The Diagnosis of Invasive Aspergillosis Using Screening Detection of Aspergillus Galactomannan After Hematopoietic Stem Cell Transplant.

Chineme Nwaichi

A thesis

Submitted in partial fulfillment of the Requirements

for the degree of

Master of Science

University of Washington 2024

Committee:

R. Scott McClelland

David Fredricks

Seth Cohen

Program Authorized to Offer Degree: Epidemiology

University of Washington

Abstract

The Diagnosis of Invasive Aspergillosis Using Screening Detection of *Aspergillus* Galactomannan After Hematopoietic Stem Cell Transplant.

Chineme Nwaichi

Chair of Supervisory Committee:

R. Scott McClelland

Department of Epidemiology

Early detection of invasive aspergillosis (IA) in hematopoietic stem cell transplant (HSCT) patients is crucial and requires early initiation of antifungal therapy. One method that has been suggested as a means for early detection of aspergillosis is serum *Aspergillus* galactomannan (GM) detection. However, there have been few formal evaluations of the performance of GM screening for early detection of aspergillosis. We performed a retrospective cohort study at the Fred Hutch Cancer Center (FHCC) that revealed a false positive serum GM result in 105/169 (62.1%) HSCT patients who screened positive between 2006 - 2010. Among all positive GM results, logistic regression analysis showed that the presence of acute gut graft vs. host disease (AGVHD) at the time of screening was not associated with a higher likelihood of a false positive result (odds ratio 1.29; 95% confidence interval 0.69, 2.45; p=0.4) compared to patients without AGVHD. These findings support the FHCC's decision in 2011 that led to the cessation of routine GM screening for invasive aspergillosis. Additionally, the results do not indicate that the screening performs better in patients without AGVHD. These data highlight the need for more specific diagnostic approaches for the early detection of IA in this vulnerable patient population.

Introduction

Aspergillus Galactomannan (GM) is a polysaccharide released from *Aspergillus* that can be detected in serum using an immunoassay and is used as an aid to diagnose Invasive aspergillosis (IA). IA is classified as proven, probable, or possible based on histologic, microbiologic, and clinical criteria. One clinical challenge is false positive *Aspergillus* galactomannan (GM) testing in the context of screening in immunocompromised patients, which limits the ability to initiate early or pre-emptive therapy[1].

Galactomannan testing is a cornerstone in diagnosing IA because it detects fungal cell wall components in bodily fluids[1]. However, concerns persist with the screening accuracy of GM testing, notably regarding the high prevalence of false positives. Recent epidemiological studies have revealed a high incidence of false positives in at least some studies of GM screening, with a reported rate as high as 85.7% [2]. These findings highlight the need to quantify the positive predictive value of GM for the screening detection of IA in HSCT patients.

Between 2006 and 2010, Fred Hutchinson Cancer Center (FHCC) implemented a protocol utilizing serum GM assays to screen for IA in patients 18 years or older after allogeneic hematopoietic stem cell transplant (HSCT). By 2011, the use of this protocol came into question, prompting its discontinuation based on a clinical consensus that it was not an effective strategy. A better understanding of the prevalence and determinants of a false positive GM is needed when GM is used for screening[1,3].

We hypothesize that the prevalence of false positive GM among patients who screen positive for GM would be high enough to limit its clinical utility in our patient population of HSCT recipients [1,4]. In addition, we hypothesize that among patients with a positive GM, those with

acute gut graft vs. host disease (AGVHD) will have higher odds of a false positive compared to patients without AGVHD because of the possible translocation and detection of dietary galactomannan in the serum [5].

This study aims to identify the prevalence of false positive GM after a positive screen in allo-HSCT recipients and investigate the relationship between AGVHD and false positive GM. Neutropenia in the absence of engraftment is a known risk factor for invasive fungal infection, and the associated mucositis from chemo-radiation around the time of neutropenia and subsequent engraftment may predispose to the detection of GM in serum [5]. In addition, neutrophil recovery may predispose to a robust inflammatory response in initially quiescent infection with clinically evident disease after engraftment [3,4]. Based on these clinical correlates related to engraftment, we have considered engraftment as a marker for possible immunologic and mucosal changes that predispose to true positive or false positive GM detection.

