Urinary d-Arabinitol/L-Arabinitol Levels in Infants Undergoing Long-Term Antibiotic Therapy

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Long-term antibiotic therapy is one of the main risk factors for mycosis. The urinary d-arabinitol/l-arabinitol (d-/l-ARA) ratio (a biomarker of several Candida species) was determined by gas chromatography with an electron capture detector in samples from 51 infants undergoing long-term antibiotic therapy. Although 47 of these children had higher d-/l-ARA ratios than healthy controls (P < 0.0003), their values nonetheless remained within upper-normal limits (d-/l-ARA ratio of <3.6). Four children with suspected invasive candidiasis had above-normal ratios that normalized with fluconazole treatment.

Modern invasive methods of treatment and current therapies are leading to increased survival rates of critically ill neonates and infants, who become potentially at risk for Candida infections (1, 8). The number of these infections was reported to have increased by 11 fold to as much as 20 fold (Candida albicans and Candida parapsilosis are the most common etiological factors) (3, 7). Aside from central venous catheters, total parental nutrition, mechanical ventilation, Candida colonization, and prolonged hospitalization in an intensive care unit (ICU) (8–10), long-term therapy with broad-spectrum antibiotics is considered to be one of the basic risk factors for fungal infections (10, 20). Infection in neonates and infants is characterized by nonspecific clinical symptoms, high mortality (18), and major diagnostic difficulties: diagnosis is based mainly on cultures, the sensitivity of which is estimated to be from 24 to 60% (12), whereas antibody determinations are unreliable due to immune system immaturity. Most studies have focused on low birth weight and premature children (8, 17); here, we present a study of full-term neonates.

d-Arabinitol is a characteristic major metabolite of several Candida species (2). The d-arabinitol/l-arabinitol (d-/l-ARA) ratio in urine can be rapidly determined by gas chromatography as a biomarker of candidiasis (15). In a previous study, we determined the urinary d-/l-ARA ratio samples from healthy children, showing that it decreases with age and that a value of 3.6 (mean ± 2 standard deviations [SD]) is the upper limit of normal values for the 0- to 1-year-old group (19). In this study, we report on urinary d-/l-ARA ratios and their changes in a group of high-risk infants undergoing long-term antibiotic therapy.

The study group encompassed 51 infants (27 males and 24 females) aged from 1.5 to 12 months (mean age ± SD = 4.1 ± 2.5 months), born at term (≥38 weeks of gestation) with birth weights of >2,400 g (mean ± SD = 2,929.6 ± 494.0 g). The children were hospitalized in the Infant Department with the following diagnoses: pneumonia, 32 cases (62.7%); bronchitis, 8 cases (15.7%); meningitis, 5 cases (9.8%); ear-nose-throat infections, 3 cases (5.9%); and diarrhea, 3 cases (5.9%). At the time of study enrollment, the children did not exhibit symptoms of fungal infection or colonization. The patients received long-term antibiotic therapy (β-lactams, cephalosporins, amphotericin B) for periods exceeding 3 weeks (mean ± SD = 34.3 ± 14.7 days). During this time, each child was given oral nystatin at a dose of 100,000 IU/kg of body weight/day. With 10 of the 51 children in the study, we were able to determine the d-/l-ARA ratio before and after antibiotic treatment. In the remaining children (who were already under treatment when they were transferred to our department), the d-/l-ARA ratio was determined after 3 to 12 weeks of antibiotic therapy. The control group (group C) comprised 30 full-term, healthy infants (17 males and 13 females) ranging in age from 1 month to 1 year old (mean age ± SD = 4.8 months ± 2.8 months) who were not hospitalized and had no clinical symptoms of either superficial or invasive candidiasis.

The assays were performed with urine samples. Samples of about 1 to 2 ml of urine were collected and were usually analyzed directly after sampling. If necessary, they were stored at −20°C until analysis. d-/l-ARA ratios were determined as trifluoroacetic derivatives by gas chromatography with an electron capture detector (19). The BacT/Alert method was used for blood cultures. Candida was cultured on Sabouraud agar from samples of blood, urine, and mucous membrane swabs. Serum from each child was tested for the Candida antigen with the Pastorex Candida test and for anti-Candida mannan antibodies with the immunofluorescence IFp test (which uses fluorescein-labeled anti-immunoglobulin G human globulin) (4).

