Guidelines on the management of invasive fungal infection during therapy for haematological malignancy

British Committee for Standards in Haematology

Address for correspondence:
BCSH Secretary
British Society for Haematology
100 White Lion Street
London
N1 9PF
e-mail bcsh@b-s-h.org.uk

Writing group: AG Prentice[1],A Glasmacher[2],RP Hobson[3], S Schey[4] , RA Barnes[5], JP Donnelly[6], G Jackson[7]

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1 Department of Haematology, Royal Free Hospital, London UK
2 Department Internal Medicine, Bonn, Germany
3 Department of Microbiology, Leeds Royal Infirmary, Leeds UK
4 Department of Haematology, Guy's Hospital, London UK
5 Department of Medical Microbiology, Cardiff University, Cardiff UK
6 Department of Haematology, Radboud University Nijmegen Medical Centre The Netherlands
7 Department of Haematology, Newcastle General Hospital UK

Introduction

The guideline group was selected to represent those with a special interest in the management of invasive fungal infection (IFI) in patients with haematological malignancy (HM) in the UK and EU. PUBMED, MEDLINE and EMBASE were searched systematically for publications in English from 1981 to 2007 and important abstracts from recent meetings have been included. The writing group produced the draft guideline which was subsequently revised by consensus by members of the Haemato-oncology Task Force of the British Committee for Standards in Haematology (BCSH). The guideline was then reviewed by a sounding board of approximately 100 UK haematologists, the BCSH and the committee of the British Society for Haematology and amended, again by consensus. Criteria used for levels of evidence and recommendations are as outlined in appendix 3 of the Procedure for Guidelines commissioned by the BCSH (http://www.bcshguidelines.com/process1.asp#App3).

The objective of this guideline is to provide healthcare professionals with clear guidance on the management of IFI in patients with HM. The guidance may not be appropriate for patients with other malignancies or AIDS and in all cases individual circumstances may dictate an alternative approach. Studies of patients in all age groups were considered and the guideline makes no distinction between paediatric, adolescent and adult patients in its recommendations. Some of the evidence is unsatisfactory making recommendations difficult.

Summary of key recommendations

- **Incidence rates of IFI and levels of risk of IFI in HM patients vary. The diagnosis of IFI should be confirmed whenever possible and every unit treating HM patients should keep up to date diagnostic records to allow both accurate definition of risk and changes in pathogens over time. Patients receiving chemotherapy for acute leukaemia may have the same risk of IFI as allogeneic haematopoietic stem cell transplant patients.**
- **All units treating HM patients at risk from IFI should have immediate access to CT scanning and mycological laboratory diagnostic methods.**

- **Empirical use of systemic antifungal agents as treatment of fever of unknown origin (FUO) which is “resistant” to broad spectrum antibacterial therapy should be discouraged.**
- **When antifungal therapy is unavoidable because of possible IFI, justification for this use should be sought from CT scans and mycological tests.**
- **In trials of empiric treatment of antibacterial-“resistant” FUO the lowest rates of toxicity are reported for caspofungin followed by liposomal amphotericin B. They have similar efficacy.**
- **For all levels of certainty of diagnosis there is no evidence that any other drug is superior in efficacy to either of these two.**
- **Prophylaxis of IFI should be confined to high risk patients. The drugs of choice are itraconazole which has clinically significant but manageable or avoidable interactions with other drugs and posaconazole which has not yet been shown to be superior in efficacy to itraconazole. Both are superior in efficacy to fluconazole. There are no data to justify the use of voriconazole for prophylaxis.**

1 EPIDEMIOLOGY

Epidemiological reporting could aid assessment of risk and identify which patients need prophylaxis and early therapy. In three of the biggest empiric therapy RCTs (Walsh et al 1999, 2002, 2004) IFI were proven in less than 6% of patients. The two most common organisms in all studies are *Candida* and *Aspergillus* spp. In the European Organisation for Research and Treatment of Cancer (EORTC) study of systemic candidosis (SC) the associated death rate was 39% (Viscoli et al 1999) and Lin et al (2001) reported invasive aspergillosis (IA)-associated death rates of 49.3% with chemotherapy and 86.7% with haematopoietic stem cell transplantation (SCT). These figures conceal wide variation in the risk of acquiring SC and IA and published incidence rates vary between centres and change over time. Chamilos et al (2006) reported autopsy-proven IFI in patients with HM from a single institute during three separate four year periods, 1989-93, 1994-98 and 1999-2003. There was a declining rate of autopsies (67%-34%-26%) but IFI were found in 314 of 1017 autopsies and most were not diagnosed in life (75%). The prevalence of invasive mould infections rose significantly (19%-24%-25%; p=0.05), as did zygomycetes (0.9%-4%-3%; p=0.03). The fall in the prevelance of all invasive candidal infections (13%-10%-9%; p=0.07) and *C. albicans* infections (3.2%-2.8%-1.8%; p=0.5) were not statistically significant but the increase in non-*albicans C.* spp. infections was (3.2%-6.7%-7.8%; p=0.01).

Risk factors for IFI have been extensively reported in patients treated for HM (Gerson et al 1984; Schwartz et al 1984; Tollemar et al 1989; Guiot et al 1994; Walsh et al 1994; Jantunen et al 1997; Wald et al 1997; Prentice et al 2000; Baddley et al 2001; De La Rosa et al 2002; Grow et al 2002; Marr et al 2002; Martino et al 2002; Glasmacher et al 2003; Mahfouz et al 2003). Some of these definitions of risk reflect the depth and duration of immunosuppression following therapy for HM.

Table 1; Risk factors

Risk factor	*Candida*	*Aspergillus*
Neutropenia	+	+
Steroids	+	+
GVHD[1]	+	+
Indwelling central IV line[2]	+	-
Bacterial infection	+	+
Antibacterials	+	-
Inadequate prophylaxis	+	+
CMV[3] infection	-	+
Mucosal colonisation	+	+
Building works	-	+

1 Graft versus host disease

2 Tunnelled indwelling central intra-venous line

3 Cytomegalovirus infection

1.1 Candidosis

The incidence of hospital IFI doubled in the US between 1980 and 1990 (Beck-Sague & Jarvis, 1993) largely due to *Candida* spp. *C. albicans* accounted for 50% of these but SC due to non-*albicans Candida* spp., especially *C. glabrata*, increased (Pfaller et al 2000) and, in the EORTC study of 1992-94 *C. albicans* was responsible for only 36% of SC in patients with HM compared to 70% in patients with solid tumours (Viscoli et al 1999). *C. glabrata* (significantly higher mortality rate), *C. tropicalis, C. parapsilosis, C. krusei* and a group of other species each accounted for 12 to 14% of the cases in patients with HM. The overall crude mortality rate was 39% at 30 days and the candidaemia-associated mortality was 24% although mortality attributable solely to candidaemia was only 8%. SC incidence rates cannot be calculated from these studies because they lacked a denominator population. One tertiary care hospital, reported 38 cases of “hepatosplenic” or systemic candidosis (6.8%) with a five-fold increase in the incidence rate in 562 patients treated for acute had leukaemia between 1980 and 1993 (Antilla et al 1997).

In a retrospective study of incidence rates of IFIs between 1999 and 2003 in 11,802 HM patients treated with chemotherapy only there was an overall rate of 1.5% of positive blood cultures for *Candida* spp. (57% non-*albicans C* spp.) with an overall mortality rate of 0.5% (OMR) and an attributable mortality rate (AMR) of 33% (Pagano et al 2006). In patients with acute myeloblastic leukaemia (AML) these rates were respectively 4%, 1.4% and 35.5% and in those with acute lymphoblastic leukaemia (ALL) 2%, 0.7% and 36%.

In 1188 patients receiving autologous SCT (470 lymphoma, 395 multiple myeloma, 132 breast carcinoma) from 1990 to 2001 there was an overall rate of candidaemia of 0.3% (Jantunnen et al 2004). In 21 cases of FI in 142 consecutive SCT patients with haematological malignancies from one unit, 4 were SC (Jantunnen et al 1997). In 395 consecutive, matched and related SCT patients with HM, Martino et al (2002) reported only 13 of 50 cases of IFI were SC. In 31 patients with a variety of haematological and non-haematological malignancies receiving non-myeloablative or low intensity conditioning SCT, there was one case of SC due to *C. glabrata* (Hagen et al 2003). This predominance of non-yeast IFI is presumed to be an effect of fluconazole prophylaxis. In one SCT centre fluconazole for 75 days post-SCT reduced the incidence rate of SC from 11.9% to 4.6% (mainly *C. krusei* and *C. glabrata*) and the SC-associated mortality rate (Marr et al 2002). These presumed effects of fluconazole prophylaxis have been reported by others (Abi-Said et al 1997; Girmenia and Martino 1998).

1.2 Aspergillosis

Incidence rates of IA in HM patients vary widely, particularly in low-intensity conditioning allograft patients from none in 76 consecutive patients (Hori et al 2004) to 11 proven IA in 31 consecutive patients given fluconazole prophylaxis (Hagen et al 2003).

In 2496 consecutive SCT patients in one centre, 158 had proven IFI (6.4%) of which most were IA (Wald et al 1997), only 30% were neutropenic and only 7% of the IA cases were alive at one year compared to 54% without IA. Jantunnen et al (1997) reported 21 cases of IFI [15 IA (10.6%)], in 142 consecutive allogeneic SCTs (131 matched-related), between 6 and 466 days after SCT (median 136 days). Only 3 (14 %) were neutropenic and only 3 survived long-term. Martino et al (2002) reported 50 cases of IFI in 395 matched, related allogeneic SCTs, of which 37 were non-candidal

and 32 (9.3%) IA. In all three of these studies presentation was bimodal at around 16 and 100 days. Chen et al (2003) reported a series of 1359 patients, 19 (6%) of whom had fungal pneumonia late after SCT. Marr et al. (2000) reported an incidence of IA increasing from 7.3% in 1992 to 16.9% in 1998 with a parallel decline in SC, a trend confirmed by others (Chandrasekar 2000).

These differences may be due to variable diagnostic criteria. In a multicentre, prospective survey of IA in haematology patients by the EORTC, the highly sensitive techniques of open lung biopsy and autopsy did not yield the expected rate of proven cases in those with suspected IA (Denning et al 1998).

As proof of IA, the Invasive Fungal Infection Cooperative Group of the EORTC and the Mycoses Study Group of the US National Institute of Allergy and Infectious Diseases (IFICG/MSG) required histopathological or cytopathological demonstration of hyphae with associated tissue damage or a positive culture in a sample collected by sterile technique from a normally sterile site which was clinically or radiologically infected (Ascioglu et al 2002). Using similar criteria, Maertens et al (1999) found 27 cases of proven IA (positive histology and culture) in a series of 186 HM patients (14.5%). Assessing the incidence rate is difficult because *Aspergillus* seldom grows in blood cultures and alternative diagnostic techniques for IA are not widely used or not accepted as adequately standardised.

Three recent studies have used these criteria to re-assess the risk of IA in HM patients given chemotherapy, an autologous SCT or an allogeneic SCT. In those treated with chemotherapy alone, of 3012 patients with AML there was an overall of 7%, an OMR of 2.6% and an AMR of 38%, of 1173 and with ALL an overall rate of 3.7%, an OMR of 1.6% and an AMR of 43% and of 596 with chronic myeloid leukaemia an overall rate of 2.5%, an OMR of 1.2% and an AMR of 50% (Pagano et al 2006). There were 310 cases of aspergillosis, 40% proven and 60% probable, with 14 of zygomycetes and 15 of *Fusarium* spp. In AML and ALL half of the cases of aspergillosis occurred during the first course of remission induction and 31 of the 112 deaths due to aspergillosis also occurred at this phase of treatment (an AMR of 20%) while the risk of death from aspergillosis during relapse was 63%. The effect of prophylaxis on these data is not reported but from 1987 to 2003 in six of the centres taking part there had been no increase in the incidence rate of systemic mould infections although the AMR for aspergillosis had dropped from 60% to 32% ($p=0.019$).

