EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Amphotericin B and *Aspergillus* spp.	Rationale for the EUCAST clinical breakpoints, V 1.0	11th January 2012

Foreword

EUCAST
The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is organised by the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), the European Centre for Disease Prevention and Control (ECDC), and the active national antimicrobial breakpoint committees in Europe. EUCAST was established by ESCMID in 1997, was restructured in 2001-2002 and has been in operation in its current form since 2002. The current remit of EUCAST is to harmonise clinical breakpoints for existing drugs in Europe, to determine clinical breakpoints for new drugs, to set epidemiological (microbiological) breakpoints, to revise breakpoints as required, to harmonise methodology for antimicrobial susceptibility testing, to develop a website with MIC and zone diameter distributions of antimicrobial agents for a wide range of organisms and to liaise with European governmental agencies and European networks involved with antimicrobial resistance and resistance surveillance.

Information on EUCAST and EUCAST breakpoints is available on the EUCAST website at http://www.EUCAST.org.

EUCAST rationale documents
EUCAST rationale documents summarise the information on which the EUCAST clinical breakpoints are based.

Availability of EUCAST document
All EUCAST documents are freely available from the EUCAST website at http://www.EUCAST.org.

Citation of EUCAST documents
This rationale document should be cited as: “European Committee on Antimicrobial Susceptibility Testing. Amphotericin B and *Aspergillus* spp.: Rationale for the clinical breakpoints, version 1.0, 2012. http://www.eucast.org.

Introduction

Amphotericin B is a polyene antifungal agent active against yeasts and moulds. In Europe it is available in four different formulations including Amphotericin B deoxycholate and three lipid formulations. The active compound is identical but the pharmacokinetics and toxicity profiles differ from formulation to formulation. Amphotericin B is active in vitro against *Candida* spp., *Aspergillus* spp., Mucorales, various other opportunistic fungi and against the endemic moulds.

The following formulations of amphotericin B are licensed for treatment of *Aspergillus* infections, yet licensed applications may differ from country to country - Amphotericin B deoxycholate (D-AmB), Amphotericin B colloidal dispersion (ABCD), amphotericin B lipid complex (ABLC) and lipsomal amphotericin B (L-AmB). L-AMB is also licensed for empirical therapy for presumed fungal infection in febrile, neutropenic patients.

The species most frequently involved are *Aspergillus fumigatus, Aspergillus flavus, Aspergillus terreus* and *Aspergillus niger.* Although *Aspergillus* species are generally susceptible to polyenes, elevated MICs have been reported for some species including *Aspergillus lentulus* and *Aspergillus fumigatiaffinis*. Amphotericin B has limited activity against *A. terreus.*

The European Committee on Antimicrobial susceptibility Testing - Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST) has determined breakpoints for amphotericin B against *Aspergillus* spp. These breakpoints are tentative and will be revised after two years.

1a. Dosage for treatment of aspergillosis: D-AmB												
	Denmark	**Germany**	**Spain**	**Sweden**	**Switzerland**	**Turkey**	**Austria**	**Norway**	**France**	**The Netherlands**	**Estonia**	**Greece**
Minimum dose (mg/kg/day)	1	1	1	1	NA	1	1	1	1	1	1	1
Most common dose (mg/kg/d)	1-1.5	1-1-5	1-1.5	1-1.5	1.5	1-1.5	1-1.5	1-1.5	1-1.5	1-1.5	1-1.5	1-1-5
1st day dose (mg/kg/day)	0.5	1-1.5[1]	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1-1.5[1]
2nd day dose (mg/kg/day)	1	1-1.5	1	1	NA	NA	NA	1	1	1	NA	1-1.5
Maximum dose (mg/kg/d)	1.5	1.5	1.5	3-5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Loading dose (mg/kg/d)	1	NA	NA	NA	0.25	NA	NA	NA	0.5	NA	NA	NA
Available formulations	iv	iv	iv	iv	iv	iv	iv	iv	iv; oral 250 mg tablet; 10% oral suspension	iv	iv	iv

NA = Not applicable;
[1] Treatment should be started at the full target dose under careful clinical monitoring for infusion related reactions.