The normality of variable distributions was verified by the Kolmogorow-Smirnov and Shapiro-Wilk tests. The results are presented as means with standard deviations. Pre- and post-treatment results within one group were compared by using Student’s t test for dependent samples. The differences between three groups were established by using the Kruskal-
The value of D-/L-ARA in the sample from each patient as determined before antibiotic therapy is represented by the beginning of the line (i.e., at the y axis). The value of D-/L-ARA in the sample from the same patient is represented by the end of the line and is marked with a circle. Each line corresponds to the results for one patient.

Wallis analysis of variance test (due to nonhomogenous variances, results were checked by Levene’s test). Discriminant analysis was performed to determine if the D-/L-ARA ratio could be used to classify the study groups.

Figure 1 presents the D-/L-ARA ratios in samples from 10 children for whom it was possible to determine this ratio before and after antibiotic therapy (lasting 21 to 53 days). The D-/L-ARA ratio rose in each child on average (± SD) from 2.24 ± 0.32 to 2.86 ± 0.16. The differences between pre- and post-treatment D-/L-ARA ratios were significant (P < 0.0001).

The D-/L-ARA ratios after long-term antibiotic therapy for the entire group of 51 children are presented in Table 1. This group was subdivided on the basis of clinical course into subgroups A (47 patients) and B (4 patients). The differences between D-/L-ARA ratios of subgroup A and the control group were significant (P < 0.0001). The discriminant analysis model was statistically significant (F = 62.409; P < 0.001). The following discriminant equations were obtained for the particular groups (ARA equals the D-/L-ARA ratio): for subgroup A, −26.502 + 18.305 × ARA; for control group C, −20.864 + 15.974 × ARA; for subgroup B, −71.375 + 29.736 × ARA. The percentage of correctly classified cases based on discriminant equations was 91.8% in group A, 100% in group B, and 42.9% in group C. The percentage of correctly classified cases based on discriminant equations was 91.8% in group A, 100% in group B, and 42.9% in group C.

Antibiotic therapy was the first risk factor to be associated with fungal infections. Samonis et al. showed that the use of broad-spectrum antibiotics increased colonization of the gastrointestinal tract by yeast-like fungal cells (16). The widespread use of broad-spectrum antibiotics has largely contributed to the increased incidence of fungal infections.

In our study, the D-/L-ARA ratios in the subgroup of children after long-term antibiotic therapy who did not exhibit symptoms of systemic infection (group A) were found to be higher than in the control group (C). Two different methods of statistical analysis, i.e., comparisons between groups and discriminant analysis, confirmed that D-/L-ARA ratios were higher after long-term antibiotic therapy. This finding is in agreement with the results of studies of animals, in which increased D-/L-ARA ratios were also found after antibiotic administration (21).

In an earlier study, we found that the D-/L-ARA ratio decreased exponentially in healthy children with age. The highest values were found in the group with the youngest children.

![Image](http://jcm.asm.org/)

**Figure 1.** The value of D-/L-ARA in the sample from each patient as determined before antibiotic therapy is represented by the beginning of the line (i.e., at the y axis). The value of D-/L-ARA in the sample from the same patient is represented by the end of the line and is marked with a circle. Each line corresponds to the results for one patient.

**Table 1.** Urinary D-/L-ARA ratios in samples from 51 infants after antibiotic therapy

| Subgroup studied (n) | D-/L-ARA | Duration of antibiotic treatment (days) | Candida antigen | Anti-Candida mannan antibodies by IF
<table>
<thead>
<tr>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (47)</td>
<td>2.84 ± 0.26</td>
<td>2.3–3.4</td>
<td>32.2 ± 12.6</td>
<td>47 (−)</td>
</tr>
<tr>
<td></td>
<td>4.59 ± 0.73</td>
<td>4.0–5.4</td>
<td>51.5 ± 21.7</td>
<td>1 (+); 3 (−)</td>
</tr>
<tr>
<td></td>
<td>2.48 ± 0.55</td>
<td>1.5–3.3</td>
<td>30 (−)</td>
<td>1:10–1:320</td>
</tr>
<tr>
<td>B (4)</td>
<td>4.59 ± 0.73</td>
<td>4.0–5.4</td>
<td>51.5 ± 21.7</td>
<td>1 (+); 3 (−)</td>
</tr>
<tr>
<td></td>
<td>2.48 ± 0.55</td>
<td>1.5–3.3</td>
<td>30 (−)</td>
<td>1:10–1:320</td>
</tr>
<tr>
<td>C (30)</td>
<td>2.48 ± 0.55</td>
<td>1.5–3.3</td>
<td>30 (−)</td>
<td>1:10–1:20</td>
</tr>
</tbody>
</table>

