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Summary

This prospective multicenter study confirms the good performance of serum Mucorales qPCR for the diagnosis of mucormycosis and argues for addition of this new tool in clinical settings and EORTC/MSGERC consensual definitions to improve the management of mucormycosis.

Abstract

Background

Early diagnosis and prompt initiation of specific antifungal treatment is essential for improving the prognosis of mucormycosis. We aimed to assess the performance of serum Mucorales quantitative PCR (qPCR) for the early diagnosis and follow-up of mucormycosis.

Methods

We prospectively enrolled 232 patients with suspicion of invasive mold disease, evaluated using standard imaging and mycological procedures. Thirteen additional patients with proven or probable mucormycosis were included to analyze DNA load kinetics. Serum samples were collected twice-a-week for Mucorales qPCR tests targeting the Mucorales species *Lichtheimia*, *Rhizomucor* and *Mucor/Rhizopus*.

Results

The sensitivity was 85·2%, specificity 89·8%, and positive and negative likelihood ratios 8·3 and 0·17, respectively in this prospective study. The first Mucorales qPCR-positive serum was observed a median of four days (IQR, 0-9) before sampling of the first mycological or histological positive specimen and a median of one day (IQR, (-2)-6) before the first imaging was performed. Negativity of Mucorales qPCR within seven days after liposomal-amphotericin B initiation was associated with an 85% lower 30-day mortality rate (adjusted hazard Ratio = 0·15, 95%CI [0·03-0·73], p = 0·02).

Conclusion

Our study argues for the inclusion of qPCR for the detection of circulating Mucorales DNA for mucormycosis diagnosis and follow-up after treatment initiation. Positive results should be added to the criteria for the consensual definitions from the European Organization for the Research and Treatment of Cancer/ Mycoses Study Group Education and Research Consortium (EORTC/MSGERC), as already done for *Aspergillus* PCR.

Key Words : mucormycosis – molecular diagnosis - Mucorales quantitative PCR – circulating DNA

Introduction

Mucormycoses are severe invasive mold diseases (IMDs) caused by fungal species belonging to the order Mucorales. Mucorales have an affinity for invading blood vessels, which results in thrombosis, tissue necrosis, and hematogenous dissemination. Mucormycosis can present as rhino-orbito-cerebral disease in uncontrolled diabetic patients and as pulmonary and disseminated diseases in severely immunocompromised patients (1). Its incidence has risen with the increase in the number of immunocompromised patients (2, 3) and the dramatic increase in diabetes mellitus, specifically in India (4). The recent epidemic of mucormycosis in the COVID-19 pandemic has further increased the number of cases (5, 6). Overall, the 90-day mortality rate varies depending on the localization and extension of the infection, from 12·5–31% for localized cutaneous mucormycosis, to 75-90% for disseminated disease (2, 7, 8).

Early diagnosis of mucormycosis in immunocompromised patients, mainly in hematology, is difficult because of clinico-radiological similarities with invasive pulmonary aspergillosis and the limited performance of mycological and histopathological tools (9). Invasive procedures for histopathology cannot always be performed on patients who are in poor condition in hematology or intensive care units, who frequently show thrombocytopenia and neutropenia. Moreover, the observation of typical broad non-septate ribbon-like hyphae in biopsy samples requires trained pathologists. Furthermore, cultures are often negative and even the molecular detection of Mucorales in paraffin-embedded tissues can be negative (10). Alternatively, quantitative PCR (qPCR) detection of Mucorales DNA in serum is a non-invasive method that may improve the therapeutic management of patients with mucormycosis (11, 12). Indeed, qPCR can be prescribed from the first clinical suspicion, without invasive procedures. Several retrospective studies have already reported that Mucorales qPCR on serum samples can anticipate the diagnosis of mucormycosis by an average of eight days in hematological and critically ill burn patients (11-17). This is of utmost interest, as the early initiation of specific antifungal treatment is essential for improving the prognosis (18), (19).

We carried out the MODIMUCOR study (Projet Hospitalier de Recherche Clinique national-PHRC 2013-0397) to confirm our previous results (11, 12). This prospective multicenter study aimed to assess the performance of serum Mucorales qPCR for the diagnosis of mucormycosis. Patients with a suspicion of IMD were prospectively enrolled in nine teaching hospitals. Mucorales qPCR was performed at the time of serum sampling in each center using the combination of genera-specific qPCR assays targeting *Lichtheimia*, *Rhizomucor*, and *Mucor/Rhizopus* we previously described (12).

