Histoplasma Antigen Clearance during Treatment of Histoplasmosis in Patients with AIDS Determined by a Quantitative Antigen Enzyme Immunoassay[▽]

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Clearance of *Histoplasma* antigen has been used as a marker for response to treatment of progressive disseminated histoplasmosis (PDH) in patients with AIDS. Advancements in *Histoplasma* antigen detection permit accurate quantification of antigen concentration. We compared the clearance of antigenemia and antigenuria during effective treatment of PDH. Urine and serum specimens were serially collected from patients with AIDS who were successfully treated for PDH as part of two prospective clinical trials. Samples were stored frozen until they were tested in the quantitative *Histoplasma* antigen enzyme immunoassay. The kinetics of antigen clearance during the first 12 weeks of therapy were assessed in urine and serum during treatment with liposomal or deoxycholate amphotericin B followed by itraconazole and, in a separate analysis, in patients receiving only itraconazole. Latent class growth analysis was performed to define patterns of antigen clearance over time. In patients receiving amphotericin B, antigen levels declined the most during the first 2 weeks of treatment and antigenemia decreased more rapidly than antigenuria (5.90 ng/ml per week versus 4.21 ng/ml per week, respectively; $P = 0.09$). Mean reductions of antigen levels from baseline at weeks 2 and 12 were greater in sera than in urine: 11.26 ng/ml versus 7.65 ng/ml ($P = 0.0948$) and 18.52 ng/ml versus 14.64 ng/ml ($P = 0.0440$), respectively. In patients who received itraconazole alone, most of the decline in antigenuria occurred later during treatment and was overall slower than that seen with amphotericin B ($P < 0.0001$). Results of latent class growth modeling showed two distinct trajectories for each parameter. With effective therapy, *Histoplasma* antigenemia decreases more rapidly than antigenuria, providing a more sensitive early laboratory marker for response to treatment. Antigenuria declines earlier with amphotericin B than with itraconazole.

Histoplasma antigen levels are often monitored as a marker for response to treatment. Antigen levels decline with effective therapy (9, 11) and increase with relapse (10). The Infectious Diseases Society of America (IDSA) guidelines for treatment of patients with progressive disseminated histoplasmosis (PDH) recommend monitoring antigen levels during and after completion of treatment (13). Prior studies of antigen clearance in PDH used the original *Histoplasma* antigen radioimmunoassay (11) and enzyme immunoassay (EIA) (2, 9). Subsequently, the assay was modified to reduce false-positive results caused by interfering substances (14) and to provide quantification (1).

In the quantitative assay, patient results are determined by comparison to calibration standards that contain known concentrations of *Histoplasma* galactomannan (1). By calculating results using a calibration curve, the interassay variation was markedly reduced, permitting comparison of results from different assays without retesting prior specimens alongside current specimens. The objective of this study using the quantitative assay was to determine the clearance of antigenemia and antigenuria during early successful treatment of PDH in patients with AIDS (1, 4, 7).

MATERIALS AND METHODS

Clinical materials. The clinical samples used in this study were collected from patients with PDH and AIDS who were enrolled in two multicenter trials. In the first study (AIDS Clinical Trials Group 120 [ACTG-120]; 1991 to 1992), patients with mild to moderate PDH were treated with 300 mg itraconazole orally twice daily for 3 days followed by 200 mg twice daily for 12 weeks (7). In the more recent trial (Mycoses Study Group 29 [MSG-29]; 1995 to 1999), patients with moderately severe to severe PDH received 3 mg liposomal amphotericin B/kg body weight/day or 0.7 mg deoxycholate amphotericin B/kg/day for 2 weeks, followed by 200 mg itraconazole twice daily for another 10 weeks (4). Clearance of positive cultures and antigen was similar in the liposomal and deoxycholate amphotericin B arms (4), so both groups were combined for this analysis and designated "amphotericin B" here. In this study, specimens were not collected

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beyond week 1 or 2 from patients who failed treatment; therefore, patients who experienced early clinical and microbiological failure were excluded from this analysis. Urine and serum samples were collected at weeks 0, 2, 4, 8, and 12 of treatment and were stored frozen at $-80°C$ since the studies were conducted. Only urine samples were available for testing from the itraconazole study, because serum specimens had been depleted during earlier testing (7, 9, 12). For the purpose of this report, we included only subjects who had baseline samples that were available for testing and that tested positive in the antigen assay.

