Quantitative Urine Cultures Do Not Reliably Detect Renal Candidiasis in Rabbits

EILEEN E. NAVARRO,1 JOSE S. ALMARIO,2 ROBERT L. SCHAUFELE,1 JOHN BACHER,3 AND THOMAS J. WALSH1*

Immunocompromised Host Section, Pediatric Branch, National Cancer Institute, 1 and Veterinary Resource Program, Division of Research Resources, National Institutes of Health, 3 Bethesda, Maryland, and Section of Nephrology, Department of Internal Medicine, Georgetown University Hospital, Washington, D.C. 2

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The significance of quantitative urine cultures in patients at risk for hematogenous disseminated candidiasis is controversial. While various concentrations of Candida spp. in urine have been suggested as critical cutoff points in the diagnosis of renal candidiasis, other investigators consider quantitative cultures less critical in diagnosing upper tract infections. To determine the significance of quantitative urine cultures in renal candidiasis, we studied serial quantitative urinary cultures of Candida albicans in a rabbit model of hematogenous infection. Of 197 urine samples from 34 infected animals, 144 were culture positive, with a sensitivity of 73.1% for urine cultures and a lower limit of detection of 10 CFU/ml. The yield of urine cultures varied according to severity and duration of infection. The mean renal and urinary concentrations of C. albicans from rabbits with subacute candidiasis differed significantly from those from rabbits with acute candidiasis (P = 0.013 and P = 0.001, respectively). During the first 4 days of subacute renal candidiasis, more than one-half of all urine cultures were negative for C. albicans. Only 12 (8.1%) of 148 urine cultures in animals with subacute renal candidiasis had concentrations of >105 CFU/ml, and none were ≥106 CFU/ml. By comparison, all urine cultures from the animals with lethal acute renal candidiasis had higher concentrations of C. albicans and were positive throughout the course of infection. Urinary concentrations of C. albicans were not predictive of the amount of Candida in the kidney (r ≤ 0.49) and did not correlate with survival (r = 0.0232). However, the renal concentration of C. albicans (in CFU/gram) inversely correlated with the duration of survival (in days) of rabbits with renal candidiasis (r = 0.76; P < 0.001). These findings indicate that a negative urine culture in rabbits does not preclude the presence of renal candidiasis. The interpretation of a urine culture positive at any concentration, on the other hand, must involve an analysis of the risk factors for renal candidiasis, for any urinary concentration of C. albicans may reflect kidney infection.

Urinary tract infections due to Candida albicans are increasingly being described in a variety of clinical situations (4, 7, 10, 13, 26, 35), particularly in intensive care units (ICUs) (9, 31). In many instances, these infections represent local bladder infection or colonization related to the presence of indwelling bladder catheters. Other cases, however, may reflect a population of immunocompromised patients with occult infection in the kidneys. Distinguishing between renal candidiasis and Candida cystitis in this population of patients carries important therapeutic and prognostic implications.

The isolation of C. albicans from blood in these settings is often considered synonymous with deep-tissue candidiasis, particularly renal infection (4, 11, 12). By comparison, the significance of positive urine cultures remains controversial. Whether positive urine cultures for Candida spp. represent renal candidiasis and whether quantitative urine cultures can distinguish between upper and lower urinary tract infections is not known. Published clinical reviews on candiduria (7, 15, 19, 43) propose various levels of colony counts per milliliter of urine as critical cutoff points for renal infection. These studies are limited by the lack of tissue-confirmed cases, the variable clinical settings studied, and the methodology of urine sampling.

In order to understand the utility and significance of quantitative colony counts in hematogenous renal candidiasis, we studied quantitative urine cultures for C. albicans in a rabbit model of this infection. Rabbits received various inoculum sizes of C. albicans intravenously (i.v.) in order to stimulate varying degrees of severity of infection. We then compared the urinary concentrations of Candida to quantitative cultures of kidney tissue and histopathologic evidence of renal candidiasis. These studies provide an experimental foundation for understanding the significance of quantitative urine cultures for C. albicans as a marker for renal candidiasis.