In 1188 adults given autologous stem cell transplants (980 for HM) over 11 years the incidence of proven or probable IA was 0.8% (Jantunen et al (2004).
In a prospective series of stem cell transplant patients studied in 19 transplant units over 22 months the aggregated cumulative incidence of IA at 12 months was 0.5% for autologous SCT, 2.3% for allogeneic SCT from a matched, related donor, 3.2% using a related, mismatched donor and 3.9% using a matched unrelated donor (MUD) (Morgan et al 2005). There was no difference according to whether a myeloablative or a non-myeloablative regimen was used (3.1% vs 3.3%) for conditioning. Mortality at 3 months following the diagnosis of IA ranged from 53.8% for autografts to 84.6% for MUDs. The influence of prophylaxis or early therapy on these data is unclear. Three recent retrospective reviews of patients who underwent SCT (predominantly allogeneic) illustrate which variables predict for progression of IA and death post-transplant (Cordonnier et al 2006; Martino et al 2006; Upton et al 2007).

1.3 Other fungi

The incidence rates and risks of IFI due to the more rare or "emerging" fungi are more difficult to determine.
IFI with the yeasts Saccharomyces spp., *Trichsporon* spp, *Malassezia* spp., *Geotrichium candidum*, *Hansenula anomola*, *Rhodotorula* spp., and *Picchia* spp. may occur in any immunocompromised patient. IFI with *Cryptococcus neoformans* is rarely seen in haematology patients.
The moulds include the two hyalohyphomycoses; over 80 cases of *Fusarium* spp. IFI mostly in patients with HM (Gupta et al 2000) and with 16 cases of *Scedosporium prolificans* IFI in neutropenic patients, 14 of which were fatal (Berenguer et al 1997); *Scedopsporium apiospermum* is more common than *prolificans* as a cause of IFI in the UK. The low prevalence of IFI with moulds in the order of Mucorales (*Rhizopus, Mucor,* and *Rhizomucor Absidia*) in less specific populations (Groll et al 1996; Rees et al 1998) are not reliable guides to the risk of IFI with these organisms in HM patients.
The incidence of dimorphic and endemic moulds is well known in their endemic areas but they can be exported with patients from these areas into the non-endemic areas where the patients are treated for HM. These include *Penicillium marneffii* from SE

Asia, histoplasmosis, the most commonly imported IFI into Europe (117 cases in France between 1970 and 1994 (Warnock et al 1998)), and coccidioidomycosis from SW USA (100,000 cases per year) and Central and Southern Americas.

Recommendations

In HM patients there is a shift from SC to IA and within SC a shift from *C.albicans* to non-*albicans C.* spp. Continuous prospective collection of incidence rates from many centres may indicate risk of IFI by diagnosis and type of HM therapy. These data should be regularly and frequently updated and conform to the IFICG-MSG definitions of levels of certainty of diagnosis (grade B, level III)

Antifungal prophylaxis is not recommended in autologous SCT because of the low risk of IFI in these patients (grade B, level IIb)

Antifungal prophylaxis is recommended in allogeneic SCT and in intensive chemotherapy for acute leukaemia because of the significant risk of IFI in both groups of patients (grade A, level Ia)

2 DIAGNOSIS

Reliable diagnosis depends on an understanding of risk factors and incidence rates, the significance of different clinical presentation and the timely use of mycological and radiological investigations. IFI is suspected in HM patients when antibacterial drugs have failed to reduce fever during a period of neutropenia or sustained immunosuppression. Most therapy is empirical.

The IFICG/MSG definitions of proven, probable and possible IFI were created to inform the conduct of future clinical research (not as guides to clinical practice), but emphasise the difficulties of obtaining timely and objective proof of IFI in HM patients (Ascioglu et al 2002). Diagnoses of probable or possible IFI require combinations of host factors and microbiological and clinical criteria. For a diagnosis of proven IFI, specimens must be obtained by a sterile technique from a normally sterile site. Invasive mould infection (e.g. IA) is proven if hyphae are seen in a histological or

cytological specimen (with evidence of tissue damage seen either in the biopsy material or “unequivocally” by imaging) or a mould is grown in culture from that specimen with clinical or radiological evidence of infection at the site from which the specimen was taken. Systemic yeast infection (e.g. SC) would be proven on the same evidence as above or if the yeast was grown in blood culture. These requirements are rational but strict, fulfilling them might be difficult in practice and results may be delayed.

2.1 Clinical Diagnosis

IFICG/MSG suggested that a diagnosis of IFI was probable with a combination of host factors (fever, neutropenia and resistance of FUO to broad-spectrum antibacterials) plus clinical, microbiological and radiological criteria (Ascioglu et al 2002).

Fever and hypothermia are no more specific signs of IFI than any other clinical symptom or sign; the temperature may be normal in any systemic infection, including IFI, in HM patients. There are many non-infective causes of an abnormal temperature.

Neutropenia of less than $0.5x10^9$/l for more than 10 days is a non-specific risk factor for all forms of systemic infection in HM patients and most cases of IA post SCT now occur late after engraftment with some recovery of the neutrophil count (Jantunen et al 1997; Wald et al 1997; Martino et al 2002). Severe or extensive and chronic graft versus host disease (GVHD) and protracted use of high doses of steroids are more significant risk factors for IA (Jantunen et al 1997; Martino et al 2002).

In patients recognised to be at risk, the unexpected acute presentation of symptoms and signs such as dyspnoea with no primary cardio-respiratory explanation, haemoptysis or epistaxis when haemostasis is otherwise adequate, pleural pain (omitted from the IFICG/MSG list), meningism and focal neurological signs, pleural rub, periorbital swelling, palatal necrotic lesions or perforation, maxillary tenderness, papular and nodular skin lesions, unexplained bone pain (skeletal aspergillosis) and evidence of deep ocular infection are important clinical features suggesting IFI. All are non-specific.

Table 2; Clinical features suggesting IFI

Any new fever during prolonged, severe neutropenia or immunosuppression
Fever resistant to broad spectrum antibacterials while neutropenic
Symptoms and signs of new, resistant or progressive lower respiratory tract infection; e.g. pleuritic pain, pleural rub
Prolonged, severe lymphocytopenia in chronic GVHD and immunosuppression
Symptoms and signs of progressive upper respiratory tract infection
Periorbital swelling
Maxillary swelling and tenderness
Palatal necrosis or perforation
Focal neurological or meningeal irritation symptoms and signs with fever
Unexplained mental changes with fever
Papular or nodular skin lesions
Intra-ocular signs of SFI

2.2 Laboratory Diagnosis

Many techniques might improve diagnostic objectivity such as microscopy (cytology and histology), culture and the detection of free protein by immuno-absorption and free DNA by PCR, but there are problems of varying sensitivity and specificity

2.2.1 Microscopy

This technique applies to the microsopy of aspirates from para-nasal sinuses, sputum, pleural fluid, broncho-alveolar lavage (BAL), urine, and CSF and to the histology of any tissue biopsy.

IFI is proven on microscopic demonstration of the hyphae of moulds (with unequivocal evidence of tissue damage) or yeast cells from needle aspiration or biopsy samples obtained by a sterile technique from a normally sterile area which is clinically or radiologically abnormal and consistent with infection (Ascioglu et al 2002) (ignoring contamination of samples from skin and ambient air).

Probable or possible IFI is supported by microscopic findings of any mould in a sinus aspirate, any mould or *Cryptococcus* spp. in sputum or BAL or candidal casts in the urine in the absence of a bladder catheter (Ascioglu et al 2002).The sensitivity or specificity of cytological examination are unclear for IFI. Heavy colonisation is sometimes described as a risk factor but “heavy” cannot be defined objectively in a cytological sample. Moulds and yeasts are ubiquitous and mucosal colonisation is seen in the normal population. Expectorated sputum is of little help in the diagnosis of IFI and is not produced by severely neutropenic patients. Cytological examination (white cell count and gram stain) and chemical analysis (protein and glucose) of the CSF may confirm a diagnosis of meningitis but they are unlikely to confirm which organism is responsible unless CSF cytospin samples are stained for all fungi especially *Cryptococcus* spp.

The diagnostic rate for microscopy of BAL is higher than that for culture of BAL in immunocompromised patients (Kahn et al 1986; Levy et al 1992; Fischler et al 1997; Yuen et al 1997). In one study of cancer and SCT patients the respective rates were 64% and 40% (Levy et al 1992). In 22 patients with IA, 5 were positive by microscopy alone (Pagano et al 1997) but low sensitivity of isolation of *Aspergillus* spp. in BAL has been reported (Yu et al 1986). Microscopy provides results rapidly and can distinguish certain infections such as those caused by the Mucorales and *Pneumocystis jeroveci* pneumonitis. The technical aspects of different staining techniques can be found in other reviews and guidelines (Richardson & Kokki 1998; Denning et al 2003).

Histological demonstration of moulds and yeasts defines proven IFI but transbronchial or percutaneous lung biopsy will be possible in a minority of patients. This definition is justified by the poor sensitivity of culture techniques for *Aspergillus* spp. and the poor specificity of colonisation for infection (Ascioglu et al 2002). In deep SC blood cultures may also be negative (Berenguer et al 1993; Antilla V-J et al 1994). In those patients who are regenerating neutrophils and whose liver function tests are deteriorating, laparoscopy-guided liver biopsy may detect SC (Antilla V-J et al 1997) but the type of hepatosplenic SC described by Antilla et al (1994) is now reported less often.

2.2.2 Culture

Growth of a yeast in blood cultures (or from biopsies from normally sterile sites provided this is temporally related to relevant clinical signs and symptoms) defines proven IFI and is associated with a higher rate of disseminated tissue infection and mortality (Anaissie et al 1998). SC is probable if yeasts are grown in two urine samples or candidal casts are seen in one urine sample in the absence of a bladder catheter in at risk patients (Ascioglu et al 2002).

Blood culture may not detect all patients with SC in deep tissue (Beruenger et al 1993) but there is a strong correlation between positive blood cultures, whether from peripheral vein or central venous catheter, and autopsy findings of disseminated candidosis (Lecciones et al 1992). In 94 patients with HM and an autopsy proven invasive candidal infection however, there was also a strong correlation, using multiple regression analysis, between reduced sensitivity of blood cultures for candidal species and the use of absorbable antifungal agents (Kami et al 2002a).

Several recent studies have shown that SC can no longer be assumed to be due to *C. albicans*, that there are as many if not more non-*albicans C.* spp. infections now, and their sensitivity to systemic anti-fungal drugs varies significantly (Abi-Said et al 1997; Viscoli et al 1999; Marr et al 2002). Referral of candidal isolates for speciation and sensitivity testing is essential.

Growth of any mould from biopsies by sterile technique of any normally sterile tissue or from fluid from any site, excluding mucous membranes and urine, with clinical or radiological signs of infection, defines proven IFI (Ascioglu et al 2002). *Aspergillus* spp. and *Penicillium* spp. (other than *Penicillium marneffei*) do not grow well in blood culture so this is excluded from IFICG/MSG definitions. Probable or possible SFI is supported by growth of any mould from a sinus aspirate or moulds (*Aspergillus*, *Fusarium* , or *Scedosporium spp.,* or Zygomycetes), *Cryptococcus neoformans*, or an endemic fungal pathogen (*Histoplasma capsulatum, Blastomyces dermatitidis, Coccidioides immitis* or *Paracoccidioides brasiliensis*) from sputum or BAL fluid (Ascioglu et al 2002).

