

Prospective evaluation of mannan and anti-mannan antibodies for diagnosis of invasive *Candida* infections in patients with neutropenic fever

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The diagnostic performance and usefulness of the Platelia antigen and antibody test (Bio-Rad) was investigated in a prospective study of haematological patients at risk for invasive *Candida* infections. Among 100 patients, 86 were eligible, of whom invasive candidiasis (IC) occurred in 12 (14%), according to the criteria of the European Organization for Research and Treatment of Cancer/Mycoses Study Group. These included candidaemia due to *Candida albicans* (one patient) or *Candida tropicalis* (four patients), and hepatosplenic candidiasis (seven patients). The comparator group of 74 patients included 50 with febrile neutropenia alone and 24 with mould infections. A strategy was developed to determine diagnostic cut-offs from receiver operating characteristic curves with maximal sensitivity and, given this sensitivity, maximal specificity, both being greater than 0. In this patient population, these values were 0.25 ng ml⁻¹ for mannan (M) and 2.6 arbitrary units ml⁻¹ for anti-mannan (AM), which are lower than those recommended by the manufacturer. All patients developed at least one positive diagnostic M or AM result during the 10 days of persistent febrile neutropenia (PFN). The optimal overall performance was found when two consecutive positive tests for both M and AM were used [sensitivity, specificity, positive predictive value and negative predictive value (NPV) (95% confidence intervals) of 0.73 (0.39–0.94), 0.80 (0.69–0.89), 0.36 (0.17–0.59) and 0.95 (0.86–0.99), respectively]. There was a positive correlation of M with β -D-glucan ($r=0.28$, $P=0.01$). The first positive M test was found up to a mean \pm SD of 8.8 ± 8.5 (range 2–23) days prior to a clinical/mycological diagnosis of IC. Day-to-day variation in quantitative M levels was high. High-level AM responses were delayed until leucopenia resolved. The low specificities of the test performance may have been due to some of the comparator patients having subclinical *Candida* infections as evidenced by the high incidence of colonization among them (60% had a colonization index of ≥ 0.5). The high NPVs suggest that the tests may be particularly useful in excluding IC. It is feasible to explore the use of serial measurements of M and AM as part of a broader diagnostic strategy for selecting PFN patients to receive antifungal drug therapy.

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Abbreviations: AM, anti-mannan; AU, arbitrary units; BDG, β -D-glucan; CI, colonization index; CO_{AM}, cut-off value for anti-mannan; CO_M, cut-off value for mannan; CT, computed tomography; EORTC, European Organization for Research and Treatment of Cancer; IATCG, International Antimicrobial Therapy Cooperative Group; IC, invasive candidiasis; IM, invasive mycosis; M, mannan; MSG, Mycoses Study Group; NPV, negative predictive value; PFN, persistent febrile neutropenia; PPV, positive predictive value; ROC, receiver operating characteristic.

INTRODUCTION

Patients with haematological malignancy developing persistent febrile neutropenia (PFN) risk infection with an invasive mycosis (IM). Invasive candidiasis (IC) accounts for up to 50% of IMs, but the incidence varies with local epidemiology, antifungal prophylaxis and other factors (Chang *et al.*, 2008). Early identification of such patients is crucial for optimizing a successful outcome, when

managed according to international guidelines (Rex *et al.*, 2000; Jones & McIntock, 2003). A treatment delay of even 1 day significantly increases mortality, particularly in patients with candidaemia (Garey *et al.*, 2006). The current practice of administering broad-spectrum antifungal treatment to all patients with PFN exposes them to drug toxicity, contributes to the emergence of resistance and is expensive, as only 10–20% of unselected patients with PFN will develop IC (EORTC IATCG, 1989; Martino & Viscoli, 2006). Therefore, identifying PFN patients more likely to benefit from antifungal drug treatment using serological markers is crucial. Tools for early diagnosis include: blood culture, which has a low sensitivity (40%) and takes time; detection of β -D-glucan (BDG), which is highly sensitive and is specific for IM but is non-discriminatory for IC; and nucleic acid determination by PCR, which is highly specific but lacks creditable standardization and validation, and is expensive (Ellepola & Morrison, 2005; Kawazu *et al.*, 2004).

Detection of candidal mannan (M) and anti-mannan (AM) antibody by latex agglutination and ELISA has been available for many years (Ellepola & Morrison, 2005; Sendid *et al.*, 1999). The latex agglutination and enzyme immunoassay-based assays have been evaluated elsewhere, but have mainly been confined to non-neutropenic patients using one or two retrospective pooled samples per patient (Ellepola & Morrison, 2005; Sendid *et al.*, 2002, 2003; Mitsutake *et al.*, 1996). Little interest has been shown in exploring the use of these assays for samples from patients with haematological malignancy, due to poor results with the older latex test, concerns that profoundly immunocompromised patients may not produce antibodies, the wide range of serum M and AM concentrations used to define negative, intermediate and positive tests, and a perception that 'older' tests may not be reliable (Kahn & Jones, 1986; Ellepola & Morrison, 2005; Yeo & Wong, 2002; Phillips *et al.*, 1990; Reiss & Morrison, 1993). Currently, the Platelia *Candida* antigen and antibody tests (Bio-Rad) are the most commonly available *Candida*-specific serological tests for diagnosing IC (Ellepola & Morrison, 2005). Recent-generation enzyme immunoassay-based M detection assays are regarded as approximately ten times more sensitive for diagnosing IC (Sendid *et al.*, 2002). M constitutes around 7% of the dry weight of *Candida*. The α -mannopentaose expressed by mannoglycoconjugated M released from *Candida* cells is detected by peroxidase-linked rat mAb EBCA-1 and detected using a peroxidase-labelled goat antibody to human immunoglobulin (Sendid *et al.*, 2002).

