Urine d-Arabinitol/L-Arabinitol Ratio in Diagnosis of Invasive Candidiasis in Newborn Infants

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Invasive candidiasis has become an important infection in preterm infants (1, 8). In one survey, the rate of candidemia had increased 11-fold between 1981 and 1995 (4). In a recently published retrospective study, the frequency of invasive candidiasis in low-birth-weight (<1,250 g) infants was 3.5% (7). Predisposing factors are indwelling vascular catheters, total parental nutrition, and long-term treatment with broad-spectrum antibiotics (7). By contrast, invasive candidiasis rarely affects full-term newborns with normal birth weight. An incidence of 0.6% was reported for infants with birth weights of >2,500 g, and 76% of the infected infants had major congenital malformations (9).

Clinical signs of invasive candidiasis can be unspecific, and diagnosis is still mostly based on blood cultures; however, blood cultures may have been assumed to be positive for Candida in only 24 to 60% of cases (9, 12). D-arabinitol, a sugar alcohol, is a major metabolite of most pathogenic Candida species (2). Both D-arabinitol (DA) and L-arabinitol (LA) are normally present in serum and urine, and the DA/LA ratio in urine can be determined with gas chromatography-mass spectrometry (GC-MS) (6). We previously determined the diagnostic value of urine DA/LA ratio for invasive candidiasis in children with cancer (3).

In this study, we report the results of a prospective study designed to evaluate the usefulness of assessing changes in urine DA/LA ratios in the diagnosis of invasive candidiasis in both premature and full-term infants.

Invasion of neonatal intensive care units suffers an increased risk for invasive candidiasis, but the diagnosis is sometimes difficult. D-arabinitol is a metabolite of most pathogenic Candida species. An elevated urine D-arabinitol/L-arabinitol (DA/LA) ratio is a sensitive sign of invasive candidiasis in children with cancer, but the method has not been previously evaluated for newborns. We therefore enrolled 117 infants in a neonatal intensive care unit, and 411 urine samples were obtained on filter paper. The DA/LA ratio was measured by gas chromatography-mass spectrometry. For 81 infants with no suspicion of superficial or invasive candidiasis, the urine DA/LA ratio was 2.7 ± 0.7 (mean ± standard deviation [SD]). The upper cutoff level was set at 4.8 (mean plus 3 SD). Of 22 infants with mucocutaneous candidiasis and not given systemic antifungal treatment, two had elevated DA/LA ratios, which normalized after removal of intravascular catheters. Eight other infants were given empiric antifungal treatment but had negative cultures; five of these had repeatedly elevated DA/LA ratios. Six infants with culture-positive invasive candidiasis all had one or more samples with elevated ratios. For seven infants, three with suspected and four with confirmed invasive candidiasis (for which follow-up samples were available), ratios normalized during antifungal treatment. In conclusion, urine DA/LA ratio determination is a rapid test and can be used for newborns. It is possibly more sensitive than fungal blood cultures in the diagnosis of invasive candidiasis and can also be used for monitoring the effect of antifungal treatment.

MATERIALS AND METHODS

The study was approved by the ethical research committee of the hospital, and informed consent was obtained from all parents.

Healthy newborn infants. Urine samples were collected from 40 healthy full-term infants. One sample was collected from each child in one of the first 4 days of life. On the same day, a urine sample was collected from 16 of the mothers.

Patients. A total of 117 newborns (66 males and 51 females) treated at the neonatal intensive care unit (NICU) at Lund University Hospital were enrolled. Gestational age was 24 to 42 weeks (median, 30 weeks), and 97 infants were premature (gestational age, <38 weeks) and 20 were full-term. Urine samples were prospectively collected from 114 infants between October 1997 and December 1998. During the first 3 months, urine was collected from all infants admitted to the NICU, but during 1998, only children requiring long-term care with central venous catheters (CVCs) and broad-spectrum antibiotics were included. Additionally, three infants with invasive candidiasis confirmed immediately before or after the sampling period were included. The following were recorded: gestational age, birth weight, medical history, skin and oral lesions likely to be caused by Candida, microbiological cultures, number of days with unbilical vein and percutaneous CVCs, antimicrobial treatment, and local and systemic antifungal treatment. Altogether, 411 urine samples were collected.

Infants with one or more blood cultures and/or urine culture obtained by suprapubic aspiration positive for Candida were considered to have invasive candidiasis.

Test groups. The infants were divided into four groups. (i) Group A, control group (n = 81). In this group, there was no clinical suspicion of mucocutaneous or invasive infection with Candida organisms, and no antifungal treatment was given. Surveillance cultures for colonization were not done. Gestational age was 25 to 42 weeks (median, 33 weeks), and birth weight was 695 to 4,430 g (median, 1,950 g).