Patients - Methods

Ethical considerations

The study was approved by the local ethics committee (CPP Est II) and obtained authorization from the National Agency for the Safety of Medicinal Products and Health Products. All patients provided informed consent. The study was documented under ClinicalsTrials.gov (NCT02845934). The study was performed in accordance with the Standards for Reporting Diagnostic Accuracy (STARD) 2015 statement (20).

MODIMUCOR protocol: recruitment – sample collection – mycological and clinical investigations

Patients with a suspicion of IMD, defined by the presence of host factors, suggestive imaging, and clinical symptoms, according to the 2008-consensus definitions from the European Organization for the Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) (21), were prospectively recruited in nine university hospitals in France from January 1, 2015, to June 30, 2017 (Cohort 1).

Mycological and clinical investigations are detailed in supplementary Data. Detection of galactomannan antigen and *Aspergillu*s PCR assay were performed in accordance with the procedures defined in each center. The day of sampling of the first histological or mycological positive specimen was defined as Day 0 (D0). Patients were classified at Month 6 as having a possible, probable, or proven IMD according to the 2020-EORTC/MSGERC criteria (22).

Mucorales qPCR - interlaboratory assay

Serum samples were collected twice-a-week and stored at 4°C until the qPCR was carried out**.** Mucorales qPCR was performed in each center, and results were reported to clinicians one to four days after sampling. Recommendations were given for DNA extraction (DNA extraction from 1 mL of serum with an elution volume of 50 µL) and qPCR amplification according to the method described previously (12). The Mucorales PCR assay enables detection of DNA from *Lichtheimia* spp. *Rhizomucor* spp., and both *Rhizopus* spp. and *Mucor* spp. (without distinguishing between the last two genera). Serum samples were collected twice-a-week from the date of recruitment until at least two consecutives negative qPCR results were obtained.

All participants were free to use extraction kits, qPCR platform and reagents available in their own laboratories. An interlaboratory quality-control trial, was organized to ensure uniformity and reliable comparisons among data generated by multiple sites. In total, nine different protocols were used, based on a combination of three different automatic DNA extraction systems and five different qPCR platforms.

Technical details are provided in Supplementary Data. Despite the diverse platforms and reagents used, we observed very low interlaboratory variability (quantification cycle: median CV, 5·02% (IQR, 4·4-6·3%) (Supplementary Tables 1 and 2).

Performance of the Mucorales qPCR was assessed by calculating the sensitivity, specificity, predictive values, and likelihood ratios.

Analysis of Mucorales DNA load kinetics

We analyzed the DNA load kinetics relative to the time to diagnosis and outcome using a larger number of patients by prospectively recruiting additional patients diagnosed with probable or proven mucormycosis and hospitalized in the same centers during the same period (January 2015-June 2017) (Cohort 2). As for patients included with a suspicion of an IMD, serum samples were collected twice-a-week for Mucorales qPCR from the date of diagnosis until at least two consecutive negative results were obtained. If available, serum samples taken in the 30 days before the day of diagnosis, mainly for serum galactomannan screening, were tested using the same Mucorales qPCR assay. Results were reported to clinicians in variable time frames depending on the center.

Statistical analysis

Statistical analyses are detailed in supplementary Data.

Results

Performance of Mucorales qPCR

Performance characteristics were calculated on cohort 1, consisting of 232 patients prospectively recruited with a suspicion of IMD (Figure 1). Twenty-seven (12%) patients were classified as proven (n=20) or probable (n=7) mucormycosis (Cohort 1-Group 1), including nine with a mixed *Aspergillus*-Mucorales infection, 67 (29%) as probable or proven invasive aspergillosis, and six (2.6%) as IMD due to other molds (*Fusarium* sp. (n=2), *Scedosporium* sp. (n=1), *Acremonium* sp. (n=1), mixed *Aspergillus-Scedosporium* (n=1) and mixed *Aspergillus–Fusarium* (n=1)). Finally, 18 patients (8%) had host factors and imaging criteria of IMD and Mucorales qPCR as the only positive mycological test. This group of 18 patients was designated as "Mucormycosis-PCR only" and analyzed apart (referred to as Cohort 1-Group 2). In addition, one patient with probable aspergillosis and two with probable fusariosis also had at least one positive-Mucorales qPCR serum

sample, and were considered as mixed infection. Clinical and mycological data of patients from Group 1 and 2 are presented in Supplementary Tables 3 and 4.

Overall, 118 patients (51%) were diagnosed with probable or proven IMD or "mucormycosis PCR only", including 12% (14/118) with a mixed fungal infection.