Interest in this assay has waned following the introduction of more recent alternative serological tests. Furthermore, there has been scant evaluation of the Platelia assay prospectively or serially in uniquely haematological populations with accurately defined IM. This study was therefore undertaken to investigate the usefulness of serial analysis for M and AM in haematological patients at risk for IM.

METHODS

Patients. Patients with haematological malignancy received chemotherapy according to international guidelines as described previously (Ellis *et al.*, 2008). No patient received antifungal or antibiotic prophylaxis. Those who developed neutropenia ($<0.5 \times 10^9$ cells l^{-1}) and fever ($>38^\circ C$) with negative blood cultures for bacterial pathogens at the time of enrolment, and had received 3 days of broad-spectrum antibiotics (piperacillin–tazobactam) without fever resolution, and had no clinical source of infection or other demonstrable cause for fever (drug reaction, haematological disease, venous thrombosis) fulfilled the criterion for PFN and were eligible for inclusion in the analysis. All PFN patients were deemed to be at risk for IM and received empirical antifungal therapy with liposomal amphotericin B ($3 \text{ mg kg}^{-1} \text{ day}^{-1}$) according to published guidelines (Hughes *et al.*, 2002).

PFN patients were monitored for the development of IM by daily clinical examination, prolonged blood culture for fungal pathogens (Bactec 9240; Becton Dickinson), and by a high-resolution computed tomography (CT) scan of the chest and a CT scan of the liver, spleen and sinuses. Investigations were performed at baseline and weekly for as long as PFN persisted, or more frequently at the start of new symptoms and signs. The diagnosis of IM was initially made according to the previous criteria of the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) (Ascioglu *et al.*, 2002) and subsequently retrospectively clarified according to revised EORTC/MSG criteria (De Pauw *et al.*, 2008).

Ten millilitres of blood was collected daily by venepuncture for M and AM assays from the day of admission until discharge or death. Sera were separated and stored at $-40^\circ C$ until analysed. The primary interest was M and AM concentrations on the first day of PFN and daily thereafter until day 10.

Twice weekly, specimens from the oropharynx, stools and urine were cultured for yeast. The colonization index (CI) was calculated (number of sites positive for *Candida* spp./number sites tested) and the proportion of patients with a CI ≥ 0.5 was determined (Pittet *et al.*, 1994).

The study was approved by the Al-Ain Medical District Research Ethics Committee, Al-Ain, UAE. All subjects provided their informed written consent.

Detection of *Candida* M antigen and AM antibodies. Venous blood samples were collected from an antecubital vein and allowed to clot for 30 min at room temperature. Samples were then centrifuged for 10 min at 1200 g. Serum was collected and stored at $-40^\circ C$ until assayed. Detection of *Candida albicans* M and AM antibodies was carried out using the commercially available Platelia *Candida*-specific antigen and antibody ELISA kits (Bio-Rad), according to the manufacturer's instructions. A positive result as defined by the manufacturer was $>0.5 \text{ ng ml}^{-1}$ for the antigen and >10 arbitrary units (AU) ml^{-1} for the antibody. All samples were run in duplicate. Analysis of the results was carried out blind to the clinical data. BDG levels were assessed using the same samples have been reported previously (Ellis *et al.*, 2008).

Statistical analysis

Diagnostic tests. In order to reduce the number of missing values, we considered a value of M or AM as pertaining to day 1 of PFN if the corresponding blood sample was taken on day 1 or, if no blood was sampled on day 1, taken on day 2. We called these values M 1.2 and AM 1.2, respectively. Likewise, we defined M 3.4, AM 3.4 up to M 9.10 and AM 9.10.

Our aim was to determine the cut-off points for M and AM in order to develop diagnostic tests for IC and to determine the performance

(sensitivity and specificity) of these tests. A cut-off value for M (CO_M) and a cut-off value for AM (CO_{AM}) was determined by applying the following strategy. We plotted a receiver operating characteristic (ROC) curve for M 1.2 and took CO_M in such a way that the sensitivity was maximal and (given this sensitivity) the specificity was maximal (with both sensitivity and specificity being >0). The reason that we used M 1.2 was that there were (almost) no missing values in these data. This procedure was applied by taking all 74 patients in the comparator group. This group comprised all patients who did not develop IC according to EORTC/MSG criteria: those with febrile neutropenia alone and patients with other IMs (Fig. 1). We repeated this procedure for M 3.4 up to M 9.10 in order to see which cut-off points would result from these test variables.

We assessed the performance of the following tests using the cut-off point resulting from the procedure described above: (i) test 1 – positive if M 1.2 $\geq CO_M$, negative if M 1.2 $< CO_M$; likewise for days 3.4, 5.6, 7.8 and 9.10. (ii) Test 2 – positive if AM 1.2 $\geq CO_{AM}$, negative if AM 1.2 $< CO_{AM}$; likewise for days 3.4, 5.6, 7.8 and 9.10. (iii) Test 3 – positive if M 1.2 $\geq CO_M$ and AM 1.2 $\geq CO_{AM}$, negative if M 1.2 $< CO_M$ or AM 1.2 $< CO_{AM}$ (or both); likewise for days 3.4, 5.6, 7.8 and 9.10. (iv) Test 4 – positive if M 1.2 $\geq CO_M$ or AM 1.2 $\geq CO_{AM}$ (or both), negative if M 1.2 $< CO_M$ and AM 1.2 $< CO_{AM}$; likewise for days 3.4, 5.6, 7.8 and 9.10. (v) Test 5 – positive if two consecutive (days 1.2–9.10) values of M $\geq CO_M$, negative if this is not the case. (vi) Test 6 – positive if two consecutive (days 1.2–9.10) values of AM $\geq CO_{AM}$, negative if this is not the case. (vii) Test 7 – positive if patient is positive for both tests 5 and 6, negative if patient is negative for test 5 or 6 (or both). (viii) Test 8 – positive if patient is positive for test 5 or test 6 (or both), negative if patient is negative for both tests 5 and 6. The specificities of these tests were also assessed for the different comparator (subgroups): 24 with other IM, 39 with PFN only and 11 with febrile neutropenia <3 days.

