Title

Prospective Evaluation of Galactomannan and (1→3) β-d-Glucan Assays as Diagnostic Tools for Invasive Fungal Disease in Children, Adolescents, and Young Adults With Acute Myeloid Leukemia Receiving Fungal Prophylaxis.

Journal

Journal of the Pediatric Infectious Diseases Society, 10(8)

ISSN

2048-7193

Authors

Fisher, Brian T
Westling, Ted
Boge, Craig LK
et al.

Publication Date

2021-09-23

DOI

10.1093/jpids/piab036

Peer reviewed

Journal of the Pediatric Infectious Diseases Society

ORIGINAL ARTICLE

Prospective Evaluation of Galactomannan and (1→3) β-D-Glucan Assays as Diagnostic Tools for Invasive Fungal Disease in Children, Adolescents, and Young Adults With Acute Myeloid Leukemia Receiving Fungal Prophylaxis

Brian T. Fisher,[1] Ted Westling,[2] Craig L. K. Boge,[1] Theoklis E. Zaoutis,[1] Christopher C. Dvorak,[3] Michael Nieder,[4] Danielle M. Zerr,[5] John R. Wingard,[6] Doojduen Villaluna,[7] Adam J. Esbenshade,[8] Sarah Alexander,[9] Suphansa Gunn,[10] Lawrence J. Wheat,[10] and Lillian Sung[9]

[1]Division of Infectious Diseases, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA, [2]Department of Mathematics and Statistics, University of Massachusetts Amherst, Amherst, Massachusetts, USA, [3]Division of Pediatric Allergy, Immunology and Bone Marrow Transplant, University of California San Francisco, San Francisco, California, USA, [4]Division of Blood and Marrow Transplant and Cellular Immunotherapy, Moffitt Cancer Center, Tampa, Florida, USA, [5]Division of Pediatric Infectious Diseases, Seattle Children's Hospital, Seattle, Washington, USA, [6]University of Florida College of Medicine, Gainesville, Florida, USA, [7]Children's Oncology Group, Monrovia, California, USA, [8]Division of Pediatric Hematology and Oncology, Vanderbilt University Medical Center, Nashville, Tennessee, USA, [9]Division of Haematology Oncology, The Hospital for Sick Children, Toronto, Ontario, Canada, and [10]Miravista Diagnostics, LLC, Indianapolis, Indiana, USA

Background. Patients receiving chemotherapy for acute myeloid leukemia (AML) are at high risk for invasive fungal disease (IFD). Diagnosis of IFD is challenging, leading to interest in fungal biomarkers. The objective was to define the utility of surveillance testing with Platelia Aspergillus galactomannan (GM) enzyme immunoassay (EIA) and Fungitell β-D-glucan (BDG) assay in children with AML receiving antifungal prophylaxis.

Methods. Twice-weekly surveillance blood testing with GM EIA and BDG assay was performed during periods of neutropenia in the context of a randomized trial of children, adolescents, and young adults with AML allocated to fluconazole or caspofungin prophylaxis. Proven or probable IFD was adjudicated using blinded central reviewers. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for Platelia and Fungitell assays alone and in combination for the outcomes of proven and probable invasive aspergillosis (IA) or invasive candidiasis (IC).

Results. Among 471 patients enrolled, 425 participants (209 fluconazole and 216 caspofungin) contributed ≥1 blood specimen. In total, 6103 specimens were evaluated, with a median of 15 specimens per patient (range 1–43). The NPV was >99% for GM EIA and BDG assay alone and in combination. However, there were no true positive results, resulting in sensitivity and PPV for each assay of 0%.

Conclusions. The GM EIA and the BDG assay alone or in combination were not successful at detecting IA or IC during periods of neutropenia in children, adolescents, and young adults with AML receiving antifungal prophylaxis. Utilization of these assays for surveillance in this clinical setting should be discouraged.

Key words. acute myeloid leukemia; fungal biomarkers; fungal disease; pediatrics; surveillance.

Current chemotherapy protocols for acute myeloid leukemia (AML) result in repeated periods of profound and prolonged neutropenia. During these neutropenic periods, patients are at high risk for invasive fungal disease (IFD), particularly invasive candidiasis (IC) and invasive aspergillosis (IA) [1, 2], which are associated with substantial morbidity and mortality [3, 4]. Early diagnosis leading to more rapid initiation of targeted antifungal therapy can improve outcomes [5, 6].

Traditional IFD diagnostic approaches have been limited to radiologic imaging and sterile site cultures. Radiologic findings are often nonspecific and unable to differentiate between fungal and nonfungal processes. Blood cultures are useful to isolate *Candida* species, but are uninformative for IC without fungemia. Tissue specimens are typically required to provide definitive evidence for IA; however, biopsy procedures to obtain these specimens are invasive and associated with morbidity and mortality [7]. Even when these traditional approaches are successful, the time needed to make the IFD diagnosis can result in delays in appropriate therapy. Thus, there has been increasing interest in noninvasive diagnostic tests surveillance protocols to diagnose IFD at its

Received 18 September 2020; editorial decision 26 April 2021; accepted 30 April 2021; Published online June 26, 2021.

