Bloodstream infections by Malassezia and Candida species in critical care patients

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Received 6 May 2013; Revised 20 September 2013; Accepted 29 October 2013

Abstract

Despite being considered an emerging yeast related to immunocompromised individuals, severe infections by Malassezia furfur have not been evaluated. During a one-year survey on yeasts fungemia, 290 neonatal and 17 pediatric patients with intravascular catheters, lipid parenteral nutrition, prolonged ward stay, and surgery were enrolled. In addition, the origin of the infection was investigated by swabbing hand skin of patients, parents, and healthcare workers and medical devices. All biological specimens and swabs were cultured on Sabouraud dextrose agar and Dixon agar. The yeasts identification was based on morphological and biochemical features and by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and confirmed by sequencing the internal transcribed spacer of nuclear ribosomal DNA. A higher prevalence of M. furfur (2.1%) over Candida spp. (1.4%) caused bloodstream infections (BSIs). Twelve fungemia episodes were recorded: 2 by M. furfur in a pediatric ward and 10 in a neonatal intensive care unit (6 caused by M. furfur and 4 by Candida spp.). M. furfur was also isolated from the skin of all patients with BSIs, from the hand skin of a parent, and from an incubator surface and sheet. Patients with Candida spp. and M. furfur BSIs were successfully treated with intravenous liposomal Amphotericin B. These findings highlight the need for a more accurate etiological diagnosis in high-risk patients by adding lipid-supplemented culture media for Malassezia in the current mycological
routine as the clinical features, patient management, and outcomes in both Candida and Malassezia fungemia do not differ.

Key words: Malassezia furfur, fungal bloodstream infection, neonates.

Introduction

The growing number of immunocompromised patients is leading to more frequent diagnoses of fungal bloodstream infections (BSIs), mostly caused by Candida spp. [1,2]. However, while fungemia caused by Candida species has been recognized as a cause of morbidity and mortality in hospitalized patients worldwide [1,3,4], the epidemiology of Malassezia spp. related fungemia, particularly in preterm neonates [5–7], remains largely underestimated. The genus Malassezia consists of 14 lipophilic species, of which M. furfur and M. pachydermatis may cause invasive human infections [5,7,8]. These yeasts are skin commensals of healthy individuals and animals that may become pathogenic under the influence of predisposing factors, ultimately causing skin and systemic diseases [9–12]. Although yeast infections by Candida and Malassezia species occur in patients with intravascular catheters, lipid parenteral nutrition, prolonged ward stay and surgery [1,5,13], the clinical manifestations of the infections and patient management have never been evaluated.

In this study we report the results of a one-year survey of Malassezia spp. and Candida spp. BSIs in a neonatal intensive care unit (NICU) and in a surgical pediatric ward of a hospital in southern Italy.

Methods

From July 2011 to July 2012, 290 neonates or preterm infants (gestational age <35 weeks) and 17 pediatric patients (aged <16 years) with complicated postsurgery features were enrolled. All patients displayed at least one of the following clinical signs: apnea caused by respiratory distress, elevated or depressed leukocyte count, increased C-reactive protein levels (CRP ≥ 5 mg/l), abdominal distension, or thrombocytopenia. A blood sample from each patient was collected using a lyses centrifugation system (Isolator; DuPont Co., Wilmington, DE, USA) at the onset of clinical signs and soon after the start of antifungal therapy. This was done until two consecutive blood cultures were negative. After the onset of fungemia, clinical data from all patients (age, gender, underlying diseases, antifungal prophylaxis and therapy, day of catheter removal, and fungemia duration) were collected in individual anamnestic files.

In addition, clinical specimens (urine, gastric aspirates, catheter tip, and conjunctival and skin swabs) were collected from each infected patient. In order to investigate the origin of the infection, medical devices (e.g., incubators, sheets) and hand skin of parents and healthcare workers were sampled using sterile cotton swabs. All biological specimens and swabs were cultured on Sabouraud dextrose agar with 0.5% chloramphenicol and incubated at 37 °C for 5 d and on Dixon agar and incubated at 32 °C for 10 d. Following preliminary yeast identifications using an automated system (Vitek2; bioMérieux, Craponne, France), Malassezia spp. were also characterized based on morphological and biochemical features (e.g., catalase reaction, the ability to assimilate Tween, growth on PEG-35 castor oil, and β-glucosidase activity) [14].