Correlation between M and BDG. In order to reduce the influence of outliers, we log-transformed the values of M and BDG (previous values available on this group of patients with IC) by taking the natural logarithm of (values + 1) (because the logarithm of 0 is not defined). We computed both the partial Pearson and the Spearman correlation (adjusting for patient level).

Earlier diagnosis by positive M test. For those patients who had blood samples available prior to the first PFN day, we compared the

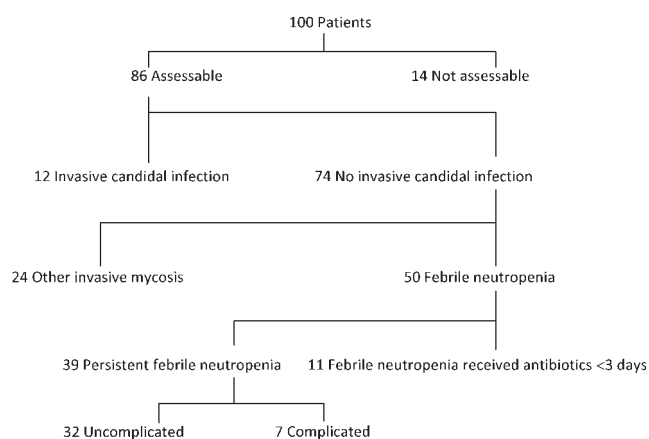


Fig. 1. Details of the patients recruited arranged according to the development of IC or no IC and subgroups thereof.

time to actual diagnosis with the time to diagnosis had this positive M test been used, by using Wilcoxon's signed rank method.

RESULTS

One hundred consecutive patients at risk for PFN were recruited over a 2 year period ending in January 2006. Fourteen patients could not be assessed due to missing blood samples. A total of 12 of the remaining 86 patients developed IC (prevalence 14 %) of which 5 had bloodstream infection alone (2 died) and 7 had hepatosplenic candidiasis. IC was diagnosed at a mean \pm SD after the start of PFN of 2.5 ± 4.8 days (median 1.0 days, range 10 to -7 days). These patient details are shown in Table 1. The characteristics were similar in each group, except that acute myeloid leukaemia was more frequent in the comparator group. A total of 74 patients without IC comprised the primary comparator group: 24 who developed IM other than IC (22 with invasive pulmonary aspergillosis and 2 with fungal sinusitis), 39 patients with PFN only without evidence of IM or other focal infection (32 were uncomplicated and 7 patients were complicated by severe abdominal pain associated with extensive mucositis and bacteraemia) and 11 patients who developed febrile neutropenia but received antibiotics for <3 days without focal infection (Fig. 1). A CI of ≥ 0.5 was found in 20/33 (61 %) of our comparator patients who had at least 2 surveillance samples taken and in 8/9 patients (89 %) with IC.

The number of blood samples available for analysis in the PFN phase was 60 from the 12 patients with IC. An additional 69 samples were available from the period immediately before PFN (pre-PFN) (Table 1) and 87 from day 10 of PFN onwards. There were 370 PFN-phase blood samples from the 74 patients without IC, who served as the comparator group.

The daily concentrations of M and AM for the PFN phase of patients with IC are shown in Table 2. Our strategy (Fig. 2) for determining cut-off values gave a cut-off value of 0.25 ng ml^{-1} for M and 2.60 AU ml^{-1} for AM. When the procedure was repeated using subgroups from the 74 comparator patient controls, the results were similar (data not shown).

During each of the sequential days of PFN, diagnostic levels of M were detected in 67–82 % of patients, AM in 73–100 %, either M or AM in 92–100 %, and both M and AM in 36–82 % (Table 2). At least 90 % of patients tested positive for either M or AM consistently from the first day of PFN.

The sensitivity and specificity for the M test over days 1–10 (for one positive result at or above the determined cut-off point) were 0.67–0.82 and 0.61–0.68, respectively (Table 3). For the AM test, the sensitivity and specificity were 0.73–1.00 and 0.53–0.63, respectively. The combination of one positive M and one positive AM result improved specificity to at least 0.80 on any day and improved sensitivity and specificity to 0.82 and 0.81, respectively, at day 9.10. Either a single positive M or a single positive AM gave the highest sensitivity but low specificity on any particular day. The

Table 1. Demographic data of patients with IC and the comparator group

| Parameter | IC | Comparator group |
|--|--------------------------------|-----------------------------|
| No. of patients | 12*† | 74 |
| Ratio male:female | 6:6 | 54:20 |
| Mean age \pm SD (years) [median, range] | 34.5 \pm 7.8 [35, 16–47] | 33.8 \pm 11.5 [31, 16–58] |
| Haematological disease (no. of patients) | | |
| AML | 3 | 42 |
| ALL | 8 | 23 |
| Others | 1 | 9 |
| Ratio AML:ALL + others | 0.3 | 1.3 |
| Duration of neutropenia (mean days \pm SD) [median, range] | 8 \pm 4.6 [9, 0–14] | 10.6 \pm 6.3 [10, 0–25] |
| Diagnosis of IC after PFN start (mean days \pm SD) [median, range] | 2.5 \pm 4.8 [1.0, 10 to –7]‡ | NA |

ALL, Acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; others, non-Hodgkin lymphoma, Burkitt's lymphoma or chronic myelocytic leukaemia.

