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 two 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 (MurcorGenius®, 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.

Conclusion

The MurcorGenius® 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.

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

![Figure 1](image1.png)

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.