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Mucorales PCR Testing in Respiratory and Biopsy Samples From Immunocompromised Patients With Invasive Pulmonary Aspergillosis and Other Mold Infections: Results From a Multicenter ECMM Study

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Background. Mucormycosis is a severe fungal infection that is challenging to diagnose as traditional methods lack sensitivity and serological testing is unavailable. This study aimed to evaluate the MucorGenius® PCR assay on respiratory and biopsy samples from high-risk patients with probable/proven invasive pulmonary aspergillosis (IPA), mucormycosis, or possible invasive mold infections (IMIs).

Methods. This multicenter cohort study was conducted across 4 sites in Austria, Belgium and the UK. A total of 132 respiratory and biopsy samples from 114 patients with IMI diagnosed in clinical routine (10 proven IPA, 13 proven mucormycosis, 62 probable IPA, 5 probable mucormycosis, and 35 possible IMI according to EORTC/MSGERC 2020 and FUNDICU criteria; 11 IPA/mucormycosis coinfections) were analyzed using the MucorGenius® PCR assay in ISO-certified laboratories. Results were compared with standard fungal diagnostics.

Results. Mucorales DNA was detected in 37/132 samples (28%) including 29 BAL fluids, 1 bronchial aspirate, 1 endotracheal aspirate, and 6 biopsies from 37 patients. Sensitivity was 94.4% (17/18) for detecting probable/proven mucormycosis (including 11 cases routinely diagnosed with IPA/mucormycosis coinfection). Among 72 patients with probable/proven IPA, 21 (29.2%) tested positive for Mucorales DNA, including 11 missed by routine diagnostics. Mucorales DNA was also detected in 9/35 (25.7%) of patients with possible IMI.

Conclusions. MucorGenius® PCR showed high sensitivity for detecting Mucorales and may support improved diagnosis of probable mucormycosis when included as a mycological criterion. It appears particularly valuable for identifying *Aspergillus*-Mucorales coinfections and detecting mucormycosis in patients with host factors, clinical or radiological evidence of IMI when routine diagnostics are negative.

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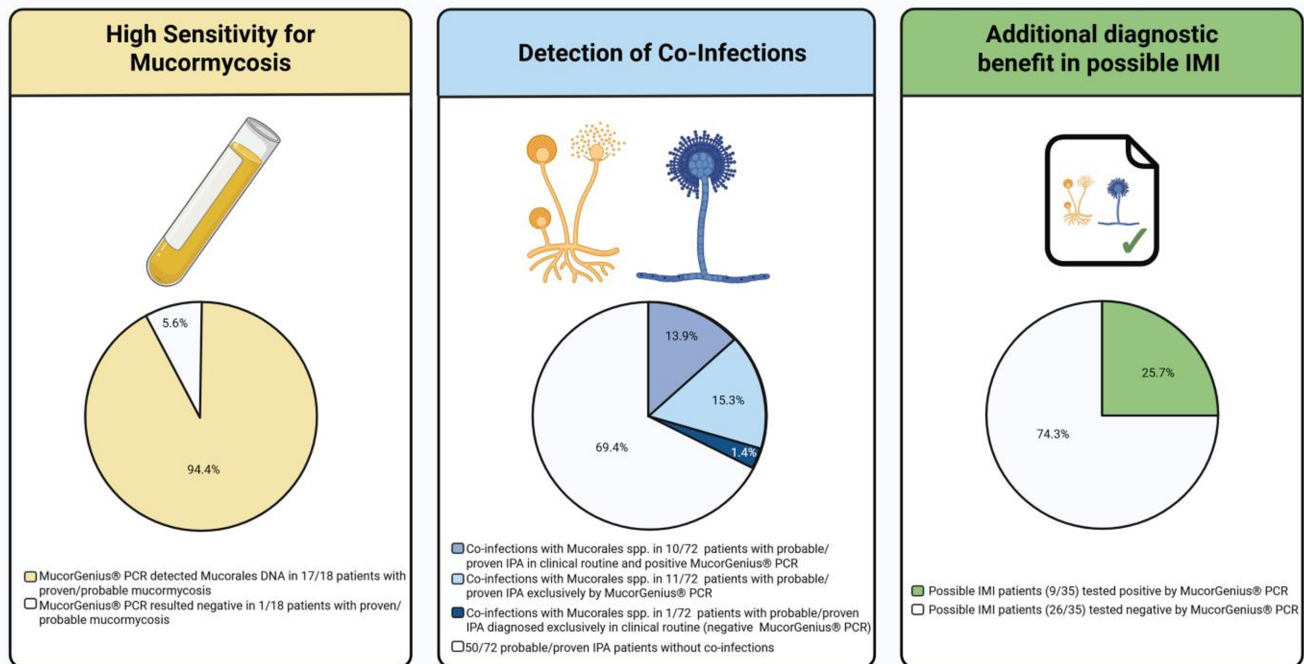
Graphical Abstract



