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BRIEF REPORT

Cryptococcus neoformans–Specific and Non–*Cryptococcus neoformans*–Specific Antibody Profiles in Organ Transplant Recipients With and Without Cryptococcosis

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Antibody immunity has not been studied in organ transplant recipients (OTRs) with cryptococcosis. We determined serum antibody levels in OTRs: 23 cryptococcosis cases and 21 controls. Glucuronoxylomannan immunoglobulin M (IgM) and laminarin IgM were lower in cases than controls, were inversely associated with cryptococcosis status, and may hold promise as markers of cryptococcosis.

Keywords. cryptococcosis; glucuronoxylomannan; IgM; laminarin; transplant recipients.

Cryptococcosis is the third most common invasive fungal disease in organ transplant recipients (OTRs) [1], with 1-year mortality approaching 30% [2]. *Cryptococcus neoformans* (CN) capsule glucuronoxylomannan (GXM)– and laminarin (Lam, a branched β-[1-3]-glucan found on the CN cell wall)–binding antibodies [3–5] have been associated with cryptococcosis status or risk in human immunodeficiency virus (HIV)–infected and HIV-uninfected persons [6], but have not been studied in OTRs. We examined posttransplant antibody profiles in OTRs with and without a history of cryptococcosis.

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METHODS

Patient Consent Statement

This case-control study was approved by the Institutional Review Board (IRB) of the University of Alabama, Birmingham (UAB). All patients provided written informed consent, and the samples were studied under an Albert Einstein College of Medicine IRB–approved protocol.

Study Population

OTRs were recruited at UAB from 1 January 2013 to 31 December 2018. Inclusion criteria included a history of organ transplantation and a history (cases) or no history (controls) of posttransplant cryptococcosis diagnosed by positive cryptococcal antigen (CrAg) test or body fluid cultures.

Demographics

Demographics were abstracted from the medical record, including the type and year of transplant, age, race, and immunosuppression regimen at enrollment. For cases, time from diagnosis to sample collection and the site of infection were recorded.

Sample Collection and Processing

Whole blood samples collected at UAB were shipped overnight to Einstein, where serum was separated by centrifugation and stored at –20°C until use.

Serologic Studies

Serum immunoglobulinM (IgM), immunoglobulin G1 (IgG1), and immunoglobulin G2 (IgG2) concentrations were measured using a Luminex platform (Austin, Texas) as previously described and quantified in units of micrograms per milliliter [3, 5]. CN GXM- and Lam-binding IgM/IgG were measured as previously described and reported as titers [3, 5]. Serum CrAg detection was done using lateral flow assay (IMMY, Norman, Oklahoma).

Statistical Analysis

Baseline characteristics were compared using the Wilcoxon rank-sum test for continuous and Fisher exact or χ^2 test for categorical variables. Univariate and multivariable logistic regression models were built with cryptococcosis status as the outcome and demographics and antibody titers as independent variables, and receiver operating characteristic (ROC) curves were calculated based on the multivariable model. Principal component analysis (PCA) was done to account for the correlation between variables. No correction was made for multiple comparisons, and all analyses were

exploratory. R and RStudio were used (Supplementary Materials).

RESULTS

Table 1 shows the demographics of the 23 posttransplant cryptococcosis cases and 21 controls. Distribution of organ recipients was as follows: kidney, 59.1%; heart, 22.7%; liver, 15.9%; and lung, 6.8%. Median age was 56 years, with no difference between groups. There were more female (39% vs 9.5%, $P = .02$) and fewer African American (8.7% vs 33%, $P = .06$) cases than controls. Most participants (43/44 [98%]) received calcineurin inhibitors (CNIs). There was no significant difference in immunosuppressant use between cases and controls. Serum CrAg was positive in 21 of 23 (91.3%) cases and negative in all 21 controls. The median number of years from most recent transplant to cryptococcosis diagnosis was 1 year (interquartile range [IQR], 0–3 years), and median days between cryptococcosis diagnosis and study enrollment was 32 days (IQR, 6–168 days).