In disseminated *Aspergillus* spp. infections the organism is seldom recovered from respiratory samples (Bodey et al 1988) and even two or more positive cultures do not exclude contamination (Rees et al 1998); nor do specialised media (eg Sabouraud dextrose agar), although they may increase the culture yield of *Aspergillus* and other species of moulds (Horvath and Dummer 1996). All isolates of *Aspergillus* spp.

should be sent for speciation and sensitivity testing at a mycology reference laboratory because of variations in their sensitivity to amphotericin and changes in their incidence rates (Denning et al 2003). Although all suspicious lesions should be biopsied and cultured for fungi, positive results of microscopy and culture may be obtained only late in the disease, sampling error results in only a 50% yield for open lung biopsies and many patients are unfit for such procedures (Denning et al 1998). The value of a positive culture result is greater in the context of other clinical, radiological and histological evidence of IFI; in an unselected hospital population the probability that a positive *Aspergillus fumigatus* culture indicated IA was only 22% (Bouza et al 2005).

The choice of antifungal therapy will depend on speciation and susceptibility testing of candidal isolates is of value in view of resistance emerging variably to some azoles. Susceptibility testing has so far proved less useful clinically for moulds (Forrest 2006).

2.2.3 Immunological and DNA assays

Spectrophotometric assays (SPA), radio-immuno assays (RIA) and enzyme-linked immunosorbent assays (ELISA) have been developed to detect fungal cell wall components and antigens and polymerase chain reaction assays (PCR) to detect fungal DNA to try to increase objectivity of diagnosis in view of the lack of specificity of clinical and radiological features, microscopy and culture. A “pan-fungal” assay of (1→3)-β-D-glucan (BG) (a cell wall polysaccharide specific for fungi except zygomycetes and cryptococci), in blood samples from a large series of HM patients, was reported to have good sensitivity, specificity and negative predictive values for systemic yeast and mould infections (Odabasi et al 2004). Other studies suggest sensitivity may vary from 50% to 87.5%, there are problems with unexplained false positive results and this test may be of use only in conjunction with others (Kami et al 2000; Perfect et al 2001; Kawazu et al 2004; Pazos et al 2005).

2.2.3.1

Candida spp.

Candidal enolase was detected by immunoassay of blood from neutropenic cancer patients in 85% with proven deep SC and in 64% with candidaemia. Multiple sampling improved sensitivity and high specificity suggesting a powerful negative

predictive value (Walsh et al 1991). Double sandwich ELISA for candidal antigen (mannan) and anti-mannan antibodies in proven SC infections (all *C. albicans*) was 40% specific for the antigen alone, and 80% specific for both combined. There was an inverse relationship between the two and repeated sampling was needed to increase sensitivity (Sendid et al 1999).

Molecular detection of candidal DNA for rapid diagnosis of SC was first reported by Buchman et al in 1990. Two small studies then suggested that PCR-restriction enzyme analysis of blood may have high specificity for SC (Morace et al 1997; Einsele et al 1997). A PCR-EIA assay of internally transcribed ribosomal DNA in 28 patients with SC was 100% sensitive but less specific when compared with patients with no evidence of SC (Burnie et al 1997). It is unclear how much any of these tests add to microscopy, culture and BG serology in SC

2.2.3.2

Aspergillus spp.

Aspergillosis develops slowly from colonisation to invasive disease and therefore should be detectable early by repeated immunological or molecular screening. Repeated studies of immunological detection of cell wall antigen (galactomannan (GM) or galactofuran) suggest correlation between positive blood results and proven IA (Dupont et al 1987; Rogers et al 1990; Stynen et al 1995; Verweij et al 1995a&b; Rohrlich et al 1996; Salonen et al 2000; Siemann & Koch-Dorfler 2001; Sulahian et al 2001). GM is detectable in blood well before the clinical diagnosis (median of 6 days), implying a benefit from surveillance in selected patients. In 243 episodes of neutropenia, 2172 serum samples were tested by ELISA for *Aspergillus* spp. antigen (Maertens et al 1999) and patients were classified as proven, probable or possible cases of IA. Sensitivity was 92.6% and specificity 95.4% with a positive predictive value (PPV) of 93% and a negative predictive value (NPV) of 95%. In over half the cases circulating antigen was detected a median of 6 days before clinical infection. Sputum and BAL cultures were positive in only 12 and 10 cases respectively. Sulahian et al (2001) also found sensitivity, specificity and predictive values of over 90%. These high detection rates have not been confirmed in more recent studies of serum GM-ELISA which show sensitivity rates varying from 32% to 50%, specificity over 90%, a PPV of 58% to 73% and a NPV over 80% (Siemann & Koch-Dorfler 2001; Herbrecht et al 2002; Becker et al 2003; Pinel et al 2003; Buchheidt et al

2004). The most consistent findings of all these reports are the strong specificity and NPV of the serum GM-ELISA. Circulating anti-GM antibodies might lower the sensitivity and PPV of this test (Herbrecht et al 2002). In some later studies systemic antifungal therapy may have been used early (based on radiological findings), suppressing circulating GM levels (Rohrlich et al 1996). The sandwich ELISA technique has given fewer false positive results and higher sensitivity than earlier latex based methods (Verweij et al 1995b; Sulahian et al 1996) but, although the specificity is high (85%), the sensitivity varies between 30% and 100% (Patterson et al 1997; Fortun et al 2001; Maertens et al 2001; Meninck-Kersten 2004) possibly because of different “cut-off” values for the assay in the US and EU. If lower “cut-off” values are adopted, the sensitivity, specificity, PPV, NPV and clinical efficacy may all be above 95% for this assay (Maertens et al 2004a). A low rate of false positivity has been described in bacteraemia (Swanik et al 1997) and false positive results remain a problem due to drugs such as cyclophosphamide (Hashiguchi et al 1994) and piperacillin/tazobactam (Viscoli et al 2004) and some food (Lescher-Bru et al 2004). If GM-ELISA latex agglutination is used early, dependent on computerised tomography (CT) scans, sensitivity of BAL samples is greater than serum (>70% vs <45%) (Caillot et al 1997, 2001). Siemann and Koch-Dorffler (2001) also found greater GM-ELISA sensitivity using BAL. Using GM-ELISA on localised BAL samples, targeted by CT scans, sensitivity was 90 – 100%, specificity 100%, PPV 100% and NPV 93% (Becker et al 2003). In CT-based studies more patients will have had GM-ELISA tests before systemic antifungal therapy. Previous studies with low BAL sensitivity did not report the relation between the test and the use of systemic antifungal therapy (Verweij et al 1995a; Salonen et al 2000). Use of the IFICG/MSG diagnostic criteria may also influence results. Sensitivity and specificity will increase if greater numbers of patients with probable and proven IA and fewer possible IA are studied and larger numbers of samples are then tested per patient (Maertens et al 2001; Becker et al 2003). Lowering the sensitivity for a positive GM-ELISA result would be useful if specificity was preserved. Pfeiffer et al (2006) reported, in a meta-analysis of 27 studies published between 1966 and 2005, that this assay overall had a sensitivity of 71% and specificity of 89%, although there was significant heterogeneity between studies. They concluded that this assay has moderate accuracy in the diagnosis of IA in immunocompromised patients and is

more useful in patients with haematological malignancies than in other groups of patients.

The first report of PCR of serum *Aspergillus* DNA in proven IA suggested 70% sensitivity and specificity (Yamakami et al 1996). When two or more blood samples were tested in 21 patients with proven IFI (13 IA), sensitivity was said to increase to 100% and specificity to 98% (Einsele et al 1997). Similar sensitivity and specificity have been confirmed in most subsequent studies of blood, serum and BAL samples (Jones et al 1998; Goldblang et al 1999; Loeffler et al 1999; Rimek et al 1999; Skladny et al 1999; Hebart et al 2000; Buchheidt et al 2001, 2002; Ferns et al 2002; Raad et al 2002), although in most the specificity has been significantly less than the sensitivity and more recent sensitivity rates are closer to 60%. As with GM-ELISA, sensitivity rises if sampling is more frequent (Williamson et al 2000; Lass-Floerl et al 2001; Buchheidt et al 2004).

The outcome of these PCR studies depends on uncontrolled clinical variables such as sample size (many are very small), changes in therapeutic strategies over time (earlier treatment reducing available circulating or pulmonary fungal DNA) (van Burik et al 1998; Lass-Floerl et al 2001), more recent selection of patients on the basis of IFICG/MSG diagnostic criteria (inclusion of “possible” cases diluting the chances of high sensitivity) (Buchheidt et al 2004), sources of sample (blood, serum, BAL) and the short half life (<5 minutes) of circulating fungal DNA (Bretagne 1998). Variable *in vitro* factors of preparation of test material, differences in PCR techniques (PCR-immunoblot, nested PCR, PCR-EIA, 2-step PCR, PCR by light cycler), and risk of sample contamination during DNA extraction and amplification (Loeffler et al 1999) will also influence results. Despite all of these difficulties there is a very high NPV in almost all reports of these PCR-based techniques and the significance of a positive result is greater if it is seen on more than one occasion (Bradstock BJH 2006). In one study single PCR positivity was never associated with clinical fungal disease and did not recur if systemic antifungal therapy was withheld (Lass-Floerl et al 2001).

Direct comparisons of ELISA and PCR in two small studies of patients at high risk of IA reported equivalent results for GM-ELISA but discrepant results for PCR, possibly due to marked differences in PCR technique (Bretagne et al 1998; Williamson et al 2000). In 19 patients with suspected IA using GM-ELISA of blood and PCR of BAL, both tests were positive in five patients and negative in nine with one or the other positive in another five (Verweij et al 1995a). Positive ELISA results were seen earlier

than positive PCR results, therefore timing of sampling may be another variable determining the result. In the largest comparative series reported so far, serum GM-ELISA testing had a sensitivity of 33.3% compared to 63.6% for PCR testing on samples from a variety of sources while specificity for GM-ELISA was high at 98.8% (Buchheidt et al 2004). PCR-based techniques are relatively more labour-intensive than immunological methods and there is insufficient evidence of inter-laboratory quality assurance for both techniques. IFICG/MSG advised against "the routine use of PCR in the diagnosis of invasive aspergillosis" and excluded it from all levels of diagnosis of SFI without reference to recent evidence on its efficacy**.**

2.2.3.3

Application of tests to clinical studies

Two studies have examined the impact of diagnostic serological tests on the use of systemic antifungal therapy. Hebart et al (2004) conducted a large randomised controlled trial (RCT) of "PCR-directed" use (n=196; group A) versus empiric use of ambisome (n=207; group B) for IFI during the first 100 days after allogeneic stem cell transplant. In group A, PCR tests were done twice weekly in hospital and once weekly following discharge and ambisome was started if two consecutive PCR results were positive or if one was positive in the presence of signs of infection (this latter group therefore receiving arguably empirical therapy). In group B, ambisome was started after 120 hours of antibacterial resistant FUO. Significantly more patients in group A received ambisome (n=109) than in group B (n=76; $p<0.05$), but there was no significant difference in the rate of proven IFI (11 vs 16), overall survival, and proven IFI-related deaths (1 candidal versus 1 aspergillosis and 4 candidal) up to 100 days or in overall deaths up to 180 days. The only significant reduction in deaths was seen at 30 days (4 vs 13; $p=0.03$).

Maertens et al (2005) examined the impact of daily serum galactomannan testing and early CT scanning on the use of amphotericin B in a single arm study of 88 patients in 136 cycles of neutropenia. In 117 episodes of FUO which was resistant to, or relapsing while on, broad spectrum antibacterial therapy, antifungal treatment was given only if two positive consecutive serum galactomannan results were confirmed on CT-directed BAL or if CT findings suggested IFI. This approach halved the potential empiric use of amphotericin B from 35% to 16% of patients with an IFI-

related mortality rate no greater than that seen in large RCTs of empiric antifungal therapy (Walsh et al 1999, 2002, 2004).