Methods

This is a retrospective cohort study of adult patients who screened positive for GM after they received their first allo-HSCT during a screening surveillance period at the FHCC from January 2006 to December 2010. Patients were 18 years or older and received conditioning chemotherapy with or without total body irradiation (TBI). All patients received graft vs host disease prevention.

We identified all cases with a positive galactomannan screen using a cutoff value of 0.5 OD through an electronic transplant database at the FHCC and considered only the first positive galactomannan results after HSCT for the case adjudication. We queried the database for patients

with fungal cultures, pathology, and corresponding radiology and adjudicated the cases as proven, probable, or possible according to international consensus criteria [5].

Infection definitions

Invasive fungal infections were graded as possible, probable, or proven according to standardized laboratory and clinical guidelines. To allow for adequate grading, we used patient identifier information from clinical records, and two investigators reviewed the medical records to adjudicate each positive GM result as a false positive, probable infection, or proven infection.

Diagnosis of IA is classified as possible if it meets a host criterion (in this case, an HSCT recipient) and microbiological criteria or compatible imaging. For the study's intent, we adjudicated patients as a false positive instead of possible infection to create a binary variable of either a true positive (probable or proven) or a false positive (possible) result [5]. A probable diagnosis generally consists of meeting a host criterion (in this case, HSCT recipient) and a positive bronchoalveolar lavage (BAL) culture and/or GM from BAL with compatible imaging. A proven infection is histopathologic evidence of hyphae with associated tissue damage and/or a positive culture from a normally sterile site with compatible imaging findings [5,6]. At the FHCC, a positive GM was defined as equal to or greater than 0.5 OD on initial and confirmatory testing with the GM enzyme immunosorbent assay [7,8]. At FHCC, we perform a confirmatory test reflexively after a positive test because of test variability.

Outcomes

The primary outcome was the proportion of false positive serum GM in patients who screened positive for GM after allo-HSCT within 60 days from transplant. The analyses had two aims; the first was to characterize the prevalence of false-positive GM in this subset of HSCT participants

with a positive GM test. The second aim was to test the hypothesis that, in patients with a positive GM result, AGVHD is associated with higher odds of a false positive result.

Statistical analyses

The false positive rate of *Aspergillus* GM screening was determined by calculating the proportion of all positive GM results that were adjudicated to be false positive, and this proportion is presented with its exact binomial 95% CI. To examine the association between AGVHD and false-positive GM, we first performed a univariate logistic regression of the association between AGVHD and a false positive vs. true positive GM. In addition, we considered engraftment as a potential confounding factor of this association [5], given it's potential for association with both AGVHD and false-positive GM results. The R software package was used for statistical analysis, and P values are 2-sided and statistically significant if <0.05.

Result

Between 2006 and 2010, our screening study utilizing *Aspergillus* galactomannan (GM) identified 169 patients with a positive GM result. Among these patients, 84 (49.0%) were male, with a median age at transplant of 51.2 years (IQR: 22.6). Notably, 70 (41.4%) patients experienced AGVHD before a positive test. The predominant cell source for transplantation was peripheral blood stem cells (PBSC), accounting for 113 (66.9%) cases, followed by bone marrow (BM) at 41 (24.3%) and cord blood (CORD) at 15 (8.9%) cases (Table 1).

Among the cases, 134 (79.3%) were caucasian. In 49 (29.0%) cases, donor-positive and recipient-negative cytomegalovirus serostatus was observed, and 108 (63.9)% patients received

total body irradiation (TBI); 116 (68.6%) had an unrelated donor. Acute leukemia was the most prevalent disease group, accounting for 86 (50.9%) cases (Table 1).

Transplant seasons were evenly distributed among spring (44 patients, 26.0%), summer (50 patients, 29.6%), fall (28 patients, 16.6%), and winter (47 patients, 27.8%). Figure 1 shows that the highest number of positive GM results were around the time when engraftment occurred. However, no clear pattern of false positives occurred at a particular time before or after engraftment transplant.

At the time of the positive GM test, engraftment had occurred in 58 patients (34.3%), while 113 patients (66.9%) had not experienced engraftment (Table 1). Regarding adjudicated results, 64 patients (37.9%) were classified as having probable infections, 105 patients (62.1%; 95% CI 55.0%, 67.9%)) had false positive results in the GM screening for IA. The positive predictive value of a positive GM result during the screening period at FHCC was 37.9%.

