Mice with Disseminated Candidiasis Die of Progressive Sepsis

Brad Spellberg,1,2 Ashraf S. Ibrahim,1,2 John E. Edwards Jr.,1,2 and Scott G. Filler1,2

1Los Angeles Biomedical Research Institute and the Division of Infectious Diseases at Harbor–University of California at Los Angeles (UCLA) Medical Center, Torrance, and 2David Geffen School of Medicine at UCLA, Los Angeles

Background. Candida species are among the most common etiologies of nosocomial bloodstream infections, causing a mortality of >40%. The murine model of hematogenously disseminated candidiasis is the standard for investigating both the activity of antifungal agents and the pathogenesis of this disease. However, despite decades of use, little is known about the physiological characteristics of the host in this model, and the cause of death remains unclear.

Methods. Using i-STAT technology, we measured blood chemistry and hemodynamic parameters to define host physiological characteristics during murine disseminated candidiasis.

Results. Mice with hematogenously disseminated candidiasis died of progressive sepsis, as manifested by worsening hypotension, tachycardia, and hypothermia. The mice developed metabolic acidosis, as well as profound acidemia and hypoglycemia. They also developed renal insufficiency, which became severe only shortly before death. Kidney fungal burden was correlated with severity of renal failure and systemic acidosis. The presence of significant weight loss, hypotension, or hypothermia was predictive of imminent death.

Conclusions. These findings indicate that the murine model of hematogenously disseminated candidiasis accurately recapitulates the progressive sepsis seen during severe clinical cases. The results underscore the validity of the model for study of the pathophysiological aspects of this disease, as well as for the evaluation of antifungal drug efficacy.

Candida species are opportunistic fungal pathogens that are among the most common causes of nosocomial infections in the United States and worldwide [1]. Candida species are now the fourth most common organisms recovered from the blood of hospitalized patients [1–3]. Even with antifungal therapy, disseminated candidiasis has an unacceptable attributable mortality of 40%–50% [4–6]. Furthermore, resistance to conventional antifungal therapies among Candida species is rising [7–11]. For these reasons, continued investigation into the pathophysiologic characteristics of disseminated candidiasis and identification of novel therapeutic agents are of paramount concern.

For several decades, the murine model of hematogenously disseminated candidiasis has been the standard for investigating mechanisms of candidal virulence and host defense, as well as for evaluating the efficacy of antifungal agents [12–33]. The primary end point in many of these studies is the duration of survival of the infected mice. Surprisingly, despite the extensive use of this model, very little is known about why candidemic mice die. We and others have speculated that mice with disseminated candidiasis die of renal failure, on the basis of the fact that the kidneys are, by far, the most heavily infected organ [15, 23, 34–36]. Nevertheless, no comprehensive evaluation of host physiological characteristics or blood chemistry parameters during murine disseminated candidiasis has been published.

One technical barrier that has, to date, limited the study of murine physiological characteristics during infection is the small volume of blood that can be obtained for evaluation of chemistry parameters. However, the recent introduction of i-STAT technology, which allows multiple chemistry tests to be performed...
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Figure 1. Rapidly or subacutely lethal infection caused by both inocula of Candida albicans. A, Time-to-death curves for 2 different inocula of C. albicans in BALB/c mice (n = 8 mice/group). B, Kidney fungal burden, by days after infection (n = 8 mice/group/time point). The Y-axis reflects the lower limit of the sensitivity of the assay. Error bars reflect interquartile ranges.