2.2.3.3

Cryptococcus

Detection of cryptococcal antigen in urine, serum or CSF by RIA suggests IFI by *Cryptococci* **(**Hamilton JR et al 1991**)**. IFICG/MSG define detection of endemic fungi in urine or serum or *Cryptococcus* spp. in CSF (despite known false positivity) by RIA as proven IFI**,** *Cryptococcus* spp. in serum by RIA as only probable or possible IFI and *Aspergillus* spp. antigen by ELISA in BAL fluid, CSF or two or more blood samples as only probable or possible IFI (Ascioglu et al 2002) despite the 90% sensitivity and specificity of this last test (Maertens et al 1999) which is similar to blood culture results for *Candida* spp.

Recommendations

Clinical features associated with IFI warrant early and thorough investigation for results needed to support the early use of systemic antifungal therapy (grade B, level IIa)

A negative blood culture does not exclude an invasive candidal infection, particularly during absorbable anti-candidal prophylaxis. Clinical suspicion should encourage biopsy for proof of SC (grade B, level IIb)

If IFI is suspected clinically, aspirated fluid, including BAL, or a biopsy specimen (in patients who can tolerate the necessary procedure) should be obtained from a suspected site of infection for cytology, histology and culture, regardless of blood culture results. Microscopic finding or growth of any yeast or mould from any normally sterile tissue or fluid source, including blood cultures, justifies systemic antifungal therapy (grade B, level III)

Routine screening of high risk patients with sandwich-ELISA for GM on samples of blood may assist earlier diagnosis of IA if the results are interpreted in the context of other evidence (i.e. clinical findings, CT scans and

targeted GM testing of BAL fluid). Targeted early therapy for IA may then replace purely empiric therapy of anti-bacterial resistant FUO (grade B, level IIb)

In all cases of meningitis or potential intracranial IFI CSF should be stained specifically for fungi, particularly *Cryptococcus* spp. All CSF samples should be examined by Gram stain and those which are negative should be tested for Cryptococcal antigen by RIA (grade B, level IIb)

All yeast isolates thought to be responsible for an episode of IFI should have speciation and susceptibility tests in a competent laboratory. Therapy need not wait for the results of these tests but may be modified by them. Speciation of all mould isolates is also recommended but the clinical role for susceptibility tests is not established for moulds and is recommended only for those IFIs in which a resistant mould is suspected (grade B, level III)

2.3 Radiological diagnosis

2.3.1 Plain chest X-ray (CXR)

A normal CXR is reported from 0% to 29% of cases of IPA and mucormycosis (Herbert & Bayer 1981; Denning et al 1998; Lee et al 1999; Kim et al 2001; Hauggaard et al 2002; Thomas et al 2003), a week or more before a definitive diagnosis and at the time of the first abnormal CT scan.

CXR appearances in IPA are non-specific, including segmental or subsegmental consolidation, patchy infiltrates, nodules (single or multiple), nodular infiltrates and cavitation (Herbert & Bayer 1981; Thomas et al 2003). Nodules are seen in 48 to 68% of proven IPA patients (Kim et al 2001; Gefter et al 1985), but in a retrospective, blinded analysis of CXR from 33 febrile SCT recipients (21 proven IFI), the sensitivity, specificity, PPV and NPV of CXR nodules in the diagnosis of IPA were 81%, 100%, 100% and 75% respectively (Mori et al 1981). Although CXR appearances are heterogeneous, the presence of nodules, cavitation and the air-crescent sign are more closely associated with IPA than other CXR findings.

Appearances progress to larger areas of consolidation with development of cavitation and the air-crescent sign in up to 90% of patients 1 to 4 weeks from the first CXR

abnormality (Kuhlman et al 1985; Potente 1989; Kim et al 2001). Cavitation and air crescents (evidence of cavitation) may be characteristic but are late findings (Potente 1989; Kim et al 2001; Lee et al 1989; Gefter et al 1985; Albelda 1985), diagnosis precedes cavitation in half of cases by almost a week and the air crescent leads to the diagnosis of IPA in only a third (Gefter et al 1985). These two signs are usually associated with recovery of the neutrophil count.
Massive haemoptysisis is associated with CXR cavitation but mortality may be higher if this occurs without cavitation (Albelda 1985) and cavitation is a favourable prognostic sign in IPA (two-month survival of 67% vs 8% if absent) (Gefter et al 1985). In pulmonary mucormycosis, cavitation is seen in a quarter of cases and the air crescent less often (8%), but the latter sign is strongly associated with fatal haemoptysis (Lee et al 1989). Cavitation is reported infrequently in paediatric IPA (Thomas et al 2003).

2.3.2 CT Scans

CT (and CXR) findings progress with time and bone marrow recovery in IPA. “Early” CT findings in IPA are single or multiple nodules or mass like infiltrates and the halo sign (a pulmonary mass or nodule surrounded by a zone of attenuation less than at the centre of the mass, but greater than that of air in the surrounding lung). “Late” signs are cavitation, with or without the air crescent sign, which correspond to the CXR findings and are also associated with bone marrow recovery and haemoptysis (Kuhlman et al 1985; Potente 1989; Hauggaard et al 2002; Mori et al 1991; Kami et al 2002b; Kuhlman 1987; Bloom et al 1994; Caillot et al 2001).
Initial CT abnormalities in IPA progress to cavitation or air crescents in 1 to 2 weeks (mean 9 days) (Kuhlman 1987). In proven, probable or possible IPA the halo sign was reported in 73 to 100% of early scans (less than 10 days after the onset of symptoms), 0% of intermediate scans (7-15 days) and 23% of late scans (more than 10 days); cavitation was not seen in early scans but was seen in 25% of intermediate scans and 23 to 100% of late scans (Potente 1989; Blum et al 1994). The overlap in these defined time scales (<10days, 7-15 days and >10days) should be noted. The time scale of this replacement of the halo sign by cavitation has been confirmed by others (Caillot et al 2001) who also showed that use of CT in diagnosis, rather than in monitoring established IPA, resulted in an increase in the incidence of the halo sign from 13% to 92% of cases.

The incidence of the CT halo sign in IPA ranges from 22 to 95% (Kuhlman et al 1985; Kim et al 2001; Hauggaard et al 2002; Mori et al 1991; Kami et al 2002; Blum et al 1994; Primack et al 1994; Won et al 1998; Ribaud et al 1999) but these values are derived from very different studies which cannot be compared directly. Lower rates were seen in a setting of "late" IPA using neither standard nor high resolution CT (HRCT) routinely (Kuhlman et al 1985) and higher rates in studies using spiral CT (8 mm slices) a median of 10 days after the start of antibiotic-resistant FUO (Hauggaard et al 2002).

These progressive CT findings suggest that the sooner after the onset of symptoms the CT examination is undertaken, the greater will be the sensitivity of the halo sign in the first week of IPA when the CXR may be normal. In several studies the halo sign has been 100% specific for the diagnosis of IPA when the diagnosis was made during neutropenia or post-BMT (Mori et al 1991; Kami et al 2002; Blum et al 1994) but around 60% of the denominator group was defined by the presence of pulmonary nodules, rather than neutropenia (Primack et al 1994; Won et al 1998). In a retrospective analysis of 37 patients with proven or highly probable IPA, early CT scanning (defined as CT used in diagnosis, as opposed to monitoring established IPA) was associated with a significant reduction in mortality, presumed due to a reduction in time to diagnosis from 7 to 2 days and earlier antifungal therapy (Caillot et al 1997).

The sensitivity and specificity of CT cavitation and air crescent formation remain unclear as they are late manifestations of IPA, predicting clinical recovery, so that the efficacy of starting antifungal therapy late on the basis of these CT findings is less predictable.

Other causes of the halo sign are Wegener's granulomatosis, metastatic angiosarcoma and Kaposi sarcoma, none of which would be likely findings in febrile neutropenic HM patients. In the study where specificity was 57%, the halo sign was caused by mucormycosis and organizing (non-fungal) pneumonia, both of which would be likely in a neutropenic setting (Won et al 1998; Jamadar et al 1995).

Characteristic CT lesions may not be seen in early stages of IPA. If the clinical suspicion of IPA persists, repeat CT scans have been recommended but with little evidence of a minimal or best time interval between scans (Denning et al 2003; Berger 1998).

Becker et al (2003) used CT scanning early on suspicion of IPA to direct sampling of BAL fluid for GM-ELISA testing and reported 100% sensitivity and specificity in contrast to all other studies testing serum. This led them to perform CT scanning more frequently when they confirmed 90% specificity, 100% specificity, 100% PPV and 93% NPV of CT-directed BAL for GM-ELISA. All these figures were obtained before systemic antifungal treatment had been started and they were based on a total of 475 CT scans in a total of 160 patients, 111 of which had been performed in 17 patients. These are very high rates of CT scanning and radiation exposure which may be neither feasible nor desirable.

The studies described above have used a range of different CT criteria. In the more recent studies, in which the halo sign especially has been most closely associated with the diagnosis of IPA, high resolution CT (HRCT) has been specified. The whole thorax is usually scanned with thick (8-10 mm) slices and suspicious lesions rescanned with thin (1-3 mm) slices (Kim et al 2001; Mori et al 1991; Kami et al 2002; Caillot 2001; Primack et al 1994; Caillot et al 1997), although an alternative has been to scan with 1 mm slices at 10 mm intervals (Won et al 1998; Heussel et al 1999). Berger (1998) has recommended scanning suspect areas using a small field of view (approx. 15 cm diameter) and imaging with wide windows (1500-2000 Hounsfield units) and at a level where both air and soft tissue. can be seen to best advantage (approximately 500 Hounsfield units), with a radiologist in attendance.

Patients with haematological malignancy are at risk of the same range of fungal sinus infections as the general population, namely allergic fungal sinusitis, sinus mycetoma, chronic invasive fungal sinusitis (IFS), granulomatous IFS and acute (fulminant) IFS (Deshazo et al 1997a). Acute fulminant IFS may be caused by a range of fungi including Zygomycetes, *Fusarium* spp., *Scedosporium* spp. and *Aspergillus* spp. The radiological diagnostic finding of “air fluid levels” in IFS (Deshazo et al 1997b) is associated with other causes of sinusitis.

Sinus CT can detect abnormalities (eg nasal cavity soft tissue oedema, sinus mucoperiosteal thickening, bone erosion, orbital invasion, facial soft tissue swelling and periantral soft tissue swelling) not seen on plain x-rays (Dykewicz 2003), although CT findings in IFS are not disease-specific (Howells et al 2001). In immunocompromised patients with invasive fungal sinusitis unilateral, severe thickening of the nasal cavity mucosa was seen in 91%, bone erosion in 35% and air-fluid levels in 9% (DelGaudio et al 2003). In immunosuppressed patients with non-

fungal sinusitis, nasal cavity soft tissue thickening without bone erosion was seen in only 20%, and was not severe. The CT technique used both axial and coronal scans at intervals of ≤ 3 mm, with both bone and soft tissue windows. In post-BMT patients with rhinocerebral aspergillosis, CT findings of mucosal thickening, sino-nasal, soft tissue, hyperdense masses and uninvolved ethmoid cells among diseased cells were reported (Saah et al 2002). Bone destruction is a late sign associated with fulminant disease and brain invasion. CT and MRI findings lack specificity but provide supportive evidence of cerebral IFI in the context of other evidence (Dietrich et al, 2001).