Three-panel summary of MucorGenius® PCR performance using respiratory specimens and biopsies. The left panel illustrates sensitivity for mucormycosis with proportions of positive and negative PCR results among patients with proven or probable disease. The middle panel shows rates and detection methods of Mucorales co-infections among patients with probable or proven invasive pulmonary aspergillosis. The right panel displays PCR positivity among patients classified as having possible invasive mold infection. Created with BioRender.com.

Keywords. *Aspergillus*; bronchoalveolar lavage; invasive mold infection; mucorales; PCR.

Invasive mold infections (IMIs), particularly mucormycosis, pose a significant threat to immunocompromised and critically ill patients, including those with severe COVID-19 or uncontrolled diabetes [1–4]. Mucormycosis may also affect immunocompetent persons with traumatic injuries during natural disasters, as well as blast injuries in settings of war [5–8].

Early detection of mucormycosis remains challenging due to the lack of reliable biomarkers [3, 9]. Current diagnostic standards—microscopy, culture, and histopathology—are limited by low sensitivity and often require invasive procedures [10]. Culture confirmation, essential for species identification and targeted treatment, succeeds in only about half of suspected cases, with many diagnoses made postmortem [11, 12].

Early targeted antifungal therapy is the prime predictor of survival in patients with mucormycosis [13, 14]; therefore, improved and more sensitive diagnostic strategies are urgently needed to enhance disease management. Molecular diagnostic methods, including Mucorales-specific Polymerase chain reaction (PCR) and pan-fungal PCR followed by sequencing, show promise in overcoming current limitations. Despite growing awareness, recent comprehensive data on the epidemiology and diagnosis of pulmonary mucormycosis are still limited. Although several recent reviews have addressed mucormycosis overall [15], very few focus specifically on the pulmonary form and the diagnostic/epidemiologic interplay in this setting. Notably, blood PCR screening has been linked to improved survival outcomes [16, 17]. Among these, the MucorGenius® multiplex PCR assay (PathoNostics®, Maastricht, The Netherlands) offers a commercially available, standardized, sensitive tool for early detection using blood, bronchoalveolar lavage, or biopsy samples. The assay targets the 28S rRNA gene of key Mucorales genera (*Rhizopus*, *Mucor*, *Lichtheimia*, *Cunninghamella*, and *Rhizomucor*), and demonstrated strong diagnostic performance and reproducibility across laboratories, representing a significant advancement in mucormycosis diagnostics [18, 19].

Importantly, the MucorGenius® assay has shown high specificity in multiple studies. In a prospective multicenter cohort, a specificity of 98% was reported in over 1300 bronchoalveolar lavage fluid (BALF) samples from immunocompromised patients [20]. Comparable specificity values have also been reported in other prospective evaluations, and an earlier

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meta-analysis found a pooled specificity of 95.8% for various Mucorales PCR assays in BALF [21, 22].

The objective of the study was to focus on sensitivity and added diagnostic value of the test, by evaluating the MucorGenius® PCR assay in respiratory and biopsy samples from a cohort of high-risk patients, diagnosed with probable/proven invasive pulmonary aspergillosis (IPA) and mucormycosis, as well as patients with possible IMI.

METHODS

This multicenter retrospective study included respiratory samples and biopsies from patients fulfilling at least possible IMI according to the revised 2020 EORTC/MSGERCcriteria [23], or FUNDICU criteria [24]. All centers obtained local Institutional Review Board approval. Eligible patients were identified from available stored sample biobanks, based on meeting the criteria for possible IMI or probable/proven IMI, if a respiratory sample or tissue biopsy was available for diagnostic evaluation.

