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Therapeutic drug monitoring (TDM) of antifungal agents: guidelines from the British Society for Medical Mycology

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The burden of human disease related to medically important fungal pathogens is substantial. An improved understanding of antifungal pharmacology and antifungal pharmacokinetics–pharmacodynamics has resulted in therapeutic drug monitoring (TDM) becoming a valuable adjunct to the routine administration of some antifungal agents. TDM may increase the probability of a successful outcome, prevent drug-related toxicity and potentially prevent the emergence of antifungal drug resistance. Much of the evidence that supports TDM is circumstantial. This document reviews the available literature and provides a series of recommendations for TDM of antifungal agents.

Keywords: triazoles, fungal pathogens, fungal diseases

Introduction

Fungal diseases exact a significant toll on human health and compromise clinical outcomes of patients. There has been a progressive understanding of antifungal pharmacology and characterization of antifungal drug exposure–response relationships. There is increased recognition that therapeutic drug monitoring (TDM) of antifungal agents is important in a wide range of clinical settings.[1–4] This document reviews the available literature and provides recommendations for antifungal TDM.

The three main classes of antifungal agents in clinical use are the polyenes, the triazoles and the echinocandins. The polyenes have a broad spectrum of activity that includes yeasts and moulds. For the triazoles, susceptibility is more variable and depends on the specific agent. Fluconazole has no activity against *Aspergillus* spp. and the mucoraceous moulds, while voriconazole lacks activity against the mucoraceous moulds. Posaconazole has the broadest spectrum of activity for all the triazoles, including activity against *Aspergillus* spp. and the mucoraceous moulds. The echinocandins are active against most medically important species of *Aspergillus* and *Candida*, but lack activity against *Cryptococcus*, *Fusarium* and the mucoraceous moulds. The key pharmacokinetic properties of each agent are summarized in Table S1 (available as Supplementary data at *JAC* Online).

Patients at risk of systemic fungal infections are varied and include those with neutropenia (caused by haematological malignancy or chemotherapy), bone marrow transplant recipients, solid organ transplant recipients and a range of critically ill patients. Other patient groups with more subtle immune dysfunction are also at heightened risk, including diabetic patients with poor glycaemic control and patients with chronic obstructive pulmonary disease receiving high-dose inhaled corticosteroids.

Methods

References for these guidelines were identified through searches of PubMed, Embase and Medline by use of the search terms 'TDM', 'therapeutic drug monitoring', 'drug monitoring', 'drug concentrations', 'tissue concentrations' and 'serum levels' and each term combined with the name of the antifungals: flucytosine, fluconazole, itraconazole, voriconazole, posaconazole, amphotericin B, caspofungin, micafungin and anidulafungin. References were retrieved and collated. Secondary references embedded in papers that were not identified in the original search were retrieved and reviewed. Following a systematic review of the literature, a series of recommendations were developed. The GRADE system (Grades of Recommendations Assessment, Development and Evaluation[5]) was used to assess the strength of evidence for each recommendation (the GRADE system is summarized in Table 1). The GRADE system uses either 'strong' or 'weak' recommendations and generally high or moderate levels of evidence resulted in a

strong recommendation, with low or very low quality evidence resulting in a weak recommendation. In areas where the quality of evidence was very variable or there was limited evidence, the recommendation was based not only on the available literature but also on the clinical judgement and experience of the authors. The recommendations for TDM for each compound are summarized in Tables 5–8. The evidence base that supports each recommendation is discussed in turn.

Overview

The importance of antifungal TDM is increasingly recognized. Nevertheless, there are no definitive data (and there are never likely to be any) from large clinical trials that address its use in every clinical context. Most evidence supporting TDM is circumstantial. Antifungal TDM is potentially expensive and time consuming, and the ultimate impact on clinical care may be difficult to estimate. There is debate as to whether TDM should be routine (as it is for some antimicrobial compounds, such as aminoglycosides) or used more selectively. This balance depends to some extent on the clinician, the patient case mix, the severity of infection, cost, and access to a TDM service. The indications for potentially recommending TDM for antifungal agents are summarized in Table 2.

There is an increased interest in the use of personalized medicines—TDM is completely consistent with this concept. Clinical input and judgement remain central to the process of TDM. Clinicians frequently forget that therapeutic concentration ranges cited by reference laboratories are derived from populations of patients. A therapeutic target that is appropriate for one patient may not necessarily be satisfactory for another. Therefore, TDM requires continuous clinical input to ensure appropriate targets are chosen rather than using a 'one size fits all' approach. The clinical circumstances that may favour the use of TDM are summarized in Table 3. The optimal frequency of TDM for patients on long-term antifungal therapy is unknown, but will largely depend upon clinical judgement. Once target concentrations have been achieved, consideration of the circumstances described in Table 3 (e.g. compliance, changing pharmacokinetics) should guide the frequency with which repeat TDM measurements are made, as well as the context in which the drug is being used.

Several methods have been used for measuring serum concentrations of antifungal agents, including bioassay, HPLC and mass spectrometry. Advantages, disadvantages and examples of each are summarized in Table 4. A key requirement for any TDM service is participation in a quality control programme and an international scheme is available for the triazole antifungals,[6] whilst the UK National External Quality Assessment Service (NEQAS) runs a scheme for the triazoles and flucytosine in the UK. A further consideration is the turn-around time. While it may be ideal to have assays performed on site, the cost of developing and running

Table 1. Quality of evidence and definitions according to the GRADE system[5]

Quality of evidence	Basis of recommendation
High quality	further research is very unlikely to change our confidence in the estimate of effect
Moderate quality	further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate
Low quality	further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate
Very low quality	any estimate of effect is very uncertain

Table 2. Overall summary of the need for therapeutic drug monitoring when using antifungal agents (see individual tables for detailed recommendations in specific indications)

Antifungal	GRADE quality of evidence and strength of recommendation[5]	Prophylaxis	Treatment	Toxicity	Table with specific details
Itraconazole	evidence quality	moderate	moderate	moderate	Table 5
	recommendation	strong	strong	weak	
Voriconazole	evidence quality	low	high	high	Table 6
	recommendation	weak	strong	strong	
Posaconazole	evidence quality	moderate	moderate	high	Table 7
	recommendation	strong	strong	strong against	
Fluconazole	evidence quality	high	high	high	see text
	recommendation	strong against	strong against	strong against	
Flucytosine	evidence quality	NA	low	moderate	Table 8
	recommendation		weak	strong	
Echinocandins	evidence quality	high	high	high	see text
	recommendation	strong against	strong against	strong against	
Polyenes	evidence quality	high	high	high	see text
	recommendation	strong against	strong against	strong against	

NA, not applicable.

Table 3. Clinical circumstances that may favour the use of TDM

Context	Example	Comment
Pharmacokinetic variability	children, neonates, elderly, obese, organ dysfunction, critical illness haemodialysis, haemofiltration, extracorporeal membrane oxygenation, cardiopulmonary bypass	pharmacokinetics of many antifungal agents very poorly defined in special populations
Changing pharmacokinetics	physiological instability, critical illness, diarrhoea, iv-to-oral switch	
Interacting drugs	antacids, histamine antagonists, proton pump inhibitors and itraconazole capsules; agents known to decrease concentrations of triazoles	drug–drug interactions well defined and documented for many antifungal compounds
Compliance		compliance may be a significant issue for longer-term consolidation therapy or secondary prophylaxis
Poor prognostis disease	extensive or bulky infection, lesions contiguous with critical structures (mediastinum), CNS disease; multifocal or disseminated infection	
Persistent and/or significant underlying immunological defects	prophylaxis versus established disease	

Table 4. Advantages, disadvantages and examples of methods for determining drug levels in serum

Method	Advantages	Disadvantages	References
Bioassay	cheap; simple to perform	subject to interference from other drugs, including other antifungals; may measure combined activity of parent and metabolites (e.g. itraconazole)	32,132
HPLC with ultraviolet fluorescence detection	technology widely available; commercially available assays; can quantify multiple drugs in single sample	subject to interference from miscellaneous substances; run times maybe slow	133–135
Liquid chromatography–mass spectrometry	very sensitive and specific; can quantify multiple drugs in single sample	expensive; not widely available	136–139

assays may mean that many TDM services are only available in specialist centres. Commercially available assays are now available from at least two manufacturers (Recipe and Chromsystems), removing the need to develop in-house assays that could facilitate the implementation of TDM services in non-specialist centres where HPLC equipment is available.

Antifungal TDM

Antifungal TDM is generally indicated for the mould-active triazoles (itraconazole, voriconazole and posaconazole) and the nucleotide flucytosine (5-fluorocytosine). There may be limited clinical circumstances in which TDM of fluconazole is warranted (e.g. critically ill patients on haemofiltration), but there is inadequate evidence to recommend the routine use of TDM for this agent. There is no evidence or indication at the current time to support the routine use of TDM for polyenes (amphotericin B deoxycholate, liposomal amphotericin B and amphotericin B lipid complex) or the echinocandins (micafungin, caspofungin and anidulafungin). Nevertheless, a better understanding of antifungal exposure–response relationships may mean that TDM becomes an important adjunct to the routine administration of these compounds in the future.

Fluconazole

Introduction

Fluconazole is a triazole antifungal that is active against most species of *Candida* (with the notable exceptions of *C. krusei* and *C. glabrata*—the latter often exhibits reduced susceptibility or overt resistance to fluconazole with MICs $\geq$32 mg/L). Fluconazole is also active against *Cryptococcus neoformans* and various dimorphic fungi.[7] Fluconazole is available as capsules, an oral suspension and an intravenous (iv) preparation.