MiraVista quantitative *Histoplasma* antigen EIA. This method was reported previously (1). Specimens were incubated in precoated plates, and antigen was attached to the capture antibody, where it is detected with a biotinylated detector antibody. Specimens yielding an optical density (OD) more than three times that of the negative control were classified as positive. Antigen concentration was determined by extrapolation from a calibration curve created by testing known concentrations of *Histoplasma* urinary antigen. Specimens with OD values that exceeded the cutoff for the assay but that were less than the 0.6 ng/ml standard were reported as positive, <0.6 ng/ml, and those with results exceeding the 39 ng/ml standard were reported as positive, >39 ng/ml. For analysis, specimens that were >39 ng/ml were assigned values of 39 ng/ml and those that were <0.6 ng/ml were assigned values of 0.6 ng/ml. Urine and serum specimens from the same week of the study were tested in the same assay with quantification standards performed with every assay run: baseline and week 1 in the first assay, weeks 2 and 3 in the second assay, weeks 4 to 6 in the third assay, weeks 7 to 9 in the fourth assay, and weeks 10 to 16 in the fifth assay (quantification standards performed over a 2-week interval). The coefficient of variation between runs was 0.078 (7.8%), consistent with earlier findings (1). Testing was performed at MiraVista Diagnostics, Indianapolis, IN.

Statistical analysis. A linear spline model was used to investigate change over time from baseline to week 12 for (i) urine antigen in the amphotericin B (MSG-29) protocol, (ii) urine antigen in the itraconazole (ACTG-120) protocol, and (iii) serum antigen in the amphotericin B (MSG-29) protocol. Individual line plots were first used to examine trends over time. Week 2 was noted to be the inflection point or common knot in the observation period. The piecewise linear model included separate parameters for the slope to capture the change from baseline to week 2 and then from week 2 to week 12. Models were performed using PROC MIXED in SAS software version 9.2 (SAS Institute, Cary, NC), specifying time as a repeated effect. The within-patient correlation among repeated measures was modeled with an unstructured variance-covariance matrix. Group comparisons of the slope estimates were determined using the P value from the interaction term between group and time. For the model comparing urine and serum clearance among the MSG-29 group, a second repeated effect of urine versus serum was included to account for the extra correlation of having two tests within each patient. The model fit was determined by comparing predicted means to observed means at each time point. Significant reduction in antigen level was defined as a reduction greater than 15% when baseline values were ≥20 ng/ml or a reduction greater than 3 ng/ml when baseline values were <20 ng/ml (1). Two other targets (4.0 and 10.0 ng/ml) were also used for assessment of antigen clearance. These targets were selected for the following reasons. The lower boundary of the moderate range of antigen values is 4.0 ng/ml. This boundary allows assessment of antigen decline in specimens that were initially moderately positive. This is also a convenient boundary for antigenuria since it serves as criteria for stopping maintenance therapy, as was shown in the itraconazole withdrawal study (3, 13). On the other hand, 10.0 ng/ml is a midpoint in the moderate range (4.0 to 19.9 ng/ml), providing a convenient boundary for assessment of antigen decline in patients with high initial results (>20.0 ng/ml). Chi-square and McNemar's tests were used to compare independent and dependent proportions, respectively. A paired t test was used to compare antigen levels at baseline.