The remaining 114 patients (49%) had no probable or proven IMD and were eventually diagnosed with either possible mold infection (host factors and consistent imaging without mycological criteria) or infection due to other fungi (*Pneumocystis*, *Candida*, *Cryptococcus*), bacterial or viral infections, or non-infectious disease.

Among the 27 patients who matched the criteria for proven/probable mucormycosis (excluding PCR as the only criterion), 23/27 had at least one positive Mucorales qPCR serum sample.

Sensitivity was 85·2%, specificity 89·8%, and positive and negative predictive values 52·3% and 97·9%, respectively. The positive and negative likelihood ratios were 8·3 and 0·17, respectively (Table 1). Serum sampling was suboptimal for the four patients with negative Mucorales qPCR (median of two serum samples per patient versus seven for the PCR-positive patients). The sensitivity would have likely been higher if the sampling protocol had been strictly applied.

Global analysis on all proven probable cases

We included 13 additional patients diagnosed with probable (n=3) or proven (n=10) mucormycosis recruited during the same period in the same centers and similarly tested (Cohort 2) to increase the number of analyzable cases to study the fungal load kinetics. All 13 patients had at least one Mucorales-positive qPCR serum sample (Supplementary Tables 3 and 4). Thus, when combined with patient from cohort 1, a total of 40 mucormycosis cases were analyzed (Table 2). Thirteen of the 40 patients (32%) presented mixed *Aspergillus*-Mucorales infection. The clinical presentations were mainly pulmonary, disseminated, and rhino-orbito-cerebral (Figure 2). Mucorales-positive cultures were obtained for 25/40 patients and yielded mainly *Rhizopus* spp. (40%) and *Lichtheimia* spp. (32%) (Figure 3). Molecular identification was performed on tissue samples for 7/16 patients with negative Mucorales cultures. For the eight remaining patients, the diagnosis relied on histological examination only.

Overall, 36/40 patients had at least one positive serum Mucorales qPCR (Figure 4). Twenty of them were given at least one mould-active antifungal (voriconazole (n=7), posaconazole (n= 4), L-AMB 3 mg/kg (n=5) or casponfungine (n=9) in the month preceding the first positive serum Mucorales qPCR**.** The genera detected in serum by the Mucorales qPCR was consistently in accordance with the species cultured or identified using qPCR or ITS sequencing for all 29 patients (100%) with a positive culture or molecular identification. For the

seven remaining cases (positive histology but no positive culture or molecular identification), serum qPCR identified *Rhizomucor* (n=5) or *Mucor/Rhizopu*s (n=2). Of note, the *Rhizomuco*r qPCR assay on serum samples was positive for 9 patients for whom mycological cultures from the infection site were negative.

The first positive qPCR sample was observed a median of four days (IQR, 0-9) before the sampling date of the first positive mycological or histological specimen and a median of one day [IQR, (-2)-6] before the first imaging (Table 2).

Among the 36 patients with positive Mucorales qPCR, three died within eight days after the first positive Mucorales qPCR without being given L-AMB. The 33 other patients were given L-AMB two days (IQR, 1-8) after the first positive Mucorales qPCR. Analysis of the outcome according to DNA load kinetics was restricted to L-AMB-treated patients who had regular twice-a-week serum sampling (n=30) (supplementary Figure 1). For 21 patients, the qPCR became negative on a serum sample collected four days (IQR, 2-6) after the start of L-AMB (Table 2).

Survival at 30 days and six months was significantly higher among patients with a qPCR becoming negative within seven days after treatment initiation than among patients for whom the qPCR remained positive (Table 2). Indeed, negativity of the Mucorales qPCR was associated with an 85% lower 30-day mortality rate (age-, sex- and primary disease-adjusted hazard ratio (aHR)=0·15; 95%CI, 0·03-0·73; p=0·02) and an 88% lower six-month mortality rate (aHR=0·12; 95%CI, 0·03-0·40; p=0·001).

Characteristics of " Mucormycosis PCR only" patients

The "Mucormycosis PCR only" group (Cohort 1-Group 2) was composed of 18 patients with one or more host factors and radiological signs compatible with IMD, without mycological criteria (possible IMD), but with at least one positive Mucorales qPCR. Mycological culture from BAL performed for 15 of the 18 patients remained negative. No mycological investigation could be performed on the three remaining patients. A median of 10 serum samples was collected per patient and 13/18 patients had at least two qPCR-positive serum samples (Supplementary Tables 3 and 4).

