Clinical impact of enhanced diagnosis of invasive fungal disease in high-risk haematology and stem cell transplant patients

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ABSTRACT

Aims: To investigate the impact of routine use of biomarkers for diagnosing fungal infection within a care pathway on antifungal usage and clinical outcomes.

Methods: A cohort of high-risk haematology and stem cell transplant patients was entered into a neutropenic care pathway in which targeted diagnostic testing replaced empiric antifungal treatment. Patients were screened twice a week by PCR and antigen testing during fever or when chronic graft versus host disease was present and were followed-up for a minimum of 1 year.

Results: No excess morbidity or mortality was seen in patients in whom empiric antifungal treatment was withheld, and there were substantial savings in antifungal drug expenditure.

Conclusions: The introduction of a comprehensive diagnostic surveillance strategy to exclude invasive fungal infection in high-risk patients with haematological malignancy and those undergoing transplantation can result in improvements in clinical management. There are also potential additional benefits of improved patient survival, decreased morbidity and decreased hospital stay.

The burden of invasive fungal disease (IFD) continues to increase as a result of improved medical intervention and supportive care.[1–4] Diagnosis is suboptimal and problematic in patients with haematological malignancy and those undergoing stem cell transplantation (SCT). Mortality is high if diagnosis is delayed,[5] and IFD may delay further chemotherapy leading to higher relapse rates.[6] This has led to the practice of empiric antifungal treatment in patients with refractory fever, although there is no evidence that this reduces invasive fungal infection in patients or confers a survival benefit.[7–9] Costs associated with this practice are considerable, and drug toxicities increase morbidity and mortality further.[10] Even lipid amphotericin B preparations are associated with nephrotoxicity,[11] which, if severe, prolongs hospital stay and increases risk of death.

Newer drugs, notably voriconazole and caspofungin, are used in empiric and definitive treatment of invasive IFD.[12–15] Acquisition costs are high and cost–effectiveness data are limited.

Inpatient antifungal drug expenditure exceeds most other categories. The major cost is for empiric therapy. Rational prescribing requires risk stratification[16] based on clinical parameters including underlying disease, duration of neutropenia, graft versus host disease and steroid use.[17]

Advances in diagnosis, including antigen and nucleic acid detection, have not impacted on empiric strategies. Antigen testing is included in the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) consensus definitions for diagnosing fungal infection[18] and should be considered part of the expected standard of care for patients at risk of IFD.[19] Commercially available enzyme immunoassays (EIAs) for the detection of mannan (M-EIA) and galactomannan (GM-EIA) are available and show good specificity but variable sensitivity.[20] Fewer data are available for the utility of β-D-glucan antigen testing, but this too is included in the modification for the EORTC/MSG consensus diagnostic criteria.[21]

DNA-based tests for the diagnosis of IFD[22] have been hampered by lack of standardisation. Semi-automated, standardised real-time *Aspergillus* and *Candida* PCR methodology has been developed,[23] and preliminary evaluation has confirmed benefit both clinically[24] and in comparison with antigen detection. Sensitivity is 95% and 92.3% for candidosis[25] and aspergillosis,[26] respectively, with specificity values (positive predictive values) of 97% and 94.6% and negative predictive values of 98.5% and 99.3%, respectively. These high negative predictive values allow IFD to be excluded and render empiric antifungal treatment unnecessary. Serial testing is necessary, and the combination of antigen and PCR testing is recommended.[27]

METHODS

Patients

Between 1 October 2005 and 31 March 2006 (test period), haematology patients admitted to University Hospital of Wales who were at high risk of IFD were entered into a care pathway for neutropenic fever management (fig 1). Patients considered high risk included those undergoing SCT, those with acute leukaemia, and those with refractory disease receiving aggressive chemotherapy. Patients undergoing SCT and patients with acute myeloid leukaemia (AML) received itraconazole prophylaxis; itraconazole concentrations were monitored weekly. Patients with acute lymphocytic leukaemia or lymphoma received fluconazole prophylaxis. Those with refractory disease undergoing aggressive chemotherapeutic regimens received itraconazole oral solution (5 mg/kg/day). Patients intolerant of itraconazole received 7 mg/kg AmBisome once a week.