Antibody Profiles

Median IgG1 (1057 vs 1516 μg/mL, $P = .03$) and IgG2 (724 vs 1097 μg/mL, $P = .055$) concentrations were lower in cases than controls. Median inverse titers of GXM-IgM (11 vs 42, $P = .001$), Lam-IgG (319 vs 589, $P = .05$), and Lam-IgM (49 vs 132, $P = .008$) were lower in cases than controls (Table 1; Supplementary Figure 1).

Logistic Regression

GXM-IgM and Lam-IgM were inversely associated with cryptococcosis status in univariate analysis (Figure 1). Neither was significant in a multivariable model including antibodies and sex due to the strong correlation between GXM-IgM and Lam-IgM (Supplementary Figure 2). Antibody levels were inversely associated with cryptococcosis status in models including sex and either GXM-IgM (odds ratio [OR], 0.40, $P = .01$) or Lam-IgM (OR, 0.33, $P = .01$) (Figure 1). To analyze the discriminatory ability of the biomarkers in predicting cryptococcosis status, we constructed two ROC curves. The first depicted the sensitivity and specificity at various thresholds for the final multivariable model that included Lam-IgM, GXM-IgM, and sex, which resulted in an area under the ROC curve of 80.3% (95% confidence interval [CI], .66–.92) (Supplementary Figure 3*A*). To ensure that sex was not driving the results of the curve, a second ROC curve was developed that included GXM-IgM and Lam-IgM titers without sex and found a 77.6% probability (95% CI, .62–.91) to predict cryptococcosis status (Supplementary Figure 3*B*), comparable to the model including sex. ROC analyses for GXM-IgM showed an optimal titer cutoff at 1:25, Lam-IgM at 1:158, and corresponding sensitivity, specificity, positive predictive value, and negative predictive value of 0.90, 0.67, 0.73, and 0.88, respectively, for GXM-IgM, and 0.95, 0.48, 0.65, and 0.91, respectively, for Lam-IgM (Supplementary Table 1).

Table 1. Demographic and Laboratory Attributes of Solid Organ Transplant Recipients With and Without Cryptococcosis

Characteristic	Control Group (n = 21)	Case Group (n = 23)	*P* Value
Age, y, median (IQR)	56 (47–60)	56 (49–66)	.5
Sex, No. (%)			.02
Male	19 (90)	14 (61)	
Female	2 (9.5)	9 (39)	
Race, No. (%)			.06
White	14 (67)	21 (91)	
Black	7 (33)	2 (8.7)	
CNS cryptococcosis, No (%)	NA	11 (48)	
Transplanted organ, No. (%)			.8
Lung	2 (9.5)	1 (4.3)	
Heart-kidney	1 (4.8)	0	
Kidney	8 (38)	12 (52)	
Liver	3 (14)	3 (13)	
Kidney-pancreas	3 (14)	1 (4.3)	
Heart	4 (19)	5 (22)	
Liver/kidney	0	1 (4.3)	
Transplant year, median (IQR)	2012.5 (2010–2015)	2011 (2010–2013)	
Time from transplant to cryptococcosis, y, median (IQR)	NA	1 (0–3)	
Time from cryptococcosis to enrollment, d, median (IQR)	NA	32 (6–168)	
Immunosuppressive agents, No. (%)			
Corticosteroids	17 (81)	17 (74)	.7
Calcineurin inhibitors	22 (100)	22 (96)	>.9
MMF	16 (76)	17 (74)	.9
mTOR inhibitors	3 (14)	2 (8.7)	.7
CN-binding antibodies (1/titer), median (IQR)[a]			
GXM-IgM	42 (15–99)	11 (6–16)	.001
GXM-IgG	298 (155–414)	163 (103–410)	.2
Lam-IgM	132 (44–346)	49 (36–105)	.008
Lam-IgG	589 (393–1199)	319 (157–959)	.05
Non-CN-specific antibodies, μg/mL, median (IQR)[a]			
IgM	777 (476–1122)	608 (228–723)	.1
IgG1	1516 (1238–2441)	1057 (709–1682)	.03
IgG2	1097 (540–1644)	724 (339–1028)	.055

Abbreviations: CN, *Cryptococcus neoformans*; CNS, central nervous system; GXM, glucuronoxylomannan; IgG, immunoglobulin G; IgG1, immunoglobulin G1; IgG2, immunoglobulin G2; IgM, immunoglobulin M; IQR, interquartile range; Lam, laminarin; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; NA, not applicable.