1b. Dosage for treatment of aspergillosis: L-AmB

	Denmark	Germany	Spain	Sweden	Switzerland	Turkey	Austria	Norway	France	The Netherlands	Estonia	Greece
Minimum dose (mg/kg/day)	3	3	1	1	1	1	1	1	3	3	NA	3
Most common dose (mg/kg/d)	3	3-5	3	3	3	3-5	3-5	3	3	3	3-5	3-5
1st day dose (mg/kg/day)	1	3	1	3	1	1	1	1	3	1	3	3
2nd day dose (mg/kg/day)	1	3	1	3	3	1	1	1	3	3	3	3
Maximum dose (mg/kg/d)	5, 7 and 10	5-10	5, 7 and 10	3	5-6	5	5	5	10	5	5-6	5-7
Loading dose	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Available formulations	iv	iv	iv	iv	iv	iv	iv	iv	iv	iv	iv	iv

NA = Not applicable

1c. Dosage for treatment of aspergillosis: ABLC												
	Denmark	**Germany**	**Spain**	**Sweden**	**Switzerland**	**Turkey**	**Austria**	**Norway**	**France**	**The Netherlands**	**Estonia**	**Greece**
Minimum dose (mg/kg/day)	5	5	3	NA		3	3	5	5	3		5
Most common dose (mg/kg/d)	5	5	5	5		5	3-5	5	5	3-5.5		5
1st day dose (mg/kg/day)	5	5	5	5		5	5	NA	5	3-5.5		5
2nd day dose (mg/kg/day)	5	5	5	5		NA	NA	NA	5	3-5.5		5
Maximum dose (mg/kg/d)	5	5	5	5		5	5	5	5	5.5		5
Loading dose	NA	NA	NA	NA		NA	NA	NA	NA	NA		NA
Available formulations	iv	iv	iv	iv	NA	iv	iv	iv	iv	iv	NA	iv

NA = Not applicable

1d. Dosage for treatment of aspergillosis: ABCD

	Denmark	Germany	Spain	Sweden	Switzerland	Turkey	Austria	Norway	France	The Netherlands	Estonia	Greece
Minimum dose (mg/kg/day)							3			3		
Most common dose (mg/kg/d)							3-5			3		
1st day dose (mg/kg/day)							3-5			1		
2nd day dose (mg/kg/day)							3-5			3		
Maximum dose (mg/kg/d)							5			4		
Loading dose							NA			NA		
Available formulations	NA	NA	NA	NA	NA	NA	iv	NA	NA	iv	NA	NA

NA = Not applicable

2. MIC distributions and epidemiological cut-off (ECOFF) values (mg/L)

	0.002	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512	ECOFF
A. flavus	0	0	0	0	1	1		13	80	92	54	21	8	3	1	0	0	0	0	4
A. fumigatus	0	0	0	1	3	13	110	589	709	267	147	6	3	2	1	0	0	0	1	1
A. nidulans	0	0	0	0	0	1	3	6	27	24	12	4	1	3	0	0	0	0	0	ND
A. niger	0	0	0	0	1	6	62	87	24	9	2	0	0	1	0	0	0	0	0	1
A. terreus	0	0	0	0	0	0	1	2	39	113	97	27	11	6	1	0	0	0	0	4
A. versicolor	0	0	0	0	0	0	2	2	6	9	3	1	0	0	0	0	0	0	0	ND
A. sydowii	0	0	0	0	1	2	1	4	11	28	7	1	0	1	0	0	0	0	0	ND

The table includes MIC distributions available at the time breakpoints were set and represent combined distributions from multiple sources and time periods. The distributions are used to define the epidemiological cut-offs (ECOFF) and give an indication of the MICs for organisms with acquired or mutational resistance mechanisms. They should not be used to infer resistance rates. When there is insufficient evidence no epidemiological cut-off has been determined (ND).

