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BRIEF REPORT

Cryptococcal Antigen Testing in an Integrated Medical System: Eastern Wisconsin

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Abstract

Cryptococcosis is a serious environmentally acquired endemic fungal infection commonly associated with immunocompromised hosts. Little is known regarding frequency or distribution in Wisconsin. We explored the geodemographic and clinical features of patients tested with cryptococcal antigen tests (CrAg) — previously shown to be >90% sensitive and >90% specific — within a large health care system located in eastern Wisconsin. To examine this, we retrospectively analyzed 1465 CrAg tests on 1211 unique patients (female: 50.2%; white race: 73.9%; mean age: 53.7 ± 16.5 years). At least one CrAg result was positive in 23 of 1211 patients (1.9%). From these, 21 of 23 were immunocompromised. Positive patients were disproportionately male (82.6%) and nonwhite (3.8% of those tested vs 1.2% of whites tested); $P < 0.01$ for both. These associations remained in multivariable models. Positive patients were not significantly older (59.1 vs 53.6 years; $P = 0.07$). Overall, 17 separate zip codes had at least one positive case. Positive patients were more prevalent in the zip codes that included the city of Milwaukee (11 of 377 [2.9% of those tested] vs 12 of 834 [1.4% of all those tested in the remaining area of the state]), but this difference was not statistically significant. No other case clustering or close proximity to waterways was observed (41% were <162 m from green space, similar to historical controls). Overall, male sex, nonwhite race/ethnicity, and immunocompromised status, not zip code, were statistically associated with positive CrAg. (*J Patient Cent Res Rev.* 2020;7:57-62.)

Keywords

cryptococcosis; *Cryptococcus*; mycoses; antigens, fungal; AIDS-related opportunistic infections

Cryptococcosis is a potentially serious endemic fungal infection.¹ The two major human-pathogenic species (often distinct geographically) are *Cryptococcus neoformans* and *Cryptococcus gattii*. Risk factors for disease include exposure to an environmental source of the organism. Environmental associations of these fungal species include certain trees and soils, potentially influenced by certain climatic or anthropic factors (and bird guano for *C. neoformans*).¹⁻⁷ *Cryptococcus*-related environments may include common human-frequented sites of potential exposure like urban parks.⁷ Except for the recently proposed association with the oral cavity,^{8,9} these

organisms are not part of the human microflora, but they may cause asymptomatic infection or colonization.¹

Cryptococcus fungus enters the body through inhalation.¹ The potential for infection increases markedly for people affected by acquired immune deficiency syndrome (AIDS) or in various immunocompromised states resulting from cancers and their treatments, corticosteroid therapy, and pediatric innate immune defects.^{1,10} Similarly, incidence rates appear to be proportional to the percentage of individuals in a population with inadequately treated AIDS or other immunocompromising conditions.¹ In fact, epidemiologic data from the World Health Organization suggest that nearly 8000 cases of opportunistic cryptococcal meningitis infection occur each year among HIV-infected individual in North America, resulting in an average of 700 annual deaths.¹¹

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Cryptococcal infection is often readily demonstrated by microscopic examination, culture, or cryptococcal antigen detection test (CrAg). The latter not only allows for simple, rapid, and low-cost testing for the diagnosis but is reportedly more than 90% sensitive and 90% specific for disease.¹ In some cases, a CrAg of blood or cerebrospinal fluid will be positive before the fungus is demonstrated in culture.^{1,12} Unfortunately, the CrAg cannot distinguish between the two human-pathogenic *Cryptococcus* species.¹³ This may become important regionally, given the 4 identified isolates of *C. gatti* in the Midwest.¹⁴

To our knowledge, nothing has been reported in the literature regarding the frequency or distribution of cryptococcosis in Wisconsin since the 1960s.¹⁵ The aims of this study were to explore the geodemographic features of eastern Wisconsin patients for whom CrAg was performed and examine the clinical features of those who tested positive for cryptococcal infection.

METHODS

A retrospective review of electronic medical record (EMR) data from the affiliated laboratory of a large, integrated health system spanning eastern Wisconsin

and northeastern Illinois, which houses medical records of more than 1.2 million unique patients, was conducted. This area contains the majority of Wisconsin's urban and suburban population. Subjects were all inpatients and outpatients who had CrAg from January 2013 to April 2017.

The laboratory used the latex agglutination-modeled Remel™ Cryptococcus Antigen Test Kits (Thermo Scientific, Lenexa, KS) for the detection of antigen. Patients with 1 or more positive CrAg were always considered positive, and patient demographic features (Table 1) were used from the first identified positive test. For patients with multiple negative CrAg results, data were taken from the first test. In addition, for comparison of individuals with any positive CrAg to those with negative CrAg, a manual EMR review of all CrAg-positive patients and the first 700 CrAg-negative patients, alphabetically, was performed. This comparison examined immunocompromising conditions listed in the EMR and type of clinical presentation prior to obtaining CrAg.

