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Prospective Evaluation of the Fungitell® (1-->3) Beta-D-Glucan Assay as a Diagnostic Tool for Invasive Fungal Disease in Pediatric Allogeneic Hematopoietic Cell Transplantation: A Report from the Children's Oncology Group

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Abstract

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Author Contribution Statement:

The concept/design of the study was led by Dr. Dvorak on behalf of Children's Oncology Group. Data was collected as part of data collection for ACCL1131, the parent randomized controlled trial from Children's Oncology Group. Dr. Otto, Mr. Boge, Dr. Fisher, Dr. Dang, and Dr. Chen were involved in data analysis/interpretation. Dr. Otto and Dr. Fisher drafted the article. All authors critically reviewed and revised the manusript. All authors approved the manuscript.

Background: Invasive fungal disease (IFD) is a major source of morbidity and mortality for hematopoietic cell transplant (HCT) recipients. Non-invasive biomarkers, such as the beta-D-glucan assay, may improve diagnosis of IFD. The objective was to define the utility of surveillance testing using Fungitell® beta-D-glucan (BDG) assay in children receiving antifungal prophylaxis in the immediate post-HCT period.

Methods: Weekly surveillance blood testing with the Fungitell® BDG assay was performed during the early post-HCT period in the context of a randomized trial of children, adolescents, and young adults undergoing allogeneic HCT allocated to triazole or caspofungin prophylaxis. Positivity was defined at the manufacturer cutoff of 80 pg/mL. IFD was adjudicated using blinded central reviewers. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for the Fungitell® BDG assay for the outcome of proven or probable IFD.

Results: A total of 51 patients (out of 290 patients in the parent trial) contributed blood specimens. In total, 278 specimens were evaluated. Specificity was 80.8% (95% confidence interval (CI): 75.6-85.3%) and NPV was over 99% (95% CI: 86.8-99.9%). However, there were no true positive results, resulting in sensitivity of 0% (95% CI: 0.0-84.2%) and PPV of 0% (95% CI: 0.0-6.7%).

Conclusions: Fungitell® BDG screening is of limited utility in diagnosing IFD in the post-HCT period, mainly due to high false positive rates. Fungitell® BDG surveillance testing should not be performed in children during the early post-HCT period while receiving antifungal prophylaxis as the pretest probability for IFD is low.

Keywords

invasive fungal disease; hematopoietic cell transplantation; beta-D-glucan; sensitivity; specificity

INTRODUCTION

Invasive fungal disease (IFD) remains an important source of morbidity and mortality for hematopoietic cell transplant (HCT) recipients [1]. The negative consequences of IFD in immunocompromised hosts are hypothesized to result in part from their non-specific clinical presentation and limited diagnostic tools for efficient detection. Collectively, this leads to delayed or missed pre-mortem diagnosis of IFD [2,3].

Traditional diagnostic modalities for IFD are limited to radiologic imaging and blood or tissue culture techniques. However, these studies all have significant limitations. Radiologic studies may show pulmonary or abdominal visceral involvement but cannot differentiate between a fungal or non-fungal process. Blood cultures may be used to isolate *Candida* species causing candidemia, but they are not helpful in the setting of invasive candidiasis without fungemia. Tissue cultures can detect mold pathogens such as *Aspergillus* species but require invasive techniques for sample procurement [4].

Non-invasive diagnostic biomarker assays, such as the Fungitell® beta-D-glucan (BDG) assay, have the potential to overcome some of the traditional diagnostic testing limitations. Furthermore, implementation of surveillance testing strategies may allow for detection of

an IFD prior to onset of clinical symptoms. Early detection could inform more targeted treatment decisions and avoid invasive diagnostic procedures. Beta-D-glucan is a cell wall polysaccharide found in many fungal genera, including *Aspergillus* spp., *Candida* spp., *Fusarium* spp., *Trichosporon* spp., *Coccidioides* spp., and *Histoplasma* spp., as well as *Pneumocystis jirovecii* [5]. Results from multiple meta-analyses have supported utility of the Fungitell® BDG assay in adult patients with hematological malignancies or other immune compromising conditions [6,7]. These studies led to increased optimism to incorporate Fungitell® BDG assay performance in patients with underlying conditions that increase risk for IFD.