Latent class growth analysis (LCGA) was used in M*plus* (6) to identify discrete subgroups with similar patterns of antigen clearance in serum and urine over time within the two treatment protocols. The objective of LCGA is to find groups of individuals who are similar and categorize them into different classes based on class probability and growth curve shapes. LCGA relaxes the single population assumption to allow for parameter differences across unobserved (latent) subpopulations, yielding an average trajectory shape for each class and estimating class membership probabilities (posterior probabilities) for each individual (5). Based on similarity in the trajectory shapes, the number of individuals in each class, and classification quality as determined by examining the posterior probabilities, a two-class model was found to provide the best fit for our data.
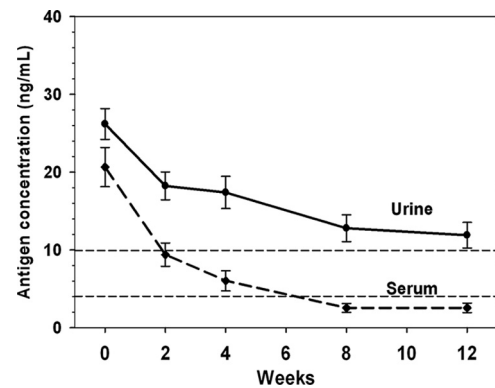

FIG. 1. Time course of *Histoplasma* antigen clearance in urine and serum during treatment with amphotericin B for 2 weeks, followed by itraconazole treatment for another 10 weeks. Data are expressed as means ± standard errors (SE). The dashed lines correspond to antigen levels of 4 ng/ml and 10 ng/ml.

RESULTS

In the amphotericin B study (MSG-29), 51 of the 81 enrolled subjects were considered successfully treated. Of those 51, 44 participants had matched pairs of urine and serum samples that were tested in the quantitative antigen assay. Of the 59 subjects enrolled in the itraconazole study (ACTG-120), 50 responded to therapy; of those patients, 40 urine samples were available for antigen testing.

Comparison of urine and serum antigen clearance during amphotericin B treatment. The mean baseline antigen levels were 26.2 ng/ml in urine samples and 20.6 ng/ml in sera from patients treated with amphotericin B ($P = 0.0002$). Using an overall model of covariate analysis, comparing urine and serum antigen levels at all time points, on average the urine antigen level was 8.5 ng/ml higher than the serum antigen level (standard deviation [SD], 1.0643; $P < 0.0001$; range, 5.6 to 10.7) throughout the study period (Fig. 1). Antigen clearance was most dramatic in the first 2 weeks of therapy with amphotericin B in both serum and urine. Two weeks after amphotericin B treatment, there was a trend toward a significant difference between urine and serum clearance slopes ($P = 0.0905$), with serum antigen levels decreasing slightly faster than urine antigen levels (-5.90 ng/ml/week versus -4.21 ng/ml/week). Antigen clearance was slower after 2 weeks and was similar between serum and urine (-0.47 ng/ml/week versus -0.54 ng/ml/week; $P = 0.5453$).

At 2 weeks of treatment with amphotericin B, a significant reduction in antigen levels, as defined above, occurred in sera in 56.8% of patients and in urine samples in 55.8% of patients (Table 1). By the end of treatment, a significant reduction occurred in sera in 77.8% of patients and in urine in 85.7% of patients. Antigen levels dropped to <10 ng/ml or 4 ng/ml in sera more often than in urine throughout the study period ($P < 0.001$). The mean reduction of antigen levels at week 2 was 11.26 ng/ml (SD, 12.89 ng/ml) in the sera and 7.65 ng/ml (SD, 8.76 ng/ml) in the urine ($P = 0.0948$). At week 4, the mean reduction of antigen levels was 15.60 ng/ml (SD, 14.73 ng/ml) in the sera and 9.73 ng/ml (SD, 10.18 ng/ml) in the urine ($P = 0.0093$). After 12 weeks of therapy, mean reductions were

TABLE 1. Comparison of the time course of antigenuria and
antigenemia during amphotericin B therapy