We compared the characteristics of the "Mucormycosis PCR only" patients to those of patients with probable or proven mucormycosis (Table 2). There was a significantly higher number of patients with a hematological malignancy in the "Mucormycosis PCR only" group. The frequency of nodules was also significantly higher in this group. No other characteristics were statistically different.

The first positive Mucorales qPCR was obtained one day (IQR, 0-3) before the first imaging sign. Sixteen of the 18 patients were treated with L-AMB within two days (IQR, 1-4)) after the first positive qPCR. Serum qPCR of 14 patients became negative three days (IQR, 0-4) after the start of L-AMB (Table 2 and supplementary Figure 2). Survival at 30 days and six months was significantly higher for patients for whom the qPCR became negative within seven days after L-AMB initiation (100% and 79%, respectively) than for those for whom the qPCR remained positive (0%), ($p<0{\cdot}05$, Table 2).

Discussion

The MODIMUCOR multicenter study confirmed the good performance of qPCR detection of circulating DNA in serum with 85·2% sensitivity and 89·8% specificity. Serum Mucorales qPCR was positive four days before mycological or histopathological examination and one day before the first imaging was performed. In addition, we confirm the poor outcome of patients for whom the qPCR remains positive, despite appropriate antifungal treatment (mortality rate of 100% at six months). By contrast, negativity of the DNA load within seven days after L-AMB initiation was associated with a far better outcome.

We believe that qPCR should be integrated as a mycological criterion for mucormycosis in the EORTC/MSGERC definitions. The cumulative experience with *Aspergillus* PCR can be used to accelerate the acceptance of Mucorales qPCR. *Aspergillus* PCR was only recently recognized as a mycological criterion for probable IPA after 20 years (22). This was made possible by the efforts of the Fungal PCR Initiative (FPCRI) to demonstrate the reliability of *Aspergillus* PCR (23-25). Low interlaboratory variability of the Mucorales qPCR was demonstrated in a large recent study from the FPCRI Mucorales Laboratory group (26). Serum Mucorales qPCR can be performed on molecular biology platforms in most teaching hospitals due to increased availability of in-house techniques and commercial kits (12, 13, 17, 27). Performing this technique on site accelerates the transmission of positive results to clinicians and the triggering of targeted antifungal treatment, which is essential for a better outcome. The new In Vitro Diagnostic Medical Devices Regulation from the European Commission (Regulation (EU) 2017/746) requires health institutions to justify the use of in-house technique when an equivalent device is available on the market. In our opinion, this may represent a potential threat as continuous development and optimization of laboratory-developed tests are essential to improve the management of mucormycosis.

The issue of patients with a positive qPCR as the only criterion for the diagnosis could not be completely resolved in this study**.** Positive results due to transient transfer of Mucorales DNA into the bloodstream in

patients without invasive infection seem possible. Nevertheless, the high index of suspicion for mucormycosis led to start L-AMB for 16 of the 18 patients with criteria for a possible IMD with at least one positive serum-Mucorales qPCR as soon as a positive result was obtained. Comparison of the clinical characteristics of these patients with those of patients with probable and proven mucormycosis showed many similarities, including better survival among patients for whom the Mucorales qPCR become negative after the initiation of antifungal treatment. This strongly argues for the initiation of specific anti-Mucorales treatment as soon as a positive Mucorales PCR is observed for patients with radiological signs of IMD.

Another positive point of the use of Mucorales qPCR is a better delineation of the epidemiology of mucormycoses. Indeed, the fungal identification could be assessed even if the culture was negative. The relative frequency of mucormycosis in our study was 12%, which is similar to a previous study (28). Reliability of the qPCR assay was confirmed by the perfect concordance between the genera identified in serum by Mucorales qPCR and conventional identification using mycological culture for various samples (pulmonary, sinus, skin, digestive). Overall, serum Mucorales qPCR allowed genus identification for 15 patients who were positive by direct examination but had negative cultures from tissue sampled at the site of infection. *Rhizomucor* DNA was identified in the serum of 9 patients with negative cultures, suggesting that positive cultures from tissue samples may be more difficult to obtain when *Rhizomucor* is the etiological agent. The lower prevalence of *Rhizomucor* (generally < 10%) reported in previous studies using culture methods should be reconsidered (1, 8, 29). Here, the detection of circulating Mucorales DNA by qPCR led to a different distribution of the main genera than previously reported, with 35% of probable/proven cases due to *Mucor/Rhizopus*, 25% *Rhizomucor*, 20% *Lichtheimia*, and 10% a mixed infection by two Mucorales genera. L-AmB is recommended as first-line treatment of mucormycosis. However, accurate identification of the causative agents might be of interest when switching to oral treatment, since compared with posaconazole, isavuconazole exhibited a lower in vitro inhibitory activity against *Rhizomucor* species (30, 31).