In the unadjusted analysis, patients with AGVHD had an odds ratio of 1.29 for a false positive GM (95% CI: 0.69 - 2.45, p = 0.4) compared to those without AGVHD, and this association was not statistically significant. After adjusting for engraftment, the odds ratio for a false positive GM increased to 1.92 (95% CI: 0.50 – 7.27, p = 0.3) but was still not statistically significant (Table 2).

A dose-response effect on the association between AGVHD and false positive GM results was evaluated by stratifying acute gut GVHD into four stages (0-3). This analysis did not provide evidence of a dose-response relationship between the stage of AGVHD and the likelihood of a false-positive GM among patients with a positive GM (Table 2).

Figures and Tables

Table 1: Characteristics of Patients in the Cohort

Variable	*Categories*	*n = 169 (%)*
Age at Transplant	Median (IQR)	51.19 [22.6]
Acute Gut GVHD at the time of screening	Yes	70 [41.4]
Cell source	BM	41 [24.3]
	CORD	15 [8.9]
	PBSC	113 [66.9]
CMV serostatus	D+/R+	49 [29.0]
	D-/R+	48 [28.4]
	D+/R-	21 [12.4]
	D-/R-	35 [20.7]
	Missing	16 [9.5]
TBI	Yes	108 [63.9]
Donor Relationship	Unrelated	116 [68.6]
	Related	53 [31.4]
Donor Sex	Female	80 [47.3]
	Male	81 [47.9]
	Unknown	8 [4.7]
Disease Group	Acute leukemia	86 [50.9]
	Chronic Leukemia	13 [7.7]
	Other	70 [41.4]
HLA matching	Matched Related	47 [27.8]
	Matched Unrelated	65 [38.5]
	Mismatched	37 [21.9]
	Haplo	5 [2.9]
	Cord	15 [8.9]
Race	Caucasian	134 [79.3]
	Non-caucasian	35 [20.7]
Recipient Sex	Female	85 [50.3]
	Male	84 [49.7]
Transplant Season	Spring	44 [26.0]
	Summer	50 [29.6]
	Fall	28 [16.6]
	Winter	47 [27.8]
Engraftment at the time of screening	Yes	58 [34.3]

GVHD, graft vs host disease; TBI, Total body irradiation; CMV, Cytomegalovirus; D, donor, R, recipient, BM, bone marrow, CORD, cord blood, PBSC, peripheral blood stem cell.

Table 2: Association between Acute gut GVHD and false positive GM results

Variables	*Unadjusted OR*	*95% CI*	*P value*	*Adjusted OR*	*95% CI*	*P value*
No AGVHD	Ref			Ref		
AGVHD	1.29	0.68-2.45	0.4	1.91	0.50-7.27	0.3
AGVHD Stage 0	1.06	0.31-3.61	0.9	1.15	0.31-4.26	0.8
AGVHD Stage 1	1.38	0.44-4.42	0.6	1.46	0.45-4.83	0.5
AGVHD Stage 2	1.00	0.25-3.92	1.0	1.00	0.26-3.94	0.9
AGVHD Stage 3	1.65	0.36-7.36	0.5	1.61	0.36-7.24	0.5

Table 2 shows the association between AGVHD and false positive GM, and the dose-response relationship by AGVHD stages (0- 3). OR-Odds ratio, Adjusted OR: Adjusted for engraftment, CI : confidence interval, AGVHD:Acute gut GVHD