Figure 2. Physiological alterations consistent with progressive sepsis, caused by both inocula of Candida albicans. Systolic blood pressure (SBP) and heart rate (HR) were measured at baseline and on days 1, 3, 5, 7, and 10 after infection. Temperature (rectal) and weight were measured at baseline and daily after infection. All values were measured serially in groups of 8 prospectively, individually marked mice/inoculum. Because of deaths over time, day 3 values were measured in 7 mice, day 4 values (temperature and weight) were measured in 6 mice, and day 5 values were measured in 5 mice. *P < .05, vs. baseline (Wilcoxon signed rank test for paired comparisons). Error bars reflect interquartile ranges. BL, baseline value (measured 3 days before infection); bpm, beats per minute.

using small volumes of blood, has enabled these types of studies to be performed in mice and other small mammals. We utilized i-STAT technology, as well as noninvasive hemodynamic and temperature measurements, to elucidate the cause of death and define the changes in host physiological and blood chemistry parameters over time in mice with hematogenously disseminated candidiasis. We found that mice infected intravenously with either a higher or a lower inoculum of Candida albicans died of progressive sepsis. Although renal insufficiency was present, markers of renal dysfunction did not become markedly elevated until shortly before death.

MATERIALS AND METHODS

Strains and culture conditions. C. albicans SC5314, a well-characterized clinical isolate, was supplied by W. Fonzi (Georgetown University School of Medicine). The strain was serially passaged 3 times by overnight growth at room temperature on a rotating drum in liquid yeast extract, peptone, and dextrose medium (YPD; Difco Laboratories), as described elsewhere [37].

Mice and experimental protocol. Male BALB/c mice (weighing 20–25 g) were infected via the tail vein with the appropriate inoculum of C. albicans blastospores. Groups of mice (n = 8) infected with either a higher (5 × 10^6) or lower (1 × 10^5) inoculum were individually marked for serial measurement of blood pressure, heart rate, weight, and temperature. Blood pressure and heart rate were measured as described below at baseline (3 days before infection) and on day 1, 3, 5, 7, and 10 after infection. Weight and temperature were measured at baseline and then daily after infection.

A different set of mice (n = 8 mice/inoculum/time point) was infected for determination of serum chemistry parameters and tissue fungal burden. Chemistry parameters and tissue fungal burden were measured at baseline (3 days before infection) and on days 3 and 5 (5 × 10^6 inoculum) or days 5, 7, and 10 (1 × 10^5 inoculum) after infection.

All infected mice were monitored at least twice daily, and moribund mice (defined as mice with an inability to ambulate or mice ambulating with staggering gait) were euthanized. Time-to-death curves included both the mice in the hemodynamic groups and the mice in the tissue fungal burden groups; mice in the tissue fungal burden groups were censored from the analysis on the day they were euthanized. All procedures involving mice were approved by the Institutional Animal Care and Use Committee, in accordance with the National Institutes of Health guidelines for animal housing and care.

Hemodynamics and temperature measurement. Hemodynamic parameters (systolic blood pressure and heart rate) were measured using an RTBP 2000 (Kent Scientific) noninvasive, piezoelectric, tail-vein blood pressure system. Mice were acclimatized to the machine for at least 3 days before the initial recordings were obtained. Mice were heated under a lamp for...
10–20 min before measurements. All hemodynamic values were measured between 11 a.m. and 2 p.m. on each day. Values for each mouse were recorded in duplicate or triplicate, and the mean values were utilized.

During the course of progressive infection, the pulse signal invariably became difficult to capture. At the time a mouse’s pulse signal could not be captured, systolic blood pressure was assigned a value of 80 mm Hg, and heart rate was assigned a value of 700 beats/min, reflective of the approximate limits of reliable detectability.

Temperature was measured using a Physitemp Model BAT-12 (Physitemp Instruments). The probe was inserted rectally to its hilt and was maintained in this position until the temperature reading stabilized. Temperatures and weights were recorded between 8 a.m. and 10 a.m. each day.

**Whole-blood chemistry parameters.** Whole-blood chemistry parameters were obtained using the i-STAT system and EC8+ and Crea cartridges (Heska Corporation). The EC8+ cartridges measure sodium, potassium, chloride, pH, pCO₂, blood urea nitrogen (BUN), glucose, hematocrit/hemoglobin, bicarbonate, and base excess. The Crea cartridges measure creatinine. To allow sufficient blood to be harvested and to prevent clotting in the i-STAT cartridges, mice were anticoagulated with heparin (100 U administered intraperitoneally; Sigma-Aldrich) 5–15 min before euthanasia. Mice were euthanized by cervical dislocation and were placed supine. The liver was separated from the diaphragm, and the inferior vena cava was incised. Whole blood was aspirated into a heparinized 25-gauge syringe, and ∼100 μL was aliquoted into the appropriate i-STAT cartridges, in accordance with the manufacturer’s instructions. Values were read on an i-STAT Portable Clinical Analyzer.