Corresponding Author: Brian T. Fisher, DO, MSCE, Division of Infectious Diseases, The Children's Hospital of Philadelphia, 2716 South Street, Room 10362, Philadelphia, PA 19146, USA. E-mail: fisherbria@email.chop.edu.

Journal of the Pediatric Infectious Diseases Society 2021;10(8):864–71

DOI: 10.1093/jpids/piab036

earliest onset, so directed therapies can be initiated early and invasive procedures avoided.

The advent of nonculture mycology biomarkers, such as the Platelia Aspergillus galactomannan (GM) enzyme immunoassay (EIA) and the Fungitell β-D-glucan (BDG) assay, provides the opportunity for surveillance testing in patients at risk for IFD. The former is designed to detect GM, a cell wall component of *Aspergillus* spp., and the latter to detect BDG, a cell wall component of some pathogenic fungi including *Aspergillus* and *Candida* spp. These commercially available assays gained approval based, in part, on diagnostic test characteristics defined by surveillance studies in adults with leukemia and those undergoing hematopoietic cell transplantation (HCT) [8, 9].

However, the utility of these biomarkers in children and adolescents has been questioned [10]. Current pediatric guidelines recommend some form of antifungal prophylaxis for patients with AML [11]. Therefore, it is important to evaluate these biomarkers in larger cohorts of pediatric patients receiving antifungal prophylaxis as this prophylaxis may alter the epidemiology of IFD reducing the frequency of pathogens that these biomarkers are designed to detect [12]. This study aimed to define the utility of GM and BDG surveillance testing in children, adolescents, and young adults with AML receiving antifungal prophylaxis. Given the anticipated low pretest probability for IFD in this cohort of patients receiving prophylaxis, it was hypothesized that the operating characteristics of these biomarkers would be poor under these surveillance conditions.

METHODS

Study Design and Population

This was a prospective observational study imbedded within a randomized controlled open-label phase III Children's Oncology Group trial (ACCL0933) comparing caspofungin prophylaxis versus fluconazole prophylaxis during neutropenic periods in children, adolescents and young adults with AML [13]. Participants were eligible for the randomized trial if they had newly diagnosed de novo, relapsed or secondary AML, or had planned treatment with standard AML chemotherapy for other diagnoses (eg, mixed phenotype acute leukemia) and were between 3 months and 30 years of age. Patients were excluded if they had acute promyelocytic leukemia, Down syndrome, juvenile myelomonocytic leukemia, documented IFD ≤30 days prior to enrollment, or were currently receiving treatment for IFD.

This study was approved by the National Cancer Institute's Central Institutional Review Board (IRB) and IRBs at each participating institution. At the time of enrollment to the randomized trial, participants were offered the option to participate in this observational study. Guardians and participants provided informed consent and assent as appropriate prior to enrollment. All participants initiated their randomized prophylaxis agent within 72 hours of completing their first cycle of systemic chemotherapy and were followed for up to 4 cycles of chemotherapy.

Specimen Collection

For each chemotherapy cycle, twice-weekly blood collection started after completion of chemotherapy administration and continued through the neutropenic period until the absolute neutrophil count was >100–500/μL following the nadir or start of the next chemotherapy cycle, whichever occurred first. Sites were instructed to collect 5 mL whole blood into a serum-separator tube, allow the blood to clot for 30–60 minutes at room temperature, centrifuge for 15 minutes at 1000–1300 × *g*, and transfer serum into BDG-free tubes. Blood specimens less than 5 mL but yielding ≥1 mL serum were included in final analyses. Maximum blood volume obtained was 3 mL/kg per 8-week period. A minimum of 2 days between specimen collections was recommended, but timing was at the discretion of the local institution. All specimens were submitted to the central laboratory (MiraVista Diagnostics, Indianapolis, Indiana) for batch testing. Details regarding specimen shipment and processing and assay performance are included in Supplementary Material S1.

Outcome

This study leveraged the outcome designation infrastructure of the randomized trial. Outcomes of proven or probable IFD were based on the 2008 criteria from the European Organisation for Research and Treatment in Cancer/Mycoses Study Group (EORTC/MSG) [14]. Of note, *Pneumocystis jiroveci* infection can cause a positive Fungitell assay. However, because this pathogen is not typically treated with antifungal agents, it was not considered an event for this study. The time at risk started on the last day of systemic chemotherapy administration of the first chemotherapy cycle and continued until the first of the following: 14 days after collection of the last specimen, diagnosis of an IFD, or the last day of observation.

A blinded central review committee systematically applied the EORTC/MSG criteria to determine the outcomes of proven or probable IFD for all enrolled patients. The committee reviewed de-identified pathology, autopsy, computerized tomography, magnetic resonance imaging, ophthalmology, bronchoscopy reports, and culture and nonculture mycology results, including molecular testing, serologies, and biomarker assays, to determine outcome. All IFD diagnostic investigations were performed at the clinician's discretion. Study biomarker assay results were not disclosed to clinicians or central reviewers and, thus, did not impact on IFD designation.

