Risk factor analysis of *Histoplasma capsulatum* fungemia

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The primary objective of the study was to investigate the risk factors for *Histoplasma capsulatum* fungemia. We conducted a retrospective case-control study among patients with histoplasmosis seen at Mayo Clinic in Rochester from 1 January 1991 through 31 December 2005. Blood cultures were prepared from specimens obtained from 111 patients with a diagnosis of histoplasmosis of which 55 had demonstrated *H. capsulatum* fungemia whereas the cultures of the remaining 56 patients were negative. The mean age of the patients was 56 years, of which 70% men and 95% were white. In univariate analysis, immunocompromised status (OR 2.9, *P*<0.008), peripheral leukocyte count (WBC) 3000 cells/mm$^3$ (OR 7.3, *P*<0.001), albumin<3.5 g/ dl (OR 3.1, *P*=0.018), and Charlson score of >4 (OR 2.9, *P*=0.022) were associated with *H. capsulatum* fungemia, but age >55 years was not (OR 1.4, *P*=0.38). In multivariable analysis, immunocompromised status (OR 2.4, *P*=0.043) and WBC<3000 cells/mm$^3$ (OR 6.5, *P*=0.001) remained significant factors associated with *H. capsulatum* fungemia. Immunocompromised status and WBC<3000 cells/mm$^3$ are independent risk factors for the development of *H. capsulatum* fungemia.

Keywords  Fungemia, disseminated histoplasmosis, *Histoplasma capsulatum*, risk factor, endemic mycosis

Introduction

Histoplasmosis is the most prevalent endemic mycosis in the United States. Most infections are asymptomatic or self-limited, but one in 2000 acute infections, often in immunocompromised hosts, results in severe and progressive dissemination [1,2].

A variety of conditions predispose to disseminated histoplasmosis (DH). A landmark study published in 1982 showed after stratification that both age >54 years and immunocompromise status were risk factors for dissemination [3]. Over the past two decades, human immunodeficiency virus (HIV) has been well-recognized as an additional risk factor for DH [4–7]. Numerous other publications have linked a variety of immunocompromised conditions to the development of either histoplasmosis or DH. These include solid organ transplantation [8,9], pediatric malignancy [10], and more recently, use of tumor necrosis factor (TNF) alpha inhibitors [11,12].

Fungemia, as one presentation of DH, has not been studied as a separate clinical entity. We therefore conducted a retrospective case-control investigation and used multivariable analysis to identify risk factors for *Histoplasma capsulatum* fungemia.

Patients and methods

The study included adult patients (age≥18 years) with a diagnosis of histoplasmosis seen between 1 January 1991 and 31 December 2005 who had lysis centrifugation fungal blood cultures drawn prior to the initiation of antifungal therapy. Cases included patients with *H. capsulatum* fungemia and controls who had negative fungal blood cultures. The case diagnosis was per patient and not per occurrence. A diagnosis of histoplasmosis was based on any of the following criteria: a positive lysis centrifugation blood, bone marrow, body fluid, or tissue culture for *H. capsulatum var. capsulatum*; histopathologic or direct microscopic demonstration of morphological forms consistent with *H. capsulatum*; a positive urine, serum, or cerebrospinal fluid
Peripheral white blood cell count (WBC)/H11350 variables were included in the regression analysis: age established data [3] and our clinical impression, the following logistic regression were used. Based on previously published data, the Mann Whitney test was obtained. To identify risk factors for fungemic groups were compared using the two sample independent 't' test for continuous variables and chi square weighting scheme were created on the basis of Cox proportional hazards modeling. Subsequently, it was adapted to the International Classification of Diseases (ICD-9-CM) diagnosis and procedure codes [13,14].

Baseline characteristics for the fungemic and non-fungemic patients. Its contents and hospitalization patients, and then validated for the same outcome in a cohort of breast cancer patients. Its contents and weighting scheme were created on the basis of Cox proportional hazards modeling. Subsequently, it was adapted to the International Classification of Diseases (ICD-9-CM) diagnosis and procedure codes [13,14].

