

## Short Communication

## Comparison of serum PCR assay and histopathology for the diagnosis of invasive aspergillosis and mucormycosis in immunocompromised patients with sinus involvement

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### Abstract

**Background and Purpose:** Invasive fungal infections cause morbidity and mortality in patients with hematologic malignancies and immunosuppression. Although these infections are commonly caused by *Candida* and *Aspergillus* species, infections caused by Mucoralean fungi are also on a growing trend. The definitive diagnosis of mucormycosis includes visualization of non-septate hyphae on pathology or growth of Mucoralean fungi culture. Polymerase chain reaction (PCR) is used to diagnose mucormycosis from paraffin blocks; however, it yields discrepant results in diagnosis of mucormycosis from blood samples. In the current study, we sought to examine the efficiency of PCR test for the diagnosis of mucormycosis and aspergillosis.

**Materials and Methods:** Thirty-one patients with suspected fungal sinus infection were recruited from the Hematology-Oncology unit in Taleghani Hospital, Tehran, Iran. DNA was extracted and semi-nested PCR was performed.

**Results:** PCR was reported negative for all the 31 serum samples. Our assay had a sensitivity of 1.3 ng and 12 pg for Mucoralean and *Aspergillus* species, respectively.

**Conclusion:** Using serum PCR, we detected *Aspergillus* and Mucoralean species in patients with suspected fungal sinus infection. While this test may have utility in diagnosis directly from biopsy site, it appears unreliable for use as a noninvasive blood test.

**Keywords:** Aspergillosis, Diagnosis, Immunocompromised patients Mucormycosis, Polymerase chain reaction

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### Introduction

Invasive fungal infections are one of the major causes of morbidity and mortality in patients with hematologic malignancies and immunosuppression [1, 2]. These infections are most commonly caused by *Candida* and *Aspergillus* species; however, the rate of infections caused by the Mucoralean fungi has increased in the recent years [3]. Remarkably, 63% of non-aspergillosis mold infections in transplant recipients were caused by Mucorales (particularly *Rhizopus*, *Lichtheimia*, and *Mucor* species) [4-6]. Mucormycosis is mostly confirmed by histopathology (characterized by non-septate hyphae, with branching at wide angles [ $>90^\circ$ ]), microscopy, or culture. However, utilizing state-of-the-art radiological techniques, as well as serological and molecular diagnostic assays can be

beneficial for improving early diagnosis and successful treatment [7-9].

Definitive diagnosis of mold infection includes visualization of non-septate hyphae on pathology or growth of the mold from a sterile culture [10]. Diagnosis and treatment of mucormycosis pose more difficulties than other fungal infections as it develops in necrotic tissues [11]. In addition, differentiating the various Mucoralean species based on their morphology is challenging [12, 13].

PCR has been applied for the tissue diagnosis of mucormycosis in paraffin blocks [14, 15]; however, there are conflicting results regarding its ability to diagnose infection from blood samples [16, 17]. In the current study, we aimed to investigate the sensitivity and specificity of blood PCR test for

diagnosis of mucormycosis and aspergillosis.

## Materials and Methods

Patients with suspected fungal sinus infection were recruited from Division of Hematology-Oncology in Taleghani Hospital of Tehran, Iran. Tissue specimens were obtained via biopsy, and serum samples (1 cc) were taken at the time of biopsy. Tissue samples were sent for fungal staining, culture, and histopathology. DNA was extracted by use of the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA), and semi-nested PCR was performed in Applied Biosystems 2720 (Foster City, CA) as previously described [18, 19].

Briefly, 12.8 µg of DNA was amplified during the 1<sup>st</sup> run, and then 10 µl of PCR Master Mix (Qiagen, Valencia, CA) was added to 1 µl of each primer (0.1 mM) and 8 µl DNA. PCR conditions for the first step were as follows: DNA was denatured at 94°C for 5 min, then amplified for 35 cycles at 94°C for 30 s, 56°C for 30 s, 72°C for 30 s, and then 72°C for 7 min. In the second step, 10 µl of Master Mix, 0.1 µl of each primers, 3 µl of the PCR product from the first step, and 5 µl sterile water were employed. The final PCR product was loaded onto a gel with 100 bp ladder; thereafter, positive samples were sequenced.

This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (official approval No.: 94-2-205-8140-8520).