[a]Data regarding antibody titers were missing for 2 patients in the case group.

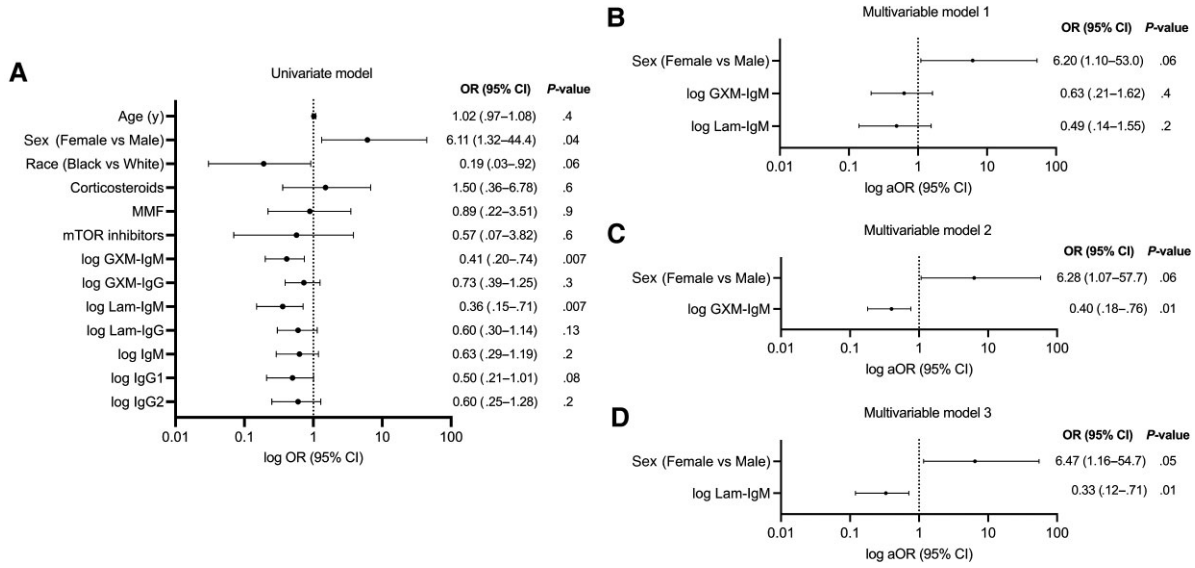

Figure 1. Forest plot of the demographic and laboratory predictors of cryptococcosis in univariate (*A*) and multivariable logistic regression models (*B–D*). Multivariable regression models were fitted with both glucuronoxylomannan (GXM) immunoglobulin M (IgM) and laminarin (Lam) IgM included (*B*) and with GXM-IgM (*C*) and Lam-IgM (*D*) separately given the collinearity between the 2 variables. Lam-IgM and GXM-IgM were significantly associated with cryptococcosis status when fitted separately (*C* and *D*). Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; GXM, glucuronoxylomannan; IgG, immunoglobulin G; IgM, immunoglobulin M; Lam, laminarin; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; OR, odds ratio.

DISCUSSION

In this retrospective single-center, case-control study, serum GXM-IgM and Lam-IgM were lower in OTRs who developed posttransplant cryptococcosis than controls without cryptococcosis and inversely associated with cryptococcosis status after adjustment for sex. OTRs who developed cryptococcosis also had lower IgG1 and IgG2 levels than those who did not.

Our data associating GXM-IgM with cryptococcosis status aligns with the study of Jalali et al, in which pretransplant GXM-IgM was lower in OTRs who developed cryptococcosis than those who did not, although posttransplant GXM-IgM was higher [7]. However, a major difference between the studies is that 75% of the OTRs reported herein received mycophenolate, which may affect B cells that produce naturally occurring and carbohydrate (GXM)–binding antibodies [8] and was not used in the Jalali et al cohort. The effect of CNIs, used in all participants in our study except 1 case, may be complicated. While CNIs inhibit T-cell proliferation, B-cell responses, and immunoglobulin production [9], cyclosporin protected mice against cryptococcal infection [10], and tacrolimus, which has anti-cryptococcal activity in vitro [11], may protect against disseminated cryptococcosis [12].