Fluconazole is used for the prevention of invasive candidiasis[8] and the treatment of cryptococcal meningitis, coccidioidomycosis and both invasive and superficial candidiasis. The licensed dose varies with the indication, but for systemic infections is usually 400–800 mg/day.[9] Higher dosages (1200–2000 mg/day) have been used for cryptococcal meningitis.

Fluconazole is highly orally bioavailable and has linear pharmacokinetics.[10] Most active drug is excreted renally,[11] and downward dose adjustment is required for patients with renal failure.[12]

TDM of fluconazole is not routinely required. Nevertheless, there is increasing information related to drug exposure–response relationships. An AUC:MIC ratio of ~100 is associated with improved clinical outcomes (when the MIC is tested using EUCAST methodology[13]). The measurement of fluconazole concentrations may be indicated in rare circumstances (e.g. CNS disease, unstable patient receiving renal supportive care, treatment of an organism with a high MIC). In this case, there is some uncertainty related to an appropriate target. One potential solution is to collect several samples throughout the dosing interval to estimate an AUC, and thereby an AUC:MIC. Sampling at 1, 4 and 24 h would enable a reasonable estimate of the AUC in the majority of patients. Dosages can be adjusted to ensure an AUC:MIC ratio of >100 is achieved.

See Table 2 for recommendations for TDM for fluconazole.

Itraconazole

Introduction

Itraconazole is a triazole antifungal with broad-spectrum antifungal activity. It is active against the commonest medically important fungal pathogens, such as *Candida* spp., *C. neoformans* and *Aspergillus* spp.[14] Current formulations include capsules, an oral solution and an iv preparation; the last two are formulated with hydroxypropyl-β-cyclodextrin. The iv formulation is no longer available in the USA. A wide range of generic formulations are available in countries outside the European Union, and pharmacokinetics may differ significantly from the original formulations developed by Janssen Pharmaceuticals (Sporonox).

Itraconazole is used for the treatment of oral and oesophageal candidosis, prevention of fungal infections in patients with profound and prolonged neutropenia, and treatment of invasive aspergillosis and cryptococcosis in patients who are refractory or intolerant to other first-line antifungal agents.[15] In addition, itraconazole is used for the treatment of allergic aspergillosis, dermatophyte infections, sporotrichosis, blastomycosis, histoplasmosis, coccidioidomycosis and infections with *Penicillium marneffei*.[16] The licensed dosage of the iv preparation in adults consists of a loading dose of 400 mg for 2 days followed by 200 mg/day. There is some uncertainty related to regimens in children that produce equivalent drug exposure to those observed in adults;[17–19] dosages of 2.5–5 mg/kg twice daily are generally used, but the pharmacokinetic studies that underpin these recommendations are not definitive.[20–22] The regimen for treatment of oropharyngeal and oesophageal candidiasis with the oral solution is 200 mg/day in one or two doses, or 200–400 mg/day for treatment of infections caused by pathogens with reduced susceptibility to fluconazole.

The extent of oral bioavailability of itraconazole is variable and dependent on the specific formulation. The oral bioavailability of itraconazole capsules is increased by food and gastric acidity.[23] Itraconazole has 30% higher bioavailability as an oral solution than as capsules, the solution is better absorbed in the fasting state, and its absorption does not appear to be pH dependent.[24] Because of the improved oral bioavailability, the oral solution of itraconazole is generally preferred for treatment, despite the increased cost and its well-documented unpalatability. The pharmacokinetics of itraconazole are non-linear (i.e. a fixed amount of drug rather than a fixed fraction is cleared per unit time), although this is relatively poorly characterized.[25] Itraconazole accumulates slowly and generally reach concentrations of 0.5–1 mg/L after 7–15 days of dosing.[26,27] The use of a loading dose or an iv preparation enables the attainment of concentrations likely to be safe and effective within the first days of therapy.[28] Itraconazole is metabolized via oxidative mechanisms and principally via the isoenzyme CYP3A4.[29] Itraconazole also inhibits CYP3A4, which leads to a number of clinically relevant drug–drug interactions. Oxidative metabolism generates a multitude of metabolites that are excreted in the urine and faeces.[30] One of these metabolites, hydroxy-itraconazole, has antifungal activity that is comparable to the parent.[31] The only practical consequence of this phenomenon is discordance in measurements of serum itraconazole using bioassay (measures both itraconazole and hydroxy-itraconazole) versus HPLC (which measures itraconazole separately)—serum concentrations measured by bioassay are ~5-fold higher compared with HPLC/mass spectrometry.[32] This may be caused by precipitation of itraconazole standards in bioassays due to poor solubility causing smaller zones and overestimation of drug concentrations.[32]

See Table 5 for recommendations for TDM for itraconazole.

Recommendation 1: TDM should be performed in the majority of patients receiving itraconazole

The evidence for the potential clinical benefits of TDM for patients receiving itraconazole is strong, but largely circumstantial. TDM should be considered in the majority of patients receiving itraconazole for both invasive and allergic disease on the basis of: (i) considerable inherent pharmacokinetic variability, a portion of which is due to variable oral bioavailability that is affected by food intake and gastric pH; (ii) clinical and experimental evidence suggesting clinically relevant drug exposure–response relationships; (iii) potential problems with compliance, especially with use of the oral solution, which is unpalatable; and (iv) clinical evidence for drug exposure–toxicity relationships.

The strongest evidence to support TDM of itraconazole is for the prevention of invasive fungal infections in profoundly immunocompromised patients. Many early clinical studies analysing the efficacy of itraconazole were inconclusive, predominantly because they were underpowered. A meta-analysis of these studies suggests that higher itraconazole serum concentrations are protective against invasive fungal infections and decrease mortality.[33] In addition, an early study of itraconazole for primary treatment of invasive aspergillosis also suggests patients with serum concentrations >8 mg/L (measured using bioassay) tend to have better clinical outcomes.[34]

Recommendation 2: A lower target concentration for TDM is a trough of >0.5–1 mg/L measured using HPLC or mass spectrometry

Breakthrough infections are more common in neutropenic patients with trough itraconazole concentrations of <0.25–0.5 mg/L.[35,36] Furthermore, mortality is significantly higher in patients with concentrations <0.5 mg/L.[37] Patients with invasive

Table 5. Recommendations for TDM for itraconazole

Patient group	Specific indication	Quality of evidence	Strength of recommendation
Immunocompromised patients receiving itraconazole for prevention of invasive fungal infection	target trough concentration for prophylaxis is 0.5 mg/L	high	strong
	measurement of trough serum concentrations 5–7 days after initiation of therapy or dose adjustment	high	strong
	when interacting drugs start or stop (either inhibiting absorption or affecting metabolism)	high	strong
	uncertain compliance with oral therapy	high	strong
	concerns about gastrointestinal absorption	low	weak
	potential clinical or laboratory manifestations of toxicity occur	moderate	strong
Patients receiving itraconazole for established invasive and allergic fungal diseases	target trough concentration for treatment is >0.5 mg/L	moderate	strong[a]
	measurement of trough serum concentration 5–7 days after initiation of therapy or dose adjustment	high	strong
	when interacting drugs start or stop (either inhibiting absorption or affecting metabolism)	high	strong
	uncertain compliance for oral therapy	high	strong
	concerns about gastrointestinal absorption, especially for prolonged periods of time	low	weak
	potential clinical or laboratory manifestations of toxicity occur	low	weak

[a]The target concentration for treatment is inferred from prophylaxis data, although there are few treatment studies that have addressed this.

infections caused by *Aspergillus* spp.,[38] *C. neoformans*[39,40] and *Histoplasma capsulatum*[41] all tend to have better clinical outcomes with higher itraconazole trough concentrations. Patients with oropharyngeal and oesophageal candidiasis also have better responses to itraconazole therapy if serum concentrations are >0.6–1 mg/L.[42,43] Collectively, therefore, a target for the prevention and treatment of invasive fungal infections is a trough concentration of 0.5–1 mg/L when measured using HPLC/mass spectrometry. The precise target that is ultimately chosen by the clinician depends on the organism, its MIC, the site of infection and overall clinical context.

Therapeutic concentration targets to optimize the antifungal effect of itraconazole have been derived exclusively in the context of prevention or treatment of invasive disease. Itraconazole is used in the treatment of other fungal diseases, such as treatment of infections with dimorphic fungi (e.g. *Blastomyces*, *Sporothrix* and *Histoplasma*), cryptococcal meningitis, chronic pulmonary aspergillosis, allergic bronchopulmonary aspergillosis (ABPA) and in some cases of severe asthma with fungal sensitization (SAFS). There is no evidence that concentration targets derived from the prevention of invasive fungal infection are necessarily optimal for these other diseases, although in the absence of specific evidence to the contrary, use of these same targets is probably reasonable.

A potential limitation of using a standard trough concentration is that it does not incorporate the MIC of the fungal pathogen in question. Experimental models of aspergillosis[44–46] and candidosis[47,48] have demonstrated that greater drug exposure is required for successful outcomes for infections caused by isolates with higher MICs. The identification of concentration targets for TDM occurred in an era when resistance to anti-*Aspergillus* triazoles was uncommon. The most appropriate target value for treatment of pathogens with elevated MICs is not known. Furthermore, the relationship between this target and the emergence of drug resistance is not known, and may be important for chronic and allergic forms of aspergillosis, both of which require long-term antifungal therapy. These areas require further research.