Empiric antifungal agents were not added for patients receiving effective prophylaxis unless there was clinical, microbiological or radiological evidence of IFD. Clinical evidence of infection included new cough with pleuritic chest pain, haemoptysis, nodular skin rash or radiological evidence of disease.

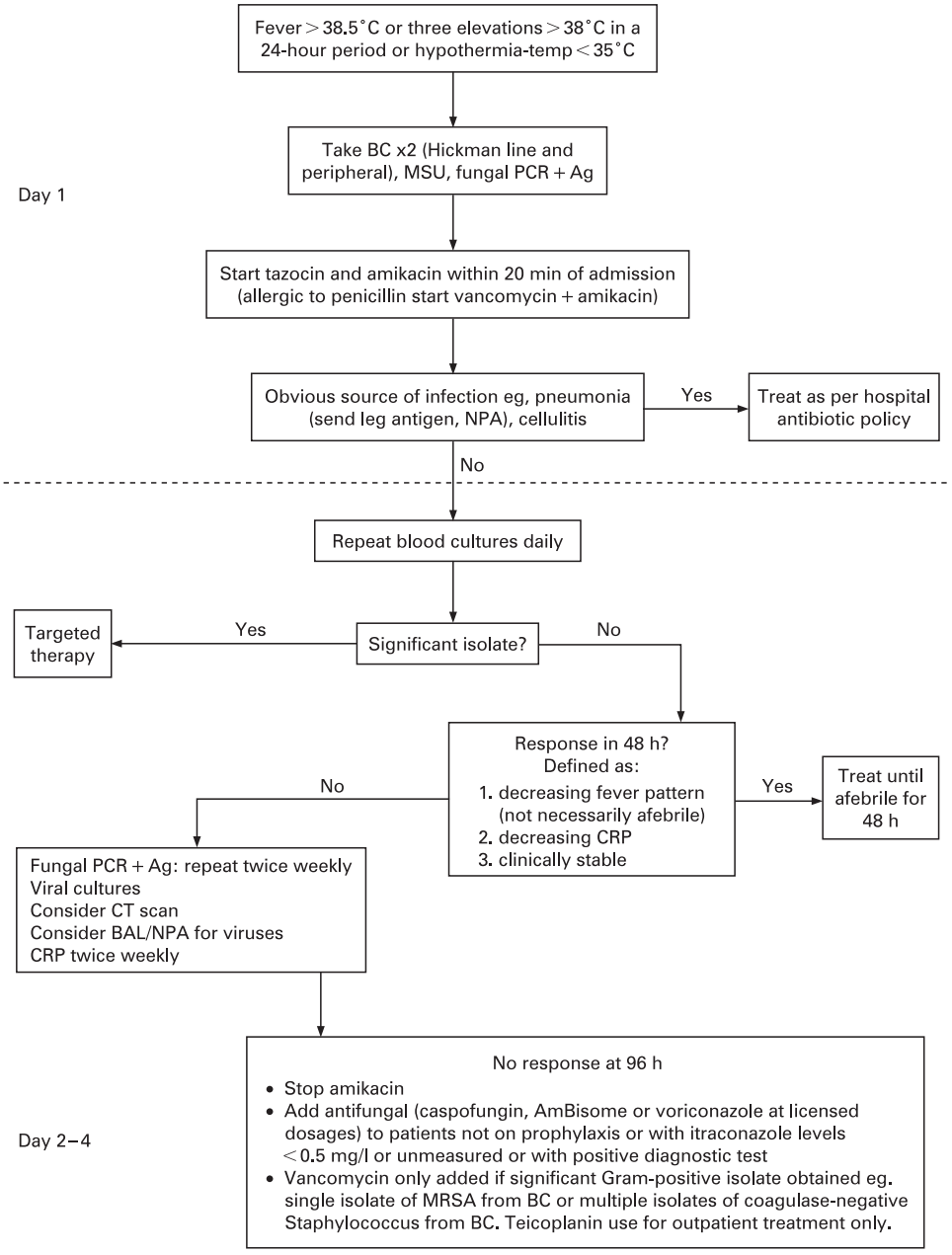

Figure 1 Diagnostic and therapeutic strategy delivered within neutropenic fever care pathway. Ag, antigen; BAL, bronchoalveolar lavage; BC, blood cultures; CRP, C-reactive protein; MRSA, meticillin-resistant *Staphylococcus aureus*; MSU, midstream urine; NPA, nasopharyngeal aspirate.