**Tissue fungal burden.** To determine the number of viable *C. albicans* cells at different time points, the right kidneys were weighed, homogenized, diluted with saline, and quantitatively cultured overnight on Sabouraud dextrose agar at 37°C. The results were expressed as log colony-forming units per gram of tissue.

**Statistical analysis.** Differences in systolic blood pressure, heart rate, weight, and temperature were compared with baseline values in each individual mouse by use of the nonparametric Wilcoxon signed rank test for paired comparisons. Differences in serum chemistry parameters were compared using the Mann-Whitney *U* test for nonparametric unpaired comparisons. Correlations were determined by use of the nonparametric Spearman rank sum test. *P* < .05 was considered to be significant.

**RESULTS**

**Rapid rise in fungal burden paralleled by rapid death during disseminated candidiasis.** As expected, 100% mortality was observed in mice infected with either inoculum (figure 1A). More than 90% of the mice infected with the $5 \times 10^5$ inoculum died within a tight cluster between days 5 and 7, and 75% died on day 6 or 7. In contrast, the time to death of mice infected with the $1 \times 10^5$ inoculum was more spread out, with 90% dying between days 9 and 14 and 75% dying between days 10 and 14. These results are in concordance with our prior experience [36].
The kidney is consistently identified as the organ with the highest fungal burden during murine disseminated candidiasis [15, 23, 35, 36]. We therefore determined the kidney fungal burden in mice infected with both inocula. In mice infected with the $5 \times 10^4$ inoculum, the renal fungal burden was very high by day 3 after infection and increased even further by day 5 (figure 1B). In mice infected with the $1 \times 10^5$ inoculum, the renal fungal burden steadily increased from day 5 to day 10 after infection, ultimately reaching levels equivalent to those at day 3 in mice infected with the higher inoculum. Similar to the survival data, the spread of results for colony-forming units, as reflected by interquartile ranges, was greater at the lower inoculum than at the higher inoculum. These results provided an outcome-based context with which to compare the host physiological data.

**Physiological parameters consistent with septic shock during disseminated candidiasis.** Mice infected with both inocula of *C. albicans* became increasingly hypotensive and tachycardic during the course of infection (figure 2). The systolic blood pressure progressively declined, falling more rapidly in the mice infected with the higher inoculum. Because of this decline in blood pressure, it rapidly became difficult to detect a pulse signal in mice infected with the higher inoculum. By day 5 after infection, no pulse signal could be reliably detected in any of these mice. Similarly, in mice infected with the lower inoculum, the pulse signal became increasingly difficult to detect by day 7 after infection and could not be detected in several mice on day 10 after infection.

Mice infected with the higher inoculum rapidly lost weight and became progressively more hypothermic (figure 2). Their temperatures were not significantly increased at any of the time points tested. All mice infected with the lower inoculum also progressively lost weight during the first 5 days of infection, albeit at a lower rate than the mice infected with the higher inoculum. The median weight in mice infected with the lower inoculum plateaued after 6 days of infection. However, the interquartile ranges widened as some mice continued to progressively lose weight while others maintained their weight for several days.

Most of the mice infected with the lower inoculum developed increased temperatures on days 1 and 2 after infection. After this time, there was a dichotomy of temperatures, with some mice becoming progressively more hypothermic and others maintaining a relatively normal temperature for several days.