Each central review was conducted by webinar with a minimum of 3 reviewers (M.N., D.M.Z., J.R.W., A.J.E., S.A.). Disagreements were resolved by consensus. The IFD onset date and causative pathogen were reported for each event. If a

clinically performed GM or BDG assay was used to establish mycology criteria, the pathogen was reported as *Aspergillus* not otherwise specified (NOS) and fungus NOS, respectively. If histopathology was used to meet proven IFD mycology criteria, the pathogen was reported as yeast NOS or mold NOS based on morphology.

Covariates

Demographic data such as age, gender, race, and planned chemotherapy were captured as part of the randomized trial. Exposures to intravenous immunoglobulin (IVIG), amoxicillin–clavulanate, and piperacillin–tazobactam, as they have been associated with false positive assay results, were captured specifically for this study [15–17].

Statistical Analysis

Sample Size

The randomized trial had a planned sample size of 550 patients; assuming an 80% consent rate, this biomarker study had a planned sample size of 440. Assuming an 8% event rate in the fluconazole group (18 events) and 2% in the caspofungin group (4 events), one-third of all IFD events would be IA, and all other non-IA IFDs would be IC [18]; we anticipated 7 IA and 15 IC events.

Primary Analysis

Supplementary Material S2 describes the prespecified primary, secondary, and sensitivity analyses as well as post hoc analyses. In brief, the primary analysis determined the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of GM EIA for the outcome of IA, BDG assay for an outcome inclusive of IA and IC, and both GM EIA and BDG assay (requiring only one to be positive) for an outcome inclusive of IA and IC. In the primary analysis, a GM EIA was considered positive if the optical density index (ODI) value was ≥0.5. The BDG assay was considered positive if the result was ≥80 pg/mL. A positive result was only considered a true positive if the specimen was collected within 7 days of the date of an IA or IC diagnosis.

The diagnostic test characteristics for each assay were calculated for the entire cohort and separately for the caspofungin and fluconazole prophylaxis groups. The area under the receiver operating characteristic curve (AUC) was computed for both assays. Sensitivity and AUC were not computed in cohorts with no observed events. Confidence intervals were constructed using the clustered, continuity-corrected nonparametric percentile bootstrap.

Secondary Analyses

Secondary analyses considered modifications to positivity thresholds, outcome definitions, and time window for IA or IC onset. Positivity thresholds of ≥1.0, 1.5, and 2.0 ODI for GM EIA and ≥100 and 120 pg/mL for BDG assay were evaluated. Since proven or probable IFD can be diagnosed without specifying pathogen genus or species, broader outcome definitions for IA and IC events, including mold NOS, yeast NOS, or fungus NOS, were considered. Finally, since the optimal time window between specimen collection and IFD diagnosis is not known for either assay, we evaluated an extended window of 14 days.

Sensitivity and Post hoc Analyses

Recent prior exposure to IVIG, amoxicillin–clavulanate, and piperacillin–tazobactam were considered in sensitivity analyses. Any positive GM EIA or BDG assay in a patient exposed to IVIG in the past 3 months or either antibiotic within the past 7 days were removed from the analysis.

Post hoc analyses considered lower positivity thresholds (ODI of 0.1, 0.2, 0.3, and 0.4) for the GM EIA, an extended time window of 28 days between specimen collection and IFD diagnosis, and consideration of any type of IFD diagnosis.

RESULTS

The randomized trial enrolled 508 evaluable patients (255 fluconazole and 253 caspofungin) at 115 participating institutions between April 4, 2011 and November 11, 2016. The 5-month IFD cumulative incidence in this trial was 3.1% for the caspofungin group and 7.0% for the fluconazole group. Of these, 471 patients (235 fluconazole and 236 caspofungin) consented to this biomarker study and 425 patients (209 fluconazole and 216 caspofungin) contributed ≥1 blood specimen. Baseline characteristics of the cohort are described in Table 1. Demographic characteristics were similar across the 2 groups and distributed similarly to the randomized trial cohort [13]. The median duration of time at risk for IFD was 115 days (range 4–231 days) and the IFD event rate was 3.3%. Table 2 displays the IFD events considered in this analysis for the study cohort and by treatment group. The majority (78.6%) of the 14 proven and probable IFD events were the result of an *Aspergillus* species or *Candida* species. The IFD rate was lower than the parent trial because fungi that could not be detected by a GM or BDG assay were not considered in this analysis. In total, 6103 specimens were collected and shipped to the central laboratory, representing a median of 15 specimens per patient (interquartile range 8–20, range 1–43).

Primary and Secondary Analyses

Test characteristics from the primary analysis for the GM EIA, BDG assay, and combination are shown in Tables 3–5, respectively. Specificity was >93% and NPV >99% for the GM EIA, BDG assay and both together. However, no specimens collected within 7 days of an IA or IC diagnosis met positivity thresholds. Therefore, calculated sensitivity and PPVs were zero, and all positive specimens were false positives.