Recommendation 3: Itraconazole TDM should be performed to minimize drug-related toxicity

Adverse events associated with itraconazole include gastrointestinal disturbances, neurological problems and hepatitis.[16] Some of the gastrointestinal intolerance may be primarily caused by the osmotic effects of the hydroxypropyl-β-cyclodextrin component of the oral or iv solution.[49] Two studies have demonstrated an increased incidence of toxicity at higher concentrations. Both studies used a bioassay to quantify itraconazole concentrations.[50,51] An average concentration of 17 mg/L (bioassay) is a reasonable upper concentration bound to minimize the probability of drug-related toxicity. The equivalent target using HPLC has not been specifically determined, but is ∼5-fold lower.[32]

Recommendation 4: Itraconazole concentrations should be measured in the first week of therapy and regularly thereafter

Because itraconazole exhibits non-linear pharmacokinetics, the time to steady state cannot be expressed in terms of half-life (i.e. itraconazole does not have a half-life). Itraconazole concentrations steadily increase and reach 0.5–1 mg/L in the first 2 weeks of therapy. One approach for TDM is to draw a pre-dose sample at the end of the first week of therapy and then at regular intervals that are appropriate to the clinical context. More frequent sampling may be required if there is great clinical urgency, or there is a change in dosage and/or formulation. Moreover, a change in other clinical

parameters, such as the development of achlorhydria, the addition of agents that decrease gastric acidity (e.g. concomitant use of itraconazole capsules with antacids, histamine antagonists or proton pump inhibitors[52]) or the addition of agents that interact via hepatic oxidative mechanisms (e.g. rifampicin, carbamazepine, phenytoin[53–55]) may also mandate more frequent sampling. The requirement for repeated sampling for a patient who is stable and on longer-term itraconazole therapy is less clear. Nevertheless, intermittent measurements may be helpful to exclude issues with compliance or unanticipated changes in pharmacokinetics.

Voriconazole

Introduction

Voriconazole is a broad-spectrum second-generation triazole antifungal agent that has activity against *Candida* (including fluconazole-resistant species), *C. neoformans*, *Aspergillus*, many dimorphic fungi and several other medically important fungi.[7] Voriconazole is a structural congener of fluconazole, but has significantly diminished aqueous solubility.[56] A number of formulations are available for clinical use, including an iv preparation (containing sulfobutyl ether β-cyclodextrin sodium) and oral capsules (available as 50 and 200 mg), as well as a suspension designed for oral use in children.[56]

Voriconazole is a first-line agent for the treatment of invasive aspergillosis, invasive candidiasis caused by *Candida* spp. with reduced susceptibility to fluconazole, and serious infections caused by *Scedosporium* or *Fusarium* spp.[15] Voriconazole is the drug of choice for CNS aspergillosis.[57] Voriconazole may potentially be used in combination with other antifungal agents for the treatment of invasive aspergillosis.[58] Despite several clinical studies demonstrating the safety and efficacy of voriconazole for the prevention of invasive fungal infections,[59,60] it is not currently licensed for this indication. The currently licensed dose is 6 mg/kg iv twice daily for two dosages, followed by 4 mg/kg iv twice daily. If therapy is initiated with oral voriconazole, a loading dose of 400 mg twice daily for two doses is used (for individuals >40 kg), followed by 200 mg twice daily, and in individuals <40 kg the maintenance dose is 100 mg twice daily. The dosage can be increased to 300 mg twice daily if clinically indicated. Recently, population-based pharmacokinetic studies have suggested that higher oral doses than those currently recommended may be needed to achieve optimal plasma concentrations and therapeutic responses.[61] There has been considerable debate about appropriate paediatric regimens that produce equivalent drug exposures to those observed in adults, for which efficacy has been established in Phase II and III clinical trials. A loading dose of 9 mg/kg twice daily for two doses followed by 8 mg/kg twice daily is now recommended for the iv preparation, with oral dosing maintained at 9 mg/kg twice daily, and reflects the higher weight-adjusted clearance of voriconazole that is observed in paediatric patients.[62,63]

Voriconazole exhibits classical Michaelis–Menten (non-linear) pharmacokinetics in adults that are related to saturable clearance mechanisms. This has important implications for dosage adjustment because of unanticipated and unpredictable changes in drug exposure (i.e. significantly greater or smaller than anticipated). Voriconazole is highly orally bioavailable, with current estimates of ~80%–86% in children and adults,[64,65] although estimates as low as 60% have recently been reported.[61] Oral bioavailability may also be lower in children, hence TDM is especially important in this setting.[64–66]

Voriconazole is metabolized via oxidative mechanisms. The predominant cytochrome P450 isoenzymes involved in this process are CYP3A4, CYP2C19 and CYP2C9.[67] CYP2C19 exhibits a number of clinically relevant polymorphisms that have been associated with differing rates of enzyme activity and therefore clearance of voriconazole. These polymorphisms account for a portion of the observed variance in serum concentrations, which is otherwise extensive (e.g. 100-fold in healthy volunteers). Voriconazole inhibits CYP3A4 activity (as well as CYP2C19 and 2C9), which results in a number of clinically relevant drug–drug interactions that have been extensively reviewed elsewhere.[68]

See Table 6 for recommendations for TDM for voriconazole.

Recommendation 5: TDM should be performed in the majority of patients receiving voriconazole

There is an increasing evidence base that supports TDM for voriconazole. The British Society for Medical Mycology (BSMM) working party recognizes that it is possible to use voriconazole without TDM and that the definitive trials used for registration were all performed using a fixed regimen. Nevertheless, the case supporting TDM as a routine adjunct to the use of voriconazole is increasing and rests with the following arguments: (i) concentration–effect and concentration–toxicity relationships are consistently reported in both experimental[69–72] and clinical contexts,[73–75] and, in patients, these relationships have been defined in both adults[73,76,77] and children;[65,78] (ii) the pharmacokinetic variability of voriconazole is extensive, and has been rigorously quantified using non-parametric population pharmacokinetic modelling techniques,[64] and a consequence of this pharmacokinetic variability is that an unacceptably low proportion of patients receiving a fixed regimen have drug exposures associated with a high probability of success and low probability of toxicity; and (iii) dosage adjustment results in fewer cases of toxicity, and may improve clinical responses.[73] More recently, a prospective, randomized controlled trial compared clinical outcomes in patients who had voriconazole dosages adjusted based on serum concentrations with the outcomes in those who received a fixed voriconazole regimen.[77] Outcomes (complete or partial response) in patients undergoing TDM (who had plasma concentrations maintained between 1.0 and 5.5 mg/L) were significantly better (81%) than those in the non-TDM group (57%).

Recommendation 6: A minimum lower target concentration for TDM for treatment of established disease is a trough concentration of >1 mg/L or a trough:MIC ratio of 2–5

The potential relationship between voriconazole serum concentrations and clinical outcome was initially described in a Phase II clinical study of voriconazole for invasive aspergillosis. In that study, a serum concentration of <0.25 mg/L was associated with a higher probability of clinical failure.[79] Subsequently, a number of retrospective studies from single centres also suggested a relationship between drug exposure and clinical outcome.[80,81] These studies are all limited by difficulties in estimating voriconazole drug exposure in individual patients and controlling for the

Table 6. Recommendations for TDM for voriconazole

Patient group	Specific indication	Quality of evidence	Strength of recommendation
Immunocompromised patients receiving voriconazole for prophylaxis of invasive fungal disease	target trough concentration for prophylaxis is >1 mg/L	low	weak
	measurement of trough serum concentration within the first 7 days after initiation of therapy, and regularly thereafter	high	strong
	when interacting drugs start or stop	high	strong
	uncertain compliance for oral therapy	high	strong
	concerns about gastrointestinal absorption, especially for prolonged periods of time	low	weak
	potential clinical or laboratory manifestations of toxicity occur	high[a]	strong
Patients receiving voriconazole for invasive fungal diseases	target trough concentration for treatment is >1 mg/L	high	strong
	measurement of serum tough concentration within 7 days of initiation of therapy or following dose adjustment	high	strong
	when interacting drugs start or stop	high	strong
	uncertain compliance for oral therapy	high	strong
	concerns about gastrointestinal absorption, especially for prolonged periods of time	low	weak
	potential clinical or laboratory manifestations of toxicity occur	high	strong

[a]This is inferred from treatment studies.

myriad of clinical factors that also have an impact upon clinical outcome. Studies variously identified target concentrations of ≥1[73,81] or ≥2 mg/L[74–76,80,82] as being associated with improved outcomes, whilst one large study found no relationship between exposure and clinical outcome.[83] Recent experimental and retrospective clinical studies have incorporated the MIC into targets for TDM;[72,75] both suggest that a trough concentration:MIC target of 2–5 (when the MIC is estimated using CLSI methodology) is tenable and this may be useful if the MIC of the invading pathogen is known.

The most appropriate concentration target for prevention of invasive fungal infections in immunocompromised patients is less clear. A study of allogeneic haematopoietic stem cell transplant recipients suggests breakthrough infections only occur in patients with serum concentrations <2 mg/L.[84] Similarly, lung transplant recipients who are colonized (with various fungi) or who develop invasive fungal infections have lower median trough concentrations compared with patients without colonization or infection (0.92 versus 1.72 mg/L).[85] More studies are required to further define these relationships.

Collectively, therefore, a trough concentration of >1 mg/L is required to maximize efficacy for patients with invasive fungal infections. The probability of a clinical response increases with higher concentrations, but only incrementally.[61,73] The target that is chosen for dosage adjustment depends on the clinical context. A higher target (e.g. 2 mg/L) should be used if there is disease with a poor prognosis (e.g. CNS infection, bulky disease, multifocal infection; see Table 3).