**Progressive renal failure in mice infected with either inoculum of *C. albicans.*** Mice infected with either the higher or lower inoculum ultimately developed marked renal dysfunction, as determined by both BUN and creatinine levels (figure 3). The renal dysfunction occurred more slowly in mice infected with the lower inoculum. Although both BUN and creatinine levels were elevated on day 3 after infection with the higher inoculum, the levels did not become extremely elevated until shortly before death. A similar phenomenon, although with a delayed time course, was observed in mice infected with the lower inoculum.

In concert with the rise in BUN and creatinine levels, serum sodium and chloride levels progressively fell in both groups (figure 3). These results, combined with the progressive weight loss (figure 2), are consistent with an overall decline in oral
intake, resulting in hypovolemia in the presence of free water replacement [38, 39].

Development of an early “contraction” alkalosis followed by late-onset metabolic acidosis. At the early time points, mice infected with either inoculum developed a metabolic alkalosis, as indicated by rising blood bicarbonate and base excess levels (figure 4). This finding is consistent with a hypochloremic, hypovolemic process. Five days after infection with the higher inoculum, the blood bicarbonate and base excess levels fell dramatically, indicating onset of a metabolic acidosis. Interestingly, on day 10 after infection with the lower inoculum, there was marked animal-to-animal variability in both blood bicarbonate and base excess levels. Some mice developed a marked metabolic acidosis, others trended toward acidosis from alkalosis, and others remained alkalotic. The blood pH values of the infected mice paralleled the blood bicarbonate and base excess levels. Finally, serum glucose levels fell progressively throughout the course of infection with both inocula of C. albicans (figure 4). The composite of low blood bicarbonate levels, negative base excess levels, low pH, and falling glucose levels was consistent with the development of metabolic acidosis from severe sepsis [40].

Indistinguishable temperatures and serum chemistry parameters in moribund mice infected with either inoculum. To determine whether mice infected with the different inocula had different physiological parameters at the time of death, we prospectively planned to compare the serum chemistry parameters and temperatures of moribund mice (i.e., those meeting the requirements for being euthanized, as defined in Materials and Methods) infected with the higher or lower inoculum. In total, the temperatures and serum chemistry parameters of 7 moribund mice infected with the higher inoculum were compared with those of 4 moribund mice infected with the lower inoculum. No differences in any of the measured parameters were detected between the 2 groups (figure 5). All moribund mice were hypothermic and had undetectable blood pressures and heart rates. They also had moderate to severe renal insufficiency and were acidemic.

Correlation of tissue fungal burden with severity of renal failure and acidemia and of several physiological parameters with day of death. Because the blood chemistry parameters and tissue fungal burden were measured in the same mice, we searched for possible correlations between these parameters. To determine whether the severity of tissue fungal burden in an individual mouse was correlated with the degree of renal dysfunction in that mouse, kidney colony-forming units and serum creatinine levels were compared. Renal fungal burden was di-

![Figure 5](image_url)

**Figure 5.** Indistinguishable serum chemistry parameters and core temperatures in moribund mice infected with either inoculum of *Candida albicans*. The $5 \times 10^6$ inoculum data were obtained from 5 mice in the hemodynamic group (euthanized on days 3, 6, 6, 7, and 7 after infection) and 2 mice in the tissue fungal burden group (both euthanized on day 5 after infection; temperature data were not available for these mice). The $1 \times 10^6$ inoculum data were obtained from 2 mice in the hemodynamic group (euthanized on days 13 and 14 after infection) and 2 mice in the tissue fungal burden group (both euthanized on day 10 after infection). BUN, blood urea nitrogen.

![Figure 6](image_url)

**Figure 6.** Correlation between kidney fungal burden and severity of renal failure and acidemia and of several physiological parameters with day of death. Results are from 16 mice infected with $5 \times 10^6$ *Candida albicans* (8 mice euthanized on day 3 or 5 after infection) and 24 mice infected with $1 \times 10^6$ *C. albicans* (8 mice euthanized on day 5, 7, or 10 after infection). $\rho$, correlation coefficient determined by the nonparametric Spearman rank sum test. *$P < .05$ for the correlation.
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Figure 7. Correlation between physiological parameters and time to death ($n = 8$ mice/group). Because of deaths before achievement of endpoints, systolic blood pressure (SBP) results reflect 7 mice for each inoculum, temperature results reflect 7 mice at the inoculum, and weight results reflect 6 mice at the $5 \times 10^5$ inoculum and 7 mice at the $1 \times 10^6$ inoculum. $\rho$, correlation coefficient determined by the nonparametric Spearman rank sum test. * $P < 0.05$ for the correlation.