a Subgroups: A, infants after antibiotic therapy, without fungal infection; B, infants after antibiotic therapy with elevated D-/L-ARA ratios and a history of systemic antifungal treatment; C, control group (healthy infants aged from 1 month to 1 year). n, number of children.

b Values are numbers of patients in each group testing positive (+) or negative (−).

c Values are ranges of antibody titers.
balance, enabling mechanisms by which antibiotics upset the bacterium-fungus ing an increase in the D-/L-ARA ratio. When we administered Candida ing the pool of bacterial antagonists to bicans filaments and kills the fungus (5). It is likely that reduc-

opportunistic it decreased (21). On the other hand, it has been shown that level rose because the pool of bacteria capable of metabolizing invading the host (14). Nevertheless, none of the children (groups A and B) received oral nystatin during antibiotic therapy. The children in subgroup B had severe congenital malformations (or intrauterine dystrophy [patient 4]) and thus belonged to risk groups for invasive candidiasis (7, 12). Three of the children in subgroup B had severe congenital malformations and their clinical condition improved, which supports our study, this average was approximately a week longer, 35.4 days (range, 21 to 85 days). It is noteworthy that the two children with mucocutaneous colonization had D-/L-ARA ratios below the pathological limits, which strongly supports our conclusion that colonization does not exclude the use of D-/L-ARA ratios as a marker of invasive candidiasis (21) or of oral or vaginal candidiasis and candiduria (6).

Fluconazole treatment in two children was monitored, and the results show that the D-/L-ARA ratios correlated well with the patients’ clinical condition, reflecting treatment effects (Fig. 2). Candida infections are associated with high mortality, especially in children of <2 years of age (11). In our study, all of the children in group B survived. Presumably, this was due in part to the children being from term pregnancies, rapid diagnosis allowing early introduction of systemic antifungal therapy, and treatment monitoring.

The results presented support the conclusion that long-term antibiotic therapy leads to an increase in the D-/L-ARA ratio in urine samples from infants at risk. The D-/L-ARA ratios determined correlated well with the patients’ clinical and treatment

### TABLE 2. Infants with elevated D-/L-ARA ratios and a history of systemic antifungal treatment (subgroup B)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>D-/L-ARA</th>
<th>Candida antigen</th>
<th>Anti-Candida mannann antibodies by IFp</th>
<th>Duration of antibiotic treatment (days)</th>
<th>CVC</th>
<th>ICU hospitalization (days)</th>
<th>Severe congenital malformation/ intrauterine dystrophy</th>
<th>D-/L-ARA ratio after fluconazole treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.3</td>
<td>+</td>
<td>1:20</td>
<td>82</td>
<td>+</td>
<td>32</td>
<td>+</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>5.4</td>
<td>-</td>
<td>1:40</td>
<td>36</td>
<td>+</td>
<td>14</td>
<td>+</td>
<td>1.7</td>
</tr>
<tr>
<td>3</td>
<td>5.4</td>
<td>-</td>
<td>1:40</td>
<td>52</td>
<td>+</td>
<td>28</td>
<td>+</td>
<td>1.9</td>
</tr>
<tr>
<td>4</td>
<td>4.0</td>
<td>-</td>
<td>1:40</td>
<td>36</td>
<td>+</td>
<td></td>
<td>+</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* Values are antibody titers.

Empirical antifungal therapy is the treatment of choice in light of the numerous diagnostic difficulties and high mortality (14). Nevertheless, none of the children (groups A and B) received empirical systemic antifungal treatment (only oral nystatin, which is not absorbed from the gastrointestinal tract) in the light of the numerous diagnostic difficulties and high mortality. In our study, this average was approximately a week longer, 35.4 days (range, 21 to 85 days). It is noteworthy that the two children with mucocutaneous colonization had D-/L-ARA ratios below the pathological limits, which strongly supports our conclusion that colonization does not exclude the use of D-/L-ARA ratios as a marker of invasive candidiasis (21) or of oral or vaginal candidiasis and candiduria (6).