Fungal diagnostic tests (antigen and PCR) were performed twice a week on febrile patients and SCT patients with graft versus host disease. All assays are run on-site twice a week, with same-day reporting for antigen tests and next-day reporting for PCR tests.

Outcomes

Data on bed occupancy, length of stay and finished consultant episodes were obtained from the hospital IT department. Case notes were audited for compliance with the pathway and administration of antifungal drugs. Patients were followed for a minimum of 1 year for evidence of late fungal infection or adverse clinical outcome.

Antigen testing

Platelia kits (Bio-Rad, Hemel Hempstead, UK) were used for the detection of galactomannan and mannan. Galactomannan results were determined using a threshold index ($A_{450/620}$ sample/$A_{450/620}$ threshold serum). Any value above 0.5 was considered significant, although 0.5–0.7 was considered indeterminate and a repeat was requested.

PCR

DNA extraction

Molecular testing was performed as described previously.[25 26] All positive specimens were repeated. Sensitivity, specificity and diagnostic odds ratios were calculated according to EORTC/MSG criteria.

RESULTS

Over the 6- month testing period, 130 patients were screened. Five patients were excluded because they did not fulfil risk stratification criteria. Table 1 shows basic and clinical characteristics of the 125 evaluable patients. Antigen and PCR

Table 1 Patient characteristics

Characteristic	Value
Age (years)	
Range	16–83
Mean	56.2
Male/female ratio	1.4 : 1 (73:52)
Underlying disease/treatment modality	
HSCT	55
Allograft	23
Autograft	23
Syngeneic	1
Matched unrelated	8
AML	39
ALL	1
CLL	4
CML	1
NHL	22
HD	1
Aplastic anaemia	2
Total	125

ALL, acute lymphocytic leukaemia; AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; HCST, haematology stem cell transplant; HD, Hodgkin disease; NHL, non-Hodgkin lymphoma.

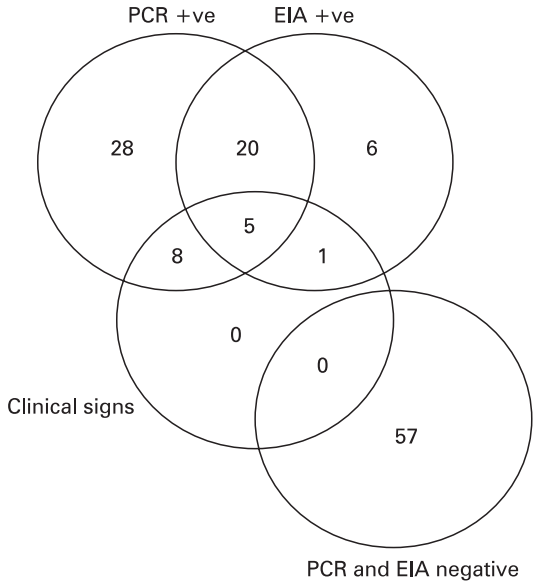

Figure 2 Number of patients positive for *Aspergillus* PCR or antigen and correlation with clinical signs of invasive aspergillosis (n = 125). EIA, enzyme immunoassay.