MATERIALS AND METHODS

Animals. Female New Zealand White rabbits (Hazleton, Rockville, Md.), weighing 2 to 3 kg, were used in all experiments. Silastic central venous catheters were inserted into all rabbits, as previously described (42). Forty-four rabbits were studied in these experiments. All animals were individually housed and provided with food and water ad libitum in accordance with National Institutes of Health (NIH) guidelines for animal care and American Association of Laboratory Animal Care criteria (8).

Organism and inoculation. C. albicans NIH 86-21h, a well-characterized isolate from a patient with autopsy-proven disseminated candidiasis, was utilized for all experiments. Organisms from stock isolates stored in skim milk at −70°C were streaked onto Sabouraud glucose agar (SGA) plates and incubated at 37°C for 24 h. Four to five well-isolated colonies were then inoculated into 90 ml of Emmon's modified Sabouraud broth (pH = 7) and incubated at 37°C for 16 h on a shaking incubator at 80 rpm. The Candida suspension was then centrifuged at 4,500 × g for 10 min, and the pellet was resuspended in sterile normal saline after serial washing. After quantitation in a hemacytometer, the inoculum was diluted to the desired concentration of blastocandinid in a 5-ml volume of saline per rabbit. The inoculum size was confirmed by plating serial dilutions onto SGA check plates.

Thirty-four rabbits each i.v. received a single inoculum, ranging from 105 to 106 blastocandinid of C. albicans depending on the dosage group, in order to simulate...
Validation of rabbit model of renal candidiasis. All study rabbits demonstrated histologic evidence of fungal infection with or without positive cultures in the kidneys, and all but one had urine cultures positive for C. albicans. Histologic criteria for assessing severity of renal involvement utilized a score of the number of low-power fields demonstrating fungal abscesses for every 100 low-power fields examined on a periodic acid-Schiff-stained section of the kidney. There was a direct relationship between the severity of renal candidiasis, the number of low-power fields demonstrating fungal abscesses for every 100 low-power fields, and the concentration (in CFU/milliliter) of C. albicansuria in rabbits with subacute renal candidiasis (Table 1) and 49 (25%) were from animals with acute renal candidiasis (Table 2). All 112 urine samples from the 10 control animals without renal candidiasis were negative for C. albicans, whereas 144 of the 197 study samples were positive for C. albicans, with a sensitivity of 73.1%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 69% for quantitative urine cultures detecting at least 10 CFU/ml of urine in this model of renal candidiasis. The yield or apparent sensitivity of quantitative urine cultures, however, varied according to the severity and duration of infection.

### RESULTS

#### Yield of quantitative urine cultures

One hundred ninety-seven urine samples were collected from all study animals over the course of the infection of these, 148 (75%) were from animals with subacute renal candidiasis (Table 1) and 49 (25%) were from animals with acute renal candidiasis (Table 2). All 112 urine samples from the 10 control animals without renal candidiasis were negative for C. albicans, whereas 144 of the 197 study samples were positive for C. albicans, with a sensitivity of 73.1%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 69% for quantitative urine cultures detecting at least 10 CFU/ml of urine in this model of renal candidiasis. The yield or apparent sensitivity of quantitative urine cultures, however, varied according to the severity and duration of infection.

### TABLE 2. Yields of urine cultures and concentrations of C. albicans in 17 rabbits with acute renal candidiasis

<table>
<thead>
<tr>
<th>Day of infection</th>
<th>No. of samples</th>
<th>% Positive cultures</th>
<th>% Positive urine samples at C. albicans concen(^a) of:</th>
<th>Survival (%)(^d)</th>
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<td>10(^a)</td>
<td>10(^b)</td>
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<td>1</td>
<td>13</td>
<td>100</td>
<td>7.7</td>
<td>69.3</td>
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<td>2</td>
<td>13</td>
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<td>7.7</td>
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<td>100</td>
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\(^a\) C. albicans blastoconidia (10\(^7\) to 10\(^8\) CFU) were administered i.v. to each rabbit.

\(^b\) Percentage of urine specimens positive by culture for C. albicans/all specimens obtained per day of infection.

\(^c\) Percentage of positive cultures with different concentrations of C. albicans per milliliter of urine.