Recommendation 7: A trough concentration to minimize drug-related toxicity is <4–6 mg/L

Concentration–toxicity relationships for voriconazole have been estimated in several key studies.[73,86] Voriconazole toxicity may manifest as visual disturbances (photopsia), liver dysfunction, skin reactions and neurotoxicity (confusion and visual hallucinations).[87,88] Trough concentrations that are associated with greater probability of toxicity vary from study to study, and include ≥4,[61,89–91] ≥5[73,76,88] and ≥6 mg/L.[82] Some studies do not define a specific cut-off value, but note a progressively higher probability of toxicity with higher voriconazole concentrations.[86,92–95] There is a statistically significant (albeit relatively weak) relationship between average voriconazole concentration and the probability of elevated bilirubin, alkaline phosphatase, aspartate transaminase and alanine transaminase.[86,93,95] Furthermore, there is a relationship between the trough concentration and the probability of encephalopathy, which manifests as confusion and hallucinations.[73,76,92] Active dosage adjustment to keep serum concentrations <5.5 mg/L prevents voriconazole-related toxicity.[77]

Recommendation 8: Voriconazole concentrations should be measured in the first 5 days of therapy and regularly thereafter

At the current time, a trough concentration is the most readily interpretable measure of drug exposure. Voriconazole concentrations change faster than those of itraconazole and posaconazole, and initial sampling in the first 2–5 days of therapy is reasonable. Some patients sampled at this time may have progressively accumulating drug concentrations even though the initial concentration is 'therapeutic'. This occurs if serum concentrations are $>K_m$ (the Michaelis constant for that individual), meaning that clearance mechanisms are saturated. Therefore, a second sample should be routinely collected to ensure voriconazole concentrations are stable and in a desired therapeutic range. The same sampling strategy is required if there is a change in dosage, a change in clinical condition or an iv-to-oral switch.

Posaconazole

Introduction

Posaconazole is a broad-spectrum triazole agent that is structurally similar to itraconazole. Posaconazole has activity against a large number of medically important fungal pathogens, including *Candida*, *Aspergillus*, *Cryptococcus* and the mucoraceous moulds.[7] Posaconazole is currently only available as an oral suspension (40 mg/mL), although other orally bioavailable and iv formulations are under development.[96,97]

The current licensed indications for the use of posaconazole include salvage therapy for aspergillosis, treatment of coccidioidomycosis, chromoblastomycosis, mycetoma or *Fusarium* infections. Posaconazole is increasingly used for the prevention of infections in patients with acute myeloid leukaemia (AML) or myelodysplastic syndrome (MDS) who are expected to become neutropenic, and stem cell transplant recipients receiving immunosuppressive agents for graft-versus-host disease[15] The dose for treatment of established infection is 800 mg/day in two to four divided doses (i.e. 200 mg four times daily or 400 mg twice daily), with four divided doses providing the best exposure. A dose of 600 mg/day in three divided doses is used for the prevention of invasive fungal infections in immunocompromised patients (i.e. 200 mg every 8 h).

Posaconazole is primarily metabolized by glucuronidation, with little involvement of oxidative mechanisms. Metabolites are excreted in the faeces and urine.[68] Posaconazole inhibits CYP3A4 activity and dosage adjustment of drugs metabolized via this pathway (most importantly cyclosporine and tacrolimus) is required. The oral absorption of posaconazole appears saturable and this may be affected by both the rate and the extent of absorption. Dosage escalation beyond 800 mg/day does not result in a proportional increase in systemic drug exposure, although some studies do suggest there may be some incremental benefit.[98]

There is a significant food effect (increased oral bioavailability with food),[99] acid effect (increased absorption with an acidic environment[100]) and fat effect (increased oral bioavailability with administration with fatty food or nutritional supplements[99,101]). All these characteristics have a potential impact on the ability to increase systemic drug exposure. Posaconazole has a long terminal half-life (~34 h) and does not achieve steady-state serum concentrations until the end of the first week of dosing. Because the dosing interval is significantly shorter than the half-life, the concentration–time profile is typically reasonably flat and there is a high degree of concordance between the average and trough concentrations. Posaconazole is generally well tolerated, but can cause nausea, vomiting and hepatotoxicity.[102] To date, there are no data that suggest any correlation between toxicity and drug exposure, but with the newer formulations of posaconazole in development this may change.

See Table 7 for recommendations for TDM for posaconazole.

Recommendation 9: TDM should be performed in the majority of patients receiving posaconazole

There is an increasing evidence base that supports TDM for posaconazole. The BSMM working party recognizes that posaconazole has been extensively used without TDM, and that the efficacy of this compound for the prevention of invasive fungal infections was established without resorting to TDM. Posaconazole TDM should be considered in the majority of cases in which it is used and this is based on the following: (i) concentration–effect relationships are apparent and have been established in experimental models of invasive fungal infection[103,104] and in clinical contexts;[105,106] (ii) the pharmacokinetic variability is extensive and has been quantified using a variety of pharmacokinetic modelling approaches;[100,107,108] and (iii) serum concentrations are potentially suboptimal in a relatively high proportion of patients receiving a fixed regimen. Many studies note the problems of achieving

Table 7. Recommendations for TDM for posaconazole

Patient group	Specific indication	Quality of evidence	Strength of recommendation
Immunocompromised patients receiving posaconazole for prophylaxis of invasive fungal disease	target for prophylaxis is >0.7 mg/L at steady state or 0.35 mg/L 48 h after initiation of therapy	low	weak
	measurement of trough serum concentration 7 days after initiation of therapy and following dose adjustment	high[a]	strong
	when interacting drugs start or stop	low	weak
	uncertain compliance	high	strong
	concerns about gastrointestinal absorption, especially for prolonged periods of time	high	strong
Patients receiving posaconazole for salvage therapy of invasive fungal diseases	target for treatment is >1 mg/L	moderate	strong
	within 7 days of initiation of therapy or following dose adjustment	high	strong
	when interacting drugs start or stop	high	strong
	uncertain compliance	high	strong
	concerns about gastrointestinal absorption, especially for prolonged periods of time	high	strong

[a]This is from a pharmacokinetic model.[113]

nominal target concentrations in patients because of suboptimal absorption that is compounded by mucositis and/or graft-versus-host disease of the gut. For example, ~50% of patients receiving posaconazole have serum concentrations <0.5 mg/L,[109–111] which are potentially subtherapeutic (see Recommendation 10). Recent studies have shown that concentrations of posaconazole associated with the cellular membranes in the lung may be many times in excess of those levels found in the blood,[112] which may in future influence the recommendations for monitoring of blood levels during prophylactic use.

Recommendation 10: A lower target concentration for TDM for patients receiving posaconazole for prophylaxis is a trough concentration of >0.7 mg/L

A target trough concentration of 0.7 mg/L for patients receiving posaconazole for prophylaxis is widely cited (see e.g. Bryant *et al*.,[109] Jang *et al*.[113] and Tonini *et al*.[114]). This target concentration is derived from analysis by the FDA of pharmacokinetic data from two Phase III prophylaxis studies that were originally used for the purposes of registration.[115,116] There is a degree of uncertainty about the relevance of this target concentration because a composite endpoint for successful clinical outcome was used—there were simply too few patients with microbiologically documented breakthrough infection in these studies to rely solely on this as a criterion for success. Because posaconazole concentrations are not at steady state until after the first week of therapy, a target concentration of 0.35 mg/L after 48 h of treatment has also been proposed.[117] Several other studies have reported a correlation between drug exposure and efficacy[98,109–111,114,118] in a range of clinical contexts. Target concentrations vary in these studies from 0.5 to 0.7 mg/L. Although many of the studies are small, retrospective in design and generally underpowered, they all show a general trend towards an increased probability of response with greater drug exposure. In the absence of more definitive data, a concentration target of 0.7 mg/L is reasonable, although the evidence that supports this is relatively weak, and the BSMM working party have graded this recommendation accordingly (see Table 7).

Recommendation 11: A lower target concentration for TDM for patients with established infection is a trough concentration of >1.0 mg/L

Patients with invasive aspergillosis who are intolerant or refractory to other licensed antifungal agents receiving posaconazole have a progressively higher clinical response with higher posaconazole drug exposures.[106] In that study, among patients with a C_{max} and C_{avg} of 0.142 and 0.134 mg/L, respectively, 24% had a successful clinical outcome. In contrast, patients with a C_{max} and C_{avg} of 1.48 and 1.25 mg/L, respectively, had a 75% response rate. Thus, there appears to be a progressive increase in the probability of a response with increasing drug exposure. A pragmatic approach for TDM is to attempt to obtain the highest possible concentration, although suboptimal and saturable absorption may mean this is simply not feasible even following progressive dosage escalation. A trough concentration of 1 mg/L can be used as a lower concentration target for TDM.

The use of a target concentration of 1 mg/L does not specifically incorporate the MIC of the invading pathogen (unlike voriconazole; see above). Experimental data suggest that the MIC and genotype of the invading pathogen are important determinants of exposure–response relationships.[103,104,119]

The Antifungal Subcommittee of EUCAST has recently set breakpoints for posaconazole against *Aspergillus* spp. and specifically incorporated TDM into the classification of isolates into 'susceptible', 'intermediate' and 'resistant' categories. Hence, isolates with MIC ≤0.125 mg/L and >0.25 are deemed susceptible or intermediate only if adequate drug exposure has been documented with TDM, with a therapeutic target serum concentration of 1 mg/L at steady state being recommended.[120]

Recommendation 12: Posaconazole concentrations should be measured in the first week of therapy and regularly thereafter

Posaconazole concentrations steadily increase in the first week and plateau thereafter.[121] A steady-state trough concentration is not apparent until the end of the first week, and changes to dosage will take a further 7 days before a new steady state is established. Repeat testing is required if the clinical condition changes or following dosage adjustment. Serum samples can be collected earlier than 7 days (before the attainment of steady state), but the use of a lower therapeutic target of 0.35 mg/L after 48 h of therapy is appropriate.

