory specimens of immunocompromised patients are highly variable, mixtures of culturable species are frequent and reproducibility over serial samples is often lacking. The frequency with which different moulds were isolated from the same sample (10.1%; 18/179) in the present study was probably greatly underestimated, because: (i) some species can interfere with the growth of other species and pure cultures are often difficult to obtain; (ii) we did not identify some isolates at the species levels (e.g. *Penicillium* spp.); (iii) some of the isolates identified actually belong to complexes of species (e.g. *A. niger*); and (iv) many species cannot grow on Sabouraud medium. In addition to being frequently mixed, moulds were also very diverse in terms of the species involved. Ten different genera/species of mould were identified morphologically by microscopy and 22 sequenced NSMs were assigned to 16 different genera/species. An additional advantage of sequencing NSMs is that it can rule out the presence of non-sporulating pathogenic species, such as a non-sporulating *A. fumigatus*.[7]

The epidemiology of IMD in haematology patients has changed in recent years with new populations at risk, such as patients with lymphoproliferative diseases,[2] long-term steroid therapy as the main risk factor[11,12] and breakthough infections under antifungal prophylaxis.[13] Galactomannan detection tends to be less sensitive in these patients. As a consequence, direct examination and culture are more often the only mycological criteria to assess probable IA.[1] Therefore, the interpretation of cultures of mixed moulds or those containing moulds with no known pathogenic role should be revised in immunocompromised patients investigated for fever or febrile neutropenia. NSMs are often deemed clinically irrelevant because the simplest and most widely accepted explanation for their presence is environmental contamination. However, some NSMs from the basidiomycetes[14] as well as hyalo- and phaeohyphomycosis[15] are now thought to be potential pathogens.

A common although unproven criterion to ascribe a pathogenic role to a new species, is the recovery of the same species in serial samples,[8] with the hypothesis that persistent species are more likely to be pathogenic. Whether a NSM was isolated more than once is rarely specified in case reports for unusual or newly reported species.[14] In our study, we did not grow NSMs of the same species in samples from the same patient more than once, which argues against the perennial presence of the species in the respiratory tract. However, persistent isolation of *A. fumigatus* from patients with a final diagnosis of probable IA was not observed in our population either. Moreover, it remains impossible to determine whether a cultivable *A. fumigatus* was recently acquired or is a long-term resident of the bronchi with higher pathogenic potential. Even with well-established genotyping methods and extensive sampling, the timing of acquisition of *A. fumigatus* cannot be elucidated[16,17] and that would be probably the same for NSMs.

An argument for considering NSMs in immunocompromised patients is the results of next generation sequencing (NGS). This technology shows that the association of several moulds is the rule in respiratory specimens and not the exception, with the coining of the term 'mycobiome'.[18,19] The mycobiome should be explored in immunocompromised patients with haematological disorders, as reported for asthma,[20] cystic fibrosis patients[21] and lung transplant recipients.[22] Currently, the main advantage of cultures over NGS, besides the lower cost and workload, is that cultures select viable moulds and not only DNA, which, on the other hand, can introduce bias in the analysis of species diversity.

The isolation of non-aspergillus moulds in respiratory specimens from haematology patients should be accorded the same significance as yeast is in some centres. Indeed, although some authors did not find any correlation between *Candida* and death from pneumonia,[23] others found that *Candida* either limits,[24] or promotes[25] bacterial lung injury. The isolation of several moulds could thus be considered an indirect sign of an altered respiratory epithelium, as imbalance in the intestinal mycobiome is thought to play a role in gastro-intestinal graft-vs.-host disease.[26] Alterations of the respiratory epithelium may also increase the risk of *A. fumigatus* conidia being trapped in the bronchi. Additionally, some non-*fumigatus* moulds may be involved in asthma or chronic cough, which can also indicate altered or sensitised bronchi.[14]

In conclusion, the fungi present in respiratory specimens of immunocompromised patients are very diverse and can be regarded as part of a mycobiome. Changes to this mycobiome over time should be considered more clinically relevant than the isolation of a particular species at a given time. In future epidemiological studies on IA (at least until more studies investigating the pulmonary mycobiome in patients with respiratory diseases are performed), the number of samples

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yielding *A. fumigatus* colonies and the presence of other moulds should be specified. This may help us to understand, at least in part, the involvement of non-aspergillus species in these patients. Protocols should be established to define the number of respiratory samples and all identified fungi should be declared. This will also help to understand the significance of the galactomannan detection in respiratory specimens since several fungal species can produce this antigen.

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Conflicts of interest

We have no conflict of interest regarding the software, products, or concepts used in this study.

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