Table 1. Patient and Clinical Characteristics of the Study Population and by Allocated Prophylaxis Group

Characteristic	Overall (N = 425)	Fluconazole (n = 209)	Caspofungin (n = 216)
Age (y)[a], median (range)	10 (0–25)	9 (0–21)	10 (0–25)
Sex, n (%)			
Female	185 (43.5)	87 (41.6)	98 (45.4)
Male	240 (56.5)	122 (58.4)	118 (54.6)
Race, n (%)			
White	300 (70.6)	146 (69.9)	154 (71.3)
Black/African American	51 (12.0)	24 (11.5)	27 (12.5)
Asian	29 (6.8)	16 (7.7)	13 (6.0)
American Indian/ Alaska Native	4 (0.9)	3 (1.4)	1 (0.5)
Unknown	41 (9.6)	20 (9.6)	21 (9.7)
Ethnicity, n (%)			
Hispanic/Latino	79 (18.6)	42 (20.1)	37 (17.1)
Non-Hispanic/ Latino	332 (78.1)	163 (78.0)	169 (78.2)
Unknown	14 (3.3)	4 (1.9)	10 (4.6)
AML type, n (%)			
De novo or newly diagnosed	362 (85.2)	177 (84.7)	185 (85.6)
Other[b]	63 (14.8)	32 (15.3)	31 (14.4)
Days at risk			
Median (range)	115 (4–231)	113 (16–231)	118 (4–205)
Number of specimens received			
Median (range)	15 (1–43)	13 (1–32)	15 (1–43)

Abbreviation: AML, acute myeloid leukemia.
[a]The number of participants 18 years of age and older were 13 for fluconazole and 18 for caspofungin.
[b]Includes secondary AML, first or subsequent relapse of AML, and treatment with institutional standard AML therapy in those without AML.

Secondary analyses evaluating increased positivity thresholds reduced the number of false positives but did not alter sensitivity and PPV (Tables 3–5). Additional secondary analyses were performed for an expanded list of IFD designations (Supplementary Tables S1–S3), a 14-day window between test positivity and IFD onset (Supplementary Tables S4–S6), and both an expanded list of IFD designations and a 14-day window between test positivity and IFD onset (Supplementary Tables S7–S9). Although sensitivity and PPV improved under some of these conditions, sensitivity never exceeded 20% and PPV never exceeded 6%. Notably, all specimens collected within14 days of IA or IC events were in patients in the fluconazole group and thus it was not possible to calculate sensitivity for these assays in the caspofungin group.

Sensitivity Analysis

Analyses accounting for recent concomitant exposure to IVIG, amoxicillin–clavulanate, and piperacillin–tazobactam are shown in Supplementary Tables S10–S12. Comparing these results to Tables 3–5 reveals a modest improvement in number of false positive specimens with little impact on the overall test characteristics.

Post Hoc Analysis

In primary and secondary analyses, the point estimate for the AUC for GM EIA exceeded 80% (Table 3 and Supplementary Table S1). This led to post hoc consideration for lower GM EIA positivity thresholds. An ODI of ≥0.1 did improve GM EIA sensitivity to >70% but also increased the number of specimens testing positive (Supplementary Tables S13 and S14). Analyses allowing for a 28-day window from test positivity to IFD onset (Supplementary Tables S15–S19) and considering any type of proven or probable IFD (Supplementary Tables S18 and S19) did not improve test characteristics.

DISCUSSION

In this observational study imbedded within a randomized controlled open-label phase III trial comparing caspofungin

Table 2. Etiology of Invasive Fungal Disease Events Considered in the Biomarker Analysis for the Study Cohort and By Study Group

	Study Cohort			Fluconazole Group			Caspofungin Group		
	Total	Prob.	Prov.	Total	Prob.	Prov.	Total	Prob.	Prov.
Mold	9	7	2	8	6	2	1	1	0
Aspergillus flavus[a]	1	1	0	1	1	0	0	0	…
Aspergillus NOS[a]	7	6	1	6	5	1	1	1	…
Mold NOS[b]	1	0	1	1	0	1	0	0	…
Yeast	3	0	3	3	0	3	0	0	0
Candida albicans[a]	1	0	1	1	0	1	…	…	…
Candida glabrata[a]	1	0	1	1	0	1	…	…	…
Candida krusei[a]	1	0	1	1	0	1	…	…	…
Fungus NOS[b]	2	2	0	2	2	0	0	0	0
All	14	9	5	13	8	5	1	1	0

Abbreviations: NOS, not otherwise specified; Prob., probable; Prov., proven.
[a]Included in primary outcome and comprehensive secondary outcome definition for invasive fungal disease.
[b]Included in comprehensive secondary outcome definition for invasive fungal disease.

Table 3. Test Characteristics[a] of GM EIA for Predicting Any Proven or Probable Invasive Aspergillosis Event Diagnosed Within 7 Days Following Specimen Collection