Flucytosine

Introduction

Flucytosine is a pyrimidine analogue that acts as a subversive substrate within the pyrimidine salvage pathway of a number of clinically important fungal pathogens. Flucytosine is active against the majority of *Candida* spp. and *C. neoformans*, but also has activity against *Aspergillus* spp. and rare dematiaceous fungal pathogens causing chromoblastomycosis.[122] Flucytosine should always be used in combination with other antifungal agents because of the significant risk of emergent drug resistance when used as monotherapy.

The advent of newer antifungal agents and classes has somewhat relegated the importance of flucytosine in many clinical settings. Nevertheless, it remains a cornerstone for induction therapy of cryptococcal meningitis, in combination with either a polyene (amphotericin B deoxycholate, liposomal amphotericin B) or fluconazole.[123,124] Flucytosine is highly orally bioavailable, making it an especially attractive option for use in resource-poor healthcare settings, although the oral preparation is not available in all countries. Furthermore, it penetrates the CSF and cerebral parenchyma. Flucytosine may also be useful in some cases of refractory infections caused by *Candida* spp., especially if there is deep infection where poor drug penetration may compromise the therapeutic response. The standard dose is 100–150 mg/kg/day, and is usually administered in three or four divided dosages. A dosage reduction is required with renal impairment (creatinine clearance >50 mL/min, 150 mg/kg/day; creatinine clearance 26–50 mL/min, 75 mg/kg/day; creatinine clearance 13–25 mL/min, 37 mg/kg/day; creatinine clearance

<13 mL/min, avoid flucytosine[125]). Alternatively, the normal dose can be given, but with an increased interval between doses.

Flucytosine is a small polar molecule that is cleared via renal mechanisms. Flucytosine is generally well tolerated, although there is well-documented associated toxicity that includes bone marrow suppression (primarily manifesting as neutropenia), gastrointestinal intolerance, hepatitis and rash. However, the more serious liver toxicity and myelosuppression are generally only seen with prolonged maintenance of high blood levels. Flucytosine has few (if any) direct drug–drug interactions. Flucytosine rapidly accumulates with the onset of renal impairment if there is not an appropriate downward revision of dosage. Historically, the most common agent that leads to renal impairment and subsequent accumulation of flucytosine is amphotericin B deoxycholate.

See Table 8 for recommendations for TDM for flucytosine.

Recommendation 13: TDM should be performed in the majority of patients receiving flucytosine

TDM for flucytosine has long been considered a standard of care.[126] A requirement for TDM is predominantly based on the well-established concentration–toxicity relationships,[125] the most important of which is myelosuppression (see below). There is also some evidence from reference centres that flucytosine concentrations are variable and frequently outside nominal concentration targets for TDM.[127,128] There is also a theoretical concern for the emergence of drug resistance, which occurs rapidly when *Candida* is exposed to flucytosine *in vitro*. There are well-described drug exposure targets for flucytosine against *Candida albicans*, with a requirement for serum concentrations to exceed the MIC for ∼45% of the dosing interval.[129,130] Nevertheless, the use of TDM to aid in the optimization of the antifungal efficacy (as opposed to prevention of toxicity) of flucytosine remains poorly elucidated.

Recommendation 14: A lower target concentration for TDM is a trough concentration of >20–40 mg/L

A number of lower target concentrations have been used to direct flucytosine dosing. The use of this target concentration is principally based on *in vitro* findings in which the emergence of drug resistance is observed when yeasts are exposed to lower concentrations.[125,131] The clinical relevance of these concentrations for patients is less clear. Furthermore, the optimal concentration targets for flucytosine in combination with other antifungal agents—

Table 8. Recommendations for TDM for flucytosine

Patient group	Specific indication	Quality of evidence	Strength of recommendation
Patients receiving flucytosine in combination with other antifungal agents for treatment of invasive fungal diseases	trough concentration of 20–40 mg/L; peak concentration should not exceed 100 mg/L	weak	weak
	within 72 h of initiation of therapy or following dose adjustment	high	strong
	when interacting drugs start or stop	high	strong
	uncertain compliance for oral therapy[a]	high	strong
	potential clinical or laboratory manifestations of toxicity occur	high	strong

[a]Oral therapy is not widely available.

Table 9. Strategies for dose adjustments for patients with low serum concentrations

Compound	Upward dosage adjustment	Additional strategies
Itraconazole	increase from 200 mg twice daily to 300 mg twice daily	• change capsules to solution • if using capsules, stop or reduce H2 antagonists or proton pump inhibitors • if using solution check it is being given in the fasting state • check compliance • stop interacting drugs
Voriconazole	increase iv therapy by 50% to a maximum of 6 mg/kg twice daily (adults); increase oral therapy from 200 mg twice daily to 300 mg twice daily	• check compliance • stop interacting drugs
Posaconazole	increase from 600 mg/day to 800 mg/day; fractionate total daily dose and administer every 6 h	• administer with food • administer with high-fat food (e.g. ice cream) • remove acid suppression if possible (i.e. stop or reduce H2 antagonists or proton pump inhibitors • check compliance • stop interacting drugs
Flucytosine	increase dose by 50%	

which is the way flucytosine is invariably administered—are not well defined.

Recommendation 15: A concentration target to minimize flucytosine drug-related toxicity is a peak concentration of 50–100 mg/L

There is strong evidence that there is an increased risk of myelotoxicity with peak concentrations of flucytosine >100 mg/L. This concentration target was defined 2 h after an oral dose of flucytosine in a cohort of patients receiving 0.3 mg/kg/day amphotericin B deoxycholate and flucytosine. A total of 23/37 patients with a peak concentration of >100 mg/L over a 2 week treatment period had flucytosine-related toxicity, whereas only 15/48 patients with concentrations <100 mg/L had drug-related toxicity. The implications of these findings for the current BSMM recommendations are slightly difficult to interpret because a peak concentration was defined as the concentration 2 h after an oral dose of flucytosine.[125] Comparable concentration targets for peak samples taken 30 min after the dose in patients receiving iv flucytosine are not known and require further study. Although the dosage of flucytosine which produced these concentrations was higher than that in current use, toxicity is still seen with current dosages, especially in the setting of renal impairment.

Recommendation 16: Flucytosine concentrations should be measured in the first 72 h of therapy and regularly thereafter

Flucytosine has a short half-life. Serum concentrations can change quickly, especially if renal function changes. Regular measurements are required to prevent persistence or recurrence of potentially toxic concentrations. Serum concentrations should be re-measured following dosage adjustment.

Strategies for dose adjustment

Dosage adjustments may be required in patients with low serum concentrations or other measures may need to be taken, such as the cessation of interacting drugs. The strategy that is required varies between drugs and is detailed in Table 9.

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Supplementary data

Table S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Andes D, Pascual A, Marchetti O. Antifungal therapeutic drug monitoring: established and emerging indications. *Antimicrob Agents Chemother* 2009; **53**: 24–34.

2 Bruggemann RJ, Donnelly JP, Aarnoutse RE *et al*. Therapeutic drug monitoring of voriconazole. *Ther Drug Monit* 2008; **30**: 403–11.

3 Hope WW, Billaud EM, Lestner J *et al*. Therapeutic drug monitoring for triazoles. *Curr Opin Infect Dis* 2008; **21**: 580–6.

4 Lewis RE. Antifungal therapeutic drug monitoring. *Curr Fungal Infect Rep* 2010; **4**: 158–67.

5 Guyatt GH, Oxman AD, Vist GE *et al*. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* 2008; **336**: 924–6.

6 Bruggemann RJ, Touw DJ, Aarnoutse RE *et al*. International interlaboratory proficiency testing program for measurement of azole antifungal plasma concentrations. *Antimicrob Agents Chemother* 2009; **53**: 303–5.

7 Sabatelli F, Patel R, Mann PA *et al*. In vitro activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large collection of clinically important molds and yeasts. *Antimicrob Agents Chemother* 2006; **50**: 2009–15.

8 Ullmann AJ, Akova M, Herbrecht R *et al*. ESCMID guideline for the diagnosis and management of *Candida* diseases 2012: adults with haematological malignancies and after haematopoietic stem cell transplantation (HCT). *Clin Microbiol Infect* 2012; **18** Suppl 7: 53–67.

9 Voss A, de Pauw BE. High-dose fluconazole therapy in patients with severe fungal infections. *Eur J Clin Microbiol Infect Dis* 1999; **18**: 165–74.

10 Bellmann R. Clinical pharmacokinetics of systemically administered antimycotics. *Curr Clin Pharmacol* 2007; **2**: 37–58.

11 Brammer KW, Farrow PR, Faulkner JK. Pharmacokinetics and tissue penetration of fluconazole in humans. *Rev Infect Dis* 1990; **12**: 318–27.

12 Cousin L, Berre ML, Launay-Vacher V *et al*. Dosing guidelines for fluconazole in patients with renal failure. *Nephrol Dial Transplant* 2003; **18**: 2227–31.

13 Rodriguez-Tudela JL, Almirante B, Rodriguez-Pardo D *et al*. Correlation of the MIC and dose/MIC ratio of fluconazole to the therapeutic response of patients with mucosal candidiasis and candidemia. *Antimicrob Agents Chemother* 2007; **51**: 3599–604.

