Mucorales-specific quantitative PCR on peripheral blood is a sensitive and early diagnostic marker for invasive mucormycosis

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Purpose

Invasive mucormycosis is still a potentially lethal infection, requiring early and aggressive therapy. However, making a timely diagnosis is a challenge due to the lack of sensitive diagnostic tests.

Recently, Mucorales-specific quantitative PCR (qPCR) assays were developed for the detection of Mucorales DNA in patient samples. These assays have already proven to be useful on biopsy specimens or on broncho-alveolar lavage fluid from infected sites. However, getting a biopsy or another sample from the affected body site is not always possible. Detection of circulating Mucorales DNA in blood could offer an attractive diagnostic tool in these patients.

We therefore evaluated the sensitivity of a commercial Mucorales-specific qPCR and kinetics of Mucorales DNA in serial blood samples from patients with culture-positive invasive mucormycosis.

Methods

We retrospectively collected serial serum, plasma or whole blood samples from the biobanks of 2 hospitals in Belgium (University Hospitals Leuven and AZ St Jan Brugge) from patients with culture-positive invasive mucormycosis.

Cases were classified according to the 2008 revised EORTC / MSG consensus definitions. We added a classification of “putative” mucormycosis for patients with well-recognized risk factors for mucormycosis (such as diabetic ketoacidosis or iron chelation therapy), but not fulfilling the EORTC/MSG-defined host criteria.

The date on which the sample that resulted in a positive Mucorales culture was taken, was defined as the date of diagnosis (D+0).

We collected all blood samples from our biobanks from 2 weeks before up to 2 weeks after the date of diagnosis (maximum 2 samples per week). We extended our search period until the samples became negative, or until there were no more stored samples available in the biobank, as applicable.

All samples were tested using a Mucorales-specific qPCR (MucorGenius®, PathoNostics, The Netherlands).

Results

We identified 16 patients with invasive mucormycosis between 2009 and 2019 and retrieved 106 blood samples for qPCR testing (Table 1). The temporal evolution of each patient is shown in Figure 1.

We found an overall sensitivity of 0.75 (95% CI 0.48 – 0.93). Serial testing of blood samples showed that DNA was present up to 81 days (median 8 days, inter-quartile range [IQR] 1.75 – 16.25) before diagnosis by culture, and up to 24 days (median 3 days, IQR -0.25 – 8.5) before the first signs of fungal infection on imaging. qPCR was positive in all patients who died within six weeks, whereas qPCR was negative in 40% of patients who survived for more than six weeks (6/6 vs 6/10, p=0.234). The evolution of qPCR after initiation of therapy is shown in Figure 2.

All patients who succumbed before week 6 died of mucormycosis. Autopsy reports in the four patients in whom this was performed showed disseminated disease in all four cases, also involving organs that showed no clear signs of infection pre-mortem such as the liver and spleen.

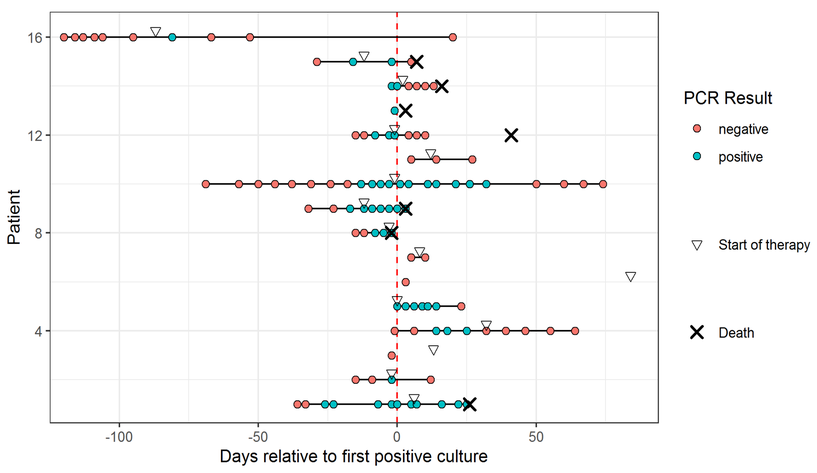

Figure 1. Temporal evolution of blood qPCR results. A white triangle denotes initiation of antifungal therapy.