Table 2 Clinical markers of IFD during test period

	Aspergillus		*Candida*			
Feature	**EIA**	**PCR**	**EIA**	**PCR**	**No of cases**	**EORTC/MSG**
C tropicalis fungaemia, major nodularity on HRCT	Positive	–	Positive	Positive	1	Proven candidosis Possible aspergillosis
Histological evidence of invasive mould infection	Positive	Positive	–	–	1	Proven mould infection (probable aspergillosis)
Cavitating lesion on chest radiograph. *A fumigatus* from sputum	Positive	Positive	–	–	1	Probable aspergillosis
Halos on HRCT of chest	Positive	Positive	–	Positive	1	Probable aspergillosis
Halos on HRCT of chest	–	Positive	–	–	1	Possible aspergillosis
Nodularity on HRCT of chest	Positive	Positive	–	Positive	1	Probable aspergillosis
Major nodularity on HRCT of chest	Positive	Positive	–	–	1	Probable aspergillosis
Major nodularity on HRCT of chest	Positive	Positive	Positive	Positive	1	Probable aspergillosis Possible candidosis
Major nodularity on HRCT of chest	–	Positive	–	–	3	Possible aspergillosis
Chest consolidation and haemoptysis	Positive	–	–	–	1	Possible aspergillosis
Chest consolidation and pleural rub	Positive	Positive	–	–	1	Probable aspergillosis
Chest consolidation and pleural rub	–	Positive	–	–	1	Possible aspergillosis
Paronychia	–	Positive	–	–	1	Possible aspergillosis
None	–	Positive	–	–	24	Unclassified
None	Positive	Positive	–	–	16	Possible aspergillosis
None	Positive	–	–	–	6	Possible aspergillosis
None	Positive	Positive	Positive	Positive	2	Possible aspergillosis Possible candidosis
None	Positive	Positive	–	Positive	1	Possible aspergillosis
None	Positive	Positive	Positive	–	1	Possible aspergillosis Possible candidosis
None	–	Positive	–	Positive	3	Unclassified
None	–	–	Positive	–	1	Possible candidosis
None	–	–	–	Positive	1	Unclassified
None	–	–	–	–	55	No evidence of IFD

EIA, enzyme immunoassay; EORTC/MSG, European Organization for Research and Treatment of Cancer/Mycoses Study Group; HRCT, high-resolution CT; IFD, invasive fungal disease.

Table 3 Performance of the *Aspergillus* PCR

	Sensitivity (%)	Specificity (%)	Positive likelihood ratio*	Negative likelihood ratio	Diagnostic odds ratio†
Single non-reproducible positive PCR result	87.5	98	2.7	0.18	15
Single reproducible positive PCR result			3.8	0.18	21.1
Multiple positive PCR results	75	99	8.3	0.27	30.7

*Likelihood of a positive result in a patient with proven/probable disease versus positive result in a patient without evidence of disease.
†Positive likelihood ratio /negative likelihood ratio.

testing were performed on 1028 specimens. By EORTC/MSG criteria, there were two definite fungal infections (one candidaemia and one invasive mould infection (probably aspergillosis)), six probable IFD (clinical features and EIA-positive) and 34 possible IFD (EIA-positive or clinical features) (table 2).

Figure 2 shows patients positive for *Aspergillus* PCR and antigen and concordance with significant clinical signs of IFD. Sixty-one patients were positive by PCR, 27 (44%) had multiple PCR-positive results, and 25 (41%) were also positive by GM-EIA for *Aspergillus* antigen.

Of the 25 patients positive by both *Aspergillus* PCR and GM-EIA, five had clinical signs consistent with probable IFD and all were treated with additional antifungal drugs. Of the 20 possible cases lacking clinical signs, 10 patients received systemic antifungal agents—one, a patient with chronic graft versus host disease and cytomegalovirus infection, subsequently had invasive mould infection (probable aspergillosis) confirmed at autopsy. Eight patients treated with antifungal agents died, including the proven case and all five with clinical signs.

Ten patients positive by both tests were not treated with antifungal agents. One of these, a patient with AML, was maintained on itraconazole prophylaxis until he went into remission and further specimens were negative. However, when he relapsed 6 months later, he was again positive for *Aspergillus* by PCR and EIA. High-resolution CT of the chest revealed nodular lesions, which progressed to cavitation. He underwent a left upper lobectomy before receiving an allogeneic SCT. Histological examination confirmed invasive mould infection. Of the remaining untreated patients, an elderly patient with refractory AML was treated palliatively. The remainder were maintained on itraconazole prophylaxis, and fevers settled with engraftment. One patient died from relapsed disease, but the remaining patients were alive at 1 year.

