Official Symposium

PCR for early diagnosis of invasive fungal infections in immunocompromised patients: where are we?

L. Klingspor* (Stockholm, SE)

Invasive Fungal infections (IFI) are important causes of morbidity and mortality in immune-compromised patients. Diagnosis relies on culture-based methods, which lack sensitivity and delay diagnosis. PCR for the diagnosis of IFI offers an attractive method for early diagnosis . However, a lack of standardization of fungal PCR assays has limited its acceptance as a diagnostic tool. There is currently no consensus on the optimal blood fraction from which to isolate Candida DNA. A PCR detection limit of <10 CFU/ml in whole blood (WB)have been associated with improved test performance for Candida. A consensus concerning the type of specimen, volume, the extraction method, target, PCR format and platform has to be reached. In 2006, the European Aspergillus PCR Initiative (EAPCRI) was formed. The aim of the initiative was to provide optimal standardized protocols for the clinical evaluation of the Aspergillus PCR to determine its diagnostic role and allow inclusion in disease diagnosis criteria. A standardisation for Aspergillus PCR assay in (WB) and serum has been proposed by the EAPCRI. For the detection of Aspergillus in WB: the importance of the nucleic acid extraction protocol in achieving satisfactory analytical sensitivity was highlighted. For PCR testing of WB: blood volumes (>3 ml) should be be efficiently lysed before bead beating to disrupt the fungal cell and use of an internal control PCR to exclude false negativity. DNA should be eluted in volumes of <100 _l. For the dection of Aspergillus in serum: A positive association between sensitivity and the use of larger sample volumes, an internal control PCR and PCR targeting the ITS region was shown. Negative association between sensitivity and the use of larger elution volumes (> 100 ul) and PCR targeting the mitochondrial genes was demonstrated. A multi-centre clinical trial is required to determine the clinical validity and utility of both Candida and Aspergillus WB and serum PCR testing, and a multi-centre comparison of WB and serum PCR is required to determine the optimal specimen for PCR diagnosis.