Finally, the Mucorales qPCR evaluated in this prospective study detects the most common genera associated with mucormycosis (*Rhizopus Lichtheimia*, *Mucor*, and *Rhizomucor)* but misses other genera that are less common in France (*Cunninghamella* and *Saksenae* or *Apophysomyces* spp.) (1, 2). Thus, the sensitivity could be less in some studies conducted in other geographical areas, where these rare species maybe more common than in Europe. Other systems currently available are: 1/ the in-house assay by Springer *et al.*, which uses a more generic approach targeting all clinically relevant Mucorales and requires sequencing to identify the genus (13), and 2/ a commercial semi-quantitative PCR kit (Mucorgenius®, PathoNostics) that also detects the main

Mucorales genera, including *Cunninghamella*, but without possible distinction between them (17). The development of techniques enabling both the generic detection of Mucorales and species identification is required to improve the molecular diagnosis of mucormycosis from serum.

In addition, the use of qPCR allowed us to detect mixed mold infections. Indeed, we found 12% mixed infections versus 2 to 6% recently reported in a series of patients with hematological malignancies (32-34). Of note, one third of mucormycoses were actually mixed *Aspergillus*-Mucorales infections. Better detection of mixed infections was already noted in previous retrospective studies based on molecular diagnosis, with a rate of approximately 20% of mucormycosis associated with aspergillosis (12, 14, 35). Therefore, we recommend to perform serum Mucorales qPCR in patients already diagnosed with invasive pulmonary aspergillosis, especially if voriconazole therapy is not rapidly effective.

Our study had several limitations, mainly due to the low prevalence of mucormycoses, which is a hurdle in prospective studies. However, although only 28 patients were ultimately diagnosed with mucormycosis among the 233 patients recruited with a suspicion of IMD, it was possible to assess the performance. With a positive likelihood ratio of 8.3 and negative likelihood ratio of 0.17, Mucorales qPCR can be considered to provide moderate evidence to confirm or rule out the diagnosis of mucormycosis, respectively (36). As other laboratory diagnostic methods require invasive procedures and are less performant, this new tool is the most promising to improve the diagnosis of mucormycosis. In addition, this figure was too low to study the fungal-load kinetics and we had to recruit additional cases of confirmed mucormycosis. However, the serum sampling protocol and time between first positive qPCR and other diagnostic tools were similar for both cohorts.

In conclusion, serum Mucorales qPCR is a non-invasive technique that can help to anticipate the diagnosis of mucormycosis and trigger early targeted antifungal treatment. Follow-up of the Mucorales DNA load in serum could also be helpful for therapeutic management. The possible standardization of the test and very good performance now demonstrated in a prospective multicenter study, argue for the addition of Mucorales qPCR in clinical settings and EORTC/MSGERC consensual definitions to improve the management of mucormycosis. There is still the issue of the diagnostic strategy: prospective screening or part of the diagnostic work-up for suspicion of a mold disease. The second strategy is likely more realistic because of the low frequency of mucormycosis in hematology patients.

NOTES

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Author's Contribution

LM and RH designed the overall study protocol. RH, DC, AB, FL, BD, DB, FB, AC, and FA were responsible for patient management and data collection. VLB, FD, SB, AA, MEB, FB, TC, DD, ES, APB, and SR performed and interpreted the analysis and collected the data. LM drafted the initial version of the manuscript with input from RH, SB, DC, AB, FL, and FM. HG and SR performed the statistical analyses of the pooled data. All authors helped to analyze or interpret data, contributed to the revision and correction of multiple versions of the manuscript, approved the final version, and agree to be accountable for all aspects of the work.