Parameter	Specimen	Value at:			
		2 wk	4 wk	8 wk	12 wk
Significant decline	Urine	55.8	63.2	84.9	85.7
$(\%)^a$	Serum	56.8	69.2	75.8	77.8
	P value	1.00	0.491	0.317	0.317
Decline to	Urine	7.0	15.8	24.2	25.7
<4 ng/ml (%)	Serum	31.8	59.0	75.8	83.3
	P value	0.0009	<0.0001	<0.0001	<0.0001
Decline to <10	Urine	30.2	39.5	42.2	51.4
ng/ml (%)	Serum	65.9	82.1	97.0	91.7
	P value	0.0001	0.0002	<0.0001	0.0002
Mean decline	Urine	7.65	9.73	14.29	14.64
(ng/ml)	Serum	11.26	15.60	17.16	18.52
	P value	0.0948	0.0093	0.1543	0.0440
Median decline	Urine	6.66	7.54	14.55	12.49
(ng/ml)	Serum	3.51	8.55	7.93	11.37

[a] Significant reduction in antigen level is defined as a reduction greater than 15% when baseline values are ≥20 ng/ml or a reduction greater than 3 ng/ml when baseline values are <20 ng/ml.

TABLE 2. Comparison of the time course of antigenuria between
amphotericin B (AmB) and itraconazole (Itra) therapy

Parameter	Treatment	Value at:			
		2 wk	4 wk	8 wk	12 wk
Significant decline	AmB	55.8	63.2	84.9	85.7
$(\%)^a$	Itra	13.9	28.1	29.0	62.2
	P value	0.0001	0.0034	<0.0001	0.023
Decline to	AmB	7.0	15.8	24.2	25.7
<4 ng/ml (%)	Itra	5.6	9.4	6.5	27.0
	P value	0.796	0.424	0.050	0.899
Decline to <10	AmB	30.2	39.5	42.2	51.4
ng/ml (%)	Itra	25.0	34.4	41.9	51.4
	P value	0.605	0.660	0.968	0.995
Mean decline	AmB	7.65	9.73	14.29	14.64
(ng/ml)	Itra	0.06	0.30	0.90	7.86
	P value	0.0002	0.0002	<0.0001	0.0114
Median decline	AmB	6.66	7.54	14.55	12.49
(ng/ml)	Itra	0.0	0.0	0.25	4.69

[a] Significant reduction in antigen level is defined as a reduction greater than 15% when baseline values are ≥20 ng/ml or a reduction greater than 3 ng/ml when baseline values are <20 ng/ml.

18.52 ng/ml (SD, 15.79 ng/ml) in sera and 14.64 ng/ml (SD, 11.10 ng/ml) in urine ($P = 0.0440$).

Comparison of antigenuria clearance during amphotericin B versus itraconazole treatment. There was no difference in the mean baseline antigen levels between the two studies ($P = 0.280$). At week 2, patients treated with amphotericin B experienced a faster decline in urine antigen (-3.96 ng/ml/week) than those treated with itraconazole (0.36 ng/ml/week) ($P < 0.0001$). The changes after 2 weeks were similar between the two groups (-0.68 ng/ml/week versus -0.87 ng/ml/week; $P = 0.2807$) (Fig. 2).

After 2 weeks of itraconazole treatment, a significant reduction in urine antigen level was noted in 13.9% of patients compared to that in 55.8% of those treated with amphotericin B ($P = 0.0001$) (Table 2). Urine antigen was slightly more likely to drop to a level below 4 ng/ml in patients treated with
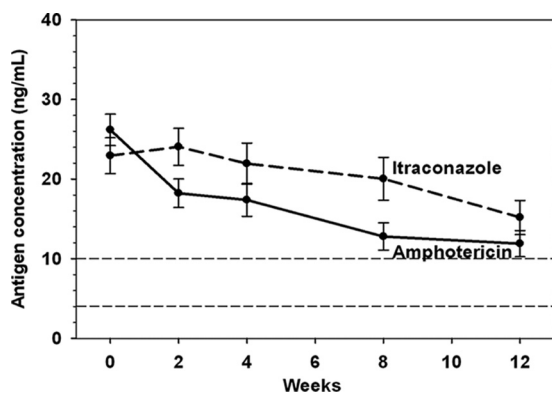