Fluconazole treatment in two children was monitored, and the results show that the D-/L-ARA ratios correlated well with the patients’ clinical condition, reflecting treatment effects (Fig. 2). Candida infections are associated with high mortality, especially in children of <2 years of age (11). In our study, all of the children in group B survived. Presumably, this was due in part to the children being from term pregnancies, rapid diagnosis allowing early introduction of systemic antifungal therapy, and treatment monitoring.

The results presented support the conclusion that long-term antibiotic therapy leads to an increase in the D-/L-ARA ratio in urine samples from infants at risk. The D-/L-ARA ratios determined correlated well with the patients’ clinical and treatment

*FIG. 2. D-/L-ARA ratios in an 8-month-old girl hospitalized for pneumonia (patient 1 with a positive antigen result). Fourteen days after completion of fluconazole therapy, a high fever recurred, the D-/L-ARA ratio increased, fluconazole († F) was reintroduced, and clinical improvement was achieved. This case illustrates the need for extended monitoring of therapy (the solid line indicates treatment time, and the dashed line indicates the upper limit of normal values).*

(Infants aged 0 to 1 year) (19). The study group in the current report was a group of infants in the same age range.

In studies with rats, Wong et al. concluded that d-arabinitol level rose because the pool of bacteria capable of metabolizing it decreased (21). On the other hand, it has been shown that opportunistic Pseudomonas aeruginosa deactivates Candida albicans filaments and kills the fungus (5). It is likely that reducing the pool of bacterial antagonists to Candida is one of the mechanisms by which antibiotics upset the bacterium-fungus balance, enabling Candida albicans to develop and hence causing an increase in the D-/L-ARA ratio. When we administered fluconazole as prophylaxis to infants, we observed a reduction in the level of this biomarker (unpublished data).

In the present study, three of four children with above-normal D-/L-ARA ratios (subgroup B) did not have microbiologically confirmed invasive candidiasis; however, for all of them, the duration of antibiotic therapy was longer than the average in subgroup A (51 and 32 days, respectively). The children in subgroup B had severe congenital malformations (or intrauterine dystrophy [patient 4]) and thus belonged to risk groups for invasive candidiasis (7, 12). Three of the children in subgroup B were hospitalized in the ICU (2 to 4 weeks).

Empirical antifungal therapy is the treatment of choice in light of the numerous diagnostic difficulties and high mortality (14). Nevertheless, none of the children (groups A and B) received empirical systemic antifungal treatment (only oral nystatin, which is not absorbed from the gastrointestinal tract) concomitantly with antibiotic therapy before determination of D-/L-ARA ratios. On the basis of clinical presentation, consideration of risk factors, and gas chromatography analysis, the children in group B received fluconazole. D-/L-ARA levels normalized and their clinical condition improved, which supports the clinical suspicion of Candida infection, although it was not microbiologically confirmed. Similar results with newborns were presented by Sigmundsdottir et al. (17). If it is assumed that three of the infants with an elevated D-/L-ARA ratio (i.e., a value higher than the cutoff in subgroup B), representing 5.9% of the entire group studied, had invasive candidiasis (13, 17), this suggests that long-term antibiotic therapy should continue to be regarded as a high-risk factor, especially since all of the children were born at term, weighed at least 2,400 g, and received oral nystatin during antibiotic therapy.

In a retrospective study, Rabalais et al. (12) reported that in children with birth weights of >2,500 g treated in ICUs (the main risk factor), the frequency of fungal infections identified by culture methods was 0.6%. In that study, the average duration of antibiotic therapy was 28.1 days (range, 7 to 84 days); in

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outcomes. Given the known difficulties in rapidly confirming systemic fungal infection in infants by culture and antibody assays, monitoring D-/L-ARA ratios seems to be a valuable aid to the physician in guiding decisions on instituting systemic antifungal treatment. Data from a large number of patients, preferably from numerous centers, supported by culture results, will show whether D-/L-ARA ratios alone can be the much-needed rapid laboratory method for diagnosing systemic fungal infection.

REFERENCES