(ii) Group B, mucocutaneous candidiasis (n = 22). Newborns included in this group were clinically diagnosed with mucocutaneous candidiasis but were not considered to have invasive candidiasis and received only local antifungal treatment. In some cases, fungal cultures were positive. Gestational age was 24 to 40 weeks (median, 26 weeks), and birth weight was 575 to 3,740 g (median, 870 g).

(iii) Group C, empirically treated infants (n = 8). Flucanazole was given empirically to these infants although all cultures were negative for Candida. Gestational age was 24 to 39 weeks (median, 24.5 weeks), and birth weight was 640 to 3,520 g (median, 850 g).

(iv) Group D, confirmed invasive candidiasis (n = 6). Infants in this group had at least one positive blood culture or a positive urine culture obtained by suprapubic aspiration. All children were treated with fluconazole or liposomal am-
photoricin B (AmBisome). Gestational age was 24 to 27 weeks (median, 24.5 weeks), and birth weight was 575 to 1,030 g (median, 825 g).

Urine samples. The aim was to collect urine samples twice weekly from the infants admitted to the NICU. Most samples were collected by placing a piece of filter paper approximately 3 cm by 4 cm in the diaper (11). The filter paper was removed and dried after the infant had urinated. A few urine samples from infants with indwelling urinary catheters were collected in culture vials. Samples in culture vials were stored at −20°C, and filter paper samples were stored at room temperature pending analysis by GC-MS. Urine culture was done on urine samples arriving in culture vials.

Sample preparation for analysis by GC-MS. Filter paper spots approximately 2 cm in diameter and containing urine were cut from the filter paper and extracted in approximately 3 ml of methanol for 30 min. Of the solution, 300- to 600-μl aliquots were transferred to 1-ml vials and evaporated to dryness under a 60°C to 2°C, and filter paper samples were stored at 20°C. Helium was used as a carrier gas. Analyses were performed in the electron impact mode by using a selected ion monitoring with an m/z of 519. The peak urine DA/LA ratio was defined as the mean of the two highest values obtained within 1 week of elevated urine DA/LA ratio.

Microbiology. The BacT/Alert method was used for blood cultures, and Candida was cultured on Sabouraud agar (10). Identification of Candida was done by testing the yeast’s ability to ferment glucose, galactose, saccharose, maltose, lactose, and trehalose and by testing for chlamydospores (5). Urine samples arriving in culture vials were cultured on blood agar and Sabouraud agar at 36°C for 48 h.

Statistical analysis. Descriptive statistics are presented as the mean ± standard deviation (SD) for normally distributed data; the median and range were used otherwise. Student’s t test or the Mann-Whitney test were therefore used when appropriate. P values of <0.05 were regarded as statistically significant.

RESULTS

Healthy newborn infants. The urine DA/LA ratio for the 40 healthy full-term newborn infants was 2.5 ± 0.6 (mean ± SD) (range, 1.6 to 4.1). For the 16 mothers, it was 1.8 (range, 0.9 to 2.9), which was significantly lower than for their respective infants (P < 0.001).

NICU patients. Eighteen infants included in the study had blood cultures positive for coagulase-negative staphylococci (n = 15), Proteus mirabilis (n = 1), Bacillus sp. (n = 1), and Staphylococcus aureus (n = 1). Malassezia furfur was isolated from a CVC tip in one patient. Urine culture on urine samples collected in culture vials showed no growth of bacteria or fungi. One hundred three infants were treated with antibiotics, and 96 had a CVC. Ten infants died.

Group A (n = 81). These infants had no symptoms of Candida infection. The mean value of the DA/LA ratios for the whole group was based on calculating mean values for each patient during the course of the study. The mean urine DA/LA ratio was 2.7 ± 0.7 (mean ± SD), which was not significantly different from the mean ratio for the healthy full-term newborn infants. The upper cutoff level for NICU infants was set at 4.8 (group mean plus 3 SD). There was no correlation between gestational age or birth weight and urine DA/LA ratio. Three infants in this group died, all of causes other than invasive candidiasis.

Because of the heterogeneity of the infants with regards to gestational age and disease severity, we selected a subgroup of very low-birth-weight infants (<1,250 g, n = 18) to serve as a control group for infants with elevated urine DA/LA ratios in groups B and C when comparing treatment times with antibiotics and time with indwelling CVCs. These control infants’ gestational ages were 25 to 29 weeks (median, 26.5 weeks), their birth weights were 695 to 1,175 (median, 910) g, and they had a total treatment time with broad-spectrum antibiotics and indwelling CVCs of 11 days (median). The mean and median urine DA/LA ratio in this subgroup was 2.3 (range, 1.4 to 3.2).