However, as with all diagnostic tests, the clinical situation and testing approach have implications on the effectiveness of the assay. A recent Cochrane review highlights this challenge, as they were not able to combine data from identified studies because of heterogeneity of study design [8]. Their summary results identified a wide range of sensitivity (27-100%) and specificity (0-100%) for the Fungitell® BDG assay. Previous systematic reviews in pediatric populations have reported similar variation in Fungitell® BDG assay operating characteristics across patient type and indication for testing [9,10]. These data underscore the importance of assessing the Fungitell® BDG assay in a defined population for a specific testing indication before incorporating the assay into clinical practice.

Data regarding utilization of serial Fungitell® BDG Assay testing in pediatric allogeneic HCT recipients receiving antifungal prophylaxis are limited. One small single center study of 34 pediatric allogeneic HCT recipients reported a baseline IFD rate of 17.6%, with estimated positive (PPV) and negative predictive value (NPV) of 12% and 100%, respectively, at the first pathological sign of illness [11]. Another single-center study examined the use of fungal biomarkers in the diagnosis of invasive aspergillosis [12]. Probable aspergillosis occurred in 11.5% of patients, with PPV of 25% and NPV of 100% for the Fungitell® BDG assay. However, the single center nature of these studies may limit their generalizability. We sought to define the test characteristics of weekly Fungitell® BDG assay testing for identifying IFD in a prospective multicenter cohort of pediatric allogeneic HCT recipients enrolled to a randomized trial comparing two antifungal prophylaxis regimens during the post-transplant neutropenic period.

METHODS

Study Design and Population

This prospective study assessed the operating characteristics of the Fungitell® BDG Assay in children above or equal to 3 months of age to younger than 21 years of age undergoing allogeneic HCT. This was an ancillary study to ACCL1131, a randomized, open-label trial of caspofungin versus either fluconazole or voriconazole for the prevention of IFD in pediatric allogeneic HCT recipients conducted by the Children's Oncology Group (COG). Centers were required to declare their institutional standard-of-care comparator triazole (fluconazole or voriconazole) prior to enrollment of patients. Patients receiving treatment for IFD were not eligible for inclusion. Details for the parent trial have been published previously [13].

All patients enrolled on ACCL1131 were offered the chance to participate in this ancillary fungal infection screening study. This study was approved by the National Cancer Institute's Central Institution Review Board (IRB) and IRBs at each participating institution. Participants or guardians provided informed consent and assent to participate in both the parent trial and this ancillary study.

Specimen Collection and Processing

Consented subjects had weekly blood collection for Fungitell® BDG Assay testing between the day of stem cell infusion until day +42 or withdrawal from the study. Five milliliters of whole blood were collected into a serum- separator tube, allowed to clot for 30-60 minutes at room temperature, and centrifuged for 15 minutes at 1000–1300 × g. Serum was then transferred into BDG-free tubes. Blood specimens with less than 5 mL but yielding ≥1.8 mL serum were included in final analyses. The maximum blood volume obtained during the 8-week period was set at 3 mL/kg. Serum specimens were collected and stored locally and batched shipped to the central laboratory for testing (Laboratory of Mycology Research, Memorial Hermann Texas Medical Center, Houston, TX).

Performance of the Fungitell® BDG Assay

Testing of specimens was performed in batches. Five microliters of thawed and vortexed specimen and 20 μL of alkaline pre-treatment solution were loaded into a well on a microwell plate. Standard points (31, 63, 125, 250, and 500 pg/mL) prepared in glucan-free tubes were added to the plate, and the plate was agitated and incubated at 37°C for 10 minutes. After incubation, 100 uL of Fungitell® reagent was dispensed in each well. The plate was read at 405 nm for 40 minutes on a microplate reader, and the result was reported in pg/ mL of patient's serum.