14 Slain D, Rogers PD, Cleary JD *et al*. Intravenous itraconazole. *Ann Pharmacother* 2001; **35**: 720–9.

15 Joint Formulary Committee. *British National Formulary*. London: BMJ Group and Pharmaceutical Press, 2012.

16 Lestner J, Hope WW. Itraconazole: an update on pharmacology and clinical use for treatment of invasive and allergic fungal infections. *Exp Opin Drug Metab Toxicol* 2013; **9**: 911–26.

17 de Repentigny L, Ratelle J, Leclerc JM *et al*. Repeated-dose pharmacokinetics of an oral solution of itraconazole in infants and children. *Antimicrob Agents Chemother* 1998; **42**: 404–8.

18 Abdel-Rahman SM, Jacobs RF, Massarella J *et al*. Single-dose pharmacokinetics of intravenous itraconazole and hydroxypropyl-β-cyclodextrin in infants, children, and adolescents. *Antimicrob Agents Chemother* 2007; **51**: 2668–73.

19 Schmitt C, Perel Y, Harousseau JL *et al*. Pharmacokinetics of itraconazole oral solution in neutropenic children during long-term prophylaxis. *Antimicrob Agents Chemother* 2001; **45**: 1561–4.

20 Simon A, Besunden M, Vezmar S *et al*. Itraconazole prophylaxis in pediatric cancer patients receiving conventional chemotherapy or autologous stem cell transplants. *Support Care Cancer* 2007; **15**: 213–20.

21 Baietto L, Rosa GD, D'Avolio A *et al*. Prophylactic drug monitoring of itraconazole in an oncohematological pediatric patient population. *Ther Drug Monit* 2012; **34**: 604–6.

22 Hennig S, Wainwright CE, Bell SC *et al*. Population pharmacokinetics of itraconazole and its active metabolite hydroxy-itraconazole in paediatric cystic fibrosis and bone marrow transplant patients. *Clin Pharmacokinet* 2006; **45**: 1099–114.

23 Barone JA, Koh JG, Bierman RH *et al*. Food interaction and steady-state pharmacokinetics of itraconazole capsules in healthy male volunteers. *Antimicrob Agents Chemother* 1993; **37**: 778–84.

24 Van de Velde VJS, van Peer A, Heykants J *et al*. Effect of food on the pharmacokinetics of a new hydroxypropyl-β-cyclodextrin formulation of itraconazole. *Pharmacotherapy* 1996; **16**: 424–8.

25 Poirier JM, Cheymol G. Optimisation of itraconazole therapy using target drug concentrations. *Clin Pharmacokinet* 1998; **35**: 461–73.

26 Hardin TC, Graybill JR, Fetchick R *et al*. Pharmacokinetics of itraconazole following oral administration to normal volunteers. *Antimicrob Agents Chemother* 1988; **32**: 1310–3.

27 Smith D, Van de Velde V, Woestenborghs R *et al*. The pharmacokinetics of oral itraconazole in AIDS patients. *J Pharm Pharmacol* 1992; **44**: 618–9.

28 Willems L, van der Geest R, de Beule K. Itraconazole oral solution and intravenous formulations: a review of pharmacokinetics and pharmacodynamics. *J Clin Pharm Ther* 2001; **26**: 159–69.

29 Isoherranen N, Kunze KL, Allen KE *et al*. Role of itraconazole metabolites in CYP3A4 inhibition. *Drug Metab Dispos* 2004; **32**: 1121–31.

30 Lass-Florl C. Triazole antifungal agents in invasive fungal infections: a comparative review. *Drugs* 2011; **71**: 2405–19.

31 Heykants J, van Peer A, Van de Velde V *et al*. The clinical pharmacokinetics of itraconazole: an overview. *Mycoses* 1989; **32**: 67–87.

32 Law D, Moore CB, Denning DW. Bioassay for serum itraconazole concentrations using hydroxyitraconazole standards. *Antimicrob Agents Chemother* 1994; **38**: 1561–6.

33 Glasmacher A, Prentice A, Gorschluter M *et al*. Itraconazole prevents invasive fungal infections in neutropenic patients treated for hematologic malignancies: evidence from a meta-analysis of 3,597 patients. *J Clin Oncol* 2003; **21**: 4615–26.

34 Denning DW. Treatment of invasive aspergillosis. *J Infect* 1994; **28** Suppl 1: 25–33.

35 Boogaerts MA, Verhoef G, Zachee P *et al*. Antifungal prophylaxis with itraconazole in prolonged neutropenia: correlation with plasma levels. *Mycoses* 1989; **32**: 103–8.

36 Tricot G, Joosten E, Boogaerts MA *et al*. Ketoconazole vs. itraconazole for antifungal prophylaxis in patients with severe granulocytopenia: preliminary results of two nonrandomized studies. *Rev Infect Dis* 1987; **9** Suppl 1: S94–9.

37 Glasmacher A, Hahn C, Leutner C *et al*. Breakthrough invasive fungal infections in neutropenic patients after prophylaxis with itraconazole. *Mycoses* 1999; **42**: 443–51.

38 Denning DW, Tucker RM, Hanson LH *et al*. Treatment of invasive aspergillosis with itraconazole. *Am J Med* 1989; **86**: 791–800.

39 Denning DW, Tucker RM, Hanson LH *et al*. Itraconazole therapy for cryptococcal meningitis and cryptococcosis. *Arch Intern Med* 1989; **149**: 2301–8.

40 Sharkey PK, Rinaldi MG, Dunn JF *et al*. High-dose itraconazole in the treatment of severe mycoses. *Antimicrob Agents Chemother* 1991; **35**: 707–13.

41 Wheat J, Hafner R, Korzun AH *et al*. Itraconazole treatment of disseminated histoplasmosis in patients with the acquired immunodeficiency syndrome. AIDS Clinical Trial Group. *Am J Med* 1995; **98**: 336–42.

42 Cartledge JD, Midgely J, Gazzard BG. Itraconazole solution: higher serum drug concentrations and better clinical response rates than the capsule formulation in acquired immunodeficiency syndrome patients with candidosis. *J Clin Pathol* 1997; **50**: 477–80.

43 Cross LJ, Bagg J, Oliver D *et al*. Serum itraconazole concentrations and clinical responses in *Candida*-associated denture stomatitis patients treated with itraconazole solution and itraconazole capsules. *J Antimicrob Chemother* 2000; **45**: 95–9.

44 Denning DW, Radford SA, Oakley KL *et al*. Correlation between *in-vitro* susceptibility testing to itraconazole and *in-vivo* outcome of *Aspergillus fumigatus* infection. *J Antimicrob Chemother* 1997; **40**: 401–14.

45 Odds FC, Van GF, Espinel-Ingroff A *et al*. Evaluation of possible correlations between antifungal susceptibilities of filamentous fungi in vitro and antifungal treatment outcomes in animal infection models. *Antimicrob Agents Chemother* 1998; **42**: 282–8.

46 Al-Nakeeb Z, Sudan A, Jeans AR *et al*. Pharmacodynamics of itraconazole against *Aspergillus fumigatus* in an in vitro model of the human alveolus: perspectives on the treatment of triazole-resistant infection and utility of airway administration. *Antimicrob Agents Chemother* 2012; **56**: 4146–53.

47 Odabasi Z, Paetznick VL, Rodriguez JR *et al*. Lack of correlation of 24- vs. 48-h itraconazole minimum inhibitory concentrations with microbiological and survival outcomes in a guinea pig model of disseminated candidiasis. *Mycoses* 2010; **53**: 438–42.

48 Uchida K, Shimogawara K, Yamaguchi H. Correlation of in vitro activity and in vivo efficacy of itraconazole intravenous and oral solubilized formulations by testing *Candida* strains with various itraconazole susceptibilities in a murine invasive infection. *J Antimicrob Chemother* 2011; **66**: 626–34.

49 Vandewoude K, Vogelaers D, Decruyenaere J *et al*. Concentrations in plasma and safety of 7 days of intravenous itraconazole followed by 2 weeks of oral itraconazole solution in patients in intensive care units. *Antimicrob Agents Chemother* 1997; **41**: 2714–8.

50 Lestner JM, Roberts SA, Moore CB *et al*. Toxicodynamics of itraconazole: implications for therapeutic drug monitoring. *Clin Infect Dis* 2009; **49**: 928–30.

51 Lestner JM, Denning DW. Tremor: a newly described adverse event with long-term itraconazole therapy. *J Neurol Neurosurg Psychiatry* 2010; **81**: 327–9.

52 Jaruratanasirikul S, Sriwiriyajan S. Effect of omeprazole on the pharmacokinetics of itraconazole. *Eur J Clin Pharmacol* 1998; **54**: 159–61.

53 Tucker RM, Denning DW, Hanson LH *et al*. Interaction of azoles with rifampin, phenytoin, and carbamazepine: in vitro and clinical observations. *Clin Infect Dis* 1992; **14**: 165–74.

54 Ducharme MP, Slaughter RL, Warbasse LH *et al*. Itraconazole and hydroxyitraconazole serum concentrations are reduced more than tenfold by phenytoin. *Clin Pharmacol Ther* 1995; **58**: 617–24.

55 Jaruratanasirikul S, Sriwiriyajan S. Effect of rifampicin on the pharmacokinetics of itraconazole in normal volunteers and AIDS patients. *Eur J Clin Pharmacol* 1998; **54**: 155–8.