Fisher exact test or t-test was used for univariable comparisons between patients with at least one

Table 1. Demographic Features of Subjects by Cryptococcal Antigen Test (CrAg) Result

Characteristic	Positive CrAg n=23	Negative CrAg n=1188	Total N=1211 ^a	P
Mean age, years ± SD	59.1 ± 13.5	53.6 ± 16.6	53.7 ± 16.5	0.066
Sex, n (%)				
Female	4 (0.7%)	603 (99.3%)	607 (50.2%)	
Male	19 (3.2%)	584 (96.8%)	603 (49.8%)	0.007 ^b
Race/Ethnicity, n (%)				
White	11 (1.2%)	883 (98.8%)	894 (73.9%)	
Black	7 (4.5%)	149 (95.5%)	156 (12.9%)	0.011 ^c
Hispanic	3 (2.8%)	103 (97.2%)	106 (8.8%)	0.178 ^c
Asian	2 (5.9%)	32 (94.1%)	34 (2.8%)	0.079 ^c
Native American	0 (0.0%)	6 (100.0%)	6 (0.5%)	1.000 ^c
Unknown	0 (0.0%)	14 (100.0%)	14 (1.2%)	1.000 ^c
All nonwhite	12 (3.8%)	304 (96.2%)	316 (26.1%)	0.002 ^c

^aDue to missing data, total number for sex and race/ethnicity categories was 1210.

^bCompared to CrAg-positive females (Fisher exact test).

^cCompared to CrAg-positive whites (Fisher exact test).

positive CrAg test and those who only tested negative. Stepwise and binary logistic regression were used for multivariable analysis.

Approval for this study was obtained from the local institutional review board. Note that this study is not a population-based survey, nor an analysis of clinician-diagnosed cryptococcosis, but rather a dissection of patients with clinician-ordered CrAg in one health system.

RESULTS

A total of 1465 unique CrAg tests (741 on serum, 723 on cerebrospinal fluid, 1 other specimen), including multiple tests on the same patient, were performed on 1211 unique patients during the study period. The CrAg-tested study population was 50.2% female, 73.9% white, and had a mean age of 53.7 years (standard deviation: 16.5 years) (Table 1).

At least one CrAg was positive in 23 of 1211 (1.9%) patients. Of these 23 individuals, 21 were immunocompromised (6 transplant patients, 5 with HIV, 4 with malignancy, 3 had chronic corticosteroid use, 2 with diabetes mellitus, and 1 with combined immunodeficiency). Further detailed analysis of all positive patients plus the first 700 negative patients (n=723) revealed that 58.2% had an immunocompromising condition listed. Of those patients with an immunocompromised condition, 53.5% had only a central nervous system (CNS) presentation, 23.2% had only a pulmonary presentation, 7.9% had both presentations, and 15.4% had neither presentation. Based on 58.2% having immunocompromising condition, 13 of 23 immunocompromised patients would have been predicted to test positive; in reality, 21 did (P<0.001).

Overall, 7 CrAg-positive patients were diagnosed with cryptococcal meningitis, and 2 additional patients had cerebrospinal fluid that tested CrAg-positive. Six patients were diagnosed with disseminated cryptococcosis; 3 patients had a pulmonary presentation (but no diagnosis); 1 patient had a combined pulmonary and CNS presentation but died before precise diagnosis could be established; and 4 patients had CrAg-positive serum but no recorded cryptococcosis diagnosis.

Of the 18 CrAg-positive patients with a recorded fungal culture, 17 were positive for *C. neoformans* or

C. gattii (our microbiology laboratory did not routinely differentiate the species at the time of this study). Of the 2 patients without apparent immunodeficiency, 1 had confirmed disseminated cryptococcosis. The other patient had an otherwise unexplained pneumonia; however, fungal culture was not done, and a *Cryptococcus* serum antibody titer was low (1:2).

CrAg-positive patients were disproportionately male (19 of 23 [3.2% of all males tested] vs 4 of 23 female [0.7% of all females tested]; P=0.002) and nonwhite (12 of 23 [3.8% of all nonwhite patients tested] vs 11 of 23 white [1.2% of all white patients tested]; P=0.007). These associations remained significant in a multivariable binary regression model (Table 2). In the 723-patient subcohort that included all 23 positive cases and 700 negative cases, detailed immune status data in the EMR was available for 678 patients. A stepwise regression model based on this population revealed that male sex, nonwhite race, and immunocompromised state remained significantly associated with positive CrAg result (P<0.02 for all).