Participating centers were the Medical University of Graz (Austria), University Hospitals Leuven (Belgium), Public Health Wales Microbiology (UK), and the Kings College Hospital London (UK). In Cardiff, 22 BALF samples were collected during 2 distinct periods: February 2019 to December 2020 and July 2023 to February 2024. In Graz, a total of 50 respiratory samples were included, comprising 48 BALF samples and 2 endotracheal aspirates, collected between December 2013 and January 2024. The Leuven cohort consisted of 55 samples obtained between May 2020 and October 2023, including 52 BALF samples, 1 bronchial aspirate, 1 gastric biopsy, and 1 skin biopsy sample. From London, 5 samples were collected between 2020 and 2021, including 1 BALF sample and 2 punch biopsy tissue sample, 1 skin biopsy and 1 meningeal biopsy sample. The observed differences in sample numbers and diagnostic categories reflect the local incidence of IMI, sample availability, and variations in clinical practice, rather than selective inclusion.

Respiratory samples were stored at −80°C until analysis, while tissue biopsies were stored according to local routine (fresh-frozen or formalin-fixed, paraffin-embedded). DNA extraction from BALF was performed using the NucliSens eMAG system (bioMérieux, Marcy-L'Étoile, France), while tissue biopsies were extracted with locally validated protocols appropriate to sample type. All samples were tested with the MucorGenius® qPCR assay targeting 28S rRNA of common Mucorales species and including an internal control. The assay was performed retrospectively and locally at each center in ISO-certified laboratories with appropriate internal and external controls.

MucorGenius® qPCR results were compared with routine fungal diagnostic methods, including microscopy, culture,

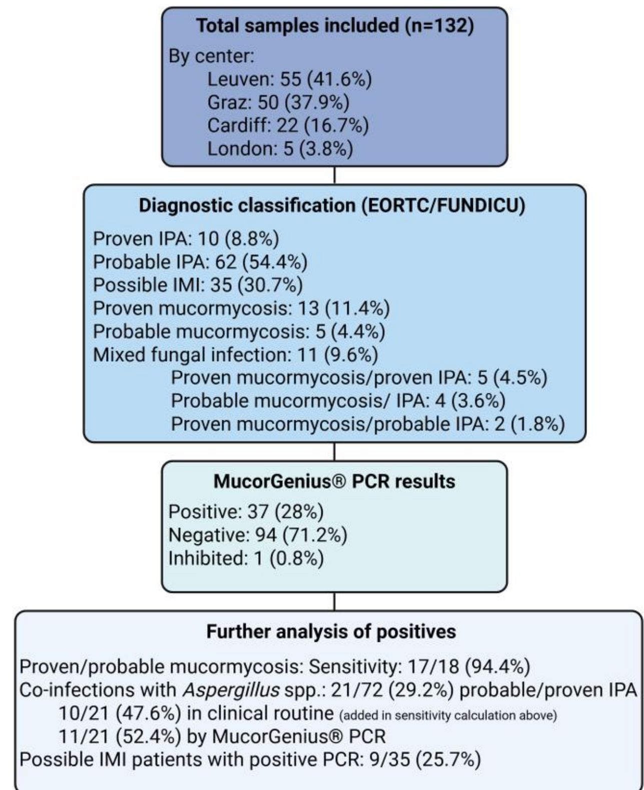

Figure 1. Overview of sample collection, diagnostic classifications, and MucorGenius® PCR results for 132 respiratory and biopsy specimens from 4 study centers. Diagnostic categories include proven and probable invasive pulmonary aspergillosis; possible invasive mold infection; proven and probable mucormycosis; and mixed fungal infections. PCR outcomes (positive, negative, and inhibited) and additional analyses of positive results, including coinfections and classification-specific positivity rates, are summarized. Created with BioRender.com.

galactomannan antigen testing, *Aspergillus* PCR, and established consensus definitions.

Detailed clinical, radiological, microbiological, and histopathological data from all included patients were collected centrally. Case classification into proven, probable, or possible IMI was performed and confirmed centrally by the study's multidisciplinary mycology and infectious diseases team. Classification followed the revised EORTC/MSGERC [23] criteria and the FUNDICU [24] criteria, as appropriate.