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	# Specimens (patients) Testing Positive
All patients; 6098 tests in 425 patients; 5 specimens obtained within 7 d of 3 IA events (0.7%)					
ODI ≥ 0.5	**0.0 (0.0–33.3)**	**98.8 (98.1–99.3)**	**0.0 (0.0–1.2)**	**99.9 (99.8–100.0)**	**73 (40)**
ODI ≥ 1.0	0.0 (0.0–33.3)	99.5 (99.0–99.8)	0.0 (0.0–3.5)	99.9 (99.8–100.0)	32 (19)
ODI ≥ 1.5	0.0 (0.0–33.3)	99.6 (99.2–99.8)	0.0 (0.0–4.8)	99.9 (99.8–100.0)	25 (15)
ODI ≥ 2.0	0.0 (0.0–33.3)	99.7 (99.3–99.9)	0.0 (0.0–7.7)	99.9 (99.8–100.0)	20 (11)
AUC = 81.6% (95% CI: 46.0–91.7)					
Caspofungin group; 3231 tests in 216 patients; no IA events within 7 d of a specimen collection in this subset					
ODI ≥ 0.5	**NA**	**98.3 (97.1–99.2)**	**0.0 (0.0–1.8)**	**100.0 (100.0-100.0)**	**55 (28)**
ODI ≥ 1.0	NA	99.1 (98.3–99.7)	0.0 (0.0–4.4)	100.0 (100.0-100.0)	28 (15)
ODI ≥ 1.5	NA	99.3 (98.6–99.8)	0.0 (0.0–5.9)	100.0 (100.0-100.0)	23 (13)
ODI ≥ 2	NA	99.4 (98.7–99.8)	0.0 (0.0–9.1)	100.0 (100.0-100.0)	19 (10)
AUC not able to be calculated					
Fluconazole group; 2867 tests in 209 patients; 5 specimens obtained within 7 d of 3 IA events (1.4%)					
ODI ≥ 0.5	**0.0 (0.0–33.3)**	**99.4 (99.0–100.0)**	**0.0 (0.0–5.9)**	**99.8 (99.6–100.0)**	**18 (12)**
ODI ≥ 1.0	0.0 (0.0–33.3)	99.9 (99.7–100.0)	0.0 (0.0–33.3)	99.8 (99.6–100.0)	4 (4)
ODI ≥ 1.5	0.0 (0.0–33.3)	99.9 (99.8–100.0)	0.0 (0.0–100.0)	99.8 (99.6–100.0)	2 (2)
ODI ≥ 2.0	0.0 (0.0–33.3)	99.9 (99.9–100.0)	0.0 (0.0–100.0)	99.8 (99.6–100.0)	1 (1)
AUC = 82.3% (95% CI: 45.8–92.7)					

Manufacturer-recommended cutoffs in bold.

Abbreviations: AUC, area under the receiver operating characteristic curve; CI, confidence interval; GM EIA, galactomannan enzyme immunoassay; IA, invasive aspergillosis; NA, not applicable; NPV, negative predictive value; ODI, optical density index; PPV, positive predictive value.

[a]Sensitivity and AUC were not calculated in cohorts with no events.

to fluconazole prophylaxis in children, adolescents, and young adults with AML, twice-weekly GM EIA and BDG assay surveillance was not effective at identifying IA or IC, either alone or in combination.

Our findings are in contrast to the initial investigation performed in adult subjects at increased risk for IFD. In 2001, Maertens et al published their experience with serial GM testing to detect IA during neutropenic periods in adults undergoing chemotherapy for hematologic malignancy or conditioning for an HCT. Patients were receiving either itraconazole (enterally) or amphotericin (aerosolized or intravenous). Among 362 neutropenic episodes, 37 (10.2%) were complicated by proven or probable IA. The GM EIA sensitivity, specificity, PPV, and NPV were 72.9%, 99.1%, 93.1%, and 70.8%, respectively [8]. In

Table 4. Test Characteristics[a] of BDG Assay for Predicting Any Proven or Probable Invasive Aspergillosis or Invasive Candidiasis Event Diagnosed Within 7 Days Following Specimen Collection

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	# Specimens (patients) Testing Positive
All patients; 5945 tests in 425 patients; 10 specimens obtained within 7 d of 6 IA or IC events (1.4%)					
pg/mL ≥ 80	**0.0 (0.0–14.3)**	**94.7 (93.7–95.7)**	**0.0 (0.0–0.2)**	**99.8 (99.7–100.0)**	**315 (147)**
pg/mL ≥ 100	0.0 (0.0–14.3)	95.5 (94.5–96.3)	0.0 (0.0–0.2)	99.8 (99.7–100.0)	270 (130)
pg/mL ≥ 120	0.0 (0.0–14.3)	96.2 (95.3–97.0)	0.0 (0.0–0.3)	99.8 (99.7–100.0)	227 (115)
AUC = 49.8% (95% CI: 40.1–66.6)					
Caspofungin group; 3149 tests in 216 patients; no IA or IC events within 7 d of a specimen collection in this subset					
pg/mL ≥ 80	**NA**	**95.0 (93.6–96.1)**	**0.0 (0.0–0.4)**	**100.0 (100.0-100.0)**	**159 (78)**
pg/mL ≥ 100	NA	95.7 (94.5–96.8)	0.0 (0.0–0.5)	100.0 (100.0-100.0)	135 (66)
pg/mL ≥ 120	NA	96.2 (95.1–97.2)	0.0 (0.0–0.6)	100.0 (100.0-100.0)	119 (62)
AUC not able to be calculated					
Fluconazole group; 2796 tests in 209 patients; 10 specimens obtained within 7 d of 6 IA or IC events (2.87%)					
pg/mL ≥ 80	**0.0 (0.0–14.3)**	**94.4 (92.6–95.9)**	**0.0 (0.0–0.4)**	**99.6 (99.3–99.9)**	**156 (69)**
pg/mL ≥ 100	0.0 (0.0–14.3)	95.2 (93.6–96.5)	0.0 (0.0–0.5)	99.6 (99.3–99.9)	135 (64)
pg/mL ≥ 120	0.0 (0.0–14.3)	96.1 (94.8–97.3)	0.0 (0.0–0.7)	99.6 (99.3–99.9)	108 (53)
AUC = 49.2% (95% CI: 39.3–65.8)					

Manufacturer-recommended cutoffs in bold.