Table 2. Multivariable Binary Regression Model of Demographics Associated With Positive Cryptococcal Antigen Test (n=1210)

Characteristic	Odds Ratio	95% CI	P
Male gender	5.00	1.67–14.29	0.007
Nonwhite	3.18	1.38–7.31	0.002

The geographic distribution of patients with positive CrAg in the eastern Wisconsin catchment area is illustrated in Figure 1. Overall, 17 separate zip codes had 1 positive case; 2 city of Milwaukee zip codes had 3 each, the closest two living 193 meters apart. No other CrAg-positive case clustering or close proximity to waterways was observed; 41% were less than 162 m (ie, <0.1 miles) from green space (parks/farms/forests). Positive patients were more prevalent in the 532xx zip codes that include Milwaukee (11 of 377 [2.9% of those tested] vs 12 of 834 [1.4% of all those tested in the remaining catchment area of the state]), but this difference was not significant (P=0.176).

Further examining the 678 Wisconsin patients with detailed immune status data in the EMR, 144 of 233

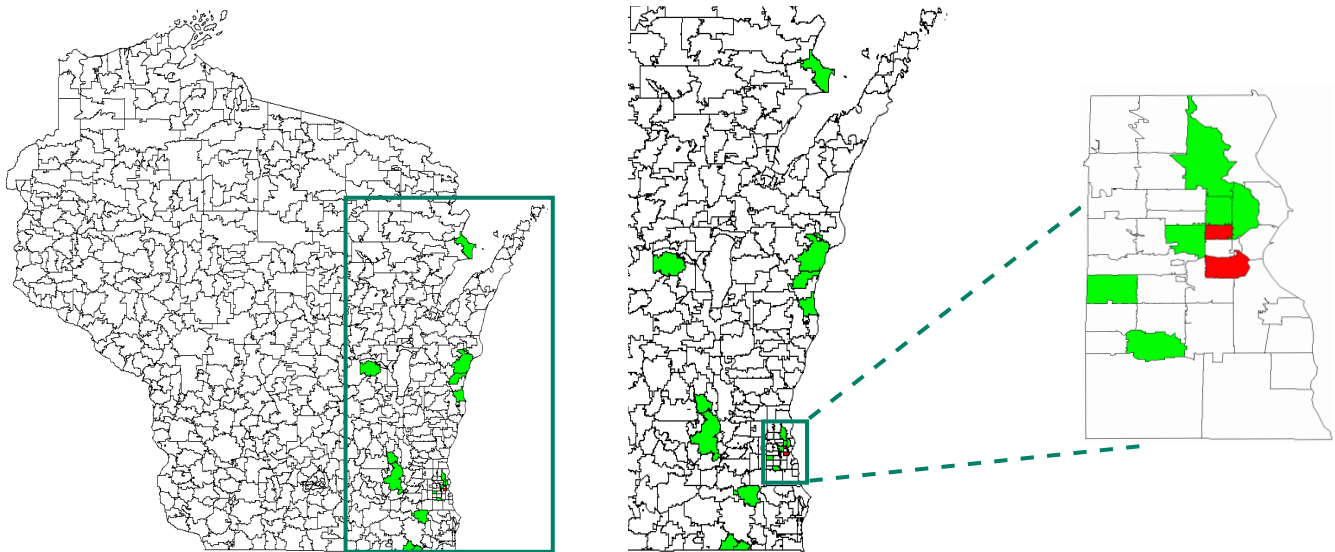


Figure 1. Map of zip code location of individuals with positive cryptococcal antigen tests. Panels show, from left to right, state of Wisconsin, eastern Wisconsin catchment area, and magnified view of Milwaukee County. Overall, 17 separate zip codes contained 1 positive case (shaded in green); 2 city of Milwaukee zip codes had 3 each (shaded in red).

in Milwaukee zip codes (61.8%) were noted to be immunocompromised compared to 251 of 445 in the rest of Wisconsin zip codes (56.4%; $P=0.190$). Similarly, examining the 17 zip codes with 1 or more CrAg-positive cases (all in Wisconsin, none in northeastern Illinois), tested individuals were more likely to be immunocompromised compared with those from zip codes without a positive case (88 of 133 [66.2%] vs 307 of 545 [56.3%], $P=0.040$). Indeed, 18 of 25 total CrAg-tested individuals residing in the pair of Milwaukee zip codes that each had 3 CrAg-positive cases were noted to be immunocompromised.