Statistical analyses were performed descriptively using IBM SPSS Statistics version 29 (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 132 clinical samples from 114 patients at risk for IMI were collected from 4 European centers: Leuven (41.6%), Cardiff (16.7%), Graz (37.9%), and London (3.8%) (Figure 1). The majority of samples were BALF ($n = 123$), followed by

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Table 1. **Baseline Characteristics of the Study Cohort**

Variables	Cohort $N = 114$ (%)
Sex	
Male	82 (71.9)
Female	32 (28.1)
Age (median and range)	62.5 (1–86)
Clinical classification	
EORTC	
Proven IPA	10 (8.8)
Probable IPA	46 (40.4)
Possible IMI	33 (28.9)
Proven mucormycosis	13 (11.4)
Probable mucormycosis	5 (4.4)
Mixed fungal infection	11 (9.6)
Proven mucormycosis/proven IPA	5 (4.4)
Probable mucormycosis/IPA	4 (3.5)
Proven mucormycosis/probable IPA	2 (1.8)
FUNDICU	
Probable IPA	16 (14)
Possible IMI	2 (1.8)
Underlying diseases/risk factors	
Hematologic malignancies	65 (57)
AML	36 (31.6)
MDS	8 (7)
GVHD	13 (11.4)
Allogeneic stem cell transplantation	19 (16.7)
Autologous stem cell transplantation	2 (1.8)
Solid organ transplantation	16 (14)
Lung transplantation	5 (4.4)
Primary immunodeficiencies	2 (1.8)
Viral respiratory diseases	13 (11.4)
COVID-19	8 (7)
Influenza	4 (3.5)
Structural lung disease	19 (16.7)
COPD	15 (15.8)
Autoimmune diseases requiring immunosuppressive treatment	8 (7)
Diabetes mellitus	3 (2.6)
Solid malignant tumors	2 (1.8)
Multiorgan failure	6 (5.3)

biopsies ($n = 6$; punch and skin, $n = 2$ each; gastric and meningeal, $n = 1$ each), endotracheal aspirates ($n = 2$), and bronchial aspirate ($n = 1$).

Patient characteristics are displayed in Table 1. Of the 114 patients, 71.9% ($n = 82$) were male and 28.1% ($n = 32$) female. The median age was 62.5 years (range 1–86). The most common underlying diseases were hematologic malignancies ($n = 66$; 57.9%), followed by structural lung disease ($n = 19$; 16.7%). Based on EORTC/MSGERC criteria, 8.8% ($n = 10$) had proven IPA, 40.4% ($n = 46$) probable IPA, and 28.9% ($n = 33$) possible IMI. Proven mucormycosis was diagnosed in 11.4% ($n = 13$), probable mucormycosis in 4.4% ($n = 5$). According to FUNDICU criteria, additionally 14% ($n = 16$) were classified as probable IPA, resulting in a total number of probable IPA of 54.4% ($n = 62$), and additional 1.8% ($n = 2$) as possible IMI. Overall mortality in this cohort of patients with mold infections was 55.3% (63/114), and did not differ between those with positive MucorGenius® PCR results (51.3%; 18/35) and those with negative results (57.9%; 44/76). Details are shown in Figure 1.

Of note, 11 of those 114 patients (9.6%) had mixed infections: 5 with proven mucormycosis/proven IPA, 4 with probable mucormycosis/probable IPA, and 2 with proven mucormycosis/probable IPA. In terms of samples, 14 samples were obtained from 13 patients with proven mucormycosis, 47 samples were obtained from 46 patients with probable IPA and 50 samples from 35 patients with possible IMI, while for all other categories 1 sample was obtained per patient.

PCR analysis detected Mucorales DNA in 28% ($n = 37$) of samples (Figure 2). A total of 71.2% samples (94 samples from 76 patients) tested negative, and 0.8% ($n = 1$) showed PCR inhibition. The 37 PCR-positive tests included 29 BAL fluids, 1 bronchial aspirate, 1 endotracheal aspirate, and 6 biopsies. The clinical classification of IMI across centers, together with the number of MucorGenius® PCR–positive samples per center, is summarized in Table 2.

The 37 MucorGenius®-positive samples were derived from 37 different patients. Overall, the sensitivity of the MucorGenius® PCR assay for detecting proven or probable mucormycosis, as diagnosed by routine clinical methods, was 94.4% (17/18; 95% CI: 72.7–99.9%). Six of those 17 MucorGenius® PCR-positive samples among patients routinely diagnosed with mucormycosis were from patients with proven mucormycosis only, one was from a patient with probable mucormycosis, while 10 were from patients with probable/proven mucormycosis IPA coinfections.

