Molecular confirmation of invasive infection caused by *Chaetomium globosum*

Identification of the causative organism in invasive fungal infections is difficult. Accurate and reliable diagnostic methods are required in light of the increasing incidence of emerging fungal infections.[1] We recently described a method for speciating fungi in formalin fixed, paraffin wax embedded tissue sections.[2] DNA is extracted using a modification of the protocol for the TaKaRa DEXPAT™ kit (TaKaRa Biomedicals), with additional steps using lyticase and ethanol precipitation.[2] Amplification of the DNA is performed using panfungal primers and a method based on that described by Einsele *et al*.[3] Identification to species level can be achieved by Southern hybridisation with a probe that binds *Aspergillus fumigatus, A flavus*, and *A versicolor*. Polymerase chain reaction products that do not hybridise with this probe are subsequently identified by sequence analysis.[2]

We have used this method to confirm the diagnosis in the case of a patient with acute myeloid leukaemia and pneumonia caused by *Chaetomium globosum*. We previously reported the details of this case and discussed the associated diagnostic difficulties.[4] A computed tomography chest scan revealed cavitating lesions characteristic of an invasive fungal infection in the right upper lobe and a right lobectomy was performed. Histology demonstrated branching hyphae invading blood vessels that were ''consistent with aspergillus''. However, many filamentous fungi resemble aspergillus species histologically, and identification relies on culturing the organism from the tissue. In this case, *C globosum* was cultured from the tissue specimen and identified six weeks later, by which time the patient had died. Moreover, such microbiological identification cannot differentiate between environmental contamination and causative organisms. Immunohistochemical staining of the tissue was subsequently carried out using a monoclonal antibody directed specifically against *A fumigatus*, *A flavus*, and *A niger*. Results were negative, confirming that the infection was not caused by these common aspergillus species. *Chaetomium globosum* is a rare pathogen but a common contaminant, so we were left with a presumptive diagnosis.

We have now achieved a definitive, molecular diagnosis of an invasive fungal infection caused by *C globosum* in this case using the method described above. Sections from the original paraffin wax embedded tissue were cut with a sterile microtome blade and two 10 μm thick sections were subjected to DNA extraction, after discarding the outer section. A positive result was obtained after polymerase chain reaction amplification with panfungal primers, but subsequent hybridisation with the aspergillus specific probe yielded a negative result. The DNA was purified using Wizard® PCR Preps DNA purification system (Promega, Madison, Wisconsin, USA), according to the manufacturer's instructions. The DNA was commercially sequenced (Cytomyx, Cambridge, UK), after which sequence analysis was performed with the EMBOSS software package (HGMP-RC; Medical Research Council, UK). The sequence was identified as *C globosum* using a BLAST search of Genbank and EMBL nucleotide sequence databases and subsequently submitted to the EMBL database (accession number AJ781794).

The *C globosum* isolate had been added to the UK National Collection of Pathogenic Fungi as NCPF 7115. We subcultured this isolate then extracted, purified, and sequenced the genomic DNA. The sequence was identical to that of the DNA extracted from the tissue sample, confirming the causative role of this organism in our case.

With the growing population of immunocompromised patients and the broadening spectrum of antifungal agents available it is imperative that we can accurately identify the organisms causing invasive fungal infections. This case illustrates the value of molecular tools to enhance the diagnostic process.

P J Paterson, S Seaton, T Yeghen, T D McHugh
Department of Microbiology, Royal Free Hospital, Pond Street, London NW3 2QG, UK

J McLaughlin
Department of Pathology, Royal Free Hospital

A V Hoffbrand
Department of Haematology, Royal Free Hospital

C C Kibbler
Department of Microbiology, Royal Free Hospital; kibbler@rfc.ucl.ac.uk

References

1 **Perfect JR**, Schell WA. The new fungal opportunists are coming. *Clin Infect Dis*. 1996;22: S112–18, .
2 **Paterson PJ**, Seaton S, McLaughlin J, *et al*. Development of molecular methods for the identification of aspergillus and emerging moulds in wax tissue sections. *Mol Pathol* 2003;**56**:368–70.
3 **Einsele H**, Hebart H, Roller G, *et al*. Detection and identification of fungal pathogens in blood by using molecular probes. *J Clin Microbiol* 1997;**35**:1353–60.
4 **Yeghen T**, Fenelon L, Campbell CK, *et al*. Chaetomium pneumonia in patient with acute myeloid leukaemia. *J Clin Pathol* 1996;**49**:184–6.

Authors Queries
Journal: **Journal of Clinical Pathology**
Paper: **cp21683**
Title: **Molecular confirmation of invasive infection caused by *Chaetomium globosum***

Dear Author
During the preparation of your manuscript for publication, the questions listed below have arisen. Please attend to these matters and return this form with your proof. Many thanks for your assistance

Query Reference	Query	Remarks
1	Please provide a location for TaKaRa Biomedicals and a town and county in the UK for the MRC.	