\(^d\) Percentage of surviving rabbits/infected rabbits.
mained at <10³ CFU/ml until day 7 of infection. Thereafter, a rise in urinary concentrations of C. albicans was seen, to a maximum of 10⁴ CFU/ml after day 9.

By comparison, all urine cultures from the animals with acute renal candidiasis were positive throughout the course of infection (Table 2). Cultures of urine from rabbits with acute renal candidiasis had higher concentrations of C. albicans than those of urine from rabbits with subacute renal candidiasis. Additionally, urinary concentrations of C. albicans rapidly rose to concentrations of >10³ CFU/ml of urine early in the course of this lethal infection.

Variation in urinary concentrations of C. albicans. Figure 1 depicts the means, SEM, and ranges of urinary concentrations of Candida from study animals given various sizes of inoculum to establish subacute and acute renal candidiasis. The mean urinary concentrations of C. albicans from rabbits with subacute renal candidiasis differed significantly from those with acute renal candidiasis.

The concentrations of C. albicans in urine of surviving animals with subacute renal candidiasis increased significantly over time (Fig. 2) (P = 0.014). However, urine cultures obtained from individual rabbits on different days were intermittently positive (Table 1). This variable yield of urine cultures, as well as the wide range of concentrations of C. albicans in urine over the duration of infection, precludes reliable prediction of the presence of renal candidiasis from a single quantitative urine culture.

Correlation between quantitative urine cultures and renal concentrations. Urinary concentrations of C. albicans were not predictive of the amount of Candida infection established in the kidney. The highest concentrations of C. albicans measured in the urine of each individual animal, as well as the colony counts in urine obtained terminally, were compared to the burden of Candida in the kidney (Fig. 3). Neither the maximal (r = 0.11; P = 0.53) nor the terminal (r = 0.49; and P = 0.004) concentrations of C. albicans in urine correlated with the amount of renal candidiasis measured in CFU of Candida per gram of tissue. Additionally, neither the maximal nor the terminal urinary concentrations of C. albicans correlated with survival.

Correlation between renal concentrations of C. albicans and survival. There was no correlation between urinary concentrations of C. albicans and survival (r² = 0.0232; P = 0.3486).
Urine cultures were only intermittently positive over time for rabbits with subacute renal candidiasis but were consistently positive for those with acute candidiasis. Moreover, the concentration of *C. albicans* in renal tissue but not that in urine correlated with survival. Thus, there appears to be no threshold of quantitative urine cultures of *C. albicans* which can reliably exclude a diagnosis of renal candidiasis, and the presence or absence of *C. albicans* in urine cultures may be all that is necessary for clinical laboratories to determine.

The method of quantitative urine cultures utilized in this study allowed detection of as little as 10 CFU of *C. albicans* per ml of urine. The sensitivity of such cultures in detecting true upper urinary tract infection was 73%, although the apparent sensitivity of urine cultures may be influenced by the size of the inoculum administered and the severity of renal infection established, as well as the duration of infection. Urine cultures were intermittently positive for *C. albicans* and, even when positive, had wide fluctuations in concentration on a daily basis, even for the same animal monitored over time. Furthermore, no evidence of lower urinary tract candidiasis was seen in the animals, which limits the translation of the findings of this animal study into humans, in whom lower urinary tract infections, particularly in hospitalized patients with indwelling Foley catheters, are common.

The kidney is among the most frequently infected organs involved in hematogenous disseminated candidiasis, and the presence of candiduria may be the first microbiologic evidence of renal infection. At the same time, asymptomatic colonization or localized infection of the urinary bladder, particularly in the catheterized patient, is very frequent. The applicability of the findings of this study to infection in humans may be limited by the fact that lower urinary tract colonization or infection with *C. albicans* was not modeled into the study. Nevertheless, these data indicate that there is no concentration of *C. albicans* below which renal candidiasis can be considered unlikely. The significance of a positive urine culture for *C. albicans* depends upon assessing the status of the host, with the most immunocompromised patient—such as the organ transplant recipient, the very low birth weight infant in the ICU, the neutropenic