FIG. 2. Clearance of *Histoplasma* antigenuria during treatment with amphotericin B or itraconazole. Data are expressed as means ± SE. The dashed lines correspond to antigen levels of 4 ng/ml and 10 ng/ml.

amphotericin B than those treated with itraconazole throughout the study period. Mean reduction of urine antigen levels was 0.06 ng/ml (SD, 8.22) for itraconazole and 7.65 (SD, 8.76 ng/ml) for amphotericin at week 2 ($P = 0.0002$), 0.30 ng/ml (SD, 9.72) for itraconazole and 9.73 ng/ml (SD, 10.18 ng/ml) for amphotericin at week 4 ($P = 0.0002$), and 7.86 ng/ml (SD, 11.03) for itraconazole and 14.64 ng/ml (SD, 11.10 ng/ml) for amphotericin at week 12 ($P = 0.0114$).

The estimates for the rates of decline in urine antigen levels in patients treated with amphotericin B shown in Fig. 1 are slightly different from those in Fig. 2 due to the addition of the second repeated effect to account for the within-patient variability of the urine tests.

Two-class latent class growth analysis (LCGA) provided the best fit and number distribution of the data, as shown in Fig. 3 and Table 3. Patients treated with amphotericin B experienced most of the drop in antigen during the first 2 weeks of treatment. In addition, patients with the highest baseline antigen levels experienced the most dramatic decrease in antigen in the first 2 weeks; afterward, the rate of drop was lower. Patients with lower baseline serum antigen levels experienced a faster clearance of antigen over time than those with higher baseline antigen levels. In the group with low baseline antigen levels (mean of 16.7 ng/ml, $n = 29$ patients), mean antigenemia dropped below 10 ng/ml by the first week of amphotericin B treatment and below 4 ng/ml by the second week of therapy. In contrast, in those with higher baseline antigen levels (mean of 32.2 ng/ml, $n = 15$ patients), it was not until week six that mean serum antigen levels dropped below 10 ng/ml. A similar trend was observed with urine antigen; patients with lower baseline antigen levels (mean of 17.2 ng/ml, $n = 21$ patients) exhibited a drop in their mean antigen levels to below 10 ng/ml before the second week of amphotericin B treatment and to 4 ng/ml by week 12. Those who started with high urine antigen levels
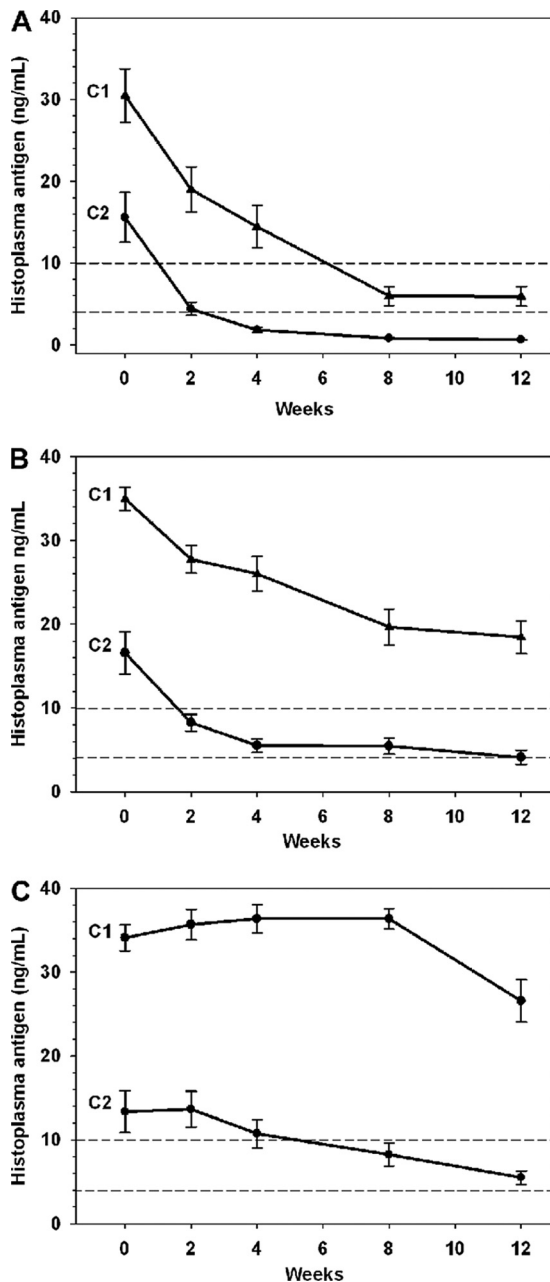
FIG. 3. Two-class model for the clearance of antigen in the sera of patients treated with amphotericin B (A), urine of patients treated with amphotericin B (B), and urine of patients treated with itraconazole (C). C1 and C2 denote LCGA class 1 and class 2, respectively. Data are expressed as means ± SE. The dashed lines correspond to antigen levels of 4 ng/ml and 10 ng/ml.