The manufacturer recommendation for a single positive Fungitell® BDG Assay result of > 80 pg/mL was used to define a positive test [14]. Positive results were re-tested for confirmation per protocol. Negative results were considered final. With each assay the percent coefficient of variation (CV) was reported. Any specimen with CV ≥30%, was retested. If the CV exceeded 30% on repeat testing, then the specimen was not resulted due to concern for interfering substances.

Outcome

Proven or probable IFD, as defined by the 2008 criteria from the European Organisation for Research and Treatment in Cancer/Mycoses Study Group (EORTC/MSG), was the primary outcome [15]. The period at-risk for primary IFD endpoint began at Day 0 (stem cell infusion date) and extended until 42 days following HCT, discharge, or death, whichever occurred first. The at-risk observation window was continued for patients who terminated protocol prophylaxis prior to Day +42.

The following data were obtained to determine presence or absence of proven or probable IFD for each subject: pathology reports (including autopsy reports), CT scan and MRI reports, direct microscopy per center standard practice, microbiology culture results, non-culture mycology testing results (such as *Histoplasma* urine antigens, cryptococcal CSF

and serum antigens), ophthalmology exams, and bronchoscopy reports. All IFD diagnostic investigations were performed at the clinician's discretion. Study biomarker assay results were not disclosed to clinicians or central reviewers. An independent data review committee (SA, AJE, MLN, WJS) reviewed prepared data packets for each subject to document the presence or absence of proven or probable IFD during the follow-up period, independent of clinical care. Central reviewers were blinded to randomized antifungal prophylaxis allocation.

Only the first IFD event for a patient with multiple episodes of IFD was considered in this analysis. *Pneumocystis jiroveci* pneumonia (PJP) was not considered an event for this study as this pathogen is not treated with antifungal agents. Data related to the occurrence of PJP were not collected.

Statistical Analysis

Sensitivity, specificity, PPV, and NPV was calculated for the Fungitell® BDG assay under *a priori* defined conditions. For the primary analysis, a true positive test was any Fungitell® BDG Assay ≥80 pg/ml obtained within +/−7 days of any proven or probable IFD diagnosis. Sensitivity, specificity, PPV, and NPV were calculated for the entire cohort, as well as for each study arm. Each test was considered an individual unit when calculating the test characteristics to mirror clinical application of the Fungitell® BDG Assay as a biomarker. In clinical practice, test results are interpreted independently of prior or subsequent results, and thus the analysis was designed to assess whether an individual test result at various points of follow-up would be meaningful. Sensitivity was not calculated for scenarios in which there were no IFD events. 95% confidence intervals for each operating characteristic were constructed using the exact binomial confidence interval method. Secondary analyses considered positivity thresholds of ≥60, ≥100, and ≥120 pg/mL for the Fungitell® BDG Assay.

Sensitivity analyses were performed that considered exposure to medications associated with false positive results for the Fungitell® BDG Assay [16]. These medications included intravenous immune globulin and amoxicillin-clavulanate. In these sensitivity analyses, all assays obtained within 90 days of receiving intravenous immune globulin or 7 days of receiving amoxicillin-clavulanate were excluded.

All statistical analyses were performed using STATA statistical software, version 15.0 (College Station, TX).

RESULTS

The randomized trial enrolled 292 patients, 145 to the caspofungin group and 147 to the triazole group, at 31 institutions between April 2013 and September 2016. As previously reported, the trial was closed early when a planned futility analysis demonstrated a low IFD rate in the comparator triazole arm [13]. Fifty-one patients (24 caspofungin and 27 triazole) from the parent trial consented to be in this ancillary study and provided 278 blood samples during their 42-day follow-up periods. Baseline characteristics of the ancillary cohort are described in Table 1.

