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Association of Fluconazole Pharmacodynamics with Mortality in Patients with Candidemia

Recent modifications to the CLSI susceptibility testing standard M27-A on fluconazole MIC determination highlight the time of incubation (48 or 24 h) and the end point criteria (80% growth inhibition for macrodilution and 50% for microdilution) (2, 4). The use of the 50% inhibition end point after 24 h resulted in one- to twofold-lower MICs than were found after 48 h, as exemplified in Fig. 1. The 24- and 48-h MIC limits for the two recommended quality control strains are lowered correspondingly: for *Candida parapsilosis* ATCC 22019, 0.5 to 4 mg/liter versus 1 to 4 mg/liter, and for *Candida krusei* ATCC 6258, 8 to 64 mg/liter versus 16 to 128 mg/liter (3). Yet, the authors conclude that this does not give rise to a need for lowering fluconazole breakpoints.

A one- to twofold-lower MIC will not alter the classification of susceptibility for the majority of *C. albicans* isolates, as the MICs are far below the breakpoint for susceptibility (MIC ≤ 8 mg/liter). However, one- to twofold-lower MICs will have a significant impact on the susceptibility of isolates belonging to the naturally more resistant species *Candida glabrata* and *Candida krusei*, with typical MICs of 4 to 64 mg/liter, as well as for isolates with acquired resistance. For instance, 3 of the 10 isolates (30%) that Baddley et al. defined as resistant (MIC ≥ 64 mg/liter) using the 48-h and 80% or 50% inhibition criteria would be reclassified as susceptible in a dose-dependent manner (SDD) if the early reading and 50% end point definition were used, while this was the case for 30/53 isolates (56.6%) in Ostrosky-Zeichner's study (2, 4). In addition, the percentage of clinical response for SDD strains would decrease from 86% to 55% when the MICs were obtained at 48 h versus 24 h (4).

Both authors conclude that even with an earlier reading and a 50% growth inhibition end point, the MIC/pharmacodynamic outcome relationship supports the use of the original breakpoints. However, we have the following concerns. Both studies pool infections caused by different species, thus assuming that the virulence levels of the yeasts and the host groups are identical. This may not be the case (1). Furthermore, at least for the Baddley study, there were fewer than 10 cases involving isolates with MICs of >1 mg/liter in most groups, making percentage calculations inappropriate (2). Classification and regression tree (CART) analysis (CART 6.0; Salford Systems, CA) produces receiver operating characteristic (ROC) area curves and relative errors which are of insufficient quality for any conclusions to be drawn (Table 1). If such analysis is performed anyway, the lowest relative error and highest ROC area for predicting mortality are obtained with a 48-h incubation, an 80% inhibition end point, and a $\log_2$ MIC of 2.5 mg/liter as the threshold (Table 1), which suggests a breakpoint for resistance of >4 mg/liter or ≥8 mg/liter (Table 2).

If data mining tools for ascertaining the statistical power of in vitro correlation with outcome do not produce results that reach statistical significance, we suggest that changes in a susceptibility test leading to one- to twofold-lower MICs should be accompanied with a similar decrease in breakpoints.

TABLE 1. CART analysis of the relationship of MIC versus mortality in patients with candidemia (data taken from reference 2)[a]

Incubation period and growth inhibition end point	MIC split (mg/liter)	Relative error[b]	ROC curve area[c]
24 h			
50%	≤32/>32	1.033	0.468
80%	≤4/>4	0.942	0.524
48 h			
50%	≤4/>4	0.883	0.560
80%	≤4/>4	0.714	0.646

[a] CART analysis (CART 6.0; Salford Systems, CA) was performed with the following methodological conditions: Gini method, minimum cost tree regardless of the size for selecting the best tree, 10 v-fold-cross-validation, equal priors, no costs, and no penalties.
[b] A relative error of 0 means no error, or a perfect fit, and 1 represents the performance of random guessing.
[c] A ROC curve area of 1 means a perfect prediction (100% sensitivity and 0% false positives), and 0.5 represents a random guess.

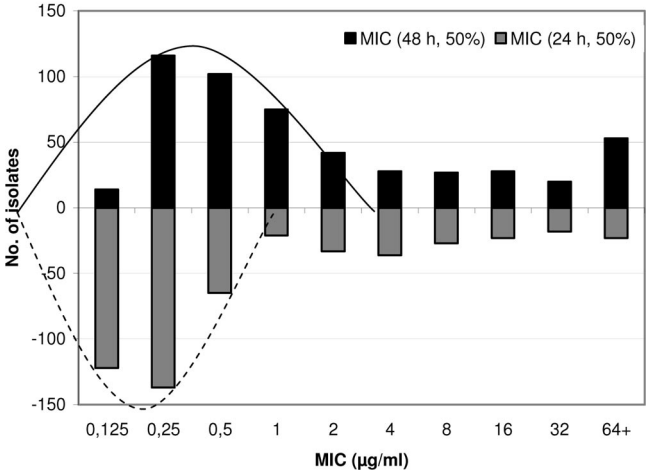

FIG. 1. MICs obtained by the CLSI method, using 48 h of incubation and 50% inhibition or using 24 h of incubation and 50% inhibition.