Among the 17 patients with Mucormycosis 11 were diagnosed by culture +/− histopathology, 1 by direct microscopy (Calcofluor-white staining) only indicating Mucorales, 1 by pan-fungal-PCR/ITS sequencing, and 4 through histopathology only from the same or different samples. Among all Mucorales culture-positive samples, *Rhizopus microsporus* was the most frequently isolated species (14.7%, $n = 5$). In one of the coinfected patients, *Lichtheimia corymbifera* and *Aspergillus niger* were coisolated, indicating a mixed infection. In other patients, *Rhizomucor pusillus* ($n = 1$) and *Rhizopus arrhizus* ($n = 2$) were detected in culture. In 2 coinfected cases, Mucorales were routinely cultured from different sample types: *Rhizomucor pusillus* from a lung biopsy and *Lichtheimia ramosa* from a skin biopsy. The patient with *Lichtheimia ramosa* also had *Aspergillus terreus* detected in BALF samples via PCR, indicating a coinfection. Table 3 displays other mycology results in PCR-positive samples. In 1/18 patients (5.6%) with routinely diagnosed mucormycosis, a patient with proven mixed infection of mucormycosis and IPA, with *Mucor circinelloides* isolated from the BALF sample in routine culture, the MucorGenius®

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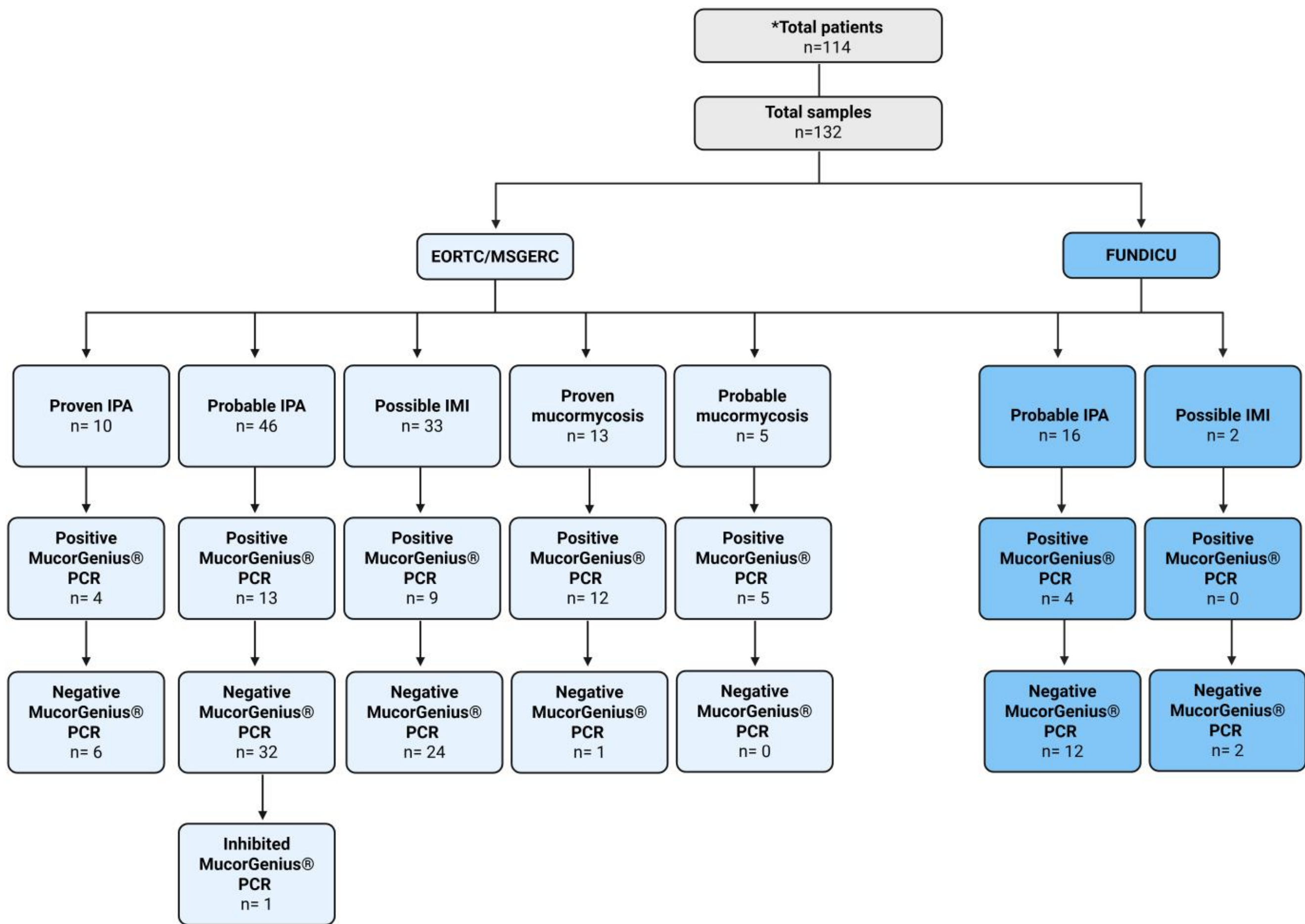

