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2-hour Oral Session

Invasive fungal infections: what is new?

Proximity ligation assay for the early detection of invasive aspergillosis

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Objective: The Proximity Ligation Assay (PLA®) combines the specificity of antibody-antigen recognition with the sensitivity of real-time PCR (qPCR) detection. Our aim was to use this technology with an *Aspergillus*-specific monoclonal antibody (MAb) to demonstrate sensitive and specific detection of *Aspergillus* mannoprotein in clinically relevant samples.

Methods: A single MAb, JF5[1], was used to generate two proximity probes (A and B) targeting *Aspergillus*-specific extracellular mannoprotein. A modified PLA® method was used, making use of Hydrolysis probe-based Protein Assay reagents (Thermo Fisher Scientific) to detect the binding of the antigen target by paired assay probes. Formation of the required proximity binding pair was achieved by utilising multiple repeat epitopes on the same antigen. Dilutions of purified mannoprotein antigen were used to establish assay sensitivity, while specificity was confirmed using culture filtrates from clinically relevant fungi. Assay performance was then evaluated by using serial dilutions of saline and serum spiked with *Aspergillus* culture filtrate and benchmarked against the Platelia™ galactomannan assay (GM) and *Aspergillus* lateral flow device (LFD)[1]. Finally, the PLA was used to test three broncho-alveolar lavage (BAL) fluid samples from patients with proven invasive pulmonary aspergillosis (IPA).

Results: PLA® detection of target antigen was demonstrated over five logs, with sensitivity 1000x greater than the LFD, which utilises the same MAb. The assay was highly specific, with no cross-reactivity demonstrated against soluble antigens from *Candida, Mucor, Fusarium, Rhizopus, Lichtheimia* or *Cryptococcus* species. PLA® detection of *Aspergillus* target protein was demonstrated in spiked serum and saline samples, with sensitivity 10x to 100x greater than the GM assay (when detecting their respective target antigens from culture filtrate). PLA® detection of *Aspergillus* target protein was demonstrated in three BAL fluids from patients with proven IPA and in 100% agreement with GM, LFD and in-house *Aspergillus* PCR tests.

Conclusion: This report is the first to describe the development of an *Aspergillus* PLA® that is significantly more sensitive than other antibody-based assays in current use. This has important implications for early diagnosis and targeted treatment of IA.

1. Thornton CR. Development of an immunochromatographic lateral-flow device for rapid serodiagnosis of invasive aspergillosis. Clinical and vaccine immunology : CVI 2008;15:1095-105.