*Four patients with *C. tropicalis* fungaemia, one patient with *C. albicans* fungaemia, six patients with probable hepatosplenic candidiasis (neutropenia plus abdominal CT scan findings of 'bull's-eye' lesions in liver/spleen plus positive BDG serology) and one patient with proven hepatosplenic candidiasis (all criteria for probable hepatosplenic candidiasis plus a positive blood culture).

†Two patients died of candidaemic shock.

‡One patient had classical lesions of hepatosplenic candidiasis that were missed on the original imaging report but were present on admission 7 days prior to neutropenic fever.

best test combination was two consecutive positive tests for both M and AM, which gave a sensitivity and specificity of 0.73 and 0.80, respectively, and a high negative predictive value (NPV) of 0.95 but low positive predictive value (PPV) of 0.36.

Different comparator subgroups gave the same sensitivities as the full comparator group of 74 patients but higher specificities. For example, in the 32 patients with uncomplicated PFN, sensitivity and specificity were 0.73 and 0.94, respectively, for two consecutive positive results,

Table 2. Individual serial M and AM concentrations for patients with IC

Results for the patients are shown as ng ml⁻¹ for M values and AU ml⁻¹ for AM values. Percentages were calculated as the number of patients with a positive result based on the derived cut-off values of 0.25 ng ml⁻¹ for M and 2.50 AU ml⁻¹ for AM/number of patients available for testing on each day.

| | Day 1.2 | | Day 3.4 | | Day 5.6 | | Day 7.8 | | Day 9.10 | |
|--------------------|---------|------|---------|------|---------|------|---------|------|----------|-------|
| | M | AM | M | AM | M | AM | M | AM | M | AM |
| Patient no. | | | | | | | | | | |
| 1 | 0.28 | 2.6 | 0.26 | 2.8 | 0.32 | 0 | 0 | 0 | 1.38 | 6.9 |
| 2 | 0.29 | 19 | 0.38 | 19.8 | 0.95 | 19 | 0.29 | 18.8 | 0.28 | 19.3 |
| 3 | 0.26 | 2.9 | 0.3 | 2.8 | 0.27 | 2.8 | 0.25 | 2.5 | 0.26 | 2.5 |
| 4 | 0.49 | 9.1 | 0.27 | 11.4 | 0.31 | 6.6 | 1.39 | 19.8 | 0.26 | 19.2 |
| 5 | 0 | 2.9 | 0.28 | 2.9 | 0 | 3.5 | 0.3 | 4.3 | 0.31 | 3.2 |
| 6 | 0.25 | 11.5 | 0.27 | 16 | 0.95 | | | | 2.31 | 5.4 |
| 7 | 0 | 26.5 | 0 | 24 | 0 | 23.2 | 0 | 20 | 0 | 22 |
| 8 | 0 | 16.6 | 0 | 4 | 0 | 5.9 | 0 | 5.2 | 0.32 | 4 |
| 9 | 0 | 0 | 0.56 | 0 | 0.96 | 0 | 0.36 | 33.8 | 0.28 | 38 |
| 10 | 0.86 | 41.5 | 0 | 8.1 | 0 | 4.3 | 0.27 | 6.5 | 0 | 5.7 |
| 11 | 0.4 | 0 | 0.52 | 0 | 7.1 | 0 | 7.4 | 0 | | |
| 12 | 0.32 | 0 | 0.38 | 0 | 0.26 | 14.5 | 0.26 | 30 | 0.49 | 35 |
| Test | | | | | | | | | | |
| M, AM | 67 % | 75 % | 75 % | 75 % | 67 % | 73 % | 73 % | 82 % | 82 % | 100 % |
| M or AM | 92 % | | 100 % | | 100 % | | 90 % | | 90 % | |
| M and AM | 50 % | | 50 % | | 36 % | | 64 % | | 82 % | |

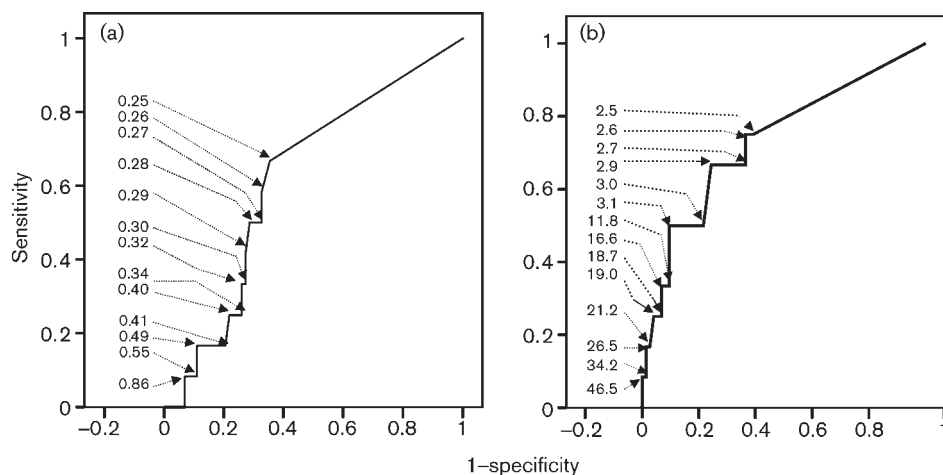


Fig. 2. ROC curves for M (a) and AM antibody (b) at day 1.2, with cut-off values (ng ml^{-1} for M and AU ml^{-1} for AM) shown by arrows. Diagonal segments were produced by ties.

compared with 0.73 and 0.80 when all 74 patients were used (other subgroup data not shown).