(mean of 35.6 ng/ml, n = 23 patients) stayed above 10 ng/ml on average throughout the 12 weeks.

Patients treated with itraconazole exhibited a different trajectory for the two identified LCGA classes. Those who started with high baseline urine antigen levels (mean of 34.4 ng/ml, n = 18 patients) did not experience any drop in urine antigen until after 8 weeks of treatment, before which the average antigen value actually increased, especially during the first 2 weeks of therapy. Those with lower baseline urine antigen

levels (mean of 13.5 ng/ml, n = 21 patients) exhibited an earlier drop in antigen and crossed to below 10 ng/ml by the sixth week of therapy (Fig. 3C).

DISCUSSION

This is the first study to describe antigen clearance during early successful treatment of PDH with either amphotericin B or itraconazole using the quantitative *Histoplasma* antigen EIA. While antigenuria and antigenemia declined by the end of the 12-week treatment period in the majority of patients, most of the decline was seen in the first 2 weeks of therapy, especially in those treated with amphotericin B and in those with the highest baseline antigen levels. Antigenemia tended to decline more rapidly than antigenuria in the first 2 weeks of therapy with amphotericin B, setting the stage for an overall earlier clearance and attainment of antigen levels below 4 ng/ml in serum than in urine. These findings suggest that reduction in antigenemia provides a more sensitive and earlier marker for response to treatment than that in antigenuria. The earlier attainment of 4 ng/ml in serum could potentially be related to the fact that at baseline serum antigen levels were lower than urine antigen levels.

Despite their ultimate recovery, a sizable proportion of patients, 44% of patients treated with amphotericin B and 86% of those treated with itraconazole, did not experience a significant decline in urinary antigen levels during the first 2 weeks of effective treatment. This was particularly true in a subset of patients with high baseline antigen levels who received itraconazole as initial treatment and in whom antigen levels increased initially and did not decline until after 8 weeks of therapy. Using the LCGA model, patients exhibited two distinct patterns of antigen clearance. We therefore defined the expected drop in antigen levels in these categories during effective antifungal therapy with amphotericin B and itraconazole. These findings indicate that a failure of antigen to decline during the first 2 weeks does not necessarily predict that treatment will be ineffective.

Reasons for the differences in the decline of antigenuria compared to that of antigenemia are unclear but may be related in part to the higher baseline levels in urine than in serum, many of which were above 39 ng/ml, although they were assigned a concentration of 39 ng/ml for this analysis. A significant decline could have occurred but was not quantifiable until the concentration fell into the range of 0.6 to 39 ng/ml measured by the assay (1). Another factor may be greater variability of antigen concentration in urine than serum caused by the effect of hydration on urine volume: if the same amount of antigen is present but the urine volume is larger, the concentration will be lower. Irrespective of the cause, quantification of antigen clearance during early treatment was possible more often in serum than in urine. For these reasons, clearance of antigenemia may be a more useful early marker for response to treatment than clearance of antigenuria.

