Poor performance of galactomannan and mannan sandwich enzyme-linked immunosorbent assays in the diagnosis of invasive fungal infection

The Platelia® *Aspergillus* and Platelia® *Candida* (BioRad Laboratories Ltd, Hertfordshire, UK) are commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits for the detection of galactomannan (GM) and mannan (M) respectively.

A number of prospective studies report excellent sensitivities (90–95%) and specificities using the Platelia® *Aspergillus* for the diagnosis of invasive aspergillosis (IA) (McLintock & Jones, 2004). The European Organisation for the Research and Treatment of Cancer and the Mycosis Study Group (EORTC/MSG) definitions of invasive fungal infection (IFI) include positive GM antigen testing as a microbiological criterion (Ascioglu *et al*, 2002). Other studies demonstrate poorer sensitivities (0–50%) (McLintock & Jones, 2004) and controversy exits over the most appropriate positive cut-off (Maertens *et al*, 2004).

There are no prospective studies which evaluate the Platelia® *Candida* and retrospective studies report modest sensitivities (30–69%) (McLintock & Jones, 2004). Positive M antigen testing is not included in EORTC/MSG definitions of IFI.

A prospective blinded study was performed to assess the value of twice-weekly screening of serum specimens, from haemato-oncology patients at high- and intermediate high-risk of IFI (Prentice *et al*, 2000), with Platelia® *Aspergillus* and Platelia® *Candida* ELISAs. Between December 2000 and December 2001, adult patients receiving allogeneic or autologous stem cell transplantation (SCT) or intensive chemotherapy were recruited. A treatment episode was defined as a single course of chemotherapy, an autologous SCT or an allogeneic SCT until day 100 following the transplant, or a course of corticosteroid therapy for graft-*versus*-host disease. An investigator blinded to the patient data performed the ELISAs according to the manufacturer's instructions. Each sample was run in duplicate. Samples were re-tested if the coefficient of variation between duplicates was more than 20%. IFI was classified as proven, probable or possible according to the EORTC/MSG definitions (Ascioglu *et al*, 2002). As the Platelia® *Aspergillus* ELISA was being evaluated it was excluded from the EORTC/MSG definitions.

The Platelia® *Candida* ELISA assay was discontinued after analysis of 82 episodes as 61% of the kits failed the manufacturer's validation criteria and results were therefore uninterpretable. Additionally, approximately 30% of the available ELISA results were non-reproducible.

A total of 1625 Platelia® *Aspergillus* ELISA results, from 125 treatment episodes, involving 78 patients, aged 16–76 years (mean 42, median 44·5 years), were analysed. IFI was documented in 19/125 (15·2%) treatment episodes: five proven (all candida); three probable (two *Candida*, one mixed infection with *Candida* and *Aspergillus*); and 11 possible (all *Aspergillus*). The Platelia® *Aspergillus* ELISA results were analysed at three different positive cut-offs: >1·5 (manufacturer's recommended positive cut-off); >1·0; and >0·5 (Table I).

Using a positive cut-off of >1·5, sequential positive (≥ 2 consecutive) results were recorded in only one of 125 treatment episodes, during which the patient had proven invasive candidiasis (IC) but no EORTC/MSG evidence of IA. In the 12 cases of EORTC/MSG-defined IA (one probable and

Table I. Sequential positive (Seq) (≥ 2 consecutive positive), non-sequential positive (Non-seq) (intermittent or single) and negative (Neg) GM ELISA results at different positive cut-offs (GMI) correlated with EORTC/MSG-defined invasive aspergillosis (IA).

IA/No IA (number of episodes)	GMI >1·5			GMI >1·0			GMI>0·5		
	Seq	Non-seq	Neg	Seq	Non-seq	Neg	Seq	Non-seq	Neg
EORTC/MSG IA (12)*	0	0	12	0	0	12	0	3†	9
No EORTC/MSG IA (113)	1‡	3	109	1‡	9§	103	11‡¶	26§**	76

*11 possible/1 probable.
†Two cases IA (possible) and one case IA/IC (possible).
‡Case 1, IC (proven).
§Case 2, IC (probable).
¶Case 3, IC (proven).
**Case 4, IC (proven).

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11 possible) no sequential, intermittent or single positive results were recorded.

Despite reducing the positive cut-off to >0·5, no sequential positive results were recorded in the 12 cases of EORTC/MSG-defined IA (one probable and 11 possible) and non-sequential results were recorded in only three cases of possible IA. In episodes with no EORTC/MSG evidence of IA (113), there were 11 sequential and 26 non-sequential positive results, including four cases of IC. As there was no evidence of EORTC/MSG IA by the time of neutrophil recovery, these positive ELISAs may represent genuine false positive results. False positive results have been associated with dietary GM, antibiotics and cross-reactivity with other fungi and bacteria (McLintock & Jones, 2004). However, it has been suggested that positive results that resolve with neutrophil recovery, or empirical antifungal therapy, may be the result of subclinical infection.

In summary, even with a reduction in the positive cut-off to >0·5, and the inclusion of single positive results, the sensitivity of the Platelia® *Aspergillus* ELISA was poor when used to detect EORTC/MSG-defined possible or probable IA. This lower positive cut-off resulted in increased numbers of false-positive results, including positive results in patients with IC.

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