## Results and Discussion

A total of 31 patients with the mean age of 42.2 years (age range: 15-82 years) were enrolled (65% male and 35% female). The patients had acute myeloid leukemia (68%), acute lymphoblastic leukemia (13%), aplastic anemia (10%), lymphoma (6%), and myelofibrosis (3%). The chief complaints were headache (42%), fever (29%), periorbital swelling (13%), cough (6%), poor vision (3%) confusion (3%), as well as dysphagia (3%). The average white blood cell (WBC) count was 2,184 cells/µl. All the patients were initially administered liposomal amphotericin B on a daily basis (5 mg/kg). After the treatment, fever resolved in 26 patients (mean duration: 2.65 days). All the patients met the indication for undergoing endoscopy, but it was only performed on 19 patients. Of the 19 patients, 18 underwent sinus biopsy, of whom 11 were found to have necrosis, with nine being pathologically negative for mold. Ultimately, 5, 2, and 2 cases of *Mucor*, *Aspergillus*, and non-specific fungal infection were diagnosed, respectively.

Serum samples were negative for PCR in all the

31 individuals (62 negative samples). The controls for these tests were positive and the sequenced PCR product confirmed the individual species. We estimated our assay to have a sensitivity of 1.3 ng for Mucoralean fungi species and 12 pg for *Aspergillus* species.

This study was undertaken to test the sensitivity and specificity of blood PCR test for diagnosis of mucormycosis and aspergillosis compared to pathology specimens. We were unable to detect any positive PCR samples in the patients. Previously, in 2008, Kasai et al. detected *Mucor* (39%) and *Cunninghamella* (58%) in plasma samples by PCR in a rabbit model of infection [20]. In a study by Badiee et al., 28 patients with suspected invasive fungal infection were studied [17]. Seven of these individuals had non-septate hyphae on direct smear of pathology specimen. The definitive diagnosis of mucormycosis was made in these patients (five had positive culture on the pathology specimen). None of the 28 blood cultures was positive for mucormycosis and none of the serum samples was positive by PCR. They recommend the direct smear as the gold standard for detecting mucormycosis [17]. In another study by Millon et al., serial blood samples were obtained from 10 patients with definitive mucormycosis. PCR was able to detect Mucorales DNA in the circulating blood of 9 of the 10 patients at 3-68 days prior to the date of definitive diagnosis [16].

In this study, we detected *Aspergillus* or *Mucor* by serum PCR in all patients. While it seems that PCR is useful for detecting these organisms in tissue samples where the organism burden is high, there are some inconsistencies in applying the same methods to serum samples. False negatives could be due to sensitivity of the assay or choosing incorrect primers, while contamination or non-specific product (not verified by sequencing) can account for false positives. Although our test did not have any false positives and the positive controls were verified by sequencing, our assay could suffer from inadequate sensitivity as it was less than what was reported by other authors [18, 19]. Another possibility is that the circulating levels of these organisms, if present, were below the level of detection. It seems that further studies are required to resolve these issues for testing the peripheral blood.

Therefore, serum PCR could not diagnose *Aspergillus* and Mucoralean fungi in patients with suspected fungal sinus infections. While this test may have utility in diagnosis directly from biopsy site, it appears unreliable for use as a noninvasive blood test.

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## Author's contribution

S. S designed and managed the study. AR. J performed PCR designing and molecular tests. J. M and MM. S analyzed the data and edited the final manuscript.

## Conflicts of interest

None declared.

## Financial disclosure

No financial interests related to the content of this manuscript are declared.