There was one proven or probable IFD event identified during the 42-day follow-up period. This was a proven mold infection (not otherwise specified) in a patient from the caspofungin group with a BDG result of <60 pg/ml. There were two possible IFD events. Operating characteristics for the Fungitell® BDG assay from the primary analysis are shown in Table 2. Specificity was 80.8% (95% CI: 75.6-85.3%) and NPV 99.1% (95% CI: 96.8-99.9%) for the entire cohort. No specimens collected within 7 days of the IFD diagnosis met positivity thresholds resulting in sensitivity of 0.0% (95% CI: 0.0-84.2%) and PPV of 0.0% (0.0-6.7%). All positive specimens were false positives, with 53/278 (19.1%) falsely positive at the cutoff value of 80 pg/mL. Nearly half of enrolled patients (25/51, 49%) had at least one false positive result during the surveillance period. Specificity and PPV results were similar within each randomized group relative to the overall cohort (Table 2). Secondary analyses evaluating different positivity thresholds showed a reduction in the number of false positives with higher thresholds for test positivity and modest increases in specificity (Table 2).

Sensitivity could not be calculated in the triazole group due to lack of IFD events.

Sensitivity Analysis

Analyses accounting for recent exposures to IVIG and amoxicillin–clavulanate are shown in Table 3. Excluding specimens obtained in proximity to IVIG and amoxicillin–clavulanate reduced the total number of false positive results, with similar specificity and NPV. Both sensitivity and PPV remained at 0.0%.

DISCUSSION

In this observational study embedded within a randomized controlled trial comparing caspofungin to azole (fluconazole or voriconazole) prophylaxis in pediatric HCT, weekly Fungitell® BDG Assay surveillance was not an effective strategy for early diagnosis of IFD in the immediate post-HCT period. There was only one proven IFD event in the follow-up period and no true positive Fungitell® BDG assay results in temporal proximity to this event, resulting in assay sensitivity and PPV of 0.0%. We also report frequent false positive results, with nearly 20% of all Fungitell® BDG assay results being false positives.

Prior studies documented higher sensitivity but lower specificity compared to our cohort [11,12]. Koltze, et al, examined the characteristics Fungitell® BDG Assay surveillance testing in pediatric HCT recipients that received polyene or echinocandin antifungal prophylaxis. A total of 34 patients contributed 702 blood specimens in the first 100 days post-HCT. The IFD event rate was 17.6%, including 2 proven and 4 probable IFD cases. At the time of the first pathological sign of infection, a single positive BDG assay had a sensitivity of 90% and specificity of 78%. Springer, et al, enrolled 26 HCT recipients that contributed 404 blood samples before, during, and after HCT, following patients for over 200 days post-HCT in some instances. Patients received antifungal prophylaxis with liposomal amphotericin B [12]. The IFD incidence was 11.5% and the sensitivity and specificity were 100% and 55%, respectively.

As with any diagnostic test, application in the setting of low pre-test probability (e.g. – low event rate) will yield false positive results. The primary analysis utilized the positivity threshold of 80 pg/mL, as recommended by the Fungitell® package insert for adult patients [14]. Nearly 20% of all Fungitell® BDG assays performed in this study were falsely positive at that cutoff value, and half of all enrolled patients had at least one false positive result during the surveillance period. Analysis of Fungitell® BDG assays in healthy children reported that the upper limit of normal may be higher in children [17]. Increasing the cutoff value did not meaningfully alter the false positive rate, as the highest cutoff (≥120 pg/ml) still have a false positive rate >10%. Excluding results for assays collected shortly after receipt of intravenous immunoglobulin (IVIG) or receipt of antibiotics like amoxicillin-clavulanate (factors associated with false positive Fungitell® BDG assays) also resulted in a reduction in false positivity rate to 14.5% (24/192). However, in both scenarios the false positivity rates remained high, the sensitivity remained zero, and specificity did not significantly improve.