3. Breakpoints prior to harmonisation (mg/L) S≤ R>		
	European breakpoints	**CLSI**
General breakpoints:		
	NA	NA
Species specific breakpoints:		
	NA	NA

NA = Not available

4. Pharmacokinetics

	D-AmB[1]	ABLC	L-AmB[2]	ABCD
Dosage (mg/kg/day)	0.6	2	3-5	3
Approx. Cmax (mg/L)	1.4	2-3	3	2-2.5
Approx. Cmin (mg/L)	0.5	0.7	0.4	NA
Total body clearance/F (L/h)	38	436	11	0.117
Terminal T ½ (h)	127	393	152	29
AUC_{0-24}h (mg.h/L)	13.9	19.2 (dosage 5 mg/kg/day)	171	45
Fraction unbound[1] (%)	<5	<5	<5	<5
Volume of central compartment, Vc (L/kg)	0.136	NA	0.05-0.1	0.089
Comments	• [1]The protein binding of amphotericin B is extremely complex and poorly understood • [2]Pharmacokinetics may vary with dosages >7.5 mg/kg • Amphotericin B exerts its antifungal effect by disruption of fungal cell wall synthesis because of its ability to bind to sterols, primarily ergosterol, which leads to the formation of pores that allow leakage of cellular components. This affinity to sterols may also account for its toxic effects against selected mammalian cells. Amphotericin B is generally considered cidal against susceptible fungi at clinically relevant concentrations. • All three lipid formulations have an improved therapeutic index and are significantly less nephrotoxic than D-AmB. • L-AmB is associated with less infusion-related reactions and less nephrotoxicity than ABLC. • ABCD is associated with increased infusion-related reactions relative to D-AmB.			
References	Hiemenz et al. Clin Infect Dis 1996; 22: S133-S144. Bellmann et al. Clin Infect Dis 2003; 36: 1500-1501. Bekersky et al. Antimicrob Agents Chemother 2002; 46: 828-833. Bekersky et al. Antimicrob Agents Chemother 2002; 46: 834-840. Walsh et al. Antimicrob Agents Chemother 2001; 45: 3487-3496. Gubbins et al. Antimicrob Agents Chemother 2009; 53: 3664-3674. Adedoyin et al. Antimicrob Agents Chemother 1997; 41: 2201-2208. Adedoyin et al. Antimicrob Agents Chemother 2000; 44: 2900-2902. Amantea et al. Chemotherapy 1999; 45(Suppl 1): 48-53.			

5. Pharmacodynamics				
*f*AUC/MIC for stasis				
*f*AUC/MIC for 2 log reduction				
*f*AUC/MIC from clinical data				
Comments	• No data are available for EUCAST MICs and *Aspergillus* spp.			
References				

6. Monte Carlo simulations and Pk/Pd breakpoints

Not available for EUCAST data because there is no clear Pk/Pd target defined.

7. Clinical data

Polyene antifungals have been the cornerstone treatment for invasive aspergillosis for over 40 years. Whilst new treatment options have somewhat changed their role, lipid-associated amphotericin B regimens remain important therapeutic options for aspergillosis due to their broad-spectrum of activity and limited cross-resistance with triazole antifungals and the need for an alternative to voriconazole in some patients who can not tolerate it. Recent studies have demonstrated the importance of accurate speciation of *Aspergillus* species during amphotericin B therapy, as some non-*fumigatus* species, particularly *A. terreus may be resistant to amphotericin B.*

There is a paucity of data to guide the administration of antifungal therapy in patients with invasive aspergillosis resistant to voriconazole. Amphotericin B formulations are alternative options for invasive aspergillosis, such as in patients who cannot tolerate voriconazole or in those with refractory aspergillosis. Selecting the most appropriate lipid-based formulation of amphotericin remains a challenge and high-quality evidence from randomized, controlled trials is limited.

In the study by Leenders et al. (1998) L-AmB 5 mg/kg/day was compared with D-AmB 1 mg/kg/day. The patient population was severely neutropenic and had proven or probably invasive fungal infections; complete responses with L-AmB were better than with D-AmB. Ellis et al. (1998) compared L-AmB 1 mg/kg/day with 4 mg/kg/day for efficacy in proven or probable invasive aspergillosis patients. There was no overall statistical difference in the survival rates at 6 months between the groups. Invasive aspergillosis was the primary cause of death for the same number of patients in both groups, but the group with definite invasive aspergillosis at the time of randomization comprised only 20 patients, and their response rate was higher on L-AmB 4 mg than on 1 mg/kg/day (37 % versus 58 %). In another study, L-AmB administered at a daily dose of 3 mg/kg was associated with similar efficacy, less nephrotoxicity, and a trend toward improved 12-week survival, as compared with a dose of 10 mg as primary therapy for invasive aspergillosis (Cornely et al. 2007); this study showed that increased doses of amphotericin B should not be equated with greater efficacy at least during invasive pulmonary aspergillosis as the number of patients with disseminated aspergillosis was low.