Recommendations

Clinical features consistent with IPA, with or without supportive microbiological evidence and regardless of CXR appearances, justify an urgent chest CT scan (not plain CXR) because the earlier a CT scan is performed the more likely it is to show the early IPA-predictive halo sign (grade B, level III)

Where IPA is suspected, HRCT should be undertaken with slices of 1 mm at regular 1 cm intervals, or through suspicious lesions detected previously on CT (grade B, level III)

In patients with clinically suspected IPA and CT abnormalities (the halo sign, the air crescent sign or cavitation) compatible with IFI, antifungal therapy should be given (grade B, level III)

Patients with inconclusive clinical or microbiological evidence of IFI and negative, non-progressive nodular changes or non-specific initial CT findings, should have a repeat CT scan after no more than 7 days (grade B, level III)

Axial and coronal CT of the sinuses and surrounding structures should be undertaken immediately on clinical suspicion of sinus infection (grade B, level III)

If CT findings are suspicious, urgent otorhinolaryngological assessment is indicated, with surgical debridement and systemic antifungal therapy if indicated by clinical, microbiological or histopathological findings (grade B, level III)

Table 3; Investigation for SFI in HM Patients

Early and repeated access to CT scans of chest and head essential for high risk patients (chest X-ray of little value)
All bacteriological samples should be investigated for fungi in HM patients **Biopsy all suspected lesions whenever possible** **BAL may be superior to blood for ELISA and PCR** **BAL should be directed by CT** **Units dealing with high risk patients need** **a) rapid access to CT scans and** **b) routine fungal ELISA and/or PCR**

3 THERAPY

3.1 Empirical therapy

3.1.1 Original placebo-controlled empirical trials

Empirical therapy was widely adopted after two placebo-controlled RCTs in the 1980s (Pizzo et al 1982; EORTC 1989) which warrant detailed examination because of their lasting influence on clinical practice. Pizzo et al (1982) conducted a single centre study over a 4 year period of 50 paediatric patients with neutropenic FUO resistant to antibacterial therapy after 7 days. Patients were randomly assigned to stop antibacterials (16) or to continue them until neutropenia and FUO resolved (16) or to receive additional amphotericin B (0.5mg/kg/day intravenously (IV)) until neutropenia and FUO resolved (18). There was no difference between the second and third arms in the time to achieve the primary end-point of resolution of fever or to achieve the secondary end-point of resolution of both fever and neutropenia or in overall survival and infective and non-infective deaths. There were 5 proven IFIs (3

candidosis, 1 aspergillosis and one mixed candidosis/aspergillosis) in the non-amphotericin B arm (2 fatal) and one fatal mould infection in the amphotericin B arm. This difference was not statistically significant.

The EORTC trial (1989) was a multi centre comparison of the introduction of 0.6mg/kg amphotericin B IV versus no additional drug at 4 to 9 days of neutropenic FUO resistant to broad spectrum antibacterial therapy in adults. There was no difference in the primary end-point of resolution of fever by per protocol and intention to treat analysis but there were two significant results in retrospective non-stratified subset analysis. The primary end-point was achieved more often with additional amphotericin B in those with a documented bacterial infection at day 4 (22/29 vs 14/31; p=0.03) and in those patients who had not received any prior antifungal prophylaxis (21/27 vs 9/20; p=0.04). The difference in fungal deaths was not statistically significant (0/69 vs 4/64 (1 *C. tropicalis*, 1 *C. albicans*, 1 *Asp.* spp., 1 *Mucor*) ; p=0.05).

These trials were too small to provide sufficient statistical power, both used a low dose of amphotericin B, both used antifungal prophylaxis of dubious efficacy and neither proved the hypothesis of the primary end point. The association of the empirical use of amphotericin B IV with fewer IFIs in the paediatric study and in retrospective subsets in the adult study has been used to justify this practice subsequently and to avoid any further placebo-controlled trials of amphotericin B and all newer drugs.

3.1.2 Non-randomised studies of IV amphotericin B

Non-randomised studies of the use of amphotericin B in HM patients with FUO have produced conflicting results which are illustrated by two extreme examples. In 15 patients treated with a high dose of amphotericin B at 1.0 to 1.5 mg/kg daily plus flucytosine in 10 for presumed IA, 87% survived (Burch et al 1987), whereas in 21 patients given IV amphotericin B for proven IA the survival rate at one month was only 6% (Kaiser et al 1998).

3.1.3 Randomised controlled trials (RCTs)

Seventeen peer-reviewed, published reports of RCTs of empiric systemic antifungal therapy are relevant to febrile neutropenia, including the two trials described above.

The trials were designed to reflect the standard clinical practice of introducing systemic antifungal therapy at a set time when antibacterial therapy had not led to resolution of FUO. One trial is excluded from consideration as it compared amphotericin B and ketoconazole which is no longer used for this indication (Walsh et al 1991). The remaining 16 trials included 3446 patients and compared amphotericin B-deoxycholate with no therapy as described above (Pizzo et al 1982; EORTC 1989), with fluconazole (Marie et al 1993; Viscoli et al 1996; Winston et al 2000; Ellis et al 1995; Malik et al 1998; Silling et al 1999), itraconazole (Boogaerts et al 2001; Ehninger et al 2002) or with lipid formulation of amphotericin B (Prentice et al 1997; White et al 1998; Walsh et al 1999). Three trials compared liposomal amphotericin B with amphotericin B lipid complex (ABLC) (Wingard et al 2000), voriconazole (Walsh et al 2002) or caspofungin (Walsh et al 2004). One trial (Ehninger et al 2002) was published only as an abstract at the time of writing but the study protocol and detailed results were available. There are weaknesses in the methodological design of all of these trials.

3.1.4 Problems of RCT design

Only two of these trials were blinded (Walsh et al 1999, 2004), the minority of patients in each were 'high-risk' (>10 days neutropenia) and these patients were usually not analysed separately. Few trials stratified prospectively by risk or any other criterion at randomisation and many included paediatric patients for whom there was no stratification prospectively or in retrospective analysis. No more than 6% of treated patients had baseline IFI (present before therapy) confirmed retrospectively in any of these trials. There was arbitrary separation of baseline IFI up to the third day after starting empiric therapy from breakthrough IFI (i.e. resistant to therapy) after three days of therapy (including breakthrough cases the rate of proven IFI rises only to 10%).

Definition of a consistent primary outcome measure is difficult. While earlier studies restricted success to the resolution of fever, later a composite outcome score was used. In this composite score each of five criteria had to be met: survival for seven days after the end of study drug; resolution of fever during the period of neutropenia; successful treatment of any base-line fungal infection, if present; the absence of breakthrough fungal infections during administration of the study drug or within seven days after the completion of treatment; the absence of premature discontinuation of

the study drug because of toxicity or lack of efficacy (Walsh et al 1999; Walsh et al 2002; Walsh et al 2004).

There are important points of criticism of this score. All trials were designed to prove non-inferiority of efficacy of the new agent in curing or at least improving IFI. The very low rates of “proven” IFI in these trials suggest, therefore, that both drugs were equally unnecessary with no failures in treating an absent condition. Most patients in most studies were low-risk, therefore, the composite end point was influenced mainly by the resolution of fever and the toxicity of the agent.

Fever was the most important criterion for entry into these RCTs and resolution of fever was an unavoidable but unsatisfactory outcome measure because many other events cause fever and resolution of fever, including bacterial infections needing prolonged antibacterial therapy (Cometta et al 2003). Toxicity of the antifungal agent was important with the simultaneous use of other toxic therapies and when most patients had no proof of the targeted disease (IFI). More toxic events than IFI were therefore likely in these empiric RCTs and including toxicity in a composite endpoint might have suggested superior efficacy for a less toxic and less effective drug. Restriction of the analysis to resolution of baseline IFI and prevention of breakthrough IFI as primary outcomes might have been more useful, but more than 1000 patients would be needed to make such a trial sufficiently statistically powerful.

There is no evidence to support alternative approaches to empirical therapy for IFI despite these methodological problems. Drug authorisation agencies in the US and Europe have accepted the use of composite endpoint analysis with some additional strict criteria in trials designed for licensing (FDA 1992; FDA-ICH 2001; CPMP:EMEA 2003). The European Agency for the Evaluation of Medicinal Products (EMEA) requires that the anti-fungal drug has an *in vitro* spectrum of activity which covers all possible causative organisms, that trials of the proof of efficacy in the treatment of proven IFI should precede studies of empirical therapy, and that judgement of efficacy based on resolution of fever is untenable (CPMP-EMEA 2003). IFICG-MSG said that their definitions of level of certainty of IFI should not be applied in clinical practice (Ascioglu et al 2002) but these definitions are equally problematic in the design and conduct of RCTs. The difference between empirical treatment and treatment of a possible IFI is too subtle for the setting of trials and everyday practice. The acceptance of CT scans plus detection of fungal antigen, protein and DNA as

evidence of infection might increase the numbers of patients available to enter RCTs with proven or probable IFI.

3.1.5 Non-placebo controlled empirical RCTs

Six trials comparing fluconazole and amphotericin B deoxycholate in 799 patients found no difference in the primary study end point (resolution of fever in five, composite endpoint in one) (Winston et al 2000)).

Two trials compared intravenous itraconazole with amphotericin B deoxycholate in 522 patients (Boogaerts et al 2001; Ehninger et al 2002). The latter trial is not yet published therefore re-analysis of data from both trials combined demonstrating a superior response in the composite outcome score for intravenous itraconazole compared to conventional amphotericin B is not permissible as evidence of efficacy in this guideline.

Three trials compared amphotericin B deoxycholate to amphotericin B lipid formulation (liposomal amphotericin B in two (Prentice et al 1997; Walsh et al 1999) and amphotericin B colloidal dispersion in one (White et al 1998)). All trials used the composite outcome score as defined above and found no difference in efficacy. The trials of liposomal amphotericin B showed that this formulation reduces the risk of the acute febrile reactions and nephrotoxicity of amphotericin B deoxycholate. The first of these trials (Prentice et al 1997) suggested that escalation of dosage of liposomal amphotericin B from 1mg/kg/day to 3mg/kg/day might be beneficial but this has yet to be confirmed in a larger, blinded RCT, although there are reports of the efficacy of higher doses (Leenders et al, 1998).

Three trials compared liposomal amphotericin B to amphotericin B lipid complex (Wingard et al 2000), voriconazole (Walsh et al 2002a) and caspofungin (Walsh et al 2004). The first of these confirms the reduced risk of toxicity using the liposomal formulation. There is no satisfactory explanation of the wide variation in the rate of overall success between the liposomal amphotericin B arms in these three trials.

The failure of the trial with voriconazole to show non-inferiority of this drug generated much discussion (Powers et al 2002a; Powers et al 2002b; Walsh et al 2002a; Walsh et al 2002b; Walsh et al 2002c). The primary end point was composite with prospective stratification (by degree of risk of IFI, receipt or not of antifungal prophylaxis and duration of neutropenia) which would allow stratified analysis of each aspect of this composite outcome. The outcomes of all parts of the composite end-

point favoured liposomal amphotericin B. Stratified analysis of the primary composite outcome for degree of risk showed equivalence for high risk patients and no non-inferiority for moderate risk patients.

Rates of “breakthrough” IFI while on therapy were presented by stratification for degree of risk and for use of antifungal prophylaxis. In high risk patients there were significantly fewer breakthrough IFI with voriconazole ($p<0.003$). It is not clear whether prior prophylaxis had any effect on this outcome. In view of the arbitrary distinction between baseline and breakthrough IFI, this outcome could be a surrogate for prophylaxis as most patients at entry would not have had IFI.