56 Johnson LB, Kauffman CA. Voriconazole: a new triazole antifungal agent. *Clin Infect Dis* 2003; **36**: 630–7.

57 Schwartz S, Ruhnke M, Ribaud P *et al*. Improved outcome in central nervous system aspergillosis, using voriconazole treatment. *Blood* 2005; **106**: 2641–5.

58 Marr KA, Schlamm H, Rottinghaus ST *et al*. A randomised, double-blind study of combination antifungal therapy with voriconazole and anidulafungin versus voriconazole monotherapy for primary treatment of invasive aspergillosis. In: *Abstracts of the Twenty-second European Conference on Clinical Microbiology and Infectious Diseases, London, UK, 2012*. Abstract LB2818. European Society for Clinical Microbiology and Infectious Disease.

59 Marks DI, Pagliuca A, Kibbler CC *et al*. Voriconazole versus itraconazole for antifungal prophylaxis following allogeneic haematopoietic stem-cell transplantation. *Br J Haematol* 2011; **155**: 318–27.

60 Wingard JR, Carter SL, Walsh TJ *et al*. Randomized, double-blind trial of fluconazole versus voriconazole for prevention of invasive fungal infection after allogeneic hematopoietic cell transplantation. *Blood* 2010; **116**: 5111–8.

61 Pascual A, Csajka C, Buclin T *et al*. Challenging recommended oral and intravenous voriconazole doses for improved efficacy and safety: population pharmacokinetics-based analysis of adult patients with invasive fungal infections. *Clin Infect Dis* 2012; **55**: 381–90.

62 Driscoll TA, Yu LC, Frangoul H *et al*. Comparison of pharmacokinetics and safety of voriconazole intravenous-to-oral switch in immunocompromised children and healthy adults. *Antimicrob Agents Chemother* 2011; **55**: 5770–9.

63 Driscoll TA, Frangoul H, Nemecek ER *et al*. Comparison of pharmacokinetics and safety of voriconazole intravenous-to-oral switch in immunocompromised adolescents and healthy adults. *Antimicrob Agents Chemother* 2011; **55**: 5780–9.

64 Hope WW. Population pharmacokinetics of voriconazole in adults. *Antimicrob Agents Chemother* 2012; **56**: 526–31.

65 Neely M, Rushing T, Kovacs A *et al*. Voriconazole pharmacokinetics and pharmacodynamics in children. *Clin Infect Dis* 2010; **50**: 27–36.

66 Karlsson MO, Lutsar I, Milligan PA. Population pharmacokinetic analysis of voriconazole plasma concentration data from pediatric studies. *Antimicrob Agents Chemother* 2009; **53**: 935–44.

67 Roffey SJ, Cole S, Comby P *et al*. The disposition of voriconazole in mouse, rat, rabbit, guinea pig, dog, and human. *Drug Metab Dispos* 2003; **31**: 731–41.

68 Bruggemann RJM, Alffenaar JWC, Blijlevens NMA *et al*. Clinical relevance of the pharmacokinetic interactions of azole antifungal drugs with other coadministered agents. *Clin Infect Dis* 2009; **48**: 1441–58.

69 Andes D, Marchillo K, Stamstad T *et al*. In vivo pharmacokinetics and pharmacodynamics of a new triazole, voriconazole, in a murine candidiasis model. *Antimicrob Agents Chemother* 2003; **47**: 3165–9.

70 Serena C, Gilgado F, Marine M *et al*. Efficacy of voriconazole in a guinea pig model of invasive trichosporonosis. *Antimicrob Agents Chemother* 2006; **50**: 2240–3.

71 van de Sande WW, Mathot RA, ten Kate MT *et al*. Combination therapy of advanced invasive pulmonary aspergillosis in transiently neutropenic rats using human pharmacokinetic equivalent doses of voriconazole and anidulafungin. *Antimicrob Agents Chemother* 2009; **53**: 2005–13.

72 Jeans AR, Howard SJ, Al-Nakeeb Z *et al*. Combination of voriconazole and anidulafungin for the treatment of triazole resistant *Aspergillus fumigatus* in an in vitro model of invasive pulmonary aspergillosis. *Antimicrob Agents Chemother* 2012; **56**: 5180–5.

73 Pascual A, Calandra T, Bolay S *et al*. Voriconazole therapeutic drug monitoring in patients with invasive mycoses improves efficacy and safety outcomes. *Clin Infect Dis* 2008; **46**: 201–11.

74 Smith J, Safdar N, Knasinski V *et al*. Voriconazole therapeutic drug monitoring. *Antimicrob Agents Chemother* 2006; **50**: 1570–2.

75 Troke PF, Hockey HP, Hope WW. Observational study of the clinical efficacy of voriconazole and its relationship to plasma concentrations in patients. *Antimicrob Agents Chemother* 2011; **55**: 4782–8.

76 Dolton MJ, Ray JE, Chen SC *et al*. Voriconazole pharmacokinetics and therapeutic drug monitoring: a multi-center study. *Antimicrob Agents Chemother* 2012; **56**: 4793–9.

77 Park WB, Kim NH, Kim KH *et al*. The effect of therapeutic drug monitoring on safety and efficacy of voriconazole in invasive fungal infections: a randomized controlled trial. *Clin Infect Dis* 2012; **55**: 1080–7.

78 Soler-Palacin P, Frick MA, Martin-Nalda A *et al*. Voriconazole drug monitoring in the management of invasive fungal infection in immunocompromised children: a prospective study. *J Antimicrob Chemother* 2012; **67**: 700–6.

79 Denning DW, Ribaud P, Milpied N *et al*. Efficacy and safety of voriconazole in the treatment of acute invasive aspergillosis. *Clin Infect Dis* 2002; **34**: 563–71.

80 Miyakis S, van Hal SJ, Ray J *et al*. Voriconazole concentrations and outcome of invasive fungal infections. *Clin Microbiol Infect* 2010; **16**: 927–33.

81 Gomez-Lopez A, Cendejas-Bueno E, Cuesta I *et al*. Voriconazole serum levels measured by high-performance liquid chromatography: a monocentric study in treated patients. *Med Mycol* 2012; **50**: 439–45.

82 Ueda K, Nannya Y, Kumano K *et al*. Monitoring trough concentration of voriconazole is important to ensure successful antifungal therapy and to avoid hepatic damage in patients with hematological disorders. *Int J Hematol* 2009; **89**: 592–9.

83 Racil Z, Winterova J, Kouba M *et al*. Monitoring trough voriconazole plasma concentrations in haematological patients: real life multicentre experience. *Mycoses* 2012; **55**: 483–92.

84 Trifilio S, Singhal S, Williams S *et al*. Breakthrough fungal infections after allogeneic hematopoietic stem cell transplantation in patients on prophylactic voriconazole. *Bone Marrow Transplant* 2007; **40**: 451–6.

85 Mitsani D, Nguyen MH, Shields RK *et al*. A prospective, observational study of voriconazole therapeutic drug monitoring among lung transplant recipients receiving prophylaxis: factors impacting levels and associations between serum troughs, efficacy and toxicity. *Antimicrob Agents Chemother* 2012; **56**: 2371–7.

86 Tan K, Brayshaw N, Tomaszewski K *et al*. Investigation of the potential relationships between plasma voriconazole concentrations and visual adverse events or liver function test abnormalities. *J Clin Pharmacol* 2006; **46**: 235–43.

87 Peman J, Salavert M, Canton E *et al*. Voriconazole in the management of nosocomial invasive fungal infections. *Ther Clin Risk Manag* 2006; **2**: 129–58.

88 Zonios DI, Gea-Banacloche J, Childs R *et al*. Hallucinations during voriconazole therapy. *Clin Infect Dis* 2008; **47**: e7–e10.

89 Hamada Y, Seto Y, Yago K *et al*. Investigation and threshold of optimum blood concentration of voriconazole: a descriptive statistical meta-analysis. *J Infect Chemother* 2012; **18**: 501–7.

90 Kim KH, Lee S, Lee S *et al*. Voriconazole-associated severe hyponatremia. *Med Mycol* 2012; **50**: 103–5.

91 Matsumoto K, Ikawa K, Abematsu K *et al*. Correlation between voriconazole trough plasma concentration and hepatotoxicity in patients with different CYP2C19 genotypes. *Int J Antimicrob Agents* 2009; **34**: 91–4.

92 Imhof A, Schaer DJ, Schanz U *et al*. Neurological adverse events to voriconazole: evidence for therapeutic drug monitoring. *Swiss Med Wkly* 2006; **136**: 739–42.

93 Lutsar I, Hodges MR, Tomaszewski K *et al*. Safety of voriconazole and dose individualization. *Clin Infect Dis* 2003; **36**: 1087–8.

94 Okuda T, Okuda A, Watanabe N *et al*. Retrospective serological tests for determining the optimal blood concentration of voriconazole for treating fungal infection. *Yakugaku Zasshi* 2008; **128**: 1811–8.

95 Trifilio S, Ortiz R, Pennick G *et al*. Voriconazole therapeutic drug monitoring in allogeneic hematopoietic stem cell transplant recipients. *Bone Marrow Transplant* 2005; **35**: 509–13.

96 Krishna G, Ma L, Martinho M *et al*. A single dose phase I study to evaluate the pharmacokinetics of posaconazole new tablet and capsule formulations relative to oral suspension. *Antimicrob Agents Chemother* 2012; **56**: 4196–201.

97 Krishna G, Ma L, Martinho M *et al*. A new solid oral tablet formulation of posaconazole: a randomized clinical trial to investigate rising single- and multiple-dose pharmacokinetics and safety in healthy volunteers. *J Antimicrob Chemother* 2012; **67**: 2725–30.