Bowden et al. (2002) compared ABCD 6 mg/kg/day with D-AmB 1-1.5 mg/kg/day against invasive aspergillosis in cancer patients and the results showed similar success rates for the two groups (52 % versus 51 %, respectively) but a better tolerance of the lipid formulation. In another study ABCD was given to 82 patients with proven or suspected aspergillosis, and was prescribed in cases of failure or intolerance to D-AmB, or to patients with a pre-existing renal insufficiency. The response rate was higher in the ABCD group compared with the D-AmB group (48.8 % versus 23.4 %) and the survival rate was nearly twice as high in the ABCD group (White et al.1997). ABCD had a similar efficacy but less nephrotoxicity than D-AmB as primary therapy for invasive aspergillosis (Hong et al. 2006).

An analysis of a large data registry on the use of ABLC as second line agent for invasive aspergillosis showed encouraging findings regarding efficacy and safety, including the drug's tolerability in patients with renal impairment (Chandrasekar et al. 2005).However, ABLC studied showed more nephrotoxic when compared to L-AMB (Hachem et al. 2008, Wingard et al. 2000), because of the higher infusional toxicity, no first line indication was granted by the FDA.

These studies did not include MICs by the EUCAST method so a correlation of in vitro MICs with clinical outcome has not been possible.

Leenders AC et al. Brit J Haematol 1998; 103: 205-212.
White MH et al. Clin Infect Dis 1997; 24: 635-642
Hong Y et al. Antimicrob Agents Chemother 2006; 50: 935-942.
Chandrasekar PH et al. Clin Infect Dis 2005; 40: Suppl 6:S392-S400.
Hachem RY et al. Cancer 2008; 112: 1282-7.
Ellis M et al. Clin Infect Dis 1998; 27: 1406-1412.
Leenders ACAP et al AIDS 1997; 11: 1463-1471.
Cornely OA et al. Clin Infect Dis 2007; 44: 1289-1297.
Bowden R et al. Clin Infect Dis 2002; 35:359-366.
Wingard JR et al. Clin Infect Dis 2000; 31: 1155-1163.

8. Clinical breakpoints	
Non-species-related breakpoints	There is insufficient evidence to set non-species-related breakpoints.
Species-related breakpoints	Breakpoints were based on microbiological data and clinical experience. *A. fumigatus,* S ≤1, R >2 mg/L *A. niger,* S ≤1, R >2 mg/L
Species without breakpoints	There is insufficient evidence (IE) to set clinical breakpoints for other species of *Aspergillus.* *A. terreus* is not considered a good target for amphotericin B, which is therefore not recommended for treatment of invasive aspergillosis caused by this species. The amphotericin B MICs for *A. flavus* are higher than those for *A. fumigatus*; the clinical relevance of this observation is uncertain and there are insufficient data to set breakpoints. There are no data available regarding the underlying mechanism of acquired amphotericin B resistance. Isolates with MICs higher than the breakpoint should be retested and send to a laboratory experienced in susceptibility testing of moulds.
Clinical qualifications	The EUCAST-AFST considers amphotericin B to be appropriate therapy for invasive aspergillosis.
Dosage	The EUCAST breakpoints apply to licensed dosing of D-AmB (1-1.5mg/kg/d), L-AmB (3-5 mg/kg), ABCD (3-5mg/kg) and ABLC (5 mg/kg) respectively.
Additional comment	The EUCAST-AFST will review breakpoints for amphotericin B when more data available for *Aspergillus* species which were not assigned breakpoints during the present review and when there are clinical data for *Aspergillus* isolates with MIC values outside the wild type distribution.

9. EUCAST clinical MIC breakpoints

All EUCAST breakpoints can be found at **http://www.eucast.org**

10. Exceptions noted for individual national committees

None