This study has several limitations. First, our ability to determine the sensitivity of the Fungitell® BDG assay was limited by our small sample size and low event rate. The incidence of proven and probable IFD in our cohort was much lower than anticipated, with only one proven IFD event presenting during the surveillance testing period. Our event rate was much lower than the two previously published single-center Fungitell® BDG assay studies. The low event rate is likely multifactorial in nature [11,12]. This study only followed patients through the first 42 days post-HCT, compared to the longer follow-up periods in previous studies. This shorter observation period missed other clinical timepoints where IFD risk could be higher, such as during treatment of graft versus host disease (GVHD). Importantly, our cohort of subjects was followed in the parent randomized trial until day 100 for the outcome of proven or probable IFD. The cumulative incidence at day 100 was only slightly higher during this extended follow-up period (2.8% in the caspofungin group and 3.6% in the azole group) [13]. Given these modest increases in incidence, it is unlikely that the operating characteristics of BDG would have been much improved in this extended follow-up window. Instead, these data suggest that universal antifungal prophylaxis reduces the risk of IFD or blunts the ability to detect BDG for occult IFDs, as suggested in previously published studies [11,12]. The Fungitell® BDG Assay may perform better in HCT cohorts that are not receiving antifungal prophylaxis. However, antifungal prophylaxis is currently recommended as standard of care for HCT recipients, making it unlikely that such patient populations will exist [18]. It is possible that true cases of proven or probable IFD had a positive Fungitell® BDG Assay but were not detected by the EORTC/MSG criteria. Such an ”imperfect gold standard” would result in a lower event rate and an increased number of false positive results. Similarly, we did not collect data related to PJP, which may potentially have explained some false positive results. However, it is standard practice for pediatric allogeneic HCT recipients to receive prophylaxis against *Pneumocystis jirovecii*. As a result, it is anticipated that PJP events would have been rare in this cohort [19].

Secondly, test characteristics were calculated using individual test results as the unit of analysis to mimic clinical practice. Each patient could contribute multiple assays, so it is possible that there may be correlation among tests from the same patient that was

not accounted for in the statistical analysis. However, with no true positive results, a patient-level analysis that accounted for repeated measures would not have improved the reported sensitivity or PPV. Additionally, adjusting for repeated measures largely impacts the estimates for the 95% confidence interval, without significant effect on the point estimate.

Another limitation is that only information on the use of intravenous immune globulin and amoxicillin-clavulanate was collected, so exposures to other agents that could cause a false positive result were not considered in the sensitivity analysis. Also, as this cohort was based on patients selected for a clinical trial, there is potential for selection bias of subjects less likely to sustain an IFD. Fifth, the testing approach required that positive results needed to be confirmed in duplicate but negative results did not. This could have resulted in fewer positive results, potentially reducing the sensitivity of the assay. This would also reduce the false positivity rate, which was unacceptably high. Finally, it is possible that patients in this study were treated pre-emptively for possible IFD, which may have prevented them from meeting the full outcome criteria. However, there were only two possible IFD events, which limits the potential impact of this scenario.

In conclusion, Fungitell® BDG assay screening in children receiving primary antifungal prophylaxis during the immediate post-HCT period is of limited utility, mainly due to a low pre-test probability of IFD and high numbers of false positive tests. Future research should evaluate the Fungitell® BDG assay during other clinical timepoints where the pre-test probability for IFD might be higher, such as at onset of prolonged fever and neutropenia, with radiographic detection of pulmonary nodules, or in patients undergoing HCT with prior history of IFD.

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CONFLICTS OF INTEREST

C.C.D. served on scientific advisory boards for Alexion Inc., Jazz Pharmaceuticals, and Atara Bio. T.E.Z provides consultant services for T2 Biosystems and Nabriva Therapeutics. B.T.F.'s institution receives funding from Merck and Pfizer for research studies not related to this project. B.T.F. also serves on a data safety monitoring board for Astellas Pharmaceuticals.

Data Availability Statement:

The data that support the findings of this study are available on request from Children's Oncology Group. The data are not publicly available due to privacy or ethical restrictions.

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Table 1.

Demographics and clinical characteristics for the ancillary study population and by randomized prophylaxis group.