Time from the first positive PFN-phase M sample to diagnosis of IC (clinicoradiologically or date of positive

blood culture) was 1.8 ± 6.4 days (mean \pm SD) (median 0, range from 12 days before to 10 days after the first day of PFN). In six patients, blood samples were available for between 0 and 23 days prior to the first day of PNF. These patients had a positive M test mean value of 8.8 ± 8.5 days

Table 3. Sensitivity, specificity, PPV and NPV for M and AM tests alone or in combination with 95% confidence intervals

Results were calculated when all 74 patients without IC were used as the comparator in the analysis. Cut-off values were 0.25 ng ml^{-1} for M and 2.5 AU ml^{-1} for AM. Numbers in parentheses indicate two-sided (*, one-sided) 95% confidence intervals. Values in bold italic indicate the best test combination.

| Day of PFN | M | | | | AM | | | | M and AM | | | | M or AM | | | |
|---|---------------------|---------------------|---------------------|---------------------|----------------------|---------------------|---------------------|----------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------|---------------------|---------------------|----------------------|
| | Sensi- tivity | Speci- ficity | PPV | NPV | Sensi- tivity | Speci- ficity | PPV | NPV | Sensi- tivity | Speci- ficity | PPV | NPV | Sensi- tivity | Speci- ficity | PPV | NPV |
| One positive result | | | | | | | | | | | | | | | | |
| 1.2 | 0.67 (0.35–0.90) | 0.65 (0.53–0.76) | 0.24 (0.11–0.41) | 0.92 (0.81–0.98) | 0.75 (0.43–0.95) | 0.61 (0.49–0.72) | 0.24 (0.11–0.40) | 0.94 (0.83–0.99) | 0.50 (0.21–0.79) | 0.81 (0.70–0.89) | 0.30 (0.12–0.54) | 0.91 (0.81–0.97) | 0.92 (0.62–1.00) | 0.45 (0.33–0.57) | 0.21 (0.11–0.35) | 0.97 (0.85–1.00) |
| 3.4 | 0.75 (0.43–0.95) | 0.64 (0.52–0.74) | 0.25 (0.12–0.42) | 0.94 (0.83–0.99) | 0.75 (0.43–0.95) | 0.60 (0.48–0.72) | 0.24 (0.11–0.40) | 0.94 (0.82–0.99) | 0.50 (0.21–0.79) | 0.80 (0.69–0.88) | 0.29 (0.11–0.52) | 0.91 (0.81–0.97) | 1.00 (0.74–1.00)* | 0.44 (0.32–0.56) | 0.23 (0.12–0.36) | 1.00 (0.89–1.00)* |
| 5.6 | 0.67 (0.35–0.90) | 0.68 (0.56–0.79) | 0.26 (0.12–0.45) | 0.92 (0.82–0.98) | 0.73 (0.39–0.94) | 0.56 (0.44–0.68) | 0.20 (0.09–0.36) | 0.93 (0.81–0.99) | 0.36 (0.11–0.69) | 0.84 (0.73–0.91) | 0.25 (0.07–0.52) | 0.90 (0.80–0.96) | 1.00 (0.74–1.00)* | 0.40 (0.29–0.53) | 0.22 (0.12–0.35) | 1.00 (0.88–1.00)* |
| 7.8 | 0.73 (0.39–0.94) | 0.61 (0.48–0.73) | 0.24 (0.11–0.42) | 0.93 (0.81–0.99) | 0.82 (0.48–0.98) | 0.63 (0.50–0.75) | 0.28 (0.14–0.47) | 0.95 (0.84–0.99) | 0.64 (0.31–0.89) | 0.86 (0.75–0.93) | 0.44 (0.20–0.70) | 0.93 (0.84–0.98) | 0.91 (0.59–1.00) | 0.38 (0.26–0.51) | 0.20 (0.10–0.34) | 0.96 (0.80–1.00) |
| 9.10 | 0.82 (0.48–0.98) | 0.63 (0.51–0.75) | 0.26 (0.13–0.44) | 0.96 (0.85–0.99) | 1.00 (0.72–1.00)* | 0.53 (0.40–0.65) | 0.26 (0.14–0.41) | 1.00 (0.90–1.00)* | 0.82 (0.48–0.98) | 0.81 (0.70–0.89) | 0.41 (0.21–0.64) | 0.96 (0.88–1.00) | 1.00 (0.72–1.00)* | 0.35 (0.24–0.48) | 0.20 (0.10–0.33) | 1.00 (0.86–1.00)* |
| Two consecutive positive results | | | | | | | | | | | | | | | | |
| | 0.75 (0.43–0.95) | 0.65 (0.53–0.76) | 0.26 (0.12–0.43) | 0.94 (0.84–0.99) | 1.00 (0.72–1.00)* | 0.38 (0.25–0.53) | 0.26 (0.14–0.41) | 1.00 (0.83–1.00)* | 0.73 (0.39–0.94) | 0.80 (0.69–0.89) | 0.36 (0.17–0.59) | 0.95 (0.86–0.99) | 1.00 (0.74–1.00)* | 0.21 (0.12–0.34) | 0.21 (0.12–0.34) | 1.00 (0.74–1.00)* |

(median 4, range 0–23 days before) prior to IC. This earlier detection time was significant ($P=0.01$).

The time course of the M and AM results are shown in Fig. 3 for selected patients. The first positive M results were found at or before clinical diagnosis of IM in most patients. Lower-level (concentrations at or near our determined cut-off values) positive AM results were likewise observed while leucopenia persisted, but an avid AM response was delayed, coinciding with leucopenia resolution. There was considerable day-to-day variability in quantitative M levels.

BDG results were available from the same 12 patients with IC and have been presented previously (Ellis *et al.*, 2008). The Pearson partial correlation between BDG and M concentrations was significant ($r=0.28$, $P=0.01$), whilst the Spearman partial correlation was not ($r=0.11$, $P=0.33$) (Fig. 4). Although these correlations were rather weak, there was 100 % agreement between the BDG and M tests as to positivity/negativity.