Antigenuria declined more slowly in patients treated initially with itraconazole than in those treated with amphotericin B. Of note is that the eligibility criteria for the two studies differed. First, more severe cases were excluded from the itraconazole study, potentially biasing the antigen clearance in favor of itraconazole. On the other hand, the itraconazole

TABLE 3. Latent class growth analysis

Protocol (no. of patients)	Class	No. of patients per class (%)	Mean baseline antigen (ng/ml)	Baseline to wk 2 slope (ng/ml/wk)	Wk 2 to wk 12 slope (ng/ml/wk)	P value[a]
MSG-29 serum antigen (44)	1	15 (34.1)	32.209	−13.937	−1.223	<0.0001
	2	29 (65.9)	16.703	−8.802	−0.924	
MSG-29 urine antigen (44)	1	23 (52.3)	35.643	−7.888	−1.036	<0.0001
	2	21 (47.7)	17.253	−4.831	−0.384	
ACTG-120 urine antigen (39)	1	18 (46.2)	34.490	+5.076	−2.052	<0.0001
	2	21 (53.8)	13.504	−0.404	−0.740	

[a] The P values correspond to the comparison between the 2 classes in the designated categories.

study preceded the amphotericin B study, possibly biasing antigen clearance in favor of amphotericin B, since more effective antiretroviral regimens were available when the amphotericin B trial was conducted, leading to improvement in immune function. However, improvement in immunity would be unlikely in the first 2 weeks of therapy. More rapid clearance of antigen is consistent with earlier findings showing more rapid clearance of fungemia in patients receiving amphotericin B than in those receiving itraconazole (9). More rapid clearance during treatment with amphotericin B than during treatment with itraconazole possibly can be explained by the greater fungicidal effect of amphotericin B and the delay in achieving effective blood concentrations of itraconazole.

Several caveats should be recognized in the application of these results to patient management. The patients received a standardized antifungal treatment regimen administered through a prospective clinical trial. Whether these findings can be applied to patients receiving treatment outside a clinical trial is unknown. However, this approach has been the accepted standard of care since the initial IDSA guideline for treatment of histoplasmosis (8), and there is no reason to expect significant differences in the treatment approach. The specimens had been stored frozen for more than 10 years. However, the *Histoplasma* galactomannan is stable, with long-term storage at −20 or −70°C (L. J. Wheat, unpublished observation). This study addresses only the first 12 weeks of treatment among patients who experienced early successful therapy; the pattern of clearance during chronic therapy requires further investigation. The pattern of antigen clearance in patients failing treatment was not studied in this report, as these patients were excluded from the analysis due to the lack of follow-up specimens. Thus, comparison of clearance levels in responding and failing patients was not possible using the stored specimens from the study (4). We assigned a value of 39 ng/ml to results that were >39 ng/ml and 0.6 ng/ml to those <0.6 ng/ml. This most likely led us to underestimate the change in antigen levels, thus making our conclusions conservative regarding the decline in antigen levels over time. Because antigen levels that are >39 ng/ml are not quantified, significant reductions in antigen concentration are likely missed when the baseline is >39 ng/ml and therefore incorrectly interpreted as failure of antifungal therapy, especially in the first 2 weeks of treatment. This limitation applies more often to urine and supports the usefulness of monitoring serum antigen clearance as well as urine clearance. Finally, these findings should be applied only to testing done at MiraVista Diagnostics.

In conclusion, during early successful treatment of PDH in patients with AIDS, antigenemia and antigenuria decline during the first 12 weeks more rapidly in serum than in urine and more rapidly in those treated with amphotericin B than in those treated with itraconazole. Most of the decline is seen during the first 2 weeks in those treated with amphotericin B and later in those treated with itraconazole. Failure of antigenemia to decline during the first month of treatment with amphotericin B, or an increase in antigen concentration, should prompt additional evaluation of the adequacy of the treatment regimen. Once antigenemia has cleared, and antigenuria concentrations have fallen into the more linear range of the assay (0.6 to 20.0 ng/ml), antigenuria could be monitored to provide additional laboratory evidence for response to treatment. Therefore, we recommend that both urine and serum antigens should be followed during treatment of PDH.

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