P0004a

Poster Session I

Diagnosis of fungal infections

Combination biomarker detection in broncho-alveolar lavage fluid: a new gold standard for clinical diagnosis of invasive pulmonary aspergillosis

G. Johnson[1], S. Sarker[2], F. Nannini[1], A. Ferrini[1], C. Lass-Flörl[3], W. Mutschlechner[3], S.A. Bustin[4], S.G. Agrawal[1]

[1]*Blizard Institute of Cell and Molecular Science, Queen Mary University of London, London, United Kingdom*

[2]*Centre for Experimental Cancer Medicine Barts Cancer Institute, Queen Mary University of London, London, United Kingdom*

[3]*Division of Hygiene and Medical Microbiology, Innsbruck Medical University, Innsbruck, Austria*

[4]*Postgraduate Medical Institute Faculty of Health Social Care and Education, Anglia Ruskin University, Chelmsford, United Kingdom*

Objectives. The objective of this study was to evaluate a combination biomarker testing approach in broncho-alveolar lavage (BAL) fluid for clinical diagnosis of invasive pulmonary aspergillosis (IPA) in haemato-oncology patients.

Methods. This retrospective study comprised a Gold Standard cohort of 11 BAL fluids from 10 adults with proven IPA, a Negative Control cohort of 16 BAL fluids from 16 adults without risk factors for invasive fungal disease (IFD) and a Study cohort of 25 BAL fluids from 24 adults with haematological malignancy at high-risk of IFD. Samples were tested using the Platelia galactomannan assay (GM), a lateral flow device (LFD) [1] and an in-house *Aspergillus* PCR[2]. Sensitivity was determined in the Gold Standard proven IPA cohort and specificity in the Negative Control cohort. These results were used to assess a combination biomarker approach in the Study cohort. Pair wise agreement between the biomarker tests was calculated using Cohen's kappa. The EORTC/MSG criteria were evaluated in the Study cohort, using the combination biomarker approach as a new clinical gold standard.

Results. In the Gold Standard cohort, the sensitivity of the PCR and the LFD were both 100%, while GM was 81.8% (cut-off, GM > 0.8).

The specificity of all 3 tests was 93.75%, with 100% pair-wise agreement of the assays in the Negative Control cohort.

In the Study cohort, the highest level of agreement was between the PCR and LFD tests, with a Cohen's kappa of 0.88 (indicating almost perfect agreement). Using dual LFD+PCR positivity as a gold standard for IPA diagnosis, 5/25 Study samples were positive for IPA, 19 were dual negative and 1 sample was inconclusive (PCR-, LFD+ [GM-]). A substantial level of agreement was seen between GM and PCR (kappa 0.62), suggesting that agreement between all 3 tests could further increase confidence in the clinical interpretation. Culture and microscopy (including calcafluor staining) in all 25 BAL were negative for *Aspergillus*. The time delay between commencing antifungal treatment and obtaining BAL fluid was 6 days (range 4-8). Using the proposed new gold standard of LFD+PCR testing, the EORTC/MSG had a sensitivity of <50% in the Study cohort.

Conclusion. LFD + PCR combination testing can be used in BAL fluid to accurately diagnose or exclude IPA, with a sensitivity of 100% and specificity of 94%. Previously reported sensitivity values for the EORTC/MSG criteria in cases of autopsy-proven IPA (15 to 40%) are comparable to the results obtained here using our combination approach as a gold standard. A dual LFD + PCR approach in BAL fluid represents a new clinical gold standard for IPA diagnosis in haemato-oncology patients.

1. Thornton (2008). Clin. Vaccine Immunol. 15(7):1095-1105

2. Johnson *et al* (2012). PLoS ONE. 7(7): e40022