DISCUSSION

This study was an evaluation of the performance of the Platelia *Candida* antigen and antibody immunoenzyme assays (Bio-Rad) in a group of patients with different characteristics from those studied previously: haematological malignancy at risk for IM in a prospective, clinical, real-time setting.

Our ROC analysis strategy generated cut-off points for M and AM that were lower than the positive value recommended by the manufacturer (0.25 vs 0.5 ng ml⁻¹ for M and 2.60 vs 10 AU ml⁻¹ for AM) for detecting IC in this particular patient population, thereby improving sensitivity without substantial loss of specificity. This finding underscores the importance of determining individual population-centred positive test criteria. A similar approach has been suggested by other investigators, for example in diagnosing aspergillosis with the galactomannan assay, where the original recommended cut-off value of 1.5 was lowered to 0.5 to capture more cases (Marr *et al.*, 2004; Pfeiffer *et al.*, 2006). Therefore, in order to achieve a reasonable PPV in our population with a low prevalence of IC, the sensitivity should be maximized by lowering the cut-off value.

During PFN, diagnostic concentrations of M or AM were seen cumulatively in all patients. The considerable day-to-day variability in the number of patients with positive M tests may reflect the transient nature of mannanaemia (Ellepola & Morrison, 2005). AM was detected less frequently, or was present at low concentrations, during leucopenia. These characteristics reduced overall sensitivities if a single test result was relied on, but the test could be improved by multiple serial testing. We found the sensitivity and specificity of the test to be optimal when a combination of one positive M and one positive AM test was used (82 and 84 %, respectively) on day 9.10 of PFN;

this complementarity of M and AM results has been observed before (Sendid *et al.*, 1999, 2002). Additionally, when two consecutive positive M and AM results were used over the PFN period, these values were 73 and 80 %, respectively. Such findings of consecutiveness are similar to those reported by Sendid *et al.* (2002) who found a sensitivity of over 80 % for all *Candida* spp. during candidaemia. Our high sensitivities (100 %) for diagnosing IC by using two consecutive positive M or AM results exceed those of 41 and 47 %, respectively, reported recently by Alam *et al.* (2007). However, their study differed from ours in that the patient population was non-neutropenic, assays were performed on a single specimen/patient (details not stated), pooled retrospective samples were evaluated and higher cut-offs were used as recommended by the manufacturer. The lower sensitivity of 25 % reported by Mitsutake *et al.* (1996) is in part explained by their use of a latex agglutination-based assay. High sensitivities (90 %) for diagnosis of IC can also be achieved if α -M is assayed at the same time as β -M (Sendid *et al.*, 2004). A recent retrospective analysis of haematological patients who had developed IC, based on one sample per patient per week, gave similar sensitivities (89 %) and NPVs (88 %), but higher specificities (86 %) and PPVs (86 %) (Prella *et al.*, 2005). However, a positive result was defined when any single positive M and AM result was found over a different and longer period of sampling (median of 51 days) compared with our study. This reflects the difficulties in comparing findings from studies that use different diagnostic criteria.

The PPV of the Platelia assay was low (36 % for two consecutive tests) in our study. This probably reflects the low prevalence of IC (14 %) in our population group, a well-established complication in such studies (Pfeiffer *et al.*, 2006; Upton *et al.*, 2006). The consistently high NPV (≥ 90 %) is similarly related to the low prevalence in the population studied. Therefore, a strong diagnostic benefit of the Platelia tests lies in excluding IC.

The population of the comparator group of 74 patients was homogeneous and similar to the patient group in terms of haematological profile, administered chemotherapy, neutropenia duration and hence risk for IM, and thus validates the use of this comparator group in the analysis. The comparator group, however, was heterogeneous with respect to subsequent disease outcomes from PFN, i.e. PFN alone, IM other than IC or low-level candidaemia secondary to chemotherapy-induced enterocyte damage (Ellis, 2004). It is possible that some of these comparator patients may have had candidal infections undetected by standard diagnostic criteria, as all received empiric antifungal drugs as part of the ward protocol. This may have resulted in a reduction in specificity of the M/AM assay. This concern is supported by the observation that specificities were somewhat higher when the subgroup of PFN alone was analysed. However, our results reflect the clinical reality of using the assay in an unselected patient group. The lower specificities generally seen with AM