Malassezia isolates were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) according to Kolecka et al. [15] and confirmed by amplification and sequencing of the internal transcribed spacer (ITS) of nuclear ribosomal DNA [16]. The nucleotide sequences have been deposited in the GenBank database under accession numbers KF682233–KF682290. Sequences were compared with those of M. furfur Centraalbureau voor Schimmelcultures (CBS) 1878 and 7019 reference strains (accession numbers HM177264.1 and HM177263.1) available in the GenBank database. Because of the observational nature of this study, approval by an ethical committee was not required. However, a written informed consent from the parents or guardian of patients was obtained according to the current Italian legislation (Art. 81–D.Lgs.vo n.196/2003).

Statistical analysis

The number of Malassezia spp. and Candida spp. BSIs was statistically compared by χ² test. The Student t test was used to compare the duration time of the fungemia with the time of catheter removal. A value of P ≤ 0.05 was considered to be statistically significant.

Results

Twelve fungemia episodes were registered: 2 (11.8%) by M. furfur in the pediatric ward and 10 in the NICU, of which 6 (2.1%) were caused by M. furfur and 4 by Candida spp. (1.4%; 2 C. parapsilosis, 1 C. albicans, and 1 C. glabrata). All Malassezia isolates were identified by MALDI-TOF MS and ITS sequencing as M. furfur (data not shown).

The clinical features and management strategies for patients with fungemia caused by M. furfur are reported in
Table 1. Clinical features of patients with M. furfur fungemia and origin of positive samples.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Mean value (standard deviation)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ward</td>
<td>NICU</td>
<td>NICU</td>
<td>NICU</td>
<td>NICU</td>
<td>NICU</td>
<td>NICU</td>
<td>Pediatric surgery</td>
<td>Pediatric surgery</td>
<td></td>
</tr>
<tr>
<td>Age/gender</td>
<td>2 months/M</td>
<td>2 months/M</td>
<td>1 month/M</td>
<td>1 month/M</td>
<td>1 month/M</td>
<td>1 year/M</td>
<td>15 years/F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underlying disease(s)</td>
<td>Severe birth asphyxia</td>
<td>Premature birth, abdominal surgery</td>
<td>Premature birth, esophageal atresia, Tetralogy of Fallot</td>
<td>Premature birth</td>
<td>Premature birth</td>
<td>Neuroblastoma, Gastrostomy, jejunostomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical signs and laboratory markers</td>
<td>CRP (30.1 mg/l), thrombocytopenia</td>
<td>CRP (170.2 mg/l), elevated leukocyte count, abdominal distension, thrombocytopenia</td>
<td>CRP (28.3 mg/l)</td>
<td>CRP (30.2 mg/l)</td>
<td>CRP (34.1 mg/l)</td>
<td>CRP (25.5 mg/l)</td>
<td>CRP (31.4 mg/l)</td>
<td>CRP (29 mg/l (3.0))</td>
<td></td>
</tr>
<tr>
<td>Antifungal prophylaxis</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>FLZ, 3 mg/kg/72 h</td>
<td>FLZ, 3 mg/kg/72 h</td>
<td>FLZ, 3 mg/kg/72 h</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Day of fungemia onset</td>
<td>62</td>
<td>18</td>
<td>24</td>
<td>16</td>
<td>11</td>
<td>22</td>
<td>19</td>
<td>36</td>
<td>26 d (16.3)</td>
</tr>
<tr>
<td>Day of catheter removal after onset of fungemia</td>
<td>7</td>
<td>17</td>
<td>5</td>
<td>6</td>
<td>9</td>
<td>6</td>
<td>No removal</td>
<td>27</td>
<td>11 d (8.2)</td>
</tr>
<tr>
<td>Human and environmental samples</td>
<td>Blood, CVC, urine, arm skin, incubator and sheet</td>
<td>Blood, CVC, urine, arm skin, conjunctival swab, gastric aspirate</td>
<td>Blood, CVC, urine, arm skin, conjunctival swab, gastric aspirate</td>
<td>Blood, CVC, urine, arm skin, conjunctival swab, gastric aspirate</td>
<td>Blood, CVC, urine, arm skin, conjunctival swab, gastric aspirate</td>
<td>Blood, arm skin and chest skin/mother’s arm skin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antifungal treatment</td>
<td>L-AMB, 5 mg/kg/d for 20 d</td>
<td>L-AMB, 5 mg/kg/d for 20 d</td>
<td>L-AMB, 5 mg/kg/d for 6 d</td>
<td>L-AMB, 5 mg/kg/d for 14 d</td>
<td>L-AMB, 5 mg/kg/d for 6 d</td>
<td>L-AMB, from 2.5 mg/kg/d for 6 d to 5 mg/kg/d for 14 d</td>
<td>L-AMB, 5 mg/kg/d for 21 d</td>
<td>L-AMB, 5 mg/kg/d for 14 d</td>
<td>14 d (6.0)</td>
</tr>
<tr>
<td>Fungemia duration, d</td>
<td>7</td>
<td>50</td>
<td>7</td>
<td>3</td>
<td>6</td>
<td>2</td>
<td>23</td>
<td>30</td>
<td>16 d (16.3)</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein; CVC, central venous catheter; F, female; FLZ, fluconazole; L-AMB, liposomal amphotericin B; M, male; NICU, neonatal intensive care unit.