Baseline characteristics for the fungemic and non-fungemic patients were compared using the two sample independent ‘t’ test for continuous variables and chi square test for categorical variables. For non-parametric variables, Mann Whitney test was obtained. To identify risk factors for H. capsulatum fungemia, univariate and multivariable logistic regression were used. Based on previously published data [3] and our clinical impression, the following variables were included in the regression analysis: age ≥55 years, immunocompromise; Charlson score >4; peripheral white blood cell count (WBC)<3000 cells/mm³; and albumin <3.5 g/dl. Receiver operating curve revealed an area under the curve of 0.7 consistent with a good discriminative property for the model. The Kaplan Meier method was used to perform the survival analysis using a log rank test to compare the survival curves (Fig. 1).

Results

Four hundred and thirty-eight patients fulfilled the diagnostic criteria for histoplasmosis. Of these, 111 had at least one blood sample drawn for fungal culture prior to the initiation of antifungal therapy. We found that cultures were positive for 55 patients indicating H. capsulatum fungemia (cases) but negative for the remaining 56 patients (controls). The 55 cases and 29 of the 56 controls were previously described in a review of systemic histoplasmosis [15]. A mean of 2.2 and a median of 2 fungal blood cultures were initiated among patients with proven Histoplasma fungemia. Patients with negative fungal blood cultures had a mean of 1.5 and a median of 2 fungal blood cultures. Therefore, the 2 groups had a comparable number of fungal blood cultures drawn.

Baseline characteristics are described in Table 1 which indicate that the mean age of patients was 56 years, of whom 70% were males and 95% Caucasians. Cases and controls had comparable age, ethnicity and gender. Significantly more patients with H. capsulatum fungemia were immunocompromised (75% vs 50%, \( P=0.008 \)) and had higher Charlson scores (33% vs 14% with score >4, \( P=0.022 \)) when compared to non-fungal patients. Immune status categories are listed in Table 1 which shows that fungemic patients included 8 solid organ transplant (SOT) recipients (5 kidney, 1 kidney/pancreas, and 2 liver), while non-fungal patients included 3 SOT recipients (2 kidney, and 1 liver). HIV patients in both groups had CD4 counts <50 cells/mm³. Fungemic patients had a significantly lower peripheral WBC (42% vs 9% WBC<3000 cells/mm³, \( P<0.001 \)), hemoglobin (Hb) (40% vs 11% Hb<10, \( P<0.001 \)), platelets (plts) (58% vs 12% plts<150,000 cells/mm³, \( P<0.001 \)) and serum albumin (alb) (31% vs 13% alb<3.5 g/dl, \( P=0.018 \)). They also had significantly higher aspartate aminotransferase (AST) (43% vs 13% AST≥60, \( P=0.001 \)) and alkaline phosphatase (AlkP) serum concentrations (62% vs 17% AlkP≥250, \( P<0.001 \)).

The rates of positive bone marrow (89% vs 22%, \( P=0.001 \)) and urine cultures (45% vs 7%, \( P=0.025 \)) were significantly higher in patients with concomitant H. capsulatum fungemia. Urine Histoplasma antigen test results were significantly more positive in fungemic patients (81% vs 50%, \( P=0.012 \)), but no statistical differences were found between the two groups with respect to CSF and serum Histoplasma antigen levels. Overall survival was not different between the 2 groups (Log Rank 0.366).

Fig. 1 Kaplan-Meier survival curve comparing mortality rates of fungemic and non-fungemic patients.
In-hospital mortality and relapse/second episode also did not differ among the 2 groups. Fungemic patients, however, were significantly more likely to be hospitalised at presentation (76% vs 41%, \( P < 0.001 \)) and had longer hospital stays (13 vs 7 days, \( P = 0.023 \)) than did controls.

Both univariate analysis and multivariate logistic regression were used to identify risk factors for \textit{H. capsulatum} fungemia. In univariate analysis (Table 2) we noted that immunocompromise (OR 2.9, \( P = 0.008 \)), peripheral leukocyte count (WBC)\(<3000 \text{cells/mm}^3 \) (OR 7.3, \( P < 0.001 \)), albumin\(<3.5 \text{g/dl} \) (OR 3.1, \( P = 0.018 \)), and Charlson score of \( \geq 4 \) (OR 2.9, \( P = 0.022 \)) were associated with \textit{H. capsulatum} fungemia but patient age of \( \geq 55 \) years was not (OR 1.4, \( P = 0.38 \)). In multivariable analysis, immunocompromise (OR 2.4, \( P = 0.043 \)) and peripheral WBC\(<3000 \text{cells/mm}^3 \) (OR 6.5, \( P = 0.001 \)) continued to be significantly associated with \textit{H. capsulatum} fungemia. Furthermore, there was a graded risk of fungemia noted with specific categories of immunocompromise (Table 3). As compared to immunocompetent patients with histoplasmosis, those with hematologic malignancy/stem cell transplantation, solid organ transplantation, and HIV not on HAART were 4, 5, and 10 times, respectively, more likely to have \textit{H. capsulatum} fungemia. While other immunocompromised patients had twice the risk of fungemia as compared to the controls, the \( P \) value was not significant.