Figure 2. Flowchart summarizing the distribution of 114 patients and 132 samples across diagnostic categories according to EORTC/MSGERC and FUNDICU criteria. Categories shown include proven and probable invasive pulmonary aspergillosis; possible invasive mold infection; proven and probable mucormycosis; and mixed fungal infections. For each category, corresponding MucorGenius® PCR results are displayed, including positive, negative, and inhibited outcomes where applicable. FUNDICU classifications for probable IPA and possible IMI are presented with their respective PCR results. Created with BioRender.com.

assay in the same BALF sample yielded a negative result. In another patient who was diagnosed with proven mucormycosis at autopsy, 2 samples were available with a BALF sample obtained 2 weeks before autopsy resulting negative, while the second sample, obtained 2 days before the patients passing away resulted positive.

In addition, Mucorales DNA was detected in 21/72 patients (29.2%) that had been routinely diagnosed with probable or proven IPA, indicating possible Mucorales *Aspergillus* coinfections. Of those 21, coinfection with mucormycosis and IPA had been detected before in clinical routine in 10 (47.6%) patients, while in 11 (52.4%) of patients routine diagnostics for mucormycosis did not yield a positive result.

Finally, Mucorales DNA was detected in 9/35 patients (25.7%; 9 samples positive) classified in routine clinical setting as having possible IMI, due to absence of other positive mycology test results in patients with host factors and clinical/radiological evidence of IMI.

DISCUSSION

In this multicenter retrospective study, we assessed the performance of the MucorGenius® PCR assay for detection of Mucorales DNA in respiratory and biopsy samples collected from 114 immunocompromised patients with possible, probable or proven IMI according to EORTC/MSGERC and FUNDICU criteria. Our findings support the utility of this molecular assay as a sensitive and rapid tool for early detection of mucormycosis, particularly in cases where traditional diagnostics fail. The study highlights 3 major findings: (1) the assay's high sensitivity for detecting proven/probable mucormycosis, (2) its ability to identify coinfections with *Aspergillus* spp. in

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Table 2. Clinical Classification of IMI and Number of Positive MucorGenius® PCR Tests per Center

Clinical Classification and Number of Patients of IMI Per Center	Leuven (%)	Graz (%)	Cardiff (%)	London (%)
Samples ($n = 132$)	55	50	22	5
Patients	55	48	6	5
EORTC				
Proven IPA	8 (14.5)	2 (4.2)	0 (0)	0 (0)
Probable IPA	36 (65.5)	8 (16.6)	1 (16.7)	1 (20)
Possible IMI	10 (18.2)	18 (37.5)	5 (83.3)	0 (0)
Proven mucormycosis	8 (18.2)	1 (20.8)	0 (0)	4 (80)
Probable mucormycosis	2 (3.6)	3 (6.3)	0 (0)	0 (0)
Mixed fungal infection	9 (16.4)	2 (4.2)	0 (0)	0 (0)
Proven mucormycosis/IPA	5 (9)	0 (0)	0 (0)	0 (0)
Probable mucormycosis/IPA	2 (3.6)	1 (2.2)	0 (0)	0 (0)
Proven mucormycosis/probable IPA	2 (3.6)	0 (0)	0 (0)	0 (0)
Probable mucormycosis (EORTC)/IPA (FUNDICU)	0 (0)	1 (2.1)	0 (0)	0 (0)
FUNDICU				
Probable IPA	0 (0)	16 (33.3)	0 (0)	0 (0)
Possible IMI	0 (0)	2 (4.2)	0 (0)	0 (0)
Number of positive MucorGenius® PCR per center	20 (36.4)	13 (27.1)	0 (0)	4 (80)
EORTC				
Proven IPA	4 (20)	0 (0)	0 (0)	0 (0)
Probable IPA	11 (55)	1 (7.7)	0 (0)	0 (0)
Possible IMI	3 (15)	6 (46.2)	0 (0)	0 (0)
Proven mucormycosis	7 (35)	1 (7.7)	0 (0)	4 (100)
Probable mucormycosis	2 (10)	1 (7.7)	0 (0)	0 (0)
Mixed fungal infection	7 (35)	2 (15.4)	0 (0)	0 (0)
Proven mucormycosis/IPA	4 (20)	0 (0)	0 (0)	0 (0)
Probable mucormycosis/IPA	2 (10)	1 (7.7)	0 (0)	0 (0)
Proven mucormycosis/probable IPA	1 (5)	0 (0)	0 (0)	0 (0)
Probable mucormycosis (EORTC)/IPA (FUNDICU)	0 (0)	1 (7.7)	0 (0)	0 (0)
FUNDICU				
Probable IPA	0 (0)	4 (30.8)	0 (0)	0 (0)
Possible IMI	0 (0)	0 (0)	0 (0)	0 (0)