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Declaration of interests

LM reports personal fees outside the submitted work and travel grants from Gilead and Pfizer. SB reports personal fees outside the submitted work and travel grants from Gilead. FL reports personal fees from Airnspace, Gilead, and F2G outside the submitted work. AA reports personal fees form Gilead and Pfizer outside the submitted work, consulting fees from Pfizer for serving on an Advisory Board; travel grants from Gilead and Pfizer; and patent licensed for Pneumocystis PCR and patent issued for Histoplasma PCR. FD reports travel grants from Pfizer to attend Trend in Medical Mycology congress, Aberdeen, 2021 and is Vice President for French Society of Medical Mycology. RH reports research grant from Gilead, personal fees form Gilead and Pfizer outside the submitted work, and travel grants from Gilead and Pfizer. APB reports support for attending meetings and/or travel from Gilead. BD reports fees for travel and as a speaker at 2 national congresses in 2021 from Gilead and consultancy fees at an expert meeting planned in 01/2022 from Pfizer. FB reports consulting fees, payment or honoraria, and support for attending meetings and/or travel from Gilead and support for attending meetings and/or travel from Pfizer and leadership or fiduciary role from the Society of French Medical Mycology. SB reports honorarium from Gilead to attend an educational workshop and support for attending meetings and/or travel from Gilead travel grant for TIMM congress Aberdeen UK. All other authors state no conflict of interest related to the content of the present study.

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Table1. Performance and diagnosis accuracy of Mucorales qPCR determined on Cohort 1 (n=232 patients)

		95% CI
Sensitivity	85·2	66·3%-95·8%
Specificity	89·8%	84·8%-93·5%
Positive predictive value	52·3%	36·7%-67·5%
Negative predictive value	97·9	94·6%-99·4%
Positive likelihood ratio	8·3	5·4-12·8
Negative likelihood ratio	0·17	0·07-0·41

	Mucormycosis	Others	Total
Positive qPCR	23	21	44
Negative qPCR	4	184	188
Total	27	205	232

Table 2. Main characteristics of the 40 patients with probable and proven mucormycosis and the 18 patients with "Mucormycosis PCR only"

	Probable and proven mucormycosis, n=40	Mucormycosis "PCR only", n=18	*P*-values
Age, years	55 (37-64)	59 (52-65)	0·28
Male gender (%)	29 (72·5%)	11 (61·1%)	0·39
Underlying conditions			
haematological malignancies	27/40 (67·5%)	17/18 (94·4%)	**0·044**
diabetes	10/40 (25%)	1/18 (5·6%)	0·15
solid organ transplantation	6/40 (15%)	0	0·16
allogenic hematopoietic stem-cell transplantation	7/40 (17.5%)	5/18 (27·8%)	0·37
autologous hematopoietic stem-cell transplantation	3/40 (7.5%)	0	0·55
trauma	4/40 (10%)	0	0·30
recent major surgery (< 30 days)	6/40 (15%)	1/18 (5·6%)	0·42
chronic kidney failure	8/40 (20%)	1/18 (5·6%)	0·25
neutropenia (< 500/mm3) in the previous 30 days	21/40 (52.5%)	9/17* (52·9%)	0·98
systemic corticosteroids ≥ 0.3 mg/kg/d , ≥ 21 days	6/40 (15%)	0*	0·16
inhaled corticosteroids	2/40 (5%)	2/18 (11·1%)	0·58
other immunosuppressive therapy in	9/40 (22.5%)	4/17* (23·5%)	>0·99

the last 3 months			
other severe immunodeficiency	6/40 (15%)	2/17* (11·8%)	>0·99
Medical history			
invasive pulmonary aspergillosis	4/40 (10%)	2/18 (11·1%)	>0·99
other invasive fungal infection	4/40 (10%)	0	0·30
tobacco	15/40 (37.5%)	4/18 (22·2%)	0·37
chronic bronchitis, emphysema, asthma	7/40 (17.5%)	0	0.09
antifungal treatment in the last month	21/40 (52.5%)	12/17* (70·6%)	0·25
chemotherapy in the last 3 months	21/40 (52.5%)	12/17* (70·6%)	0·25
Biology			
creatinine, µmol/L	72 (53-102), n=39	67 (55-82), n=17	0·96
C Reactive Protein, mg/L	142 (56-211), n =34	94 (27-150), n=15	0·13
fibrinogen, g/L	5·7 (4·6-6·8), n=31	6.1 (4·5-6·9), n=14	0·52
Imaging			
abnormal sinus CT	15/23 (65·2%)	3/5 (60%)	>0·99
abnormal thoracic CT	26/30 (86·6%)	18/18 (100%)	0·28
Presence of nodules	15/26 (57.7%)	17/18 (94·4%)	**0·01**
Number of nodules	1 (1-2), n=20	1 (1-2), n=15	0·51
Presence of halo sign	5/26* (19·2%)	8/17* (47·1%)	0·05
Presence of reverse halo sign	5/26* (19·2%)	6/17* (35·3%)	0·24
Micronodule(s) (< 1 cm)	9/26* (34·6%)	6/18 (33·3%)	0·93
Condensation(s)	19/26* (73·1%)	10/18 (55·6%)	0·23
Time to diagnosis and treatment **			
time between first positive qPCR and first mycological/histological test (days)	4 (0-9)[a], n=36	(··)	(··)