Our findings parallel previous studies. GXM-IgM was lower in HIV-infected than HIV-uninfected persons [13, 14] and in HIV-infected persons with than without cryptococcosis [15, 16] or cryptococcal antigenemia [3]. Lam-IgM was inversely associated with cryptococcal antigenemia [3] and cryptococcal immune reconstitution inflammatory syndrome [5] in HIV-infected individuals. Our data in another high-risk population suggest the hypothesis that GXM/Lam-IgM may enhance resistance to cryptococcosis. In support of this concept, GXM-IgM can phagocytose and kill CN in vitro [17] and in mice [18, 19]. β-glucan–binding antibodies inhibit CN growth in vivo and in vitro [20, 21] and Titan-like cell formation in vitro [22], and dampen CN-mediated inflammation [5, 23]. Furthermore, reduced levels of IgM memory B cells, the major source of serum IgM, including naturally occurring antibodies to β-glucans like Lam [4, 24], was associated with cryptococcosis in an HIV-infected [16] and HIV-uninfected cohort from the same center [6]. Thus, it is logical to posit that GXM/Lam IgM may enhance resistance to cryptococcosis in OTRs, perhaps by helping to maintain CN in a latent state [25].

We did not find a significant difference in GXM-IgG between groups. However, IgG1 levels of the entire cohort were 8-fold lower than normal adult levels, perhaps reflecting a loss of potentially responding B-cell precursors. Perturbations in GXM/Lam-IgG in HIV-associated cryptococcosis have been noted previously [3, 5, 6, 14, 15]. Differences in naturally occurring GXM/Lam-binding antibodies in high-risk (eg, HIV, OTR) and control populations, along with experimental evidence of their ability to control CN replication and dissemination, suggest that they might have diagnostic or prognostic significance. Given the need for tools for earlier diagnosis of OTR-associated cryptococcosis, this concept warrants investigation.

The strengths of this study include the use of well-established assays to measure CN-binding antibodies [3, 5–7]. We also used ROC curves to estimate the discriminatory ability of combinations of antibody markers to predict disease status and PCA to correct the multicollinearity by reducing the dimension but preserving the maximum variance. ROC analysis and unsupervised machine learning algorithms such as PCA may provide valuable insights into predictive markers in exploratory studies. Serum CrAg testing, a well-studied screening tool in HIV-infected individuals, has not been studied in OTRs [26]. Our data call for prospective studies to test the association of antibody profiles and cryptococcosis risk. This may provide a host-based screening test that could also inform the development of novel therapeutics and vaccines.

Limitations include the retrospective, single-center nature of the study. Variable time had elapsed between time of transplantation, cryptococcosis, and enrollment; the 2 groups were not recruited concurrently; and blood samples were obtained at 1 nonstandardized time. There were no pre-cryptococcosis sample and we cannot draw causal associations between antibody levels and cryptococcosis status. We did not examine B-cell subsets, which might have shed light on differences in IgM/IgG populations. We did not have a cohort not receiving immunosuppressants to assess the effect of immunosuppressants on antibody or B-cell levels, although perturbation in CN-specific antibody levels were identified in HIV-uninfected persons with cryptococcosis from the same center [6]. Standard immunosuppressants affect T cells and we did not assess cellular immunity, which could be a confounder. We did not control for possible cirrhosis, hematological malignancy, or active chemotherapy, which may have been confounders. There were proportionally more female cases than controls, likely by chance given small sample size, but sensitivity analysis without sex as a variable did not significantly change the ROC analysis.

CONCLUSIONS

OTRs with cryptococcosis had lower GXM-IgM and Lam-IgM antibody levels compared to controls. Together with previous studies, our findings suggest the hypothesis that GXM/Lam-IgM may be beneficial in resistance to OTR-associated cryptococcosis. A larger prospective study is needed to investigate antibody markers as risk-stratifying tools for earlier diagnosis of OTR-associated cryptococcosis.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. L. P. conceived of the study and wrote the study protocol. P. G. P. obtained patient consent and samples. A. N. performed the laboratory studies. V. S. H. performed the statistical analysis. A. N., V. S. H., and H. Y. wrote the first draft of the manuscript. All authors contributed to the final draft of the manuscript.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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