**Discussion**

The current study describes our experience with \textit{H. capsulatum} fungemia over a 15-year period. The relatively large number of \textit{H. capsulatum} fungemia cases at Mayo Clinic allowed us to conduct a risk factor analysis of such infections which, to our knowledge, has not been previously described in the literature. We found that immunocompromise and peripheral WBC\(<3000 \text{cells/mm}^3 \) were independent risk factors for the development of \textit{H. capsulatum} fungemia among patients from whom blood samples were drawn for fungal culture. In addition, we were able to determine the strength of association between different conditions or treatments that contribute to immunocompromise status and \textit{H. capsulatum} fungemia. HIV-infected patients not receiving HAART, solid organ and stem cell transplant recipients were found to be patients at highest risk.
Table 2 Univariate analysis of purported risk factors for fungemia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>P value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;55 years</td>
<td>1.4</td>
<td>0.379</td>
<td>0.66, 2.99</td>
</tr>
<tr>
<td>Immunocompromised status</td>
<td>2.9</td>
<td>0.008</td>
<td>1.31, 6.52</td>
</tr>
<tr>
<td>Charlson score &gt;4</td>
<td>2.9</td>
<td>0.022</td>
<td>1.14, 7.45</td>
</tr>
<tr>
<td>WBC* &lt;3000 cells/mm³</td>
<td>7.3</td>
<td>&lt;0.001</td>
<td>2.53, 21.23</td>
</tr>
<tr>
<td>Albumin &lt;3.5 g/dl</td>
<td>3.1</td>
<td>0.018</td>
<td>1.18, 8.32</td>
</tr>
</tbody>
</table>

*Peripheral white blood cell count.

Table 3 Risk of fungemia by immunocompromised category

<table>
<thead>
<tr>
<th>Category</th>
<th>Odds Ratio</th>
<th>P value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV* no HAART†</td>
<td>10</td>
<td>0.044</td>
<td>1.1, 94</td>
</tr>
<tr>
<td>Solid Organ Transplant</td>
<td>5.3</td>
<td>0.026</td>
<td>1.2, 23.3</td>
</tr>
<tr>
<td>Hematologic cancer/ transplant</td>
<td>4</td>
<td>0.046</td>
<td>1.0, 15.6</td>
</tr>
<tr>
<td>Other immunocompromised</td>
<td>2.1</td>
<td>0.458</td>
<td>0.86, 5.2</td>
</tr>
</tbody>
</table>

*Human immunodeficiency virus, †highly active antiretroviral therapy.

Immunocompromise is a known risk factor for DH. In the outbreak study, immunocompromised patients were on corticosteroids or cytotoxic drugs, HIV infected, solid organ transplant recipients, or had hematologic malignancy, solid tumor, asthma, or sarcoidosis [3]. In that study, individual immunocompromise categories were not examined as separate entities to determine if there were graded risks to the different conditions due to the limited number of patients in each category. More recent reports of histoplasmosis in solid organ transplant recipients, cancer patients, and those on immunosuppressive medications, including TNF alpha inhibitors, did not include a statistical analysis for risk factors [8–12].

All HIV patients in both of our cohorts had extremely low CD4 counts (<50 cells/mm³). The risk of developing *H. capsulatum* fungemia was lower in patients who were receiving HAART as compared to those who were not on this combination of medications. These results parallel findings from previous studies [45] in which HIV patients receiving HAART were less likely than patients not on HAART to develop histoplasmosis [4] and had a better response to antifungal therapy and lower mortality among those who developed DH [5].