*CRP value (170.2 mg/l) is not included in the mean.

Table 1; clinical features and management strategies for patients with fungemia caused by Candida spp. are reported in Table 2. No significant differences (P = 0.1) were observed in the CRP values in patients with BSI by M. furfur (29 ± 3.0 mg/l) and Candida spp. (33 ± 5.1 mg/l) measured on the same day as blood cultures were positive (Tables 1 and 2). All patients with fungemia received total lipid parenteral nutrition (mean = 38 days, from 24 to 60 days) via a central venous catheter (CVC). As a consequence of yeast isolation from the peripheral and CVC blood culture, catheters were removed from 11 (91.7%) of 12 patients. The delay of CVC removal despite amphotericin treatment resulted in a significantly prolonged duration of fungemia (Table 3, P<0.001). Tip culture was consistently positive for the yeast species isolated from the blood samples (Tables 1 and 2).

Four of six neonates with M. furfur fungemia were preterm, one had a very low birth weight (VLBW; 1001–1500 g) and three (triplets) had extremely low birth weight (ELBW; ≤ 1000 g). Similarly, candidemia was diagnosed in three preterm infants, of whom two were VLBW and one ELBW. The triplets with M. furfur fungemia and two preterm infants with candidemia had received systemic antifungal prophylactic treatment with fluconazole (FLZ; 3 mg/kg/72 h) from the day of birth (Tables 1 and 2). Fungemia by M. furfur occurred after an average of 26 days and Candida spp. occurred after an average of 42 days of hospitalization. Following the positive blood
Table 2. Clinical features of patients with candidemia.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>Mean value (standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ward</td>
<td>NICU</td>
<td>NICU</td>
<td>NICU</td>
<td>NICU</td>
<td></td>
</tr>
<tr>
<td>Age/gender</td>
<td>1 month/M</td>
<td>1 month/F</td>
<td>4 months/M</td>
<td>1 month/M</td>
<td></td>
</tr>
<tr>
<td>Underlying conditions</td>
<td>Premature birth</td>
<td>Premature birth</td>
<td>Hirschsprung’s disease</td>
<td>Premature birth</td>
<td></td>
</tr>
<tr>
<td>Clinical signs and laboratory markers</td>
<td>CRP (29.6 mg/l)</td>
<td>CRP (38.9 mg/l)</td>
<td>CRP (36.8 mg/l), thrombocytopenia</td>
<td>CRP (28.6 mg/l)</td>
<td>33 mg/l (5.1)</td>
</tr>
<tr>
<td>Antifungal prophylaxis</td>
<td>FLZ, 3 mg/kg/72 h</td>
<td>No</td>
<td>No</td>
<td>FLZ, 3 mg/kg/72 h</td>
<td></td>
</tr>
<tr>
<td>Day of fungemia onset</td>
<td>6</td>
<td>31</td>
<td>120</td>
<td>10</td>
<td>42 d (53.3)</td>
</tr>
<tr>
<td>Day of catheter removal from the day of positive blood culture</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>3 d (1.9)</td>
</tr>
<tr>
<td>Human samples</td>
<td>Blood, CVC, gastric aspirate</td>
<td>Blood, CVC</td>
<td>Blood, CVC</td>
<td>Blood, CVC</td>
<td></td>
</tr>
<tr>
<td>Antifungal treatment</td>
<td>L-AMB, 5 mg/kg/d for 14 d</td>
<td>L-AMB, 5 mg/kg/d for 10 d</td>
<td>L-AMB, 5 mg/kg/d for 13 d</td>
<td>L-AMB, 5 mg/kg/d for 17 d</td>
<td>13 d (1.9)</td>
</tr>
<tr>
<td>Fungemia duration</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>6 d (1.7)</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein; CVC, central venous catheter; F, female; FLZ, fluconazole; L-AMB, liposomal amphotericin B; M, male; NICU, neonatal intensive care unit.

Table 3. Duration of M. furfur fungemia after different times of CVC removal.