DISCUSSION

To our knowledge, this is the first comprehensive description of the physiological characteristics of mice with disseminated candidiasis or any other fungal infection. Although seminal investigations by Leunk and Moon confirmed the development of renal failure during high-inoculum disseminated candidiasis in mice [35], they did not evaluate hemodynamic parameters or other chemistry parameters, such as those reflecting acid-base status. Therefore, despite the fact that the murine model of hematogenously disseminated candidiasis is the standard animal model for investigating the pathogenesis and treatment of this life-threatening disease, to date, the cause of death in this model has been unknown.

One major barrier to the study of murine physiological characteristics during infection has been the inability to measure a variety of laboratory parameters in small volumes of blood. The present study was made possible by the recently introduced i-STAT technology, which enables the measurement of multiple blood chemistry parameters simultaneously from a small volume of blood. We report that mice infected with both acutely and subacutely lethal inocula of $C. albicans$ died of progressive sepsis, as evidenced by hypotension, tachycardia, hypothermia, metabolic acidosis, acidemia, and hypoglycemia. Furthermore, tissue fungal burden was strongly correlated with severity of acidemia. Therefore, this model appropriately mimicked the in vivo situation during clinical Candida sepsis [41]. These data support the relevance of this model for both antifungal studies and investigations into the pathophysiological characteristics of and host defense against Candida infections.

We and others have previously speculated that mice with candidemia die of renal failure [23, 35, 36]. Although the candidemic mice in this study did develop renal failure, the onset of markedly impaired renal function was likely too proximate to the day of death to be the attributable cause of death. The cause of renal failure in this model may be multifactorial, including prerenal azotemia from volume contraction (as is evidenced by the early development of a hypochloremic metabolic alkalosis), acute tubular necrosis from hypotension, and/or fungal pyelonephritis. The strong correlation between kidney fungal burden and serum creatinine level suggests that the infection did directly participate in reduced renal function. Finally, the profound weight loss that occurred in these mice suggests that a component of the marked elevation in serum BUN levels may be secondary to increased protein catabolism. Given the implications for dose adjustment of renally cleared compounds, the steady decrease in renal function during infection is of considerable significance to the use...
of the murine model to study the efficacy of drugs during infection. Measurement of creatinine levels by use of i-STAT technology requires only 50–100 μL of whole blood/mouse, which would make dose adjustment in real time feasible during future drug-efficacy experiments.

We found no evidence of differing causes of death in mice infected with the rapidly lethal or subacutely lethal inoculum. To the contrary, moribund mice initially infected with either inoculum had indistinguishable physiological parameters. As expected, sepsis occurred more rapidly in the mice infected with the higher inoculum. There was markedly greater mouse-to-mouse variability in severity of infection in animals infected with the lower inoculum, in terms of day of death, tissue fungal burden, and physiological parameters related to sepsis (i.e., blood bicarbonate level, base excess, and pH). One limitation of this study was that correlations between these values and day of death could not be determined, because tissue fungal burden and serum chemistry parameters were not checked in the mice tracked for survival.

We found no evidence that baseline physiological parameters, including weight, predicted the day of death during subsequent infection. These data likely reflect the complex interactions between host and pathogen during sepsis, as well as the limited animal-to-animal variability in this inbred strain of mouse. It illustrates the progressive sepsis that occurs during severe clinical cases of disseminated candidiasis. The application of i-STAT technology makes facile the determination of a variety of host parameters, even in the murine model. Hence, the present study could serve as a model for description of host physiological characteristics in virtually any murine model of infection.

References

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