DISCUSSION

The incidence of cryptococcosis is not well known in the United States, as it is not a reportable disease in many states.¹⁶ Limited data suggest an overall incidence of 0.4–1.3 cases/100,000 annually, and a 2.9% prevalence of cryptococcal antigen in serum of patients with HIV and low CD4 counts.¹⁶ Thus, it seems that our CrAg-positive rate (1.9%) is consistent with national statistics given a tested population with half or more having an immunocompromising condition and with cryptococcosis in the differential diagnosis. A hospital discharge-based survey of 18 states, including Wisconsin, indicated a 1997–2009

cryptococcal meningitis hospitalization rate of <2 per 1 million population for HIV-infected individuals and 2–4 per 1 million rate for those without HIV.¹⁷ Extrapolating our data and considering 1.2 million patients in our health system at the time of the study, we would estimate 3 per 1 million persons have CNS-based *Cryptococcus* infections indicated by CrAg, per year, a figure consistent with those reported numbers.

Serum CrAg may be falsely negative in patients with pulmonary cryptococcosis unless the disease has disseminated.¹⁸ Thus, the 3 patients who had only a pulmonary presentation in our study may underestimate this manifestation in our population. Pulmonary cryptococcosis may spread to the CNS, worsening the prognosis of an already serious disease but increasing the likelihood of detection.¹⁸

The preponderance of male patients with positive CrAg is consistent with previous literature.¹ This elevated male cryptococcosis prevalence predates the HIV outbreak and may be due to gender differences in macrophage response to *Cryptococcus*.¹⁹ A sample of the wide variety of reported immunocompromising conditions predisposing to cryptococcosis was seen in our study.^{1,17,18}

The proportion of CrAg-positive patients in our study who were within 162 m (<0.1 miles) from green space (41%) was similar to a historical control group of 250 randomly selected adult patients with community-acquired pneumonia taken from this same catchment area (39.6%) for a 2006–2013 blastomycosis study.²⁰ Our data suggest that Wisconsin zip codes having 1 or more patients with positive CrAg contained a higher proportion of individuals considered to be immunocompromised and that the higher proportion of individuals with positive CrAg in and near Milwaukee reflect this fact. Taken together, perhaps this suggests that the proportion of persons in a given eastern Wisconsin locale who are immunocompromised has more influence on the incidence of cryptococcal infections than does any geographic feature. Further research is required to test this hypothesis.

Interestingly, recent molecular studies indicate that *Cryptococcus* species may be members of the human oral microbiota in some individuals.^{8,9} It has been suggested that oral carriage could lead to opportunistic cryptococcal infections.⁸ We are currently undertaking a culture-based study to determine if pathogenic *Cryptococcus* species indeed reside within the human oral cavity. If this proves to be the case, the path to cryptococcal infection in some persons could be a two-step process, first from the environmental source to oral colonization, then spreading to other organ systems.

The limitations of this descriptive study are those inherent to a retrospective EMR review in a single health system. Therefore, statistical comparisons must be interpreted with caution pending further research. We included performance of CrAg as the sole entry criteria for the study and did not base the comparison of positive and negative cases on additional confirmation such as culture.

In addition, CrAg does not distinguish between *C. neoformans* and *C. gattii*. This distinction may be clinically important, as there was some evidence that the latter may lead to more pulmonary and CNS morbidity than the former and may require longer treatment.²¹ *C. gattii* appears to commonly infect normal hosts; however, it is recognized as also causing serious disease in HIV-

infected persons.²² In addition to the known endemicity of *C. gattii* in Washington and Oregon, this species is now being recognized in other regions of the United States, including the southeast,²² where it may have been present for several thousand years.²³ *C. gattii* is likely present in the environment just south of southeastern Wisconsin, meaning it may be endemic to the catchment area of our study.¹⁴ Initial attempts to culture *C. gattii* from natural environments in Wisconsin have been unsuccessful.²⁴ There is need for routine testing to differentiate between both *Cryptococcus* species and to increase clinician awareness of the nuances regarding *C. neoformans* and *C. gattii*.²²

Patient-Friendly Recap

- Cryptococcosis is a fungal infection acquired through inhalation from the environment. It can cause meningitis, pneumonia, or widespread damage to cells and body tissue, usually in people with weakened immune systems.
- The authors identified cases of cryptococcal infection contracted by people living in eastern Wisconsin and studied the characteristics of these individuals and features of their respective zip codes.
- They found that among patients testing positive for cryptococcosis, male sex, nonwhite race/ethnicity, and a weakened immune system — but not geographic features such as proximity to water — were associated with having a positive test.

Author Contributions

Study design: Baumgardner. Data acquisition or analysis: all authors. Manuscript drafting: Klumph, Baumgardner. Critical revision: Klumph, Baumgardner.

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Conflicts of Interest

None.

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