respiratory samples, and (3) its diagnostic yield in possible IMI cases lacking other mycological evidence.

The MucorGenius® assay detected Mucorales DNA in 17/18 patients with proven or probable mucormycosis, resulting in a per-patient sensitivity of 94.4% (95% CI: 72.7%–99.9%). This is consistent with previously reported sensitivity ranges of 75%–90% in similar high-risk cohorts [19, 25]. The only false-negative case was a proven mixed infection, where *Mucor circinelloides* was also isolated from the BALF sample. Possible reasons include low fungal load, suboptimal sample site, or DNA degradation [26]. Importantly, 23/32 (71.9%) of PCR-positive samples were culture-negative, which was even slightly higher than the 64.7% reported previously, confirming that conventional mycological methods—microscopy, culture, and histopathology—may miss a substantial proportion of infections [27, 28]. These findings reflect the known challenges of spatial sampling error in pulmonary infections and further emphasize the utility of molecular diagnostics in capturing low-level or localized infection. Our study also demonstrated a high rate of *Aspergillus*-Mucorales coinfections. Our study included 11 coinfections, which were diagnosed in clinical routine, with 10/11 also testing positive in the MucorGenius® assay. In total, Mucorales DNA was detected in 21/72 (29.2%) patients with probable/proven IPA, including 11 patients in whom routine diagnostics for Mucorales turned out negative. If considered true positive, our study would indicate that 30.6% (22/72) patients with probable/proven IPA had Mucorales coinfections. These findings may highlight the potential added diagnostic value of PCR in revealing clinically relevant coinfections that would otherwise remain undiagnosed [23, 29]. While it remains difficult to determine retrospectively whether both fungal pathogens contributed equally to disease, experimental and clinical data suggest that coinfections can be synergistically pathogenic in severely immunocompromised hosts [30, 31]. Although speculative, this may have therapeutic implications, particularly where antifungal regimens with limited activity against Mucorales are used [3].

While derived from a multicenter study, the high MucorGenius® PCR positivity rates in patients with probable/proven IPA should still be interpreted with caution. Although molecular detection of Mucorales in these cases may indeed reflect clinically relevant coinfections—as supported by reports of

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Table 3. Results of Routine Mycological Work up of MucorGenius® PCR-Positive BALF Samples

	Test	MucorGenius® PCR-Positive Samples ($n = 37$)
Fungal culture (same sample)	Any mold growth	10 (27)[a]
	Rhizopus microsporus	5 (13.5)
	Rhizomucor pusillus	1 (2.7)
	Rhizopus arrhizus	2 (5.4)
	Lichtheimia corymbifera	1 (2.7)
	Aspergillus fumigatus	2 (5.4)
	Aspergillus niger	1 (2.7)
	Culture negative	23 (62.2)[b]
Other diagnostic tests	ITS sequencing (*Rhizopus microsporus*)	1 (2.7)
	Calcofluor positive, possible Mucorales	1 (2.7)
Aspergillus diagnostic tests	GM-tested	27 (73)
	BALF GM ≥ 1	15 (40.5)
	Aspergillus PCR Tested positive (BALF)	9 (24.3)[c]
	Aspergillus fumigatus	4 (10.8)
	Aspergillus terreus	2 (5.4)
	Aspergillus species not further defined	4 (10.8)

Absolute numbers (%) presented.