Blood levels of orally administered voriconazole are variable and unpredictable, may not relate to efficacy, may be associated with elevated levels of liver enzymes if high, and there are no dose modification guidelines (Denning et al 2002; Walsh et al 2002a; Walsh et al 2002b; Johnson & Kauffman 2003). The FDA concluded that the risk of hepatotoxic reactions associated with voriconazole was no greater than that associated with any other systemic antifungal drug (2001). A validated HPLC assay is available but plasma levels are said not to predict abnormal liver function tests and plasma level monitoring is said to be unnecessary (Lutsar et al 2003). There is no explanation of how lower and higher levels of drug (<250 ng/ml, implying relatively poor bioavailability compared to those with levels >500 ng/ml) could be equally clinically effective while unresponsive patients with low levels might have improved responses on dose escalation (Denning et al 2002).

The largest ever RCT of empiric therapy compared liposomal amphotericin-B with caspofungin (an ecchinocandin). In a prior open label, single arm study of caspofungin, overall responses of 50% were seen in a mixed group of patients, mainly with haematological malignancy, who had been treated for proven or probable IA but were resistant to prior therapy. Only 5% had a complete response to caspofungin by intention to treat analysis (Maertens et al 2004b). The RCT of empiric therapy of presumed IFI (1095 patients) comparing caspofungin with liposomal amphotericin B (Walsh et al 2004) was double-blinded and stratified according to risk and for previous antifungal prophylaxis; outcome was judged by a five-part composite end-point. Overall success (OS) rates were equivalent (33.9% vs 33.7%) and caspofungin fulfilled the statistical criteria for non-inferiority. Adjustment for strata made no significant difference to this result. OS was significantly greater for caspofungin in base-line SFI (51.9% vs 25.9%, $p=0.04$) but these were only 5% of all

presumed IFI. Survival at 7 days was numerically greater (92.6% vs 89.2%, p=0.05) and premature discontinuation of therapy (10.3% vs 14.5%, p=0.03), nephrotoxicity (2.6% vs 11.5%, p<0.001) and infusion-related toxic events (35.1% vs 51.6%, p<0.001) were all significantly less with caspofungin. The rate of breakthrough IFI and resolution of fever were similar for the two drugs.

The data suggest that the majority of patients who receive empirical systemic antifungal therapy do not have an IFI. The choice of drug in empirical practice depends on anticipated toxicity, the ability of the patient to cope with this, potential drug interactions or impaired drug metabolism and the cost of drug acquisition. Despite its low acquisition cost and the lack of clearly superior efficacy of any other drug, the significantly greater toxicity (and therefore secondary costs) of IV amphotericin B deoxycholate suggest that safer lipid-based alternatives should be used for empiric therapy (Personn et al 1992; Boogaerts et al 1996). Caspofungin appears to reduce the risk of toxicity further (Walsh et al 2004). The proportion of patients treated needlessly will vary with the level of risk of IFI in a given population (Prentice et al 2000; McLintock et al 2004), the level of certainty of diagnosis of IFI (EORTC 1989; Heussel et al 1999; Ascioglou et al 2002), and the use of effective antifungal prophylaxis (Glasmacher et al 2003; Bow et al 2002). IFICG-MSG said that their definitions of level of certainty of IFI should not be applied in clinical practice (Ascioglu et al 2002) but these definitions are equally problematic in the design and conduct of RCTs.

Maertens et al (2005) replaced empiricism with therapy guided by CT and galactomannan results and thus halved the use of amphotericin B with no greater IFI-related mortality than that seen in large RCTs of empiric antifungal therapy (Walsh et al 1999, 2002, 2004).

3.1.6 Non-trial evidence to support empirical therapy

Most therapy for IFI is still empirical. Delaying antifungal therapy until diagnosis is proven increases the risk of death (Verweij et al 1994). Autopsy studies have shown that about 15% of SC infections are not diagnosed in life (Bodey et al 1992), diagnosis of IA by culture is rare, biopsies are difficult to obtain and established IA is rapidly fatal in most cases (Lin et al 2001).

3.2 Therapy for proven/probable IFI

Although the growth of yeasts (and rarely moulds) in blood culture is an indication for systemic antifungal treatment (Anaissie et al 1998; Ascioglu et al 2002), few published RCTs of the treatment of proven IFI in HM patients have been adequately powered to show superiority of any agent over another, none have been placebo controlled and some include patients whose SFI was not proven.

Four RCTs compared different ways of using amphotericin B.

In the first of these 28 patients with microbiologically or histologically proven IFI were randomised to 0.5 mg/kg of amphotericin B alone per day or the same plus 150mg/kg of flucytosine per day (Verweij et al 1994). The duration of neutropenia ($<0.5x10^9$/l) was 18 and 20 days respectively and only 2 and 3 patients respectively survived the SFI. This dose of amphotericin B is inadequate if used alone and most patients were probably treated late in their infection.

In the second of these trials there were no differences in response in 103 patients with proven IA post-allogeneic ASCT, randomised to amphotericin B or amphotericin B colloidal dispersion (ABCD) (Bowden et al 2002).

The third in this group was an EORTC study in which 87 patients with HM, neutropenia and proven IA were randomised to 1 mg/kg or 4 mg/kg of liposomal amphotericin B (Ellis et al 1998). The clinical response (64% v 48%), radiological response (58% v 52%) and survival at 6 months (43% v 37%) were all significantly superior in the lower dose arm but the sample size does not support these conclusions.

In the fourth trial in this group 66 neutropenic patients received either amphotericin B deoxycholate (1mg/kg IV daily) or liposomal amphotericin B (5mg/kg IV daily) (Leenders et al 1998). Forty had suspected IA, 9 had proven fungaemia and 17 had proven invasive mould infections. The complete response rate was higher (44% vs 18%, p=0.03) and the mortality rate lower (p=0.03) in the patients given liposomal amphotericin B. This study's conclusions were weakened by too many unproven infections, a small sample size and the need to exclude deaths unrelated to IFI, but it did suggest that a relatively high dose of liposomal amphotericin B may be superior to conventional amphotericin B and nephrotoxicity was significantly less ($p<0.001$) with the liposomal preparation.

In two other RCT amphotericin B was compared with a different class of drug. In the first of these trials van't Wout et al (1991) randomised 32 neutropenic ($<0.5x10^9$/l)

patients to receive either amphotericin B (0.6mg/kg alone or 0.3mg/kg with flucytosine IV daily) or itraconazole (200mg orally twice daily) for a median of 20 days**.** There were no significant differences in any measure of outcome but the sample size was too small, amphotericin B was under-dosed and concomitant flucytosine was used in a non-randomised and unstratified way in the amphotericin B arm.

In the second of these trials 391 patients were randomised to receive either voriconazole or amphotericin B deoxycholate at a dose of 1.0 to 1.5mg/kg IV daily (Herbrecht et al 2002). Neutropenia was defined as “at some point” (e.g. 1 day only) in the fourteen days prior to entry to the study and patients were included if at least one dose of test drug was given. If diagnostic criteria were insufficient or if neutropenia or an immunocompromised state were not documented patients were excluded retrospectively and independently. A modified intention to treat (MITT) analysis of 277 patients was reported.

More patients with proven IA were randomised to voriconazole (67 vs 41, $p<0.01$). Patients were randomised separately to two parallel protocols (150-602 and 150-307). Those entering 150-602 were more likely to have had a SCT (38.3 vs 15.3%, $p<0.001$), more likely to have GVHD (29 vs 9.4%, $p<0.001$), more likely to have proven IA (47.7 vs 33.5%, $p<0.02$) and less likely to have neutropenia (26.2% vs 55.9%, $p<0.001$). The median duration of treatment with voriconazole was 77 days and with amphotericin B 10 days. Other licensed antifungal drugs were given to 52 patients (36.1%) in the voriconazole group and to 107 patients (80.5%) in the amphotericin B group. The treatment in the amphotericin B arms was interrupted frequently in the first 14 days of the trial and there were major reductions in dose (0.27 mg/kg the lowest reported).

Overall success (OS) was defined as a partial or a full response. At 12 weeks significantly more patients in the voriconazole arm versus the amphotericin B arm had an OS outcome (52.8% vs 31.6%, 95% CI 10.4 to 32.9%), complete responses did not differ significantly (20.8% vs 16.5%) and the difference in partial responses (31.9% vs 15%) determined the difference in OS. Retrospective stratification by diagnostic criteria, site of infection, underlying disease or neutropenia did not change these overall conclusions. Stable disease and indeterminate outcome did not differ between the two arms and survival was significantly better in the voriconazole arm (70.8% vs 57.9%, hazard ratio 0.59, 95% CI 0.4 to 0.88). It is unclear whether the

differences in OS and survival were due to a superior effect of voriconazole, to inadequate dosing of amphotericin B, to less IA in the amphotericin B arm or to any combination of these three factors.

Voriconazole may be the drug of choice in the treatment of proven or probable intracerebral aspergillosis because of its good CNS penetration (Schwartz et al 2005).

Breakthrough IFI have been reported in SCT patients with voriconazole-resistant fungi during treatment of IA but only in one series and in small numbers (13/139 isolates; including 6 zygomycetes and 4 *Candida glabrata*) (Imhoff et al 2004). This risk has not been confirmed in other studies.

In the latest study reported on therapy for proven or probable IFI (filamentous infections) Cornely et al (2005) failed to show increased efficacy of initially higher doses of liposomal amphotericin B in a RCT of empiric therapy comparing 10mg/kg/d for 14 days followed by 3mg/kg/d (n=94) with 3mg/kg/d from the outset (n=107). This study showed no difference in the rate of favourable response at the end of treatment but a “non-significant” reduction in overall survival with the higher dose (59% vs 72%) presumably related to significantly greater nephrotoxicity (31% vs 14%; $p<0.01$) and hypokalaemia (30% vs 16%; $p<0.02$). The overall response rate does not appear to be better than reported in previous trials of conventional amphotericin B.

3.3 Combination therapy

Published reports to date are retrospective analyses of the use of the combinations of antifungal agents. In patients whose proven or probable IA was resistant after 7 or more days of IV conventional or lipid amphotericin B, 31 were given voriconazole and 16 voriconazole plus caspofungin (Marr et al 2004a). At 3 months from date of diagnosis and from start of salvage IFI therapy the survival was significantly better in the combination patients. On later analysis at 1 year however there was no difference in overall survival.

A retrospective comparison was made of 179 HM patients who had been given lipid amphotericin (101 evaluable) or that plus IV or oral solution of itraconazole (11 evaluable) and there was no difference in mortality rates in the two groups regardless of prior azole prophylaxis (Kontoyannis et al 2005).

The outcomes for first-line combined amphotericin B plus caspofungin (n=17) were compared with those for progressive IA (n=31) but about half of the cases in each

group were only possible IA (Kontoyannis et al 2003). Response rates were low in the cases of proven or probable IA (3/17 in the progressive IA group and 2/6 in the primary therapy group).

There are several other meetings abstracts of retrospective and non-randomised prospective studies of small numbers of patients with variable results. These studies suggest that combination therapy may have a place, but few show significant advantages for combinations and there is a need for large, randomised prospective trials in patients with proven and probable IA in both the primary and the resistant setting.

3.4 Duration of therapy and switching to oral therapy on discharge from hospital

There is insufficient evidence to make a recommendation on the duration of therapy and this will vary greatly between individual patients. Nor is there sufficient evidence to make a recommendation on the common practice of switching therapy from an IV drug to an oral triazole following discharge from hospital. For mould infections, there is no evidence which favours the use of any one of itraconazole, voriconazole or posaconazole in this context, other than the very large amount of data from pharmacokinetic (PK) studies in favour of the effective bioavailability of oral itraconazole in a prophylaxis context (Prentice et al 1993, 1994; Glasmacher et al 1996, 1999a, 1999b, 2003).