In the current study, age was not detected as a risk factor for fungemia. The two patient groups had a similar mean age and the cut off age of ≥55 years was not a significant risk factor in univariate and multivariate analyses. In the initial study by Wheat et al. [3] where age >54 years was found to be a risk factor for DH, the cohort was comprised of residents in an outbreak in Indianapolis. Perhaps under non-outbreak conditions where there is a broad temporal range of exposure to *H. capsulatum*, factors other than age may be operative in the development of fungemia. In addition, the outbreak study [3] included children (8 of 61 patients were less than 10 years old) who might have influenced the age distribution.

In our analysis, peripheral WBC<3,000 cells/mm³ was a risk factor for *H. capsulatum* fungemia. Among AIDS patients, WBC<4,000 cells/mm³ [6] and a peripheral neutrophil count <1,700 cells/mm³ [16] have not been previously found to be a marker of severe histoplasmosis and early death, respectively. Perhaps AIDS patients have specific characteristics other than low peripheral WBC that are critical for the development of severe disease. Our patient population included HIV-infected patients but also others patients who were immunocompromised due to other factors, including organ transplantation and malignancy. Among the other endemic mycosis, lymphocytopenia was shown to correlate with risk of dissemination of coccidioidomycosis [17].

Survival (91% vs 91%, P=1), in-hospital mortality (9% vs 7%, P=0.742), and relapse/second episode of histoplasmosis (11% vs 5%, P=0.442) were not different between the two groups. Fungemic patients were significantly more likely to be hospitalized at presentation (76% vs 41%, P<0.001) and to have a longer hospital stay (13 vs 7 days, P=0.023) than did non-fungemic patients. The outcomes were possibly affected by a more aggressive treatment regimen and closer follow-up that was prompted by the complicating fungemia. A total of 40 cases (73%) vs 17 controls (30%) received an amphotericin B as part of the treatment regimen (P<0.001), whereas itraconazole was the treatment chosen in 14 cases (25%) vs 24 controls (43%) (P=0.053). However, a chi square test showed no association between blood culture results and the type of initial antifungal therapy (P=0.275). If survival was affected by the selected treatment regimen, then use of validated risk factors for fungemia would assist in the early identification and initiation of empiric aggressive treatment to hopefully improve survival rates.

A change in the standard operating procedure for ordering fungal blood cultures at our institution could have potentially impacted the findings of this study. Prior to 1 November 2000, all blood culture ordered at Mayo Clinic automatically included bacterial and fungal cultures. After this date, fungal blood cultures were not automatically initiated for each blood culture request and a separate order for fungal blood cultures had to be provided. This change could potentially result in a selection bias because the clinicians would have to initiate an additional order. We do not think, however, that this impacted our results because the rates of fungal blood cultures obtained among patients...
diagnosed with histoplasmosis prior to and after 1 November 2000 were comparable (28% vs 24%, P=0.369). Similarly, the rates of positive fungal blood cultures for *H. capsulatum* did not differ between the two time periods despite the difference in the standard operating procedure (48% vs 52%, P=0.642).

There are several limitations in our investigation. It is a retrospective analysis that does not permit calculation of incidence or control for unknown variables. There is also a selection bias, i.e., among 438 patients with histoplasmosis, only 111 (25%) had fungal blood cultures done. The risk factors we identified are attributable to only those patients for whom fungal blood cultures were initiated. In addition, this is a single center design with a patient population that mainly represents Minnesota and the neighboring states. For instance, most patients were Caucasians and there is possible under-representation of minority populations. This is in contrast to the population described in the Indianapolis outbreaks where 40% of patients were African Americans and this was identified as a risk factor for fatal and systemic histoplasmosis by univariate analysis [3]. In addition, the number of patients with HIV and histoplasmosis was limited due to the relatively low prevalence of HIV in the upper Midwest area. In the quantitative risk factor analysis, the statistical analyses are weakened by small numbers of patients (0–8) in each subcategory of immunocompromised patients which accounted for the accompanying large 95% confidence intervals.

In conclusion, risk factors for *H. capsulatum* fungemia include peripheral WBC<3000 cells/mm³ and immunocompromise. Patients with HIV not receiving HAART are at highest risk, followed by solid organ transplant recipients and patients with hematologic cancer or stem cell transplantation. Patients with these risk factors are not only more susceptible to histoplasmosis but also *Histoplasma* fungemia. This should necessitate more aggressive early therapy with an amphotericin B-containing product in an effort to improve morbidity and mortality in this patient population.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

This paper was first published online on Early Online on 12 February 2009.

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