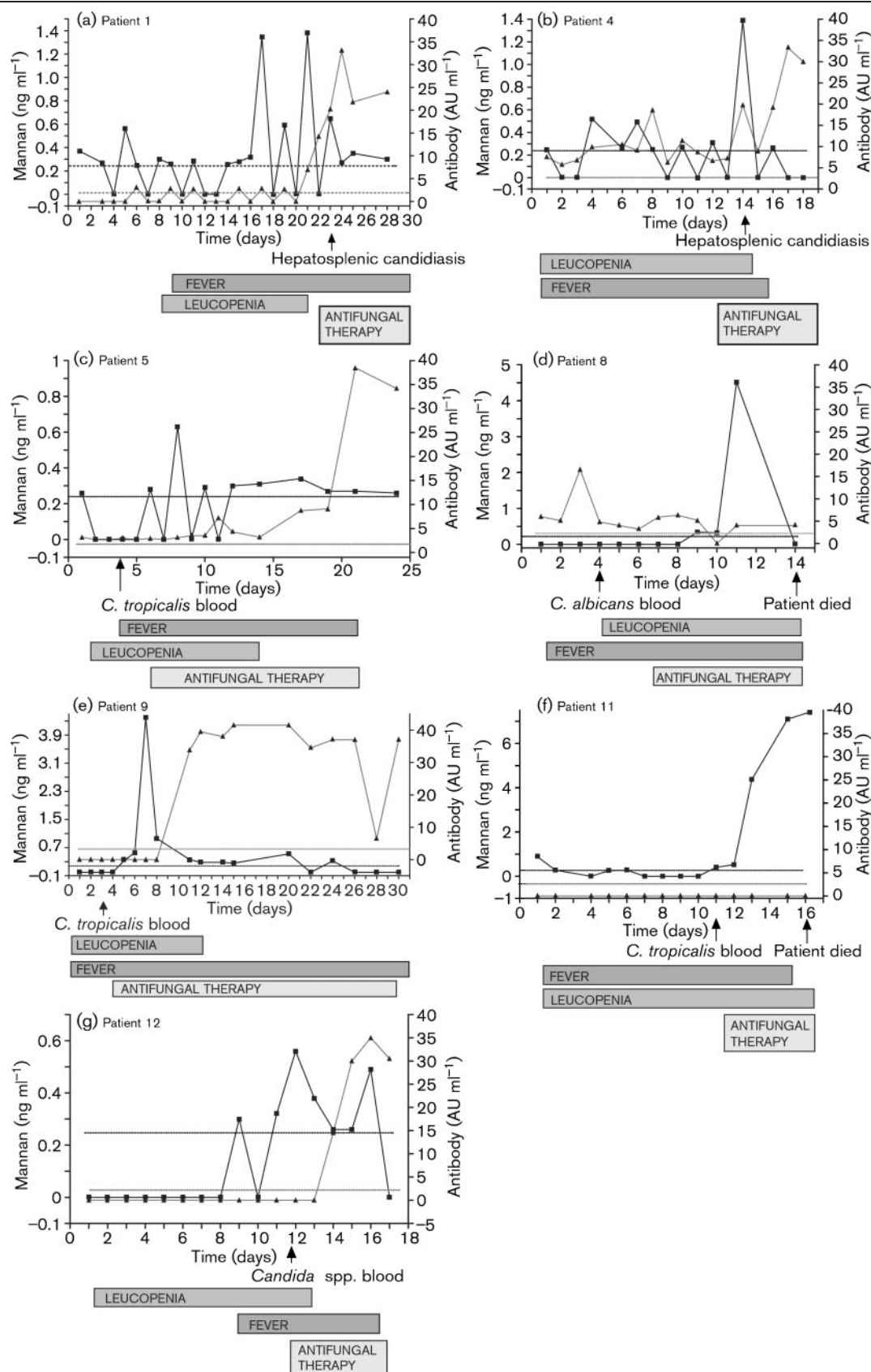


Fig. 3. Kinetics of M (left y-axis) and the AM antibody (right y-axis) in relation to fever and leucopenia for selected patients (1, 4, 5, 8, 9, 11 and 12). The test cut-off values are indicated by horizontal dashed lines for M (black) and AM (grey). The durations of fever, leucopenia and antifungal therapy are indicated by boxes. ■, M; ▲, AM antibody.

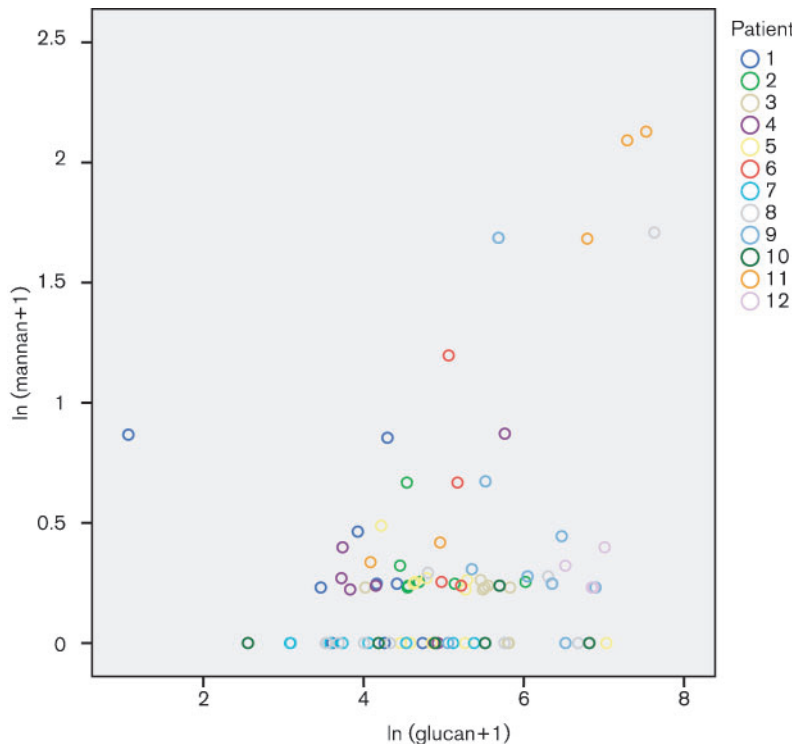


Fig. 4. Log plot of BDG and M concentrations in patients with IC. Each patient is identified by a specific colour as indicated.

compared with M results may also reflect an influence of candidal colonization (CI ≥ 0.5 in 60 % of the patients) among the comparator group, a phenomenon known to produce detectable AM in approximately one-third of uninfected, colonized patients (Sendid *et al.*, 1999).