**DISCUSSION**

Clinical case reviews of candiduria have suggested a range of urinary concentrations of *C. albicans* as being correlated with significant renal infection (15, 19, 24, 32, 39), but this issue has never been addressed under controlled conditions in animal models of renal candidiasis (3, 5, 29). Quantitative urine cultures have been historically utilized in the evaluation of urinary tract infections (2, 17, 27). As a standard of care, leading textbooks of infectious diseases, nephrology, and clinical microbiology cite the importance of 10⁵ CFU/ml as a critical breakpoint for significant bacteriuria (14, 34, 36), and algorithms in the classic texts suggest that workup for urethritis be pursued when members of the family *Enterobacteriaceae* are detected at concentrations below this. Such breakpoints, however, may have limited validity for different patient populations or organisms other than the *Enterobacteriaceae* (21, 25), organisms vastly different from *Candida* in their size, biology, virulence, and pathogenicity. Furthermore, clinical laboratories process urine specimens and report results in a standard fashion, and these CFU/milliliter thresholds are therefore extrapolated to candiduria. The recommended procedure for culturing fungal pathogens requires concentrating a sample to improve the yield (14, 34, 36), but this procedure is not routine and is time- and labor-intensive. In this animal model of upper urinary tract candidiasis, however, quantitative urine cultures were not sufficiently sensitive to detect renal candidiasis, even when the lowest detectable concentration was 10 CFU/ml.
cancer patient, or the bone marrow transplant recipient—at highest risk for renal candidiasis. The clinical microbiologist may suggest that other diagnostic studies, such as blood cultures, urine cytology to demonstrate yeast in renal casts, and imaging techniques to demonstrate renal lesions or fungal bezoars, be pursued in the appropriate setting. Non-culture-based diagnostic systems have been studied with urinary tract infections due to bacteria (6) and Candida spp. (30, 38, 40); however, these methods remain investigational.

Quantitative urine cultures did not reliably reflect the concentration of C. albicans in the kidneys. The presence of renal abscesses on gross and histopathologic examination, as well as the microbiologic quantitation of Candida per gram of kidney homogenate, was not geometrically proportional to the urinary concentration of C. albicans. That some rabbits demonstrated sterile urine terminally despite large Candida abscesses persisting in the kidneys underscores the lack of predictive power of urine cultures for renal candidiasis.

The low urinary concentrations of C. albicans in this study are unlikely to be attributable to the saline- and furosemide-induced diuresis. Concentration occurs efficiently in urine, even as solute cycling and water absorption vary over wide limits. In saline diuresis, urine/plasma osmolar ratios do not drop below unity. Even in diseased states, such as diabetes insipidus, the urine/plasma osmolar ratios do not drop below 0.4 (18). While some degree of urinary dilution may be attributed to saline- and furosemide-induced diuresis, it is unlikely that this would result in a dilution of 100- to 1,000-fold, given the physiologic limits of the countercurrent mechanism. In addition, the practice of hydration and saline diuresis is standard in ICUs, where candiduria is being reported with greater frequency.

The urinary concentration of C. albicans met or exceeded the traditional benchmark of 10⁵ CFU/ml for diagnosing urinary tract infection only in the animals with acute renal candidiasis established with the larger inocula (10⁷ to 10⁸ CFU per animal). This concentration, however, accounted for a mere 16.7% (8 of 49) of the total urine specimens obtained from these rabbits, all of which died within 4 days of infection, with bacteremia noted or exceeded in 92% of urine cultures discarded concentrations well below 10⁵ CFU/ml, and as much as one-quarter of urine specimens had less than 10 CFU of C. albicans/ml. Furthermore, the urinary concentration of C. albicans was often less than 10³ CFU/ml and would theoretically not be evident by regular quantitative urine culture techniques. The extrapolation of these findings to humans suggests that negative urine cultures or low colony counts of C. albicans do not reliably exclude significant renal infection, a clinical phenomenon inferred by several case reports (16, 22, 24, 29). Thus, a negative urine culture in this rabbit model does not reliably exclude a diagnosis of renal candidiasis, and no threshold of urinary concentration of C. albicans can be established to reliably identify renal candidiasis.

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REFERENCES