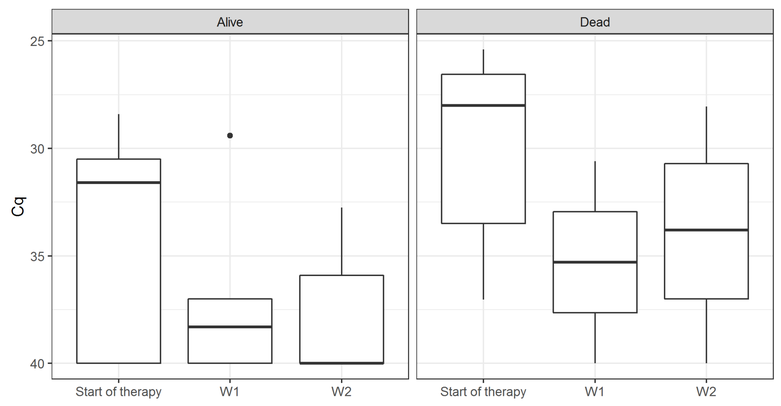

Figure 2. Boxplots of qPCR values at initiation of treatment, and after one and two weeks relative to the initiation of adequate anti-fungal therapy.

Conclusion

The MucorGenius® assay in blood proves to be a sensitive and early diagnostic tool for invasive mucormycosis. It allows to make the diagnosis before cultures are positive and before typical signs are visible on imaging

Patient	Sex, Age (Years)	Underlying Disease	Localization	Classification	Identification in Culture	*Aspergillus spp*. Co-Infection	Survival at Week 6	Survival at Week 12	Start of Appropriate Therapy, Antifungal
1	M, 57	ALL	Disseminated (cutaneous, lung)	Proven	Rhizopus microsporus	Yes (Culture *A. fumigatus/A. terreus*. BDG > 500 pg/mL. Serum GM 6.0)	Dead	Dead	D+6, L-AmB 10 mg/kg
2	M, 8	AML	Disseminated (cerebral, lung)	Probable	Rhizomucor pusillus	No	Alive	Alive	D-2, L-AmB 5 mg/kg
3	F, 34	Lung transplant	Lung	Probable	Mucor species	No	Alive	Alive	D+13, L-AmB 5 mg/kg
4	M, 29	Lung transplant	Lung	Probable	Rhizopus species	Yes (Culture *A. fumigatus*)	Alive	Alive	D+32, posaconazole
5	F, 54	Crohn’s disease	Sinus	Probable	Lichtheimia species	No	Alive	Alive	D+0, L-AmB 5 mg/kg
6	F, 50	COPD	Lung	Proven	Rhizopus rhizopodiformis	Yes (Culture *Aspergillus sp*p., BAL GM 0.5)	Alive	Alive	D+84, isavuconazole
7	M, 54	Diabetic ketoacidosis	Lung	Putative	Rhizopus microsporus	Yes (Culture *A. fumigatus*, BAL GM 5.0)	Alive	Alive	D+8, L-AmB 5 mg/kg
8	M, 61	ALL	Disseminated (pleura, pericardium, lungs, myocardium, spleen)	Proven	Rhizomucor pusillus	Yes (Culture *A. fumigatus*, BAL GM 2.2)	Dead	Dead	D-3, L-AmB 5 mg/kg
9	M, 58	AML	Disseminated (lung, liver)	Proven	Lichtheimia species	Yes (Culture and PCR *A. fumigatus*, BAL GM 5.1, Serum GM 3.9)	Dead	Dead	D-12, L-AmB 5 mg/kg
10	M, 54	Lung transplant	Lung	Probable	Rhizopus species	No	Alive	Alive	D-1, posaconazole
11	M, 78	MDS	Lung	Putative	Rhizopus species	Yes (Culture *A. fumigatus*, BAL GM 5.6)	Alive	Alive	D+12, L-AmB 10 mg/kg
12	M, 66	Allogeneic SCT	Lung	Probable	Rhizomucor pusillus	No	Alive	Alive	D-1, L-AmB 5 mg/kg
13	F, 63	Solid tumor	Lung	Probable	Lichtheimia species	Yes (Culture *A. fumigatus*, BAL GM 5.1, Serum GM 0.7)	Dead	Dead	None
14	F, 63	Aplastic anemia	Disseminated (lung, spleen)	Proven	Rhizopus species	No	Dead	Dead	D+2, ABLC 10 mg/kg
15	F, 63	AML	Lung	Proven	Rhizopus species	No	Dead	Dead	D-12, posaconazole
16	F, 64	AML	Disseminated (lung, liver, diaphragm)	Proven	Rhizopus microsporus	No	Alive	Alive	D-87, L-AmB 5 mg/kg

Table 1. Patient population.
D+0, Day on which the sample was taken which resulted in growth of Mucorales in culture. ABLC, Amphotericin B Lipid Complex; ALL, Acute Lymphoblastic Leukemia; AML, Acute Myeloid Leukemia; BAL, Bronchoalveolar Lavage; BDG, beta-D-glucan; COPD, Chronic Obstructive Pulmonary Disease; F, Female; GM, Galactomannan; L-AmB, Liposomal Amphotericin B; M, Male; MDS, Myelodysplastic Syndrome; SCT, Stem Cell Transplantation.