Abbreviations: AUC, area under the receiver operating characteristic curve; BDG, beta-D-glucan; CI, confidence interval; IA, invasive aspergillosis; IC, invasive candidiasis; NA, not applicable; NPV, negative predictive value; PPV, positive predictive value.

[a]Sensitivity and AUC were not calculated in cohorts with no events.

Table 5. Test Characteristics[a] of a Combination of Both GM EIA and BDG Assay for Predicting Any Proven or Probable Invasive Aspergillosis or Invasive Candidiasis Event Diagnosed Within 7 Days Following Specimen Collection

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	# Specimens (patients) Testing Positive
All patients; 5940 tests in 425 patients; 10 specimens obtained within 7 d of 6 IA or IC events (1.4%)					
ODI ≥ 0.5 or pg/mL ≥ 80	0.0 (0.0–14.3)	94.0 (92.7–95.1)	0.0 (0.0–0.2)	99.8 (99.7–100.0)	359 (154)
ODI ≥ 1.0 or pg/mL ≥ 100	0.0 (0.0–14.3)	95.2 (94.2–96.2)	0.0 (0.0–0.2)	99.8 (99.7–100.0)	284 (131)
ODI ≥ 1.5 or pg/mL ≥ 120	0.0 (0.0–14.3)	96.0 (95.1–96.8)	0.0 (0.0–0.3)	99.8 (99.7–100.0)	238 (115)
Caspofungin group; 3146 tests in 216 patients; no IA or IC events within 7 d of a specimen collection in this subset					
ODI ≥ 0.5 or pg/mL ≥ 80	NA	93.8 (92.1–95.4)	0.0 (0.0–0.3)	100.0 (100.0-100.0)	194 (82)
ODI ≥ 1.0 or pg/mL ≥ 100	NA	95.2 (93.8–97.0)	0.0 (0.0–0.5)	100.0 (100.0-100.0)	150 (68)
ODI ≥ 1.5 or pg/mL ≥ 120	NA	95.8 (94.6–97.0)	0.0 (0.0–0.5)	100.0 (100.0-100.0)	131 (63)
Fluconazole group; 2794 tests in 209 patients; 10 specimens obtained with 7 d of 6 IA or IC events (2.9%)					
ODI ≥ 0.5 or pg/mL ≥ 80	0.0 (0.0–14.3)	94.1 (92.4–95.6)	0.0 (0.0–0.4)	99.6 (99.3–99.9)	165 (72)
ODI ≥ 1.0 or pg/mL ≥ 100	0.0 (0.0–14.3)	95.2 (93.7–96.5)	0.0 (0.0–0.5)	99.6 (99.3–99.9)	134 (63)
ODI ≥ 1.5 or pg/mL ≥ 120	0.0 (0.0–14.3)	96.2 (94.9–97.3)	0.0 (0.0–0.7)	99.6 (99.3–99.9)	107 (52)

Abbreviations: BDG, beta-D-glucan; CI, confidence interval; GM EIA, galactomannan enzyme immunoassay; IA, invasive aspergillosis; IC, invasive candidiasis; NA, not applicable; NPV, negative predictive value; ODI, optical density index; PPV, positive predictive value.

[a]Sensitivity was not calculated in cohorts with no events.

2004, Odabasi et al reported the results of twice-weekly BDG screening during neutropenic periods in adults with AML receiving either caspofungin or itraconazole prophylaxis. Of 283 enrolled subjects, 20 (7.1%) developed proven or probable IFD. Sensitivity, specificity, PPV, and NPV of the BDG assay were 100%, 90%, 43%, and 100% [9].

These adult studies led to optimism for these assays as IFD screening tools in patients with prolonged neutropenia; recent publications are less compelling. Duarte et al assessed twice-weekly GM EIA screening in adults with high-risk hematologic malignancy on posaconazole prophylaxis [19]. Among 182 neutropenic periods, there were 5 (2.7%) episodes of proven or probable IA, with resultant sensitivity, specificity, PPV, and NPV of 100%, 85.5%, 11.8%, and 100%. GM EIA sensitivity and specificity in this cohort were similar to the sentinel study but the low IA event rate predisposed to a poor PPV. Lehrnbecher et al summarized the experience of GM and BDG screening in contemporary cohorts of children with cancer or receiving an HCT [10]. The conclusion from this systematic review suggested that GM and BDG assays were not useful for IFD screening in children. This conclusion was somewhat limited as the individual study cohorts were often small and heterogeneous in the clinical indications studied. Our observational study confirms these assays are not useful as screening tools during neutropenic periods in pediatric AML patients receiving antifungal prophylaxis.

The etiology for the poor function of these assays during surveillance testing from more recent cohorts may in part be related to the low IFD pretest probability. The earlier adult reports had IFD incidence rates higher (7%–10%) than that reported by Duarte et al [19] and our cohort. Low pretest probability translates into reduced PPV, with concomitant increased probability for a false positive. The lower pretest probability may be related to different antifungal prophylaxis approaches and better overall supportive care.