## References

- Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mold infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis*. 2002; 34(7):909-17.
- Sun QN, Fothergill AW, McCarthy DI, Rinaldi MG, Graybill JR. In vitro activities of posaconazole, itraconazole, voriconazole, amphotericin B, and fluconazole against 37 clinical isolates of zygomycetes. *Antimicrob Agents Chemother*. 2002; 46(5):1581-2.
- De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group [EORTC/MSG] Consensus Group. *Clin Infect Dis*. 2008; 46(12):1813-21.
- Park BJ, Pappas PG, Wannemuehler KA, Alexander BD, Anaissie EJ, Andes DR, et al. Invasive non-*Aspergillus* mold infections in transplant recipients, United States, 2001–2006. *Emerg Infect Dis*. 2011; 17(10):1855–64.
- Skiada A, Lanternier F, Groll AH, Pagano L, Zimmerli S, Herbrecht R, et al. Diagnosis and treatment of mucormycosis in patients with hematological malignancies: guidelines from the 3rd European Conference on Infections in Leukemia (ECIL 3). *Haematologica*. 2013; 98(4):492-504.
- Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B-based frontline therapy significantly increases mortality among patients with hematologic malignancy who have zygomycosis. *Clin Infect Dis*. 2008; 47(4):503-9.
- Badiee P, Alborzi A, Karimi M, Pourabbas B, Haddadi P, Mardaneh J, et al. Diagnostic potential of nested PCR, galactomannan EIA, and beta-D-glucan for invasive aspergillosis in pediatric patients. *J Infect Dev Ctries*. 2012; 6(4):352-7.
- Badiee P, Alborzi A. Detection of *Aspergillus* species in bone marrow transplant patients. *J Infect Dev Ctries*. 2010; 4(8):511-6.
- Badiee P, Alborzi A, Vojdani R, Shakiba E, Rasouli M, Ravanfar P, et al. Early diagnosis of systemic candidiasis in bone marrow transplant recipients. *Exp Clin Transplant*. 2010; 8(2):98-103.
- Chayakulkeeree M, Ghannoum MA, Perfect JR. Zygomycosis: the re-emerging fungal infection. *Eur J Clin Microbiol Infect Dis*. 2006; 25(4):215–29.
- Frater JL, Hall GS, Procop GW. Histologic features of zygomycosis: emphasis on perineural invasion and fungal morphology. *Arch Pathol Lab Med*. 2001; 125(3):375–8.
- Germer JJ, Lins MM, Jensen ME, Harmsen WS, Ilstrup DM, Mitchell PS, et al. Evaluation of the MagNA pure LC instrument for extraction of hepatitis C virus RNA for the COBAS AMPLICOR Hepatitis C Virus Test, version 2.0. *J Clin Microbiol*. 2003; 41(8):3503–8.
- Hata DJ, Buckwalter SP, Pritt BS, Roberts GD, Wengenack NL. Real-time PCR method for detection of zygomycetes. *J Clin Microbiol*. 2008; 46(7):2353–8.
- Hammond SP, Bialek R, Milner DA, Petschnigg EM, Baden LR, Marty FM. Molecular methods to improve diagnosis and identification of mucormycosis. *J Clin Microbiol*. 2011; 49(6):2151–3.
- Rickerts V, Just-Nübling G, Konrad F, Kern J, Lambrecht E, Böhme A, et al. Diagnosis of invasive aspergillosis and mucormycosis in immunocompromised patients by seminested PCR assay of tissue samples. *Eur J Clin Microbiol Infect Dis*. 2006; 25(1):8–13.
- Millon L, Larosa F, Lepiller Q, Legrand F, Rocchi S, Daguindau E, et al. Quantitative polymerase chain reaction detection of circulating DNA in serum for early diagnosis of mucormycosis in immunocompromised patients. *Clin Infect Dis*. 2013; 56(10):e95-101.
- Badiee P, Arastefar A, Jafarian H. Comparison of histopathological analysis, culture and polymerase chain reaction assays to detect mucormycosis in biopsy and blood specimens. *Iran J Microbiol*. 2013; 5(4):406-10.
- White PL, Mengoli C, Bretagne S, Cuenca-Estrella M, Finnstrom N, Klingspor L, et al. Evaluation of *Aspergillus* PCR protocols for testing serum specimens. *J Clin Microbiol*. 2011; 49(11):3842-8.
- Bialek R, Konrad F, Kern J, Aepinus C, Cecenas L, Gonzalez GM, et al. PCR based identification and discrimination of agents of mucormycosis and aspergillosis in paraffin wax embedded tissue. *J Clin Pathol*. 2005; 58(11):1180–4.
- Kasai M, Harrington SM, Francesconi A, Petraitis V, Petraitiene R, Beveridge MG, et al. Detection of a molecular biomarker for zygomycetes by quantitative PCR assays of plasma, bronchoalveolar lavage, and lung tissue in a rabbit model of experimental pulmonary zygomycosis. *J Clin Microbiol*. 2008; 46(11):3690-702.