<table>
<thead>
<tr>
<th>Day of CVC removal</th>
<th>Number of patients</th>
<th>Mean fungemia duration (d)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;17</td>
<td>3 (patients 2, 7, 8)</td>
<td>34</td>
<td>14.01</td>
</tr>
<tr>
<td>&lt;9</td>
<td>5 (patients 1, 3, 4, 5, and 6)</td>
<td>5</td>
<td>2.35</td>
</tr>
</tbody>
</table>

CVC, central venous catheter.

culture results, the CVC was removed after an average of 11 days (range, 5–27) for M. furfur and 3 days (range, 2–6) for Candida spp.

M. furfur was also isolated from the arm skin of all patients with BSIs and from the chest skin of another patient (patient 8 who also presented with dermatitis) and her mother’s hand skin (Table 1). The incubator surface and sheet of another patient (patient 1) were contaminated by M. furfur (Table 1). Conversely, Candida spp. were never isolated from the skin of patients, parents, and healthcare workers (Table 2). M. furfur was also isolated from urinary tract \( (n = 4) \), gastric aspirate \( (n = 3) \), and conjunctiva \( (n = 3) \) in four preterm infants and C. parapsilosis was isolated from gastric aspirate of one patient (patient 9).

After the onset of fungemia, all patients were treated with intravenous liposomal amphotericin B (L-AMB) at a dosage of 5 mg/kg/d for different time periods according to their clinical status (Tables 1 and 2). All patients responded well to the therapy and recovered from Candida spp. after 6 and from M. furfur BSIs after 16 days, with the exception of one patient (patient 1) who died of causes not related to the yeast infection.

Discussion

The results of the 12-month survey on fungal BSIs in critical care patients indicate that in patients with very complicated underlying diseases or in preterm infants, M. furfur fungemia is more common (2.1%) than candidaemia (1.4%), even if statistically not significantly different. Although no clinical features that would allow discrimination between candidemia and M. furfur fungemia were identified, the presence of CVC, parenteral lipid nutrition, preterm birth, and an extended length of hospitalization are predisposing factors for both BSIs [1,5,7,17]. Although the authors reported the predictive value of the CRP response for differentiating fungal and bacterial etiologies in patients with BSIs [18], the results of this study showed increased levels of CRP in patients with candidemia and M. furfur BSI, indicating a host inflammatory response;
however, no significant differences ($P = 0.1$) were observed. These findings suggest that the CRP levels were not specific enough to clearly identify the fungal yeast etiology in BSIs. The highest CRP value recorded (170.2 mg/l; patient 2) has been attributed to an inflammatory response due to surgery and was not included in the statistical analysis. The antifungal prophylactic treatment with FLZ might be ineffective in preventing these Malassezia infections but might have contributed to reduction of the duration of M. furfur fungemia (Table 1). This hypothesis is also supported by previous data that show a low susceptibility of M. furfur to FLZ [19].

The detection of M. furfur on the skin of infants with BSIs indicates that M. furfur fungemia appeared earlier than candidaemia (average day 26 vs. day 42) and that it is an effect of its exogenous origin. Indeed, M. furfur skin colonization of neonates occurs during hospitalization [20, 21], the NICU environment represents a major risk factor. Finally, contact with parents and healthcare workers may contribute to infection or colonization in infants [22]. In addition, the isolation of M. furfur from an incubator and the sheets of one patient indicates that these environments may also represent sources of infection [10].

Since the presence of a catheter for more than nine days increases the risk of infection [22], the longer duration of M. furfur (16 days) fungemia than of Candida spp. (6 days) might be due to the late removal of the CVC (11 days for M. furfur and 3 days for Candida spp.) as well as to the lower efficacy of the fungal therapy. Additionally, even if a standardized in vitro antifungal susceptibility test has not been defined for Malassezia spp., reports indicate that the Minimum Inhibitory Concentration (MIC) values of some antifungal drugs (including FLZ) are higher than those recorded for Candida [19, 23]. Nevertheless, in both patients with BSIs by Malassezia and Candida species, treatment with L-AMB was efficacious.

Given that the clinical features, laboratory markers, strategies of patient management, and outcomes in Candida and Malassezia fungemia do not differ, a more accurate etiological diagnosis is needed in high-risk patients. This could be performed by adding lipid-supplemented culture media for Malassezia in the current mycological routine. Similar to infections by Candida spp., the management of Malassezia-related fungemia requires the removal of any catheter as soon as the first positive blood culture occurs and the temporary discontinuation of parenteral nutrition in combination with an early intravenous antifungal therapy.

Acknowledgments

We thank Dr Bronwyn Campbell for revising the English text. Bart Theelen (CBS) is acknowledged for making available the ITS sequence data.

Declaration of interest

The authors report no conflicts of interest. T. B. was supported by the Qatar National Research Fund grant NPRP 5-298-3-086. The authors alone are responsible for the content and writing of the paper.

References