Thirty-six patients were positive by *Aspergillus* PCR alone. Eleven patients had multiple positive results (range 2–9), and eight had clinical signs. All patients with clinical signs and 10 patients without clinical signs received additional antifungal treatment. Ten patients who received treatment subsequently died, and IFD was felt to be a contributory factor in four. Sixteen untreated patients had only a single positive PCR specimen. The performance of the assays (table 3) was similar to previous reports,[25 26] with a sensitivity of 88% and specificity of 92.3%.

Thirty-two patients were positive by GM-EIA. All but seven were also positive by PCR. Evaluation of the cut-off index showed that most fell into the indeterminate range of 0.5–0.7. However, most patients had repeated positive results with rising indices, positive PCR or significant clinical signs. Only four patients remained for whom an indeterminate GM-EIA was the only suggestive criterion for IFD, and these may represent false-positive results.

Eleven patients were positive by *Candida* PCR, and nine were also positive by *Aspergillus* PCR and/or EIA. Three patients had multiple positive results, and five patients were simultaneously positive by M-EIA. There was one confirmed invasive candidal infection in the patient with *Candida tropicalis* fungaemia, who was positive by both EIA and PCR. Only nine of the 125 patients showed evidence of candidal colonisation. Two of these patients gave positive molecular results, one being the confirmed candidosis case and the other a patient with *Candida* isolated from a urine sample.

Fifty-five patients had no positive results, and none displayed significant clinical signs. Only six patients in this group received additional antifungal agents. Thirteen patients have died over the subsequent 12 month period, but none with proven or probable IFD.

Over the 6-month test period, the overall antifungal expenditure fell by more than £124 572 set against an increased

Table 4 Overall survival

Test	Crude mortality	Attributable mortality	Fungal-free survival	Patient with ongoing IFD
Aspergillosis				
PCR + GM-EIA positive	10/25 (40.0)	6/25 (24.0)	10/25 (40.0)	6
PCR positive	16/36 (44.4)	4/36 (8.3)	19/36 (52.8)	1
GM-EIA positive	4/7 (57.1)	0/7 (0)	3/7 (42.9)	
Candidosis				
PCR + M-EIA positive	7/9 (77.8)	0/9 (0)	2/9 (22.2)	
PCR positive	1/2 (50)	0/1 (0)	0 (0)	(1*)
M-EIA positive	1/1 (100)	0/1 (0)	0/1 (0)	
Negative by all tests	13/55 (23.6)	0/55 (0)	42/55 (76.4)	
Total	42/125 (33.6)	10/125 (8.0)	76/125 (60.8)	7/125 (5.6)

Values in parentheses are percentages.
*Patient with invasive pulmonary aspergillosis already included in PCR + GM-EIA positive group.
EIA, enzyme immunoassay; IFD, invasive fungal disease; GM, galactomannan; M, mannan.

diagnostic testing cost of £71 733. Bed occupancy was not significantly different between the two time periods, although there was a trend towards decreased length of stay (average 6.6 days vs 7.2 days for the same period in the previous year), and finished consultant episodes increased (376 vs 366). Adverse events were rare in this cohort (2.4%), and all were attributed to antifungal drug usage. Two patients experienced nephrotoxicity attributed to AmBisome, and one patient experienced hepatotoxicity attributed to caspofungin. Previous audit of a similar patient population showed that, in 203 at-risk patients with febrile neutropenia, half received empirical antifungal drugs, although only 26 had any evidence of IFD.[25] Reducing antifungal usage by this strategy is likely to reduce adverse events and the potential for drug interactions.

DISCUSSION

Several retrospective studies have indicated the potential value of PCR in clinical decision making[28] using the high negative predictive value to exclude IFD. This is the first study to look prospectively within a care pathway. Although not a randomised clinical trial, this work provides proof of concept and allows evaluation of enhanced diagnostic strategies in a real-time clinical setting.

The 6-month test period was chosen for evaluation of cost–effectiveness and clinical usefulness of the new care pathway. Owing to the variable incubation period of IFD, it was necessary to perform long follow-up to detect undiagnosed cases or excess morbidity and mortality in patients in whom empiric therapy was withheld.