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TABLE 2. Relationship of MIC to mortality in patients with candidemia (data taken from reference 2)

Susceptibility classification	No. of nonsurvivors/total no. in group (%) for indicated breakpoint criteria, incubation period, and growth inhibition end point							
	Alternative[a]				CLSI[b]			
	24 h		48 h		24 h		48 h	
	50%	80%	50%	80%	50%	80%	50%	80%
Susceptible	17/65 (26.1)	16/58 (27.6)	12/55 (21.8)	12/52 (23)	20/74 (27)	20/74 (27)	18/71 (25)	17/67 (25.4)
Intermediate/SDD	3/8 (37.5)	1/12 (8.3)	3/11 (27.3)	1/9 (11.1)	0/3 (0)	0/2 (0)	0/3 (0)	1/5 (20)
Resistant	4/10 (40)	7/14 (50)	9/18 (50)	11/21 (52.3)	4/7 (57.1)	4/8 (50)	6/10 (60)	6/10 (60)

[a] Susceptibility, MIC of <2 mg/liter; resistance, MIC of >4 mg/liter.
[b] Susceptibility, MIC of ≤8 mg/liter; resistance, MIC of ≥64 mg/liter.

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Authors' Reply

Arendrup and colleagues comment on recent developments in fluconazole susceptibility testing, specifically, MIC determination at the 24- versus 48-hour incubation periods (3, 7). They highlight the difficulties in fluconazole susceptibility testing and suggest lowering the MIC breakpoints by 1 or 2 dilutions. We believe that the data from our two reports suggest that no change in breakpoints is warranted (3, 7).

An important issue that Arendrup and colleagues discuss is that in many susceptibility studies, isolates are "pooled," perhaps under the assumption that the virulence levels of yeasts and host groups are identical. The paper by Baddley and colleagues was a prospective study enrolling consecutive patients with candidemia at a tertiary care medical center (3). At the enrolling institution, and probably most institutions, there is not one single species causative of candidemia. These concerns of virulence and host differences are precisely why a detailed multivariable analysis was indicated and should be performed in future studies. The presence of *Candida* spp. was not an independent predictor of outcome in this study. However, the results demonstrated that host factors, such as severity of illness and age, were more predictive of mortality than fluconazole MICs. The MIC end point for fluconazole, read at 50% inhibition at 48 h, was the MIC end point parameter most strongly associated with mortality.

Arendrup and colleagues also point out that fewer than 10 cases of isolates had fluconazole MICs of >1 mg/liter and thus that our percentage calculations were "inappropriate." We assume that Arendrup and colleagues meant that resistant isolates (MICs ≥ 64 μg/ml) were few in our study, which is correct. The interpretation of percentages and statistical testing in a study with a small sample of resistant isolates is an important issue. As is mentioned in Discussion in that paper, we acknowledge that our study was underpowered (3). The lack of a large number of fluconazole-resistant isolates is not uncommon in MIC studies and underscores the relatively low frequency of these isolates and the difficulties in detailing outcome data. Therefore, pooling of studies, with the caveat of careful data analysis, is a reasonable approach (7). Clearly, future studies need to be powered effectively to demonstrate MIC end point validity.

An important fact that we wish to emphasize is that our own data showed that the majority of microbiologically and clinically relevant disparities in MICs occur in isolates with MICs in the higher range of the scale, such as in the case with *Candida glabrata*. We clearly recommend extending the observation period and reporting results with a cautionary note at 24 h or waiting to report at 48 h for such isolates.

In addition to the use of appropriate statistical tools, conduct of exposure-response analyses necessitates a high degree of scientific judgment. This is especially true when using exploratory tools, such as classification and regression tree (CART) analysis, for identifying MIC- and exposure-related breakpoints that are predictive of outcome. As Arendrup and colleagues point out, the results obtained using this approach are less precise when studies of small sample size are evaluated (3). However, when considered together with prior knowledge, as in the case of this analysis, where CART-derived breakpoints were evaluated in the context of previous nonclinical and clinical pharmacokinetic-pharmacodynamic evaluations (1, 2, 4–6, 8–10), such breakpoints may still be of value despite the limited sample size of the study. We identified the same CART-derived breakpoints for area-under-the-curve/MIC ratio and MIC (11.5 and 64 mg/liter, respectively) with the data obtained for the 24- and the 48-hour MIC determinations at 50% inhibition (3). With respect to ROC curve analyses, we obtained areas under the ROC curve for 24- and 48-hour MICs (at 50% inhibition) of 0.57 and 61, respectively, which differ from the values of 0.47 and 0.56, respectively, obtained by Arendrup and colleagues. Logistic regression analyses with

mortality as the outcome and log MIC as the independent variable yielded *P* values of 0.28 and 0.045 for 24- and 48-hour MICs, respectively. These area-under-the-ROC-curve and logistic regression results represent reasonable evidence of relationships between MICs and mortality and support the exploratory use of CART to obtain the above-described breakpoints. Given the sensitivity associated with different settings for algorithms used for CART and the default minimum size for a terminal node in different statistical packages, the identification of different breakpoints from one package to another may not be unexpected. We suspect that different settings associated with CART may explain the differences in CART-derived breakpoints between our results and those of Arendrup and colleagues. In summary, we believe it is appropriate to use CART to explore optimal MIC breakpoints with these data. Our results are consistent with currently established breakpoints, though the work of Arendrup and colleagues demonstrates that with a sample of this size, the CART-derived breakpoints from this analysis may have a substantial amount of inherent variability. Thus, additional contextual information and data must be considered when identifying an optimal breakpoint.

In our opinion, the difference of 24 hours in reporting laboratory results to the clinician outweighs the small impact on survival gained by evaluating MICs at 48 h. Our recommendations also make the provision that data be reported at 24 h with a cautionary note or at 48 h for isolates with higher MICs. Larger studies are needed to confirm this approach.

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