Group B (n = 22). These infants had clinical signs of mucocutaneous candidiasis. For four infants, cultures from various locations were positive for Candida albicans, but no systemic antifungal treatment was given. Two infants had elevated urine DA/LA ratios, and one of them had positive fungal cultures of urine obtained via an indwelling catheter. For both of these infants, urine DA/LA ratios fell to normal values after their CVCs had been removed or exchanged. These two infants had total times with indwelling CVCs of 41 and 12 days and total treatment times with broad-spectrum antibiotics of 41 and 21 days, respectively. Two infants in this group died, all from causes other than invasive candidiasis.

Group C (n = 8). Eight patients received empiric treatment with fluconazole (Table 1). Five infants (no. 2, 4, 6, 7, and 8) had DA/LA ratios above the cutoff level. Three of these infants (nos. 2, 4, and 6) had several elevated DA/LA ratios which returned to normal during antifungal treatment, as shown by patient no. 2 in Fig. 1. The two other infants (nos. 7 and 8) received empiric antifungal treatment only after urine sampling was discontinued. These infants (nos. 5, 6, and 7) had major congenital malformations. Five infants died, all from causes other than invasive candidiasis.

The infants with elevated DA/LA ratios in groups B and C had significantly longer times with indwelling CVCs and longer treatment times with broad-spectrum antibiotics than the very low-birth-weight (<1,250 g) infants in group A (P < 0.01 and P < 0.001, respectively).

### Table 1. Infants with empiric antifungal treatment (group C)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gestational age at birth (weeks)</th>
<th>Mean peak DA/LA ratio</th>
<th>No. of positive samples/ no. of all samples</th>
<th>CVC (days)</th>
<th>Antibiotics (days)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>4.0</td>
<td>0/11</td>
<td>29</td>
<td>36</td>
<td>Survived</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>9.5</td>
<td>4/9</td>
<td>29</td>
<td>29</td>
<td>Survived</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>4.4</td>
<td>0/5</td>
<td>20</td>
<td>20</td>
<td>Died</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>15.3</td>
<td>2/7</td>
<td>22</td>
<td>37</td>
<td>Survived</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>3.8</td>
<td>0/20</td>
<td>93</td>
<td>61</td>
<td>Died</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>6.7</td>
<td>3/7</td>
<td>51</td>
<td>95</td>
<td>Died</td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>7.9</td>
<td>6/14</td>
<td>26</td>
<td>55</td>
<td>Died</td>
</tr>
<tr>
<td>8</td>
<td>24</td>
<td>9.8</td>
<td>7/16</td>
<td>74</td>
<td>74</td>
<td>Died</td>
</tr>
</tbody>
</table>

* a Number of samples with a urine DA/LA ratio of >4.8/total number of samples.
* b Time with indwelling CVC.
* c Treatment time with broad-spectrum antibiotics.
* d Peak DA/LA ratio; no relevant samples were obtained within 1 week of elevated urine DA/LA ratio.
Group D (n = 6). Six infants had invasive candidiasis based on clinical symptoms and C. albicans isolated from blood (n = 5) or urine obtained by suprapubic aspiration (n = 1). All six infants had at least one elevated DA/LA ratio (Table 2). In four cases (no. 9, 10, 12, and 13), urine DA/LA ratios normalized during treatment with fluconazole (Fig. 2, patient no. 13) while the clinical conditions also improved. One patient (no. 11) improved during treatment, but follow-up DA/LA ratios were not obtained. The infants in group D had significantly longer total treatment times with CVCs and broad-spectrum antibiotics than the control infants in group A (P < 0.01 and P < 0.01, respectively).

DISCUSSION

All six infants with confirmed invasive candidiasis had at least one positive urine DA/LA ratio. This finding is in accordance with our previous results for children with cancer (3). For all of the five premature infants in the present study for which appropriate samples were available, the DA/LA ratios declined during antifungal treatment, suggesting that the urine DA/LA ratio can be used to monitor treatment effect. This is in accordance with previous animal studies in which the tissue burden of Candida was found to correlate to serum DA/creatinine ratios (13). In a study of adult patients with cancer, serum DA/creatinine ratios correlated with the therapeutic response to antifungal treatment (14). However, it should be noted that, as shown for patient 13 in Fig. 2, CVC-associated fungal colonization may still be present although urine DA/LA ratios decline due to decreased Candida tissue burden. Therefore, DA/LA determinations should not replace but complement fungal blood cultures.