The decision to use a test positivity window of 7 or 14 days prior to IFD onset was based on prior adult data, suggesting sensitivity of the GM EIA assay is optimized in these time windows [20]. Constraining to these windows did reduce the number of collected specimens that could provide a true positive result. They also limited the number of IFD events considered as not all patients with an IFD had a specimen collected in the prior 7–14 days. A post hoc analysis extending the window to 28 days allowed for assessment of an increased number of specimens that could have resulted as a true positive. Unfortunately, this did not appreciably improve the test characteristics for either assay alone or in combination. Therefore, it is unlikely the chosen window for a true positive result led to the poor operating characteristics of the biomarkers.

The proportion of total positive GM EIA assays and not BDG assays appeared to be higher among patients in the caspofungin group (55/3231, 1.7%) as compared to the fluconazole group (18/2867, 0.6%). This was an unexpected finding that could just exist by chance or be the result of a real difference between study groups such as differences in exposure to an agent that leads to a false positive test. The main difference in exposure by study group was caspofungin prophylaxis but a review of the literature could not identify prior reports associating caspofungin with false positive GM EIA assay results. When accounting for factors that are associated with false positive results such as IVIG, amoxicillin–clavulanate, and piperacillin–tazobactam, the false positivity rate remained different between the 2 groups. Data on exposure to other agents that can cause false positive results (eg, albumin) were not collected and thus might still explain this difference.

Our analysis not only identified a high frequency of false positive results but it also revealed the presence of false

negative results (ie, poor sensitivity). Earlier adult investigations suggested that a GM EIA performed at the time of IFD diagnosis may have increased false negative results in patients already receiving mold-active antifungal therapy [20]. It is possible that antifungal prophylaxis administered to our patients blunted the ability to detect GM and/or BDG at time points that were in close proximity to the actual IFD diagnosis.

The strength of this study is that it is one of the largest studies to assess the utility of twice-weekly surveillance GM EIA and BDG assay testing among a high-risk group of pediatric patients receiving antifungal prophylaxis. Additionally, the rigorous, blinded central review mechanism used for IFD designation increased confidence in the outcome reference standard. These findings are specific to the clinical scenario of surveillance testing during periods of neutropenia in leukemia patients receiving antifungal prophylaxis. The results should not be generalized to other clinical conditions such as prolonged fever and neutropenia or pulmonary nodules on imaging. A small study of adults with clinical suspicion of IFD found significant improvement in the function of the GM EIA assay [19], but this result needs to be confirmed in larger cohorts.

These results need to be interpreted in light of limitations. First, assays were not performed in real time. As specimens were stored frozen and batch-tested, concern may be raised regarding the degradation of GM or BDG antigen. However, prior literature has documented that GM measurements from long-term frozen-stored serum specimens are similar to real-time measurements, significantly reducing this concern [21]. Second, the primary analysis positivity threshold for both assays was based on the package insert recommendation, which was derived from adult data. Prior assessment of BDG levels in healthy children suggests that the positivity threshold should be higher in children [22]. Nonetheless, the operating characteristics of both assays remained poor regardless of the threshold for a positive test. In post hoc analyses, reducing the GM positivity threshold improved sensitivity but increased false positive numbers. Third, the central review process required systematic review of all relevant medical record data, but may have missed events if the data were not provided by the sites or if aggressive testing was not pursued by clinicians. Fourth, the outcome of *P. jiroveci* infection was not captured and thus could not be considered in this study. It is possible that some BDG results deemed false positive could actually be positive secondary to *P. jiroveci* infection. Fifth, because of limitations in data capture resources, data for only a few of the concomitant exposures that contribute to false positive results were considered, namely IVIG, amoxicillin–clavulanate, and piperacillin–tazobactam. It is possible that exposure to other medications or interventions could have explained some of the false positive assay results. Finally, there were small numbers of specimens collected within the prespecified time windows preceding relatively few IFD events. This resulted in a high degree of uncertainty of estimated operating characteristics. This was particularly true for the primary analysis of the caspofungin arm as there were no IFD events within 14 days of biomarker attainment.

In conclusion, GM and BDG surveillance testing should not be done in children undergoing chemotherapy for AML while receiving antifungal prophylaxis as the pretest probability for IFD is low. Future research should evaluate these biomarkers in settings with higher pretest probability of IFD, such as children and adolescents at high risk for IFD who present with prolonged fever and neutropenia or pulmonary nodules.

Supplementary Data

Supplementary materials are available at the *Journal of the Pediatric Infectious Diseases Society* online.

Notes

Financial support. This work was supported by the National Cancer Institute's Biomarker, Imaging and Quality of Life Studies Funding Program (HHSN261200800001E), and the National Cancer Institute's support of the Children's Oncology Group (U10CA 180886, U10CA 180899, and UG1CA 189955).