Factor	Overall	Azole	Caspofungin
N	51	27	24
Age (median, interquartile range)	9 (5, 13)	8 (4, 13)	9.5 (5, 12.5)
Sex			
Female	14 (27%)	5 (19%)	9 (38%)
Male	37 (73%)	22 (81%)	15 (62%)
Race/Ethnicity			
American Indian or Alaska Native	1 (2%)	1 (4%)	0 (0%)
Asian	2 (4%)	0 (0%)	2 (8%)
Hispanic or Latino	10 (20%)	3 (11%)	7 (29%)
Non-Hispanic Black	4 (8%)	2 (7%)	2 (8%)
Non-Hispanic White	29 (57%)	17 (63%)	12 (50%)
Unknown	5 (10%)	4 (15%)	1 (4%)
Indication for HCT			
ALL	13 (25%)	6 (22%)	7 (29%)
AML/MDS	10 (20%)	6 (22%)	4 (17%)
Other Leukemia	6 (12%)	3 (11%)	3 (12%)
HD/NHL	3 (6%)	1 (4%)	2 (8%)
Primary Immunodeficiency	2 (4%)	2 (7%)	0 (0%)
Bone Marrow Failure	2 (4%)	2 (7%)	0 (0%)
Hemoglobinopathy	5 (10%)	2 (7%)	3 (12%)
Metabolic Syndrome	10 (20%)	5 (19%)	5 (21%)
Donor Type			
Matched related donor	12 (24%)	8 (30%)	4 (17%)
Mismatched family donor	3 (6%)	1 (4%)	2 (8%)
Unrelated donor	36 (71%)	18 (67%)	18 (75%)
Graft Source			
Bone Marrow	29 (57%)	17 (63%)	12 (50%)
Umbilical Cord Blood	12 (24%)	5 (19%)	7 (29%)
Peripheral Blood Stem Cells	10 (20%)	5 (19%)	5 (21%)
HLA Match			
BM/PBSC: 8/8	29 (57%)	17 (63%)	12 (50%)
BM/PBSC: ≤7/8	10 (20%)	5 (19%)	5 (21%)
UCB (≤6/6)	3 (6%)	1 (4%)	2 (8%)
UCB (≤5/6)	4 (8%)	3 (11%)	1 (4%)
UCB (≤4/6)	5 (10%)	1 (4%)	4 (17%)

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome, HD, Hodgkin's disease; NHL, Non-Hodgkin's lymphoma; HCT: hematopoietic cell transplant; BM, bone marrow; PBSC, peripheral blood stem cells; HLA, human leukocyte antigen; UCB, umbilical cord blood

Table 2.

Test characteristics of Fungitell® BDG assay for predicting any proven or probable invasive fungal disease event diagnosed within +/−seven days following specimen collection

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Total False Positive Results (# patients)
All patients; 278 tests in 51 patients; 2 specimens obtained within 7 days of IFD event (0.7%)					
pg/ml ≥60	0.0 (0.0, 84.2)	77.2 (71.8, 82.0)	0.0 (0.0, 5.7)	99.1 (96.7, 99.9)	63 (27)
pg/ml ≥80	**0.0 (0.0, 84.2)**	**80.8 (75.6, 85.3)**	**0.0 (0.0, 6.7)**	**99.1 (96.8, 99.9)**	**53 (25)**
pg/ml ≥100	0.0 (0.0, 84.2)	84.8 (80.0, 88.8)	0.0 (0.0, 8.4)	99.2 (0.97, 99.9)	42 (23)
pg/ml ≥120	0.0 (0.0, 84.2)	87.3 (82.8, 91.0)	0.0 (0.00, 10.0)	99.2 (97.1, 99.9)	35 (20)
Caspofungin group; 131 tests in 24 patients; 2 specimens obtained within +/− 7 days of IFD event (1.5%)					
pg/ml ≥60	0.0 (0.0, 84.2)	78.3 (70.2, 85.1)	0.0 (0.0, 12.3)	98.1 (93.2, 99.8)	28 (11)
pg/ml ≥80	**0.0 (0.0, 84.2)**	**83.7 (76.2, 89.6)**	0.0 (0.0, 16.1)	98.2 (93.6, 99.8)	**21 (11)**
pg/ml ≥100	0.0 (0.0, 84.2)	86.8 (79.7, 92.1)	0.0 (0.0, 19.5)	98.2 (93.8, 99.8)	17 (11)
pg/ml ≥120	0.0 (0.0, 84.2)	89.1 (82.5, 93.9)	0.0 (0.0, 23.2)	98.3 (94.0, 99.8)	14 (9)
Triazole group; 147 tests in 27 patients; no IFD events within 7 days of a specimen collection in this subset					
pg/ml ≥60	NA	76.2 (68.5, 82.8)	0.0 (0.0, 10.0)	100.0 (96.8, 100.0)	35 (16)
pg/ml ≥80	**NA**	**78.2 (70.7, 84.6)**	0.0 (0.0, 10.9)	100.0 (96.8, 100.0)	**32 (14)**
pg/ml ≥100	NA	83.0 (75.9, 88.7)	0.0 (0.0, 13.7)	100.0 (97.0, 100.0)	25 (12)
pg/ml ≥120	NA	85.7 (79.0, 90.9)	0.0 (0.0, 16.1)	100.0 (97.1, 100.0)	21 (11)