[a]Two samples showed more than 1 mold in culture (*Aspergillus niger, Lichtheimia corymbifera* in BALF and *Aspergillus fumigatus, Rhizopus microsporus* in endotracheal aspirate). Besides 1 gastric and 1 punch biopsy, all samples were BAL fluid.

[b]Culture not available from 4 biopsy samples.

[c]In 1 sample more than 1 *Aspergillus* species (*Aspergillus fumigatus* and *Aspergillus terreus*).

increased dual-mold infections in ICU, COVID-19, and hematologic populations [1, 32, 33], alternative explanations must be considered. Transient airway colonization by airborne Mucorales spores, sampling contamination, or false-positive amplification events, although uncommon, cannot be fully excluded. However, previous evaluations of the MucorGenius® assay reported consistently high specificity (>95%) [34]. In a recent prospective cohort study including 1407 BALF samples from 1330 at-risk patients, the MucorGenius® assay demonstrated a per-patient specificity of 98.6% (95% CI: 97.8–99.2) [20]. A Bayesian meta-analysis including 36 studies with 6627 participants and 16 613 samples demonstrated a pooled specificity of 97% (95% CI, 95%–98%) for Mucorales PCR across blood and BALF specimens [35]. Likewise, a systematic review covering 5147 samples from 819 patients with proven/probable mucormycosis and 4266 controls reported a specificity of 95.8% (95% CI, 89.6%–98.4%) for BALF and 95.5% (95% CI, 87.4%–98.5%) for blood [22]. While these previous studies comprising over 20.000 BALF samples outline that false-positive events are expected at a much lower rate (1.4%–4.5%) than the 18% positivity observed in this study in patients with probable/proven IPA lacking other mycological evidence for mucormycosis, some false-positive results may still have occurred and the actual rate of Mucorales coinfections may be lower than the 30.6% observed here.

Comparing our results with historic data, this study detected Mucorales DNA in 28% of samples from patients with IMI, which is significantly higher than the ∼8% proportion of mucormycosis among IFIs reported in the TRANSNET study 15-years ago [36, 37]. This difference can mainly be explained by the fact that historically, the suspicion and diagnosis of Mucormycosis relied exclusively on conventional diagnostic methods which underestimate the true burden of mucormycosis, as supported by multiple studies demonstrating low sensitivity of culture and even histopathology [27, 28].

One of the most significant findings was the detection of Mucorales DNA in 9/35 patients (25.7%) classified as having possible IMI. These patients met host, clinical and radiological criteria of IMI, but lacked conventional mycological evidence. Such cases are clinically challenging and frequently undertreated due to uncertainty around the causative pathogen. The ability of the MucorGenius® assay to provide fungal identification in this subgroup represents a major advancement, potentially enabling earlier, evidence-based therapeutic interventions. This may be particularly important given the rapid progression and high mortality of mucormycosis in immunocompromised hosts [3, 23, 29]. The use of a standardized, commercially available assay targeting the 28S rRNA gene of key Mucorales genera further ensures reproducibility and comparability across laboratories [18, 38].

This study has several limitations. First, its retrospective design and the use of heterogeneous samples from multiple centers may introduce selection bias. Although, it was not part of the objectives of the study to preferably include patients with *Aspergillus*-Mucorales coinfections, but rather all patients with IMI, the high rate of coinfections will have to be interpreted with caution and needs to be confirmed in future prospective studies with standardized sampling and comprehensive reference diagnostics including histological confirmation and autopsies [39]. Second, the number of proven/probable pulmonary mucormycosis cases, although a strength given the rareness of the disease, was still relatively small, limiting statistical power. Third, routine diagnostics—used as the reference standard—are known to have low sensitivity, which may lead to misclassification and underestimation. Fourth, detailed data on antifungal prophylaxis at the time of sample collection as well as antifungal treatment and outcomes were not systematically available for all our patients, and also given that there was only 1 single false negative result, we could not perform a formal analysis of prophylaxis impact on PCR sensitivity. Fifth, our study could not evaluate specificity of Mucorales PCR detection,

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where airway colonization or contamination may cause false positive results. However, recent large studies and meta-analyses have reported very high specificity of the MucorGenius® PCR assay [20, 22].

In conclusion, our findings indicate that the MucorGenius® PCR assay enhances the detection of mucormycosis in high-risk patients, including those with negative cultures and probable/proven IPA, where mucormycosis coinfections may occur relatively frequently. Integrating Mucorales PCR into diagnostics may improve recognition and management of this often-overlooked fungal infection.

Notes

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