time between first positive qPCR and first imaging sign (days)	1 ((-2)-6)[b], n=34	1(0-3), n=18	0·95
time between first positive qPCR and start of L-AMB (days)	2 (1-8), n=33	2 (1-4), n=16	0·81
time between start of L-AMB and first negative qPCR (days)	4(2-6), n=21	3 (0-4), n=14	0·25
Outcome			
survival: 30 days			
All patients with mucormycosis	27/40 (67.5%)	(··)	(··)
All patients with *Aspergillus*-Mucorales infection	9/14 (64.2%)	(··)	(··)
All patients with positive Mucorales qPCR	24/36 (66.6%)	16/18 (89%)	0·11
survival: 6 months			
All patients with mucormycosis	14/40 (35%)	(··)	(··)
All patients with *Aspergillus*-Mucorales infection	5/14 (35.7%)	(··)	(··)
All patients with positive Mucorales qPCR	14/36 (38.8%)	12/18 (67%)	0·05
DNA load and outcome			
Patients who received L-AMB treatment and had regular twice-a-week sampling	30/36 (83.3%)	16/18 (89%)	0·70
survival: 30 days			
Patients for whom qPCR become negative within 7 days after treatment initiation	18/21 (86%)	14/14 (100%)	0·27
Patients for whom qPCR remained positive	3/9 (33%)	0/2 (0%)	>0·99
Survival: 6 months			
Patients for whom qPCR become	12/21 (57%)	11/14 (79%)	0·31

negative within 7 days after treatment initiation			
Patients for whom qPCR remained positive	0/9 (0%)	0/2 (0%)	-

Data are presented as medians (interquartile ranges) or frequencies (%) or n/N (%)

(··): not applicable

*Missing data

** if time > 0, qPCR was positive before mycology/imaging

[a] Time did not differ when considering only the 23 patients from Cohort 1- Group 1 (median=4 [IQR, 1-15]) or the 13 patients from Cohort 2 (median=2 [IQR, (-2)-9]) (p=0.25)

[b] Time did not differ when considering only the 21 patients from Cohort 1- Group 1 (median=0.5 [IQR, (-2.5)-2.5]) or the 13 patients from Cohort 2 (median=4.5 [IQR, (-1)-7]) p=0.23)

Figure Legends

Figure 1 Patients flow diagram

IMD: invasive mould diseases;

Mucormycosis “PCR only” refers to cases with radioclinical signs consistent with invasive mould diseases and with only positive PCR in serum and no other pathogen identified

Cohort 1: patients recruited with suspicion of an IMD and ultimately classified as probable or proven mucormycosis (Group1, n = 27), ultimately classified as mucormycosis "PCR only" (Group 2, n = 18), or ultimately classified with another IMD and positive Mucorales PCR.

Cohort 2: patients recruited with a diagnosis of mucormycosis (n = 13)

Figure 2. Clinical presentation of mucormycosis (n = 40)

[a] Including 8 mixed Aspergillus-Mucorales infection

[b] Including 3 mixed Aspergillus-Mucorales infection

[c] Including 1 mixed Aspergillus-Mucorales infection

Figure 3. Identification of Mucorales from tissue or BAL samples (culture and molecular identification).

Figure 4. identification of Mucorales DNA from serum samples by Mucorales qPCR

Mixed: *Lichtheimia* + *Rhizomucor* (n = 2), *Lichtheimia* + *Mucor/Rhizopus* (n = 1), *Rhizomucor* + *Mucor*/*Rhizopus* (n = 1)