Figure 1

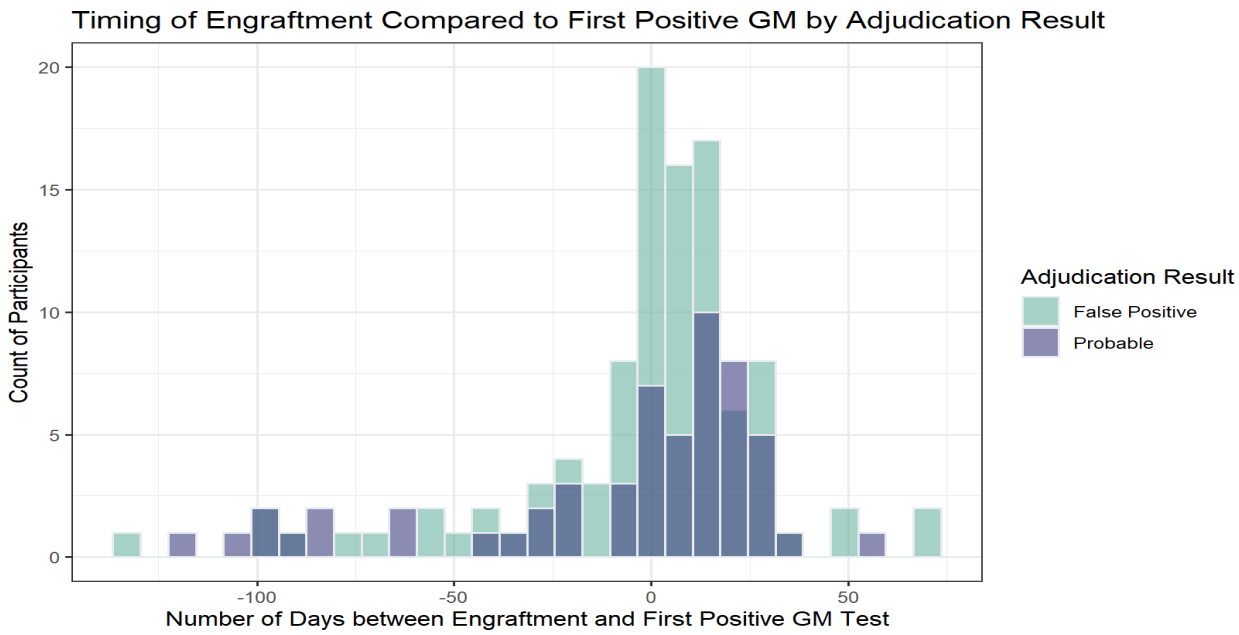

Figure 1 shows the galactomannan-positive test distribution relative to the engraftment occurrence.

Discussion

This study showed that during a period when GM was used to screen for IA in HSCT patients at FHCC, nearly two-thirds of initial positive tests were ultimately adjudicated to be false positives. The observed prevalence of false positive proportion was 62% among patients who screened positive for GM, and there was no statistically significant increase in the likelihood of a false positive result among patients with AGVHD.

This study's observed false positive proportion of 62% warrants comparison with findings from a previous study that utilized GM to screen high-risk hematology patients undergoing chemotherapy for acute myeloid leukemia and allogeneic transplant patients. That study reported a false positive prevalence of 85.7% among all patients who tested positive for GM, which is somewhat higher but broadly comparable to our cohort's observed prevalence of false positives [4].

The false positive proportion was high among all positive GM, and there was no association between AGVHD and false positive GM. While the study may have been underpowered to see minor differences, the confidence intervals suggest that the difference in performance, even in the subset of patients without acute gut GVHD, is unlikely to be good enough to recommend the test as a screening tool [9].

These results suggest that while GM may remain important in diagnosing IA in patients with suspected disease, it should not be used for screening in the absence of signs or symptoms suggesting IA in this patient population. In the setting of signs or symptoms, the testing would be considered diagnostic rather than screening. These results support the 2011 decision to stop routine GM screening in FHCC HSCT patients.

Despite the unacceptably low positive predictive value of GM screening in our study, there may be a role for using GM as part of a multi-test screening strategy by using a combination of GM and *Aspergillus.* This was investigated in a study that used both serum *Aspergillus* GM and *Aspergillus* PCR to screen for IA in a high-risk population [1]. That study found a positive predictive value of 88% if both tests were positive. If both tests were negative, the negative predictive value for IA was 100% [2].

The retrospective nature of our study design introduces some inherent limitations. We could not practically abstract data from all HSCT patients, so we focused only on those with a positive GM. This challenge meant that we could not calculate all operating characteristics of GM, including sensitivity, specificity, and negative predictive value, though this would be possible with additional data. In addition, only considering the 1st GM means that we only adjudicated a single GM test and not subsequent interval testing. This study design feature could have led us to underestimate of the overall likelihood of a false-positive GM result for patients during the screening period from 2006 to 2010.