In the present study, 7 out of 111 infants (6.3%) without microbiologically proven invasive candidiasis had a DA/LA ratio above the cutoff, five in group C and two in group B. However, all infants with elevated DA/LA ratios in groups B and C were either premature with a birth weight below 1,250 g or had severe congenital malformations and thus belonged to risk groups for invasive candidiasis (1, 4, 8). In addition, these infants had a significantly longer total treatment time with broad-spectrum antibiotics and indwelling CVCs than the very low-birth-weight controls. DA/LA ratios normalized after removal or exchange of CVC in the two patients in group B with elevated DA/LA ratios, indicating a possible microbiologically undiagnosed CVC-associated Candida infection. One of these patients also had growth of C. albicans in urine, although obtained by indwelling catheter. Furthermore, urine DA/LA ratios normalized during empiric treatment in three infants in

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gestational age at birth (weeks)</th>
<th>Basis of diagnosis</th>
<th>Mean peak DA/LA ratio</th>
<th>No. of positive samples/ no. of all samples</th>
<th>CVC (days)b</th>
<th>Antibiotics (days)c</th>
<th>Outcome (cause of death)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>25</td>
<td>3 blood cultures</td>
<td>5.4</td>
<td>2/6</td>
<td>23</td>
<td>23</td>
<td>Survived</td>
</tr>
<tr>
<td>10</td>
<td>24</td>
<td>1 urine cultured</td>
<td>7.9</td>
<td>4/12</td>
<td>24</td>
<td>24</td>
<td>Survived</td>
</tr>
<tr>
<td>11</td>
<td>24</td>
<td>1 blood culture</td>
<td>5.8e</td>
<td>1/7</td>
<td>46</td>
<td>41</td>
<td>Survived</td>
</tr>
<tr>
<td>12</td>
<td>27</td>
<td>2 blood cultures</td>
<td>9.3</td>
<td>3/20</td>
<td>35</td>
<td>87</td>
<td>Died, candida endocarditis</td>
</tr>
<tr>
<td>13</td>
<td>27</td>
<td>3 blood cultures</td>
<td>9.1</td>
<td>3/7</td>
<td>24</td>
<td>24</td>
<td>Survived</td>
</tr>
<tr>
<td>14</td>
<td>24</td>
<td>2 blood cultures</td>
<td>30.0</td>
<td>3/3</td>
<td>19</td>
<td>19</td>
<td>Died, complications of prematurity</td>
</tr>
</tbody>
</table>

a Number of samples with a urine DA/LA ratio of >4.8/total number of samples.

b Time with indwelling CVC.

c Treatment time with broad-spectrum antibiotics.

d Sample was obtained by suprapubic aspiration.

e Peak DA/LA ratio; no relevant samples were obtained within 1 week of elevated urine DA/LA ratio.
group C (no. 2, 4, and 6), which supports the clinical suspicion of invasive candidiasis, although it was not microbiologically verified. These results are similar to those of our previous study of children with cancer, in which 12 of 23 empirically treated children had elevated urine DA/LA ratios (3).

Earlier studies have estimated a sensitivity of blood cultures for Candida of 24 to 60% (9, 12). If patients with a positive DA/LA ratio in group C are assumed to have had invasive Candida infections, the sensitivity of blood culture was only 46% (5 of 11) in the present study.

The filter paper sampling technique makes urine collection very convenient, even allowing voiding in a diaper. The urine DA/LA ratio can be used as a screening test for infants at high risk or as a part of a “sepsis work-up” in suspected cases and could possibly result in earlier diagnosis of Candida infection. However, infections with Candida krusei can be missed by using the urine DA/LA ratio for diagnosis, due to the small amounts of DA produced by C. krusei (2). All the infections in our study were, however, caused by C. albicans.

We have previously shown that urine DA/LA ratios found for children are higher than those for adults (3, 6). The present study shows that newborn infants, whether healthy full-term infants or non-Candida-infected premature infants in NICU, also have significantly higher DA/LA ratios than healthy adults, here exemplified by the mothers of the full-term infants.

We conclude that the urine DA/LA ratio can be used in the diagnosis of invasive candidiasis in newborn infants. This method is more sensitive than culture and is probably highly specific. Empiric antifungal treatment should, however, never be postponed in cases with a negative urine DA/LA ratio when invasive candidiasis is strongly suspected. An elevated urine DA/LA ratio should lead to new fungal cultures and repeated samples for the urine DA/LA ratio, and immediate initiation of systemic antifungal treatment should be considered.

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REFERENCES