Manufacturer-recommended cutoffs in bold.

Abbreviations: BDG: beta-D-glucan; PPV, positive predictive value; NPV, negative predictive value; pg, picograms; IFD, invasive fungal disease; NA, not applicable; CI, confidence interval.

Table 3.

Test characteristics of Fungitell® BDG assay for predicting any proven or probable invasive fungal disease event diagnosed within seven days following specimen collection, excluding assays collected within 7 days of receipt of amoxicillin/clavulanate or 90 days within receipt of intravenous immune globulin

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Total False Positive Results (# patients)
All patients; 192 tests in 38 patients; 2 specimens obtained within 7 days of 1 proven or probable IFD event (1.0%)					
pg/ml ≥60	0.0 (0.0, 84.2)	82.1 (75.9, 87.3)	0.0 (0.0, 10.3)	98.7 (95.5, 99.8)	34 (17)
pg/ml ≥80	**0.0 (0.0, 84.2)**	**85.3 (79.4, 90.0)**	0.0 (0.0, 12.3)	**98.8 (95.7, 99.9)**	**28 (15)**
pg/ml ≥100	0.0 (0.0, 84.2)	87.4 (81.8, 91.7)	0.0 (0.0, 14.2)	98.8 (95.8, 99.9)	24 (14)
pg/ml ≥120	0.0 (0.0, 84.2)	88.9 (83.6, 93.0)	0.0 (0.0, 16.1)	98.8 (95.8, 99.9)	21 (12)
Caspofungin group; 86 tests in 17 patients; 2 specimens obtained within 7 days of 1 IFD event (2.3%)					
pg/ml ≥60	0.0 (0.0, 84.2)	85.7 (76.4, 92.4)	0.0 (0.0, 26.5)	97.3 (90.6, 99.7)	12 (7)
pg/ml ≥80	**0.0 (0.0, 84.2)**	**89.3 (80.6, 95.0)**	0.0 (0.0, 33.6)	97.4 (90.9, 99.7)	**9 (7)**
pg/ml ≥100	0.0 (0.0, 84.2)	91.7 (83.6, 96.6)	0.0 (0.0, 41.0)	97.5 (91.2, 99.7)	7 (7)
pg/ml ≥120	0.0 (0.0, 84.2)	94.0 (86.7, 98.0)	0.0 (0.0, 52.2)	97.5 (91.4, 99.7)	5 (5)
Triazole group; 106 tests in 20 patients; no IFD events within 7 days of a specimen collection in this subset					
pg/ml ≥60	NA	79.2 (70.3, 86.5)	0.0 (0.0, 15.4)	100.0 (95.7, 100.0)	22 (10)
pg/ml ≥80	**NA**	**82.1 (73.4, 88.8)**	0.0 (0.0, 17.6)	100.0 (95.8, 100.0)	**19 (8)**
pg/ml ≥100	NA	84.0 (75.6, 90.4)	0.0 (0.0, 19.5)	100.0 (95.9, 100.0)	17 (7)
pg/ml ≥120	NA	84.9 (76.6, 91.1)	0.0 (0.0, 20.6)	100.0 (96.0, 100.0)	16 (7)

Manufacturer-recommended cutoffs in bold.

Abbreviations: BDG: beta-D-glucan; PPV, positive predictive value; NPV, negative predictive value; pg, picograms; IFD, invasive fungal disease; NA, not applicable; CI, confidence interval.