98 Shields RK, Clancy CJ, Vadnerkar A *et al*. Posaconazole serum concentrations among cardiothoracic transplant recipients: factors impacting trough levels and correlation with clinical response to therapy. *Antimicrob Agents Chemother* 2011; **55**: 1308–11.

99 Courtney R, Wexler D, Radwanski E *et al*. Effect of food on the relative bioavailability of two oral formulations of posaconazole in healthy adults. *Br J Clin Pharmacol* 2004; **57**: 218–22.

100 Krishna G, Moton A, Ma L *et al*. Pharmacokinetics and absorption of posaconazole oral suspension under various gastric conditions in healthy volunteers. *Antimicrob Agents Chemother* 2009; **53**: 958–66.

101 Krishna G, Ma L, Vickery D *et al*. Effect of varying amounts of a liquid nutritional supplement on the pharmacokinetics of posaconazole in healthy volunteers. *Antimicrob Agents Chemother* 2009; **53**: 4749–52.

102 Raad II, Graybill JR, Bustamante AB *et al*. Safety of long-term oral posaconazole use in the treatment of refractory invasive fungal infections. *Clin Infect Dis* 2006; **42**: 1726–34.

103 Andes D, Marchillo K, Conklin R *et al*. Pharmacodynamics of a new triazole, posaconazole, in a murine model of disseminated candidiasis. *Antimicrob Agents Chemother* 2004; **48**: 137–42.

104 Mavridou E, Bruggemann RJ, Melchers WJ *et al*. Efficacy of posaconazole against three clinical *Aspergillus fumigatus* isolates with mutations in the *cyp51A* gene. *Antimicrob Agents Chemother* 2010; **54**: 860–5.

105 Dolton MJ, Ray JE, Chen SC *et al*. Multicenter study of posaconazole therapeutic drug monitoring: exposure-response relationship and factors affecting concentration. *Antimicrob Agents Chemother* 2012; **56**: 5503–10.

106 Walsh TJ, Raad I, Patterson TF *et al*. Treatment of invasive aspergillosis with posaconazole in patients who are refractory to or intolerant of conventional therapy: an externally controlled trial. *Clin Infect Dis* 2007; **44**: 2–12.

107 Gubbins PO, Krishna G, Sansone-Parsons A *et al*. Pharmacokinetics and safety of oral posaconazole in neutropenic stem cell transplant recipients. *Antimicrob Agents Chemother* 2006; **50**: 1993–9.

108 Courtney R, Pai S, Laughlin M *et al*. Pharmacokinetics, safety, and tolerability of oral posaconazole administered in single and multiple doses in healthy adults. *Antimicrob Agents Chemother* 2003; **47**: 2788–95.

109 Bryant AM, Slain D, Cumpston A *et al*. A post-marketing evaluation of posaconazole plasma concentrations in neutropenic patients with haematological malignancy receiving posaconazole prophylaxis. *Int J Antimicrob Agents* 2011; **37**: 266–9.

110 Eiden C, Meniane JC, Peyriere H *et al*. Therapeutic drug monitoring of posaconazole in hematology adults under posaconazole prophylaxis: influence of food intake. *Eur J Clin Microbiol Infect Dis* 2012; **31**: 161–7.

111 Hoenigl M, Raggam RB, Salzer HJ *et al*. Posaconazole plasma concentrations and invasive mould infections in patients with haematological malignancies. *Int J Antimicrob Agents* 2012; **39**: 510–3.

112 Campoli P, Al AQ, Robitaille R *et al*. Concentration of antifungal agents within host cell membranes: a new paradigm governing the efficacy of prophylaxis. *Antimicrob Agents Chemother* 2011; **55**: 5732–9.

113 Jang SH, Colangelo PM, Gobburu JV. Exposure-response of posaconazole used for prophylaxis against invasive fungal infections: evaluating the need to adjust doses based on drug concentrations in plasma. *Clin Pharmacol Ther* 2010; **88**: 115–9.

114 Tonini J, Thiebaut A, Jourdil JF *et al*. Therapeutic drug monitoring of posaconazole in allogeneic hematopoietic stem cell transplantation patients who develop gastrointestinal graft-versus-host disease. *Antimicrob Agents Chemother* 2012; **56**: 5247–52.

115 Ullmann AJ, Lipton JH, Vesole DH *et al*. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. *N Engl J Med* 2007; **356**: 335–47.

116 Cornely OA, Maertens J, Winston DJ *et al*. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *N Engl J Med* 2007; **356**: 348–59.

117 Green MR, Woolery JE. Posaconazole serum level on day 2 predicts steady state posaconazole serum level. *Ther Drug Monit* 2012; **34**: 118–9.

118 Lebeaux D, Lanternier F, Elie C *et al*. Therapeutic drug monitoring of posaconazole: a monocentric study with 54 adults. *Antimicrob Agents Chemother* 2009; **53**: 5224–9.

119 Howard SJ, Cerar D, Anderson MJ *et al*. Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. *Emerg Infect Dis* 2009; **15**: 1068–76.

120 Arendrup MC, Cuenca-Estrella M, Lass-Florl C *et al*. EUCAST technical note on *Aspergillus* and amphotericin B, itraconazole, and posaconazole. *Clin Microbiol Infect* 2012; **18**: E248–E250.

121 Howard SJ, Felton TW, Gomez-Lopez A *et al*. Posaconazole: the case for therapeutic drug monitoring. *Ther Drug Monit* 2012; **34**: 72–6.

122 Hope WW. Flucytosine. In: Grayson ML, ed. *Kucers' The Use of Antibiotics*. Boca Raton: ASM Press, 2010; 1957–63.

123 Brouwer AE, Rajanuwong A, Chierakul W *et al*. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. *Lancet* 2004; **363**: 1764–7.

124 Nussbaum JC, Jackson A, Namarika D *et al*. Combination flucytosine and high-dose fluconazole compared with fluconazole monotherapy for the treatment of cryptococcal meningitis: a randomized trial in Malawi. *Clin Infect Dis* 2010; **50**: 338–44.

125 Stamm AM, Diasio RB, Dismukes WE *et al*. Toxicity of amphotericin B plus flucytosine in 194 patients with cryptococcal meningitis. *Am J Med* 1987; **83**: 236–42.

126 British Society for Antimicrobial Chemotherapy Working Party. Laboratory monitoring of antifungal chemotherapy. *Lancet* 1991; **337**: 1577–80.

127 Soltani M, Tobin CM, Bowker KE *et al*. Evidence of excessive concentrations of 5-flucytosine in children aged below 12 years: a 12-year review of serum concentrations from a UK clinical assay reference laboratory. *Int J Antimicrob Agents* 2006; **28**: 574–7.

128 Pasqualotto AC, Howard SJ, Moore CB *et al*. Flucytosine therapeutic monitoring: 15 years experience from the UK. *J Antimicrob Chemother* 2007; **59**: 791–3.

129 Hope WW, Warn PA, Sharp A *et al*. Derivation of an in vivo drug exposure breakpoint for flucytosine against *Candida albicans* and impact of the MIC, growth rate, and resistance genotype on the antifungal effect. *Antimicrob Agents Chemother* 2006; **50**: 3680–8.

130 Andes D, van Ogtrop M. In vivo characterization of the pharmacodynamics of flucytosine in a neutropenic murine disseminated candidiasis model. *Antimicrob Agents Chemother* 2000; **44**: 938–42.

131 Normark S, Schönebeck J. In vitro studies of 5-fluorocytosine resistance in *Candida albicans* and *Torulopsis glabrata*. *Antimicrob Agents Chemother* 1972; **2**: 114–21.

132 Kim H, Kumari P, Laughlin M *et al*. Use of high-performance liquid chromatographic and microbiological analyses for evaluating the presence or absence of active metabolites of the antifungal posaconazole in human plasma. *J Chromatogr A* 2003; **987**: 243–8.

133 Muller C, Arndt M, Queckenberg C *et al*. HPLC analysis of the antifungal agent posaconazole in patients with haematological diseases. *Mycoses* 2006; **49** Suppl 1: 17–22.

134 Chhun S, Rey E, Tran A *et al*. Simultaneous quantification of voriconazole and posaconazole in human plasma by high-performance liquid chromatography with ultra-violet detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 2007; **852**: 223–8.

135 Gage R, Stopher DA. A rapid HPLC assay for voriconazole in human plasma. *J Pharm Biomed Anal* 1998; **17**: 1449–53.

136 Rochat B, Pascual A, Pesse B *et al*. Ultra-performance liquid chromatography mass spectrometry and sensitive bioassay methods for quantification of posaconazole plasma concentrations after oral dosing. *Antimicrob Agents Chemother* 2010; **54**: 5074–81.

137 Farowski F, Cornely OA, Vehreschild JJ *et al*. Quantitation of azoles and echinocandins in compartments of peripheral blood by liquid chromatography-tandem mass spectrometry. *Antimicrob Agents Chemother* 2010; **54**: 1815–9.

138 Kousoulos C, Tsatsou G, Apostolou C *et al*. Development of a high-throughput method for the determination of itraconazole and its hydroxy metabolite in human plasma, employing automated liquid-liquid extraction based on 96-well format plates and LC/MS/MS. *Anal Bioanal Chem* 2006; **384**: 199–207.

139 Araujo BV, Conrado DJ, Palma EC *et al*. Validation of rapid and simple LC-MS/MS method for determination of voriconazole in rat plasma. *J Pharm Biomed Anal* 2007; **44**: 985–90.