Diagnosis of invasive fungal infections through detection of a circulating pan fungal disaccharide by mass spectrometry.

A European multicentre study

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Introduction. We recently described a mass spectrometry (MS) method based on the detection and quantification of a serum disaccharide (MSDS) in sera from patients with invasive fungal infections (IFIs), candidiasis (IC), aspergillosis (IA) and mucormycosis (MM). In this investigation concerning patients from our tertiary hospital, MSDS seemed to provide interesting performance in terms of panfungal diagnosis. We aimed here to confirm these results through a blind study involving European expert centers in the clinical management of IC, IA and MM.

Methods. IFI patients were selected in each center according to availability of sera drawn around the time that clinical/mycological/imaging evidence of IFI was obtained. This study included 26 patients (57 sera) with IC, 19 (52 sera) patients with IA, 23 patients (72 sera) with MM patients. Hospitalized controls consisted in 20 neutropenic patients (40 sera) and 20 bacteraemic patients (20 sera). Detection of MSDS was performed blindly according to the procedure previously published. Clinical information and serological tests results were completed secondarily. Detection of glucans (Fungitell test) was used for the diagnosis of IC and IA in parallel with detection of mannan (Mnn) and Galactomannan (GM) respectively (Platelia Candida and Aspergillus Ag tests). Q-PCR was performed for MM.

Test	Cut-off	Sensitivity	Specificity	AUC
BDG	80	79/ 88.5	73.9/ 73.9	0.922
Mnn	62.5	47.3/ 56	100/ 100	0.889
MSDS	325	50.9/ 61.5	87/ 87	0.859

Fig. 1. ROC curves per patient for IC. Sensitivity/specificity values according to pre-established cut-offs of each biomarkers, analysis per serum and per patient (**bold**).

Test	Cut-off	Sensitivity	Specificity	AUC
BDG	80	50/ 57.9	100/ 100	0.921
GM	0.5	53.9/ 71.1	100/ 100	0.937
MS-DS	325	63.5/ 63.2	95/ 95	0.813
MS-DS*	325	63.5/ 63.2	100/ 100	0.862

Fig. 2. ROC curves per patient for IA. Sensitivity/specificity values according to pre-established cut-offs of each biomarkers, analysis per serum and per patient (**bold**).

Fig. 3. Repartition of MS-DS in relation to the day of mycological diagnosis. (Empty and full circles represent negative and positive Mucorales q-PCR results, respectively).

Results. All serological tests significantly discriminated IC patients from controls with bacteraemia ($p \leq 0.0001$). MS-DS Se/Sp per serum presented intermediate values in comparison with BDG and Mnn tests (Fig. 1). The analysis per patient (**in bold**) showed better sensitivity without altering the specificity. MS-DS complemented more positively high specificity of Mnn monitoring than BDG. For IA, all serological tests significantly discriminated IA patients from controls with neutropenia ($p \leq 0.0001$). MS-DS Se/Sp per serum were intermediate values in comparison with BDG and GM tests (Fig. 2). The analysis per patient showed higher sensitivity without altering specificity. Interestingly the lower specificity attributed to MS-DS was related to two neutropenic controls who developed IA few weeks later. For both IC and IA groups, follow up of the 3 glycan biomarkers evidenced complex profiles of release and degradation with independent or simultaneous circulation. For MM, 36% of sera showed concordant MSDS and q-PCR results. Among discordant results, 13 sera were positive with MSDS alone. Q-PCR and MSDS contributed similarly to the establishment of early MM diagnosis (Fig. 3). Remarkably, all the patients could be diagnosed by at least one test. For patients having long term monitoring, DNA circulation was shorter than that of MSDS.

Conclusion. This study confirms the contributions of MSDS to early diagnosis of IFI with sensitivities and specificities similar to those of tests recommended by EORTC and IDSA. Its panfungal nature and complementarity with other tests may justify its use in combination for a better management of IFI.