In most patients with IC, the AM response to IC was very close to the cut-off point while leucopenia persisted (Fig. 3). Presumably, the absence of circulating B lymphocytes due to chemotherapy was responsible for this phenomenon. Once leucopenia resolved, the AM response became avid. An increase in AM levels post-aplasia has also been recognized in a small cohort of patients with candidaemia due to *Candida tropicalis* (Sendid *et al.*, 2003). Thus, production of antibodies occurs even in this immuno-compromised patient group. This is most likely to be due to the persistence of long-lived plasma cells in the bone marrow, which are known to be relatively more resistant to chemoradiotherapy compared with circulating B lymphocytes (Tarlinton *et al.*, 2008). In contrast, M levels were high during leucopenia and displayed an inverse relationship to AM levels (Fig. 3), a phenomenon noted before (Sendid *et al.*, 2003). The late antibody response in the course of IC limits its early diagnostic usefulness. However, by adopting a CO_{AM} of 2.5 AU ml⁻¹, which is lower than that recommended by the manufacturer (10 AU ml⁻¹), low-level antibody activity earlier in the PFN phase was captured, which is useful diagnostically (Tables 2 and 3, Fig. 3).

M and AM detection performed equally well in patients with candidaemia resulting from *C. albicans*, *C. tropicalis* or non-specified *Candida* spp. and also in patients with

hepatosplenic candidiasis without positive blood cultures. This is consistent with the fact that the mAb EBCA-1 used in the assay detects β 1–5 oligosaccharides present in several non-*albicans* *Candida* spp. (Jacquinot *et al.*, 1998). However, IC due to *Candida parapsilosis*, *Candida kefyr* or *Candida krusei* may remain difficult to detect (Sendid *et al.*, 2002).

Although it might seem logical to initiate sampling at the start of PFN, the first positive M test during the PFN phase was found to occur at or close to the diagnosis of IC. It is therefore more appropriate to begin sampling earlier, for example at neutropenia commencement. Such an approach would extend the interval between any first positive M test and the diagnosis of IC by a median of 4 days and hence identify more patients at an earlier stage of IC, given the day-to-day variability in positive results, and would hence improve survival as earlier treatment could be instigated (Garey *et al.*, 2006).

The first positive M or AM result occurred at a mean of 2–9 days prior to the clinical diagnosis of IC. In patients with hepatosplenic candidiasis, M was often detected many days prior to radiological diagnosis, suggesting a particular usefulness of this test in an otherwise difficult diagnostic IM category. However, the exact timing of the first positive test was highly variable; for example, patient 1 with hepatosplenic candidiasis had a positive M test 23 days prior to clinical diagnosis whilst patient 8 was positive 5 days after candidaemia was confirmed (Fig. 3). As a positive M and AM result does not always occur at an identical time, analysis for both M and AM over several days optimizes the diagnostic rate (Fig. 3).

The absence of either M or AM in the first 4 days of PFN appeared to confidently exclude IC (NPV $\geq 96\%$; Table 3). Therefore, the feasibility of withholding anti-candidal treatment in such patients should be explored.

The reason for the absence of M in patient 7 and its delayed appearance in patient 8 is unclear (Table 2). In both patients, peak AM levels were very high (26.5 and 16.6 AU ml⁻¹, respectively), which may have resulted in efficient formation of antigen-antibody complexes and their rapid clearance.

It is interesting that the two patients dying from candidaemia (8 and 11) had very low (mean 2.9 AU ml⁻¹, range 0–4 AU ml⁻¹) terminal AM levels compared with survivors (mean 6.0 AU ml⁻¹, range 2.5–38 AU ml⁻¹). Corresponding M levels were conversely higher in those who died (mean 6.0 ng ml⁻¹, range 4.5–7.4 ng ml⁻¹) compared with survivors (mean 0.77, range 0–2.31) (Table 2, Fig. 3). Persistently high M levels might therefore indicate poor prognosis, particularly when associated with low or absent AM responses, and such data could be utilized in modifying antifungal treatment.

Although the correlation between the M and BDG concentrations was weak, and dependent on the statistical method used, concordance between these two serological tests was nevertheless absolute. However, BDG cannot differentiate IC from other IMs. One approach to a patient with PFN might therefore be to test serially for BDG from the onset of neutropenia. Patients with a positive BDG result could then be tested for the presence of M and AM. If negative, treatment with an antifungal drug effective against invasive mould infections, such as liposomal amphotericin B, would seem appropriate. If positive, then antifungal therapy with drugs directed towards *Candida* spp., such as high-dose fluconazole, would be more appropriate and would potentially offer a less-costly treatment option (Fig. 5). An alternative strategy might be to commence empirical antifungal treatment at the start of PFN in all patients, even those with negative M and AM

results, and then modify or stop it if serial serology remains negative. This might reduce the consequences inherent in missing some cases of IC due to the modest PPV.

Our results suggest that serial assays for M and AM in patients at risk for IM may usefully contribute to the management of such patients. It appears feasible to explore further the utility of these assays in managing PFN patients within a comprehensive diagnostic approach, for example by using BDG serological testing. However, this single centre's findings with a small number of proven IC cases should be confirmed in a larger multicentre setting that also explores the health economics to define the most efficient approach towards sampling frequency given the laboratory costs and labour-intensity of testing.

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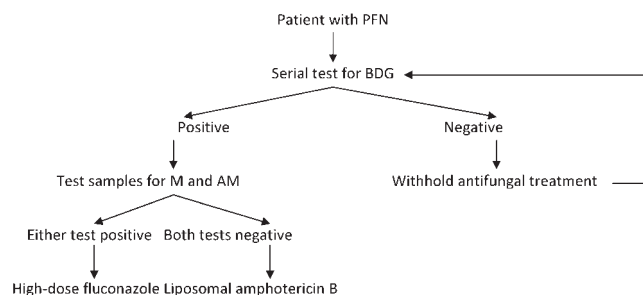


Fig. 5. Suggested algorithm for selecting antifungal therapy. Note that diagnosis of an IM also incorporates data from clinical, radiological and microbiological sources, in addition to serological tests. Fluconazole is only indicated when the *Candida* spp. are known or likely to be susceptible. Voriconazole is an alternative therapy for invasive aspergillosis.

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