Figure 1

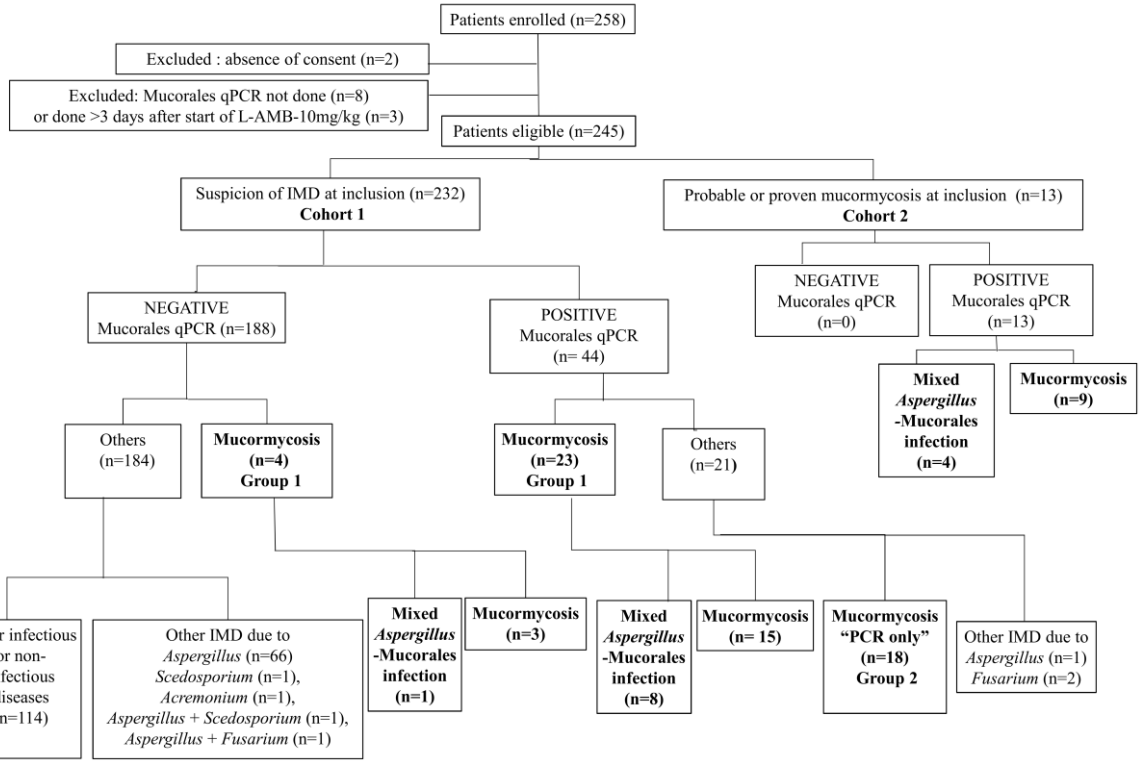

Figure 2

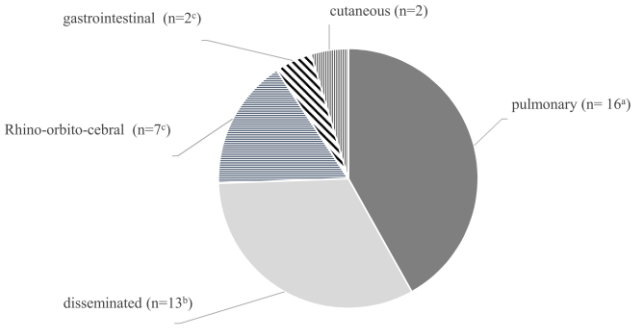

Figure 3

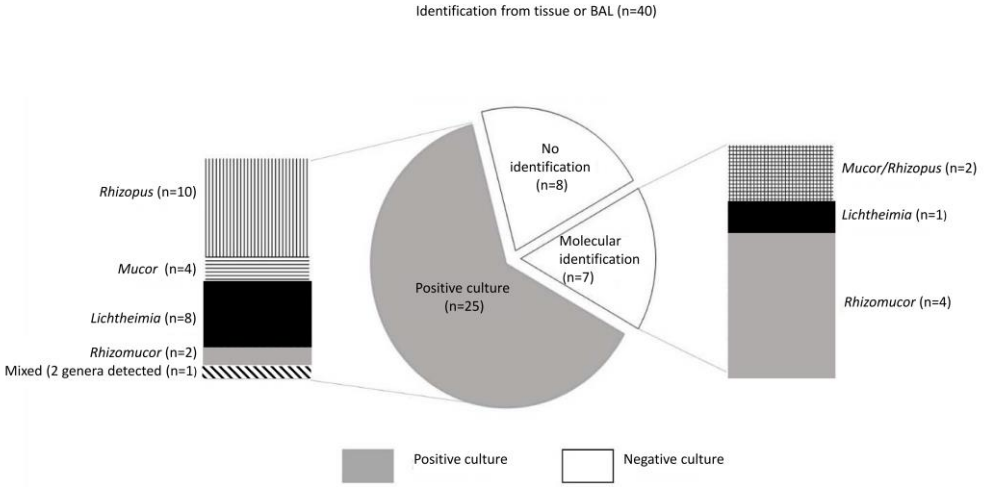

Figure 4