The prevalence of proven and probable infection during the test period was 6% (8/125) by EORTC/MSG criteria. This is lower than a previous report[29] and reflects the stringency of consensus definitions that are designed for clinical trial entry and not patient management. In the follow-up period, two additional patients had IFD confirmed, and two possible patients (*Aspergillus* PCR and GM-EIA positive) moved into a probable category with clinical signs consistent with IFD. Thus prevalence rose to 8%. Had PCR been included, prevalence would be 12% during the test period, rising to 15% to include the 12-month follow-up. Modifications to the EORTC/MSG criteria[21] will downgrade EIA-positive patients without specific radiological features to an unclassified category. Many consider that antigenaemia should be used to define IFD,[30] and the paucity of clinical features in this cohort would support that view.

Maertens and colleagues[31] used daily GM-EIA testing combined with early CT scanning in neutropenic febrile episodes to guide antifungal use. This approach suggested that it was possible to reduce empiric amphotericin B without increasing mortality or fungal-related death. The limitations of antigen include fluctuating concentrations leading to poor sensitivity[32] and false-positive results in patients receiving antibiotics, particularly piperacillin-tazobactam.[33]

We found antigen testing to be relatively specific, but with poorer sensitivity than PCR. The sensitivity of PCR in detecting proven/probable cases of invasive aspergillosis was 87.5–75.0% for single and multiple specimens, respectively. This sensitivity was lower than in our previous study (92.3%) and reflects the lower disease prevalence. With both tests, the combined sensitivity was 100% and 87.5% for single and multiple results, respectively, and specificity rose to 100%.

Performance is affected by disease prevalence.[34] Use of biomarkers as a screening test is unlikely to be cost–effective if the pretest probability of disease is low. The impact of effective antifungal prophylaxis should not be ignored. Newer agents such as posaconazole have the potential to reduce prevalence to a level at which these assays would be hard to justify.[35] Cost–effectiveness studies of prophylaxis versus diagnostic surveillance are needed.[36]

Clinical interpretation of PCR results remains difficult. Halliday *et al*[28] proposed interpretive guidelines incorporating single and intermittent PCR positive results, because the DNA load in patients with invasive aspergillosis may be below reproducible limits.[37] In our study, 34 patients had a single *Aspergillus* PCR-positive result. However, even a single non-reproducible positive PCR result was a useful marker, with a diagnostic odds ratio of 15.

All cases of proven disease were positive by both PCR and EIA, and an additional eight patients developed clinical signs consistent with IFD, giving a disease prevalence of 40% when both biomarkers are positive. Six patients were positive simultaneously by both assays, PCR preceded EIA in 15 cases, and the antigen was the first biomarker in four. Clinical signs were a late marker of disease.

No non-aspergillus mould infections were encountered during this study. We are developing a strategy that uses a panfungal followed by specific *Aspergillus* and *Candida* PCRs. This enables the high negative predictive value of the panfungal probe to screen out negative patients and has potential to detect emerging non-*Aspergillus* mould infections while maintaining the superior sensitivity of the species-specific PCRs.

Compliance with the care pathway was good, although six patients with no evidence of IFD received unnecessary antifungals. There was no excess morbidity or mortality in patients in whom empiric antifungal agents were withheld (table 4).

This pre-emptive strategy reduced antifungal drug expenditure by more than the outlay for implementation of the diagnostic testing. It has the potential to optimise pre-emptive treatment of developing invasive fungal infection and decrease empiric usage. Additional benefits through earlier targeted treatment—decreased drug toxicity, decreased hospital stay and less hospital-acquired infection—may also be realised, although formal pharmacoeconomic evaluation of the role of these tests in clinical management of these patients is required.

Take-home messages

- Biomarkers (antigen and nucleic acid detection) can be used to screen for invasive fungal disease in high-risk patients.
- The high negative predictive value of these assays enables *Candida* and *Aspergillus* infections to be ruled out and makes empirical antifungal therapy unnecessary.
- This targeted approach is safe and allows more rational use of antifungal drugs.

Competing interests: RAB serves or has served on UK advisory boards for a variety of antifungal agents, including voriconazole (Pfizer), caspofungin (MSD), AmBisome (Gilead) and posaconazole (Schering-Plough), and received sponsorship to attend international meetings and honoraria for educational lectures from these companies. JK serves or has served on a UK advisory board for caspofungin (MSD).

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