Potential conflicts of interest. B.T.F.'s institution receives funding from Merck and Pfizer for research studies. He serves on a Data Safety Monitoring Committee for Astellas. These studies are not related to this project. T.E.Z. provides consultant services for T2 Biosystems and Nabriva Therapeutics in projects unrelated to this study. C.C.D. provides consulting for Alexion and Jazz Pharmaceuticals. J.R.W. serves on Data Committees for Astellas, Shire, Merck, and Celgene. None of these projects are related to this study. L.J.W. is a Director of MiraVista Diagnostics. T.W., C.L.K.B., M.N., D.M.Z., D.V., A.J.E., S.A., S.G., and L.S. report no other potential conflicts of interest. 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.

References

1. Sung L, Gamis A, Alonzo TA, et al. Infections and association with different intensity of chemotherapy in children with acute myeloid leukemia. Cancer **2009**; 115:1100–8.
2. Lehrnbecher T, Robinson P, Fisher B, et al. Guideline for the management of fever and neutropenia in children with cancer and hematopoietic stem-cell transplantation recipients: 2017 update. J Clin Oncol **2017**; 35:2082–94.
3. Zaoutis TE, Heydon K, Chu JH, et al. Epidemiology, outcomes, and costs of invasive aspergillosis in immunocompromised children in the United States, 2000. Pediatrics **2006**; 117:e711–6.
4. Zaoutis TE, Argon J, Chu J, et al. The epidemiology and attributable outcomes of candidemia in adults and children hospitalized in the United States: a propensity analysis. Clin Infect Dis **2005**; 41:1232–9.
5. Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B-based frontline therapy significantly increases mortality among patients with hematologic malignancy who have zygomycosis. Clin Infect Dis **2008**; 47:503–9.
6. Barnes AJ, Oppenheim BA, Chang J, et al. Early investigation and initiation of therapy for invasive pulmonary aspergillosis in leukaemic and bone marrow transplant patients. Mycoses **1999**; 42:403–8.
7. Chellapandian D, Lehrnbecher T, Phillips B, et al. Bronchoalveolar lavage and lung biopsy in patients with cancer and hematopoietic stem-cell transplantation recipients: a systematic review and meta-analysis. J Clin Oncol **2015**; 33:501–9.
8. Maertens J, Verhaegen J, Lagrou K, et al. Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. Blood **2001**; 97:1604–10.

9. Odabasi Z, Mattiuzzi G, Estey E, et al. Beta-D-glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. Clin Infect Dis **2004**; 39:199–205.
10. Lehrnbecher T, Robinson PD, Fisher BT, et al. Galactomannan, β-D-glucan, and polymerase chain reaction-based assays for the diagnosis of invasive fungal disease in pediatric cancer and hematopoietic stem cell transplantation: a systematic review and meta-analysis. Clin Infect Dis **2016**; 63:1340–8.
11. Science M, Robinson PD, MacDonald T, et al. Guideline for primary antifungal prophylaxis for pediatric patients with cancer or hematopoietic stem cell transplant recipients. Pediatr Blood Cancer **2014**; 61:393–400.
12. Maron GM, Hayden RT, Rodriguez A, et al. Voriconazole prophylaxis in children with cancer: changing outcomes and epidemiology of fungal infections. Pediatr Infect Dis J **2013**; 32:e451–5.
13. Fisher BT, Zaoutis T, Dvorak CC, et al. Effect of caspofungin vs fluconazole prophylaxis on invasive fungal disease among children and young adults with acute myeloid leukemia: a randomized clinical trial. J Am Med Assoc **2019**; 322:1673–81.
14. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis **2008**; 46:1813–21.
15. Marty FM, Koo S. Role of (1→3)-beta-D-glucan in the diagnosis of invasive aspergillosis. Med Mycol **2009**; 47(suppl. 1):S233–40.
16. Wheat LJ, Walsh TJ. Diagnosis of invasive aspergillosis by galactomannan antigenemia detection using an enzyme immunoassay. Eur J Clin Microbiol Infect Dis **2008**; 27:245–51.
17. Marty FM, Lowry CM, Lempitski SJ, et al. Reactivity of (1→3)-beta-D-glucan assay with commonly used intravenous antimicrobials. Antimicrob Agents Chemother **2006**; 50:3450–3.
18. Sung L, Lange BJ, Gerbing RB, et al. Microbiologically documented infections and infection-related mortality in children with acute myeloid leukemia. Blood **2007**; 110:3532–9.
19. Duarte RF, Sánchez-Ortega I, Cuesta I, et al. Serum galactomannan-based early detection of invasive aspergillosis in hematology patients receiving effective antimold prophylaxis. Clin Infect Dis **2014**; 59:1696–702.
20. Marr KA, Laverdiere M, Gugel A, Leisenring W. Antifungal therapy decreases sensitivity of the *Aspergillus* galactomannan enzyme immunoassay. Clin Infect Dis **2005**; 40:1762–9.
21. Wheat LJ, Nguyen MH, Alexander BD, et al. Long-term stability at −20 °C of *Aspergillus* galactomannan in serum and bronchoalveolar lavage specimens. J Clin Microbiol **2014**; 52:2108–11.
22. Smith PB, Benjamin DK Jr., Alexander BD, et al. Quantification of 1,3-beta-D-glucan levels in children: preliminary data for diagnostic use of the beta-glucan assay in a pediatric setting. Clin Vaccine Immunol **2007**; 14:924–5.