Conclusion

Our study found that during a period when *Aspergillus* GM testing was used to screen for IA in post-HSCT patients, 62% of positive *Aspergillus* GM results within 60 days of transplant were false-positives. No association was detected between AGVHD and false positive results, suggesting that the screening test would not perform better in the subset of patients without AGVHD. These findings support the decision to discontinue routine galactomannan screening for all HSCT recipients at FHCC. Future research should focus on refining screening strategies using alternative preventive approaches for early detection of invasive aspergillosis in HSCT recipients.

References

1. Aguado, J. M., L. Vazquez, M. Fernandez-Ruiz, T. Villaescusa, I. Ruiz-Camps, P. Barba, J. T. Silva, et al. “Serum Galactomannan Versus a Combination of Galactomannan and Polymerase Chain Reaction-Based Aspergillus DNA Detection for Early Therapy of Invasive Aspergillosis in High-Risk Hematological Patients: A Randomized Controlled Trial.” *Clinical Infectious Diseases* 60, no. 3 (February 1, 2015): 405–14. https://doi.org/10.1093/cid/ciu833.

2. Arvanitis, Marios, Theodora Anagnostou, and Eleftherios Mylonakis. "Galactomannan and Polymerase Chain Reaction–Based Screening for Invasive Aspergillosis Among High-Risk Hematology Patients: A Diagnostic Meta-Analysis." *Clinical Infectious Diseases* 61, no. 8 (October 15, 2015): 1263–72. https://doi.org/10.1093/cid/civ555.

3. Donnelly, J Peter, Sharon C Chen, Carol A Kauffman, William J Steinbach, John W Baddley, Paul E Verweij, Cornelius J Clancy, et al. "Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium." *Clinical Infectious Diseases* 71, no. 6 (September 12, 2020): 1367–76. https://doi.org/10.1093/cid/ciz1008.

4. Duarte, R. F., I. Sanchez-Ortega, I. Cuesta, M. Arnan, B. Patino, A. Fernandez De Sevilla, C. Gudiol, J. Ayats, and M. Cuenca-Estrella. "Serum Galactomannan-Based Early Detection of Invasive Aspergillosis in Hematology Patients Receiving Effective Antimold Prophylaxis." *Clinical Infectious Diseases* 59, no. 12 (December 15, 2014): 1696–1702. https://doi.org/10.1093/cid/ciu673.

5. Marr, Kieren A., S. Arunmozhi Balajee, Lisa McLaughlin, Marc Tabouret, Christopher Bentsen, and Thomas J. Walsh. "Detection of Galactomannan Antigenemia by Enzyme Immunoassay for the Diagnosis of Invasive Aspergillosis: Variables That Affect Performance." *The Journal of Infectious Diseases* 190, no. 3 (August 1, 2004): 641–49. https://doi.org/10.1086/422009.

6. Marr, Kieren A., Rachel A. Carter, Michael Boeckh, Paul Martin, and Lawrence Corey. "Invasive Aspergillosis in Allogeneic Stem Cell Transplant Recipients: Changes in Epidemiology and Risk Factors." *Blood* 100, no. 13 (December 15, 2002): 4358–66. https://doi.org/10.1182/blood-2002-05-1496.

7. Marr, Kieren A., Rachel A. Carter, Fulvio Crippa, Anna Wald, and Lawrence Corey. "Epidemiology and Outcome of Mould Infections in Hematopoietic Stem Cell Transplant Recipients." *Clinical Infectious Diseases* 34, no. 7 (April 1, 2002): 909–17. https://doi.org/10.1086/339202.

8. Mercier, Toine, Elio Castagnola, Kieren A. Marr, L. Joseph Wheat, Paul E. Verweij, and Johan A. Maertens. “Defining Galactomannan Positivity in the Updated EORTC/MSGERC Consensus Definitions of Invasive Fungal Diseases.” *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* 72, no. Suppl 2 (March 12, 2021): S89–94. https://doi.org/10.1093/cid/ciaa1786.

9. Musher, Benjamin, David Fredricks, Wendy Leisenring, S. Arunmozhi Balajee, Caitlin Smith, and Kieren A. Marr. "Aspergillus Galactomannan Enzyme Immunoassay and Quantitative PCR for Diagnosis of Invasive Aspergillosis with Bronchoalveolar Lavage Fluid." *Journal of Clinical Microbiology* 42, no. 12 (December 2004): 5517–22. https://doi.org/10.1128/JCM.42.12.5517-5522.2004.