3.5 “Second-line” or “salvage” therapy

There may be a perceived need to change the drug first chosen for empirical treatment or for treatment of a proven IFI when response to that drug is thought to be inadequate or its toxicity is unacceptable. There is little to choose between the reported response rates in this context for amphotericin B lipid complex (42%) (Walsh et al 1998), voriconazole (38%) (Denning et al 2002), caspofungin (45%) (Maertens et al 2004) and posaconazole (42%) (Walsh et al 2007). The design of these trials is so heterogeneous that it is not possible to make a recommendation on the basis of their evidence.

Recommendations

Empiric antifungal therapy for episodes of febrile neutropenia which are resistant to antibacterial drugs is not of proven efficacy and should be discouraged (grade A, level Ib)

The need for systemic antifungal therapy should be confirmed by CT scans and mycological testing for fungal wall components (GM and /or BG) in blood or BAL. If these are non-confirmatory, empirical therapy may be unnecessary and could be avoided or stopped. It is unclear whether PCR detection of fungal DNA is as reliable as fungal antigen tests (grade B, level IIa)

If empirical antifungal therapy is given it is desirable to minimise the toxicity of this therapy since the majority of patients never have IFI confirmed. Therefore the choice of empirical therapy is between liposomal amphotericin B (but not in escalated initial doses) and caspofungin, the latter having the superior (ie lower) toxicity profile (grade A, level Ib)

Amphotericin B dexycholate is not recommended for the eradication of proven or suspected IFI because of its unacceptable toxicity (see table 4) (grade A, level Ib)

In proven or probable CNS IFI voriconazole is recommended because of its superior CNS penetration (grade A, level Ib)

Combination therapy in primary or resistant IFI is not of proven efficacy and should be discouraged (grade A, level Ib)

Table 4; Recommended therapy

Agent	Indication
Amphotericin-B dexoycholate	**Not recommended because of avoidable toxicity**
Liposomal amphotericin-B	**Empirical and proven IFI to reduce acute febrile reactions and nephrotoxicity of conventional form**
Caspofungin	**Empirical and proven IFI; least toxic choice**
Voriconazole	**Restrict to proven IFI where specific sensitivity is greatest to this agent. Intracerebral aspergillosis**

4 PROPHYLAXIS

4.1 Meta-analyses of Prophylaxis RCTs.

There have been over fifty RCTs of antifungal prophylaxis in neutropenic patients and these are best summarised by the following systematic (Gotzsche & Johansen 1997; Kanda et al 2000; Bow et al 2002; Gotzsche & Johansen 2002; Glasmacher et al 2003; Prentice et al 2005; Vardakas et al 2005) and narrative (Cornely et al 2003) reviews.

Gøtzsche and Johannsen published the first of these (1997) and it is now available largely unchanged in the Cochrane Library. This systematic review demonstrated a statistically significantly reduced risk of IFI with the use of amphotericin B (relative risk 0.39, 95%CI 0.20-0.76), fluconazole (0.39, 0.27-0.57) and itraconazole (0.51, 0.27-0.96**)** but not with ketoconazole and miconazole. The authors concluded that intravenous amphotericin B "should be preferred when prophylactic or empiric therapy in cancer patients with neutropenia is considered". The other key points in this review are as follows.

Trials were included only if they compared an antifungal agent (amphotericin B, ketoconazole, miconazole, fluconazole and itraconazole) to placebo or no therapy and were excluded if they compared an active antifungal substance to oral polyenes which are poorly absorbed, affect only gut colonisation or infection, may have no

significant or direct effect on systemic infection, and could be regarded as a surrogate placebo.

Three trials that compared intravenous amphotericin B to no intervention in patients with FUO unresponsive to several days of antibacterial therapy (Pizzo et al 1982; EORTC 1989; Goldstone & O'Driscoll 1994) were included; these trials of empirical therapy differ significantly in intent and design from trials of prophylaxis and should be excluded from any analysis of prophylaxis. All oral drugs were assumed to deliver active therapy and included one trial of amphotericin B (not absorbed), two trials of miconazole (no clinically relevant systemic activity against IFI) and eight trials of ketoconazole (only limited activity in IFI and considerable side effects). A sensitivity analysis was not performed to detect methodological, clinical or pharmacological influences on the results of the meta-analysis**.**

In 2000 Kanda et al published a meta-analysis on 16 trials with fluconazole and found a significant reduction of the overall incidence of invasive fungal infections, but not of IA infections and invasive infections by non-*albicans Candida* spp. Analysis of these trials by the dose of fluconazole and the patient group showed that a statistically significant treatment effect was seen only with 400 mg or more of fluconazole daily, only in patients after allogeneic SCT and only where there was a high base-line rate of candidal IFI and was not seen in neutropenic patients given chemotherapy for acute leukaemia.

The meta-analysis of Bow et al. (2002) included most trials of all azoles available at the time of writing (fluconazole, itraconazole, ketoconazole and miconazole) and intravenous amphotericin B as active substances and oral polyenes or no treatment as control group. Ten trials with ketoconazole and two trials with miconazole were included. The key points were as follows. Azoles significantly reduced IFI and IFI-related mortality, but not that due to invasive *Aspergillus* infections. Significant reduction of IFI was achieved by fluconazole, itraconazole, ketoconazole and low-dose amphotericin B. Fungal infection-related mortality was reduced only by fluconazole. All trials of head to head comparison of itraconazole with fluconazole were excluded from this meta-analysis despite the known difference in their activity against *Aspergillus* and some non-*albicans Candida* spp.

Glasmacher et al published a meta-analysis on the prophylactic use of only itraconazole in these patients (2003). The key points were as follows. The risk of IFI was reduced only at doses equivalent to at least 200 mg/d of bioavailable drug and

the higher dose group (i.e. doses of at least 400 mg/d oral solution or 200 mg/d intravenous solution) had significant reductions in proven or probable IFI risk was reduced by only 53%, fungal infection-related morality was reduced by only 42%, the risk of invasive *Aspergillus* infections was reduced by only 48%, the risk of invasive yeast infections was reduced by only 57% and 53% respectively for *C. albicans* and non-*albicans C.* spp. The effectiveness of the antifungal prophylaxis did not vary between trials in patients treated for acute leukaemia with myelosuppressive chemotherapy and in patients receiving allogeneic haematopoietic stem cell transplantation. This meta-analysis implies that monitoring of itraconazole levels may be required where it is critical to sustain bioavailability in high risk patients, although PK studies have shown that loading and maintenance dosage can achieve this in the majority of patients (Glasmacher et al 1999a; Prentice et al 1995; Prentice et al 1994). The outcomes of the most recent comparison of itraconazole versus fluconazole prophylaxis, showing equivalent efficacy and safety (Glasmacher et al 2006), are unlikely to influence the conclusions of the above meta-analysis.
Vardakas et al (2005) reanalysed a selection of those itraconazole versus fluconazole RCTs previously analysed by Glasmacher et al (2003) and concluded that fluconazole was the drug of choice for prophylaxis of IFI in HM patients. Prentice et al (2005/6) reanalysed the data presented by Vardakas et al and confirmed the superiority of itraconazole if given in a formulation providing greater bioavilability.
Cornely et al (2003) assessed primary antifungal prophylaxis in patients with HM from 50 RCTs without meta-analysis so that recommendations were based on whether single trials achieved statistical significance. They concluded that there was no indication to use any kind of antifungal prophylaxis but most single trials cannot achieve adequate statistical power to detect a significant difference and this problem can only be overcome by meta-analysis. Statistical evaluation of meta-analysis is valid if methodological and clinical requirements for combining the data from trials are observed (Egger et al 1997).

4.2 Newer prophylactic drugs

There are few publication on newer agents as prophylaxis. In one blinded RCT in 882 adult and paediatric HSCT patients (autologous, syngeneic and allogeneic) micafungin was compared with fluconazole (van Burik et al 2004). This was said to show superior overall efficacy of micafungin. There were no significant differences in

candidaemia (4 vs 2), proven IA (0 vs 4), probable IA (1 vs 3) or the use of systemic antifungals after the end of the study period (until engraftment plus 4 weeks). The superiority claimed for micafungin depended on the differences in suspected IFI and in “treatment failure” (64 vs 98, p=0.024; 78 vs 112, p=0.026). This result is of limited value because in RCTs of empirical treatment less than 6% of patients have subsequently proven IFI, suspected or “possible” IFI are unsatisfactory end-points and most cases of IA in SCT occur well beyond the end of the study period. An open label randomised trial of caspofungin versus itraconazole during induction chemotherapy for a total of 99 patients with AML or MDS showed no difference in efficacy (Mattiuzzi et al 2006). No comparitive prophylaxis trials using voriconazole or ravuconazole have been completed.

Ullmann et al (2007) compared posaconazole (n=301) with fluconazole (n=299) as prophylaxis of IFI in patients post-SCT with severe graft-versus-host disease (grade I/II acute GVHD 138 vs 137; extensive/chronic 99 vs 100). The post-SCT study end-point period was 112 days when there was no significant difference in all proven and probable IFI (16 vs 27) or in overall deaths (76 vs 84) so superiority was not demonstrated; but a significant difference was seen in the rate of IA (7 vs 22; p=0.004) and in the mean days to proven or probable IFI (102 vs 88; p=0.048). On more detailed analysis of treatment-related adverse events and all-cause mortality, in the posaconazole arm there were significantly fewer deaths as a complication of infection or due to proven or probable IFI but the numbers in this analysis were small.

Cornely et al (2007) compared posaconazole in the same dose as in the previous study (n=304) in a RCT against “standard” prophylaxis (flucanozole, n=240 or itraconazole, n=58; centre’s choice not randomised) for IFI in patients with acute myeloblastic leukaemia or myelodysplastis syndrome expected to be neutropenic ($<0.5x10^9$/l) for at least 7 days. During the treatment period there were significantly fewer cases of all proven or probable IFIs (7 vs 25; p<0.001) and of proven and probable invasive aspergillosis (2 vs 20; p<0.001) in the posaconazole arm. Probability of IFI or death were significantly lower in the posaconazole arm over 100 days (p=0.003 and 0.04 respectively). It is not possible to determine the impact of the use of IV amphotericin B as substitute prophylaxis when patients were unable to take oral posaconazole. In the other arm of the study the IV formulations of fluconazole or itraconazole were used for the same indication.

The design of this trial does not allow a three way randomised comparison between the drugs used, only 20% of patients in the “standard” arm received itraconazole and the spectra of activity of itraconazole and fluconazole differ significantly, therefore the conclusion that posaconazole is superior to both fluconazole and itraoconazole is open to question. The results tend to support those of the trial described above indicating posaconazole’s predictable superiority over fluconazole in the prevention of aspergillosis; however there were no proven cases of invasive aspergillosis in the itraconazole patients versus two in the posaconazole arm illustrating further that no useful statistical comparison of these two drugs can be drawn from the results of this study.

4.3 Combined prophylaxis

There is little evidence to support combined prophylaxis. No antagonistic effect has been seen in clinical studies when amphotericin B has been given to patients who had been taking itraconazole prophylaxis (Boogaerts et al 1989; Schaffner & Bohler 1993; Thunninssen et al 1991). In one study the mortality of IFI was lower in those patients who started amphotericin B with higher itraconazole plasma levels (Boogaerts et al 1989). *In vitro* data suggest antagonism of amphotericin B by itraconazole and fluconazole, since the triazoles block synthesis of fungal ergosterol which is the main target for the former polyene (Schaffner & Bohler 1993) but this has not been confirmed *in vivo*.

4.4 IV Amphotericin as prophylaxis

Of the two randomised, double-blinded, placebo-controlled trials of IV low dose (0.1mg/kg/d) of conventional amphotericin B deoxycholate as prophylaxis one was too small to provide a useful answer (Riley et al 1994) and the other showed no benefit (Perfect et al 1992). Two open randomised trials showed that IV amphotericin B was more toxic than fluconazole and no more effective (Wolff et al 2000; Bodey et al 1994).

Four trials of various forms of lipid-associated amphotericin B versus either placebo or combined fluconazole and itraconazole or fluconazole alone showed no benefit from the polyene and in one of these trials marked excess toxicity due to amphotericin B (Tollemar et al 1993; Kelsey et al 1999; Timmers et al 2000; Mattiuzzi et al 2003).

4.5 Toxicity of triazoles

The triazoles have some significant toxicities which are largely due to their binding to cytochrome P450 resulting in competitive metabolism with other drugs. The resultant clinical interactions have been summarised for itraconazole by Glasmacher et al (1996). All azoles can potentially increase levels of ciclosporin and tacrolimus but the levels of these drugs are regularly monitored and doses can be adjusted to compensate. Itraconazole should not be used if significant plasma levels are present with co-administration of vinca alkaloids because of the risk of potentiation of severe neurotoxicity of the latter. Potentially dangerous interactions may occur with the antihistamines, terfenadine and astemizol, so these are best avoided and acute allergic reactions to blood products can be treated with low doses of IV pethidine instead.

One important interaction has been described in allogeneic SCT patients by Marr et al (2004 b&c). In a RCT of prophylaxis of IFI comparing itraconazole with fluconazole, the patients given itraconazole had higher levels of serum bilirubin and creatinine in the 20 days after SCT with the highest levels in those given concomitant cyclophosphamide. Some of the itraconazole patients were also found to have higher exposure to toxic metabolites of cyclophosphamide. Survival was reduced initially in the itraconazole arm. There was no longer any significant difference between these toxicities or survival when the protocol was amended to delay the start of itraconazole until the day of stem cell infusion. On ITT analysis fewer patients in the itraconazole arm developed IFI on prophylaxis than in the fluconazole arm (7% vs 15%; $p<0.03$). More withdrew from the itraconazole arm because of gastrointestinal (GI) intolerance (36% vs 16%; $p<0.001$) which is the major toxicity of the oral cyclodextrin solution of this drug.

This study illustrates the difficulty of calculating the risk-benefit ratio when deciding whether to use antifungal prophylaxis. The GI problem can be avoided, however, by giving IV drug and the doses of itraconazole in this trial were unusually high at 2.5mg/kg three times daily orally, possibly unnecessarily according to PK studies (Glasmacher et al 1999a; Prentice et al 1994; Prentice et al 1995). With the exception of hypokalaemia (for itraconazole) and nausea (for oral solutions of fluconazole and itraconazole, not for capsules) no significant adverse events have been noted in the meta-analyses of 1,777 patients treated with fluconazole (Kanda et

al 2000) and 1,812 patients treated with itraconazole (Glasmacher et al 2003). No clinically significant renal, liver or cardiac toxicity was noted in any trial other than that of Marr et al (2004).

All azoles in use in current practice interact in some way with other drugs through the CYP cytochrome system of drug metabolism. These interactions are reviewed in detail by Lewis (2006) and physicians using these drugs should be familiar with potential interactions most of which can be managed with careful monitoring.

4.6 Patient selection for antifungal prophylaxis

The need for prophylaxis against IFI depends on the predicted risk. Several authors have presented risk assessment categories (Uzun & Anaissie 1995; Prentice et al 2000; Mahfouz & Anaissie 2003; O'Brien et al 2003). The highest risk patients are those given an allogeneic SCT, especially those who develop GVHD and require protracted steroid therapy (Jantunnen et al 1997; Martino et al 2002) and these patients may benefit from antifungal prophylaxis. There is consensus that patients with a low risk of an invasive fungal infection, such as those with lymphoma given standard chemotherapy, should not routinely receive antifungal prophylaxis. Patients who have received autologous peripheral stem cells are not considered to be at high risk unless they have recently had T-cell suppressive purine analogues or total body irradiation (Ketterer et al 1999; Offidani et al 1999; Reich et al 2001; Toor et al 2001; Auner et al 2002). Those patients with acute leukaemia and who will have protracted, cumulative neutropenia and cumulative immunosuppression due to chemotherapy are described as intermediate high risk. There is some evidence that they too may benefit from prophylaxis (Glasmacher et al 2003) but there is no concensus on this.

4.6.1 Secondary Prophylaxis

The current consensus view in practice is that patients who have had a probable or proven IFI during prior intensive therapy (eg chemotherapy for acute leukaemia) will benefit from systemic antifungal prophylaxis if they are to have further intensive therapy (eg SCT). The published evidence for this view is based on only two small, single arm, prospective studies, one using IV conventional amphotericin B in 16 patients (Karp et al 1988) and one using voriconazole in 11 patients (Cordonnier et al 2004) and one report from a multinational case registry of the use of caspofungin in 31 patients (Cornely et al). Previous IFI was proven in a minority and the recurrence rate of IFI was low in all three reports.

4.7 Duration of antifungal prophylaxis

Routine primary antifungal prophylaxis during conventional chemotherapy for acute leukaemia has been restricted to the duration of neutropenia. There is no evidence in this clinical setting that it needs to be continued for longer. This recommendation does not apply to secondary prophylaxis (i.e. prophylaxis in patients who have previously suffered from an invasive fungal infection) where prolonged use may be needed.

In SCT patients several studies have shown that invasive fungal infections occur up to and beyond 180 days after transplantation (Wald et al 1997; Baddley et al 2001; Grow et al 2002; Marr et al 2002). These patients should therefore receive prophylaxis for at least 100 days, and, if still immunosuppressed at that time (e.g. high-dose corticosteroids for graft-versus-host disease), antifungal prophylaxis should be continued as long as is necessary. Such prolonged prophylaxis has been successfully used in two randomized controlled trials in this setting (Winston et al 2003; Marr et al 2004). There is at least a theoretical protracted risk of IFI in patients given nucleic acid analogues of a potency or in doses sufficient to induce T-cell suppression as seen in protracted GVHD post-SCT or AIDS.

4.8 Dose intensity of triazole prophylaxis.

The meta-analysis of Glasmacher et al (2003) and other studies (Glasmacher et al 1999a) have shown that adequate dosing is necessary to ensure efficacy. At least 400 mg/d oral solution or 200 mg/d intravenous solution are needed for a bioavailable dose of at least 200 mg/d. Several *in vitro* (Manavathu et al 1998; Odds et al 1998; Johnson et al 1998) and *in vivo* (Berenguer et al 1994) trials and evaluation of clinical data (Glasmacher et al 1999b; Rex et al 1997; Poirier & Cheymol 1998) have demonstrated a clear dose-response relationship. A loading dose (e.g. 800 mg/d capsules for seven days or 400 mg/d intravenous solution for two days) can achieve a steady state quickly (Glasmacher et al 1999a).

Bioavailability of itraconazole from capsules may be low and unpredictable. A trough level (determined by HPLC) of at least 500 ng/ml should be reached within the first week of prophylaxis. The dose of itraconazole should be reduced if the trough level is above 2000 ng/ml although trough levels as high as 4000 ng/ml are seen without toxicity. An important advantage of having an intravenous formulation of itraconazole

is the possibility of switching between formulations according to the patients ability to tolerate oral medications.
There are insufficient data to determine if there could be a similar dose-response relationship with voriconazole.

4.9 Protective isolation and ventilation

Early prospective, randomised trials of isolation and decontamination of air did not show improved overall survival (Rodriguez et al 1978; Buckner et al 1978) but repeated subsequent studies have shown that the introduction of nursing at risk patients in rooms ventilated by laminar air flow (LAF) or HEPA filtration reduces significantly both *Aspergillus* spore counts and also cases of IA (Rhame et al 1984; Sherertz et al 1987; Loo et al 1996). In one retrospective multicentre study of SCT patients (matched/related and unrelated donors) the use of LAF or HEPA filtration was associated with a significantly higher one year survival rate and a decreased mortality rate at 100 days post-SCT (Passweg et al 1998). Two further studies of SCT patients have failed to confirm these benefits (Russell et al 2000; Svahn et al 2002).

Recommendations

HM patients at high risk for IFI should receive antifungal prophylaxis with itraconazole. Posaconazole may be equally effective but this has not yet been proven by RCT (grade A, level Ia)

All azole drugs should be avoided when there is a risk of serious drug interaction; the apparently exclusive risk of fatal interaction of itraconazole with vincristine does not exclude a class effect mediated by liver microsomal metabolism. There is a potential role for intermittent IV amphotericin B in this context but further data are needed (grade B, level IIa)
A loading dose of 800 mg/d capsules for seven days or 400 mg/d intravenous solution of itraconazole for two days is necessary to achieve steady state and thereafter a daily dose of at least 400 mg/d oral solution or 200 mg/d intravenous solution should be used to maintain the trough plasma level above

500 ng/ml. Levels should be measured weekly in high risk patients (grade B, level IIa)

Secondary prophylaxis may be effective for patients who have had a prior proven IFI (grade C, level IV)

It is not possible to recommend an exact duration of prophylaxis for all patients because of the multifactorial nature of severe immunosuppression. In patients treated for acute leukaemia with chemotherapy, prophylaxis should continue until the neutrophil count exceeds $0.5x10^9$/l; in SCT patients prophylaxis should continue if patients remain at high risk of IFI (grade B, level III)

Azoles need not be stopped when starting IV amphotericin B or any other systemic antifungal agent (grade B, level III)

No recommendation can be made on protective isolation and decontamination of air because evidence for the efficacy is conflicting (grade B, level III)

4 SURGERY

Effective and safe surgical resection of aspergilloma is well documented in non-randomised retrospective reports of carefully selected patients without HM or known immunosuppression (Babatasi et al 2000; Regnard J-F et al 2000). The extent of the surgical resection was very variable and there was a risk of failure of re-expansion of the lung if there was residual infection. Invasive aspergillosis may not respond completely to systemic antifungal chemotherapy. This may compromise the successful outcome of therapy for HM or prevent or delay necessary progression from remission induction chemotherapy to potentially curative SCT. Retrospective, non-randomised reviews also suggest that surgical resection of lung tissue suspected of harbouring residual IA is feasible in such HM patients who can then proceed to further cytotoxic therapy without recurrence of IPA (Pidhorecky et al 2000; Yeghen et al 2000).

Recommendation

Patients with resectable IPA should be considered for surgery if that would either improve the overall outcome of the therapy for their HM or allow them to proceed to further cytotoxic therapy (grade B, level III)

5 GROWTH FACTORS

A single arm study of recombinant M-CSF in 24 SCT patients with IFI reported a survival of 27% compared to 5% in historical controls (Neumanitis 1991) but this advantage was not confirmed in a subsequent placebo-controlled RCT of prophylactic molgrastim (GM-CSF) in allogeneic SCT patients (Neumanitis 1995). In a prospective RCT of filgrastim (G-CSF) versus no growth factor during intensive consolidation chemotherapy for AML the only benefit for IFI was a significant reduced of the median duration of antifungal therapy but no reduction in documented IFIs (Harousseau et al 2000).

Recommendation

Current evidence does not support use of growth factors as either prophylaxis or supportive therapy of IFI (grade B, level III)

Acknowledgements and declarations of interest

The authors have completed BCSH declaration of interest forms which have been reviewed by the Chair of the Task force and the Chair of the BCSH and have not been deemed to represent a conflict of interest. Task force membership at time of writing this guideline was Dr Simon Rule (chair), Dr Patrick Carrington, Dr Graham Jackson, Dr Andrew McMillan, Dr Anne Parker, Dr Andrew Pettitt, Dr Jonathan Cullis and Dr Jennie Wimperis

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