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MAJOR ARTICLE

Limited Positive Predictive Value of β-D-Glucan in Hematologic Patients Receiving Antimold Prophylaxis

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Background. Diagnostic value of β-D-glucan (BDG) in populations with low prevalence of invasive fungal infection (IFI), such as hematologic patients receiving antimold prophylaxis, should be re-evaluated.

Methods. We retrospectively reviewed episodes with BDG results in hematologic patients receiving antimold prophylaxis from January 2017 to August 2019 in a tertiary hospital. The episodes were classified as true positive ([TP] positive BDG with IFI), true negative ([TN] negative BDG without IFI), false positive ([FP] positive BDG without IFI), false negative ([FN] negative BDG with IFI), and nonevaluable.

Results. A total of 203 episodes were analyzed: 101 episodes (49.8%) were from stem cell transplants, 89 (43.8%) were from induction chemotherapy, and 13 (6.4%) were from graft-versus-host disease treatment. There were 62 nonevaluable episodes. Among 141 evaluable ones, there were 8 (5.7%) episodes of probable/proven IFI. True positive, TN, FP, and FN cases were 4 (2.8%), 112 (79.4%), 21 (14.9%), and 4 (2.8%) episodes, respectively. Sensitivity, specificity, positive predictive value, and negative predictive value were 50.0%, 84.2%, 16.1%, and 96.5%, respectively. Positive predictive value was 26.7% and 0.0% in diagnostic and surveillance episodes, respectively.

Conclusions. β-D-glucan test should be used to exclude IFI rather than for diagnosis in these patients.

Keywords. antimold prophylaxis; β-d-glucan; fungal diagnostics; hematologic diseases.

Invasive fungal infections (IFIs) cause significant morbidity and mortality in hematologic patients [1, 2]. Those who receive induction chemotherapy for acute myelogeneous leukemia (AML), allogenic stem cell transplantation (SCT), or treatment for graft-versus-host disease (GVHD) have higher risk of IFIs and tend to have poorer clinical outcomes [3, 4].

Several studies demonstrated that the use of prophylactic antimold agents, such as posaconazole or micafungin, improved clinical outcomes in high-risk hematologic patients [5–7]. Incidence of invasive aspergillosis has declined since the introduction of this prophylaxis, from more than 10% to approximately 2% [8–10].

Serologic markers such as serum galactomannan antigen and BDG have been used for the early diagnosis of IFIs [11]. In previous studies, the BDG assay had fair positive predictive value (PPV) (30%–89%) and negative predictive value (NPV) (73%–97%) for IFI diagnosis in hematologic patients [8, 12–14]. However, these studies included many patients who did not receive antifungal prophylaxis, leading to a high incidence of IFIs (12%–32%). The diagnostic value of BDG testing could be different in hematologic patients receiving antimold prophylaxis who may have a lower prevalence of IFIs, because PPV is largely dependent on the prevalence of a disease [15]. In this study, we aimed to re-evaluate the diagnostic value of the BDG assay for IFIs in hematologic patients under antimold prophylaxis.

METHODS

Study Setting and Participants

We conducted a retrospective study at Seoul National University Hospital (1779 patient beds). Among all hospitalized hematologic patients between January 2017 and August 2019, all episodes of induction chemotherapy, SCT, or GVHD treatment that had BDG results during posaconazole or micafungin prophylaxis were reviewed. The sensitivity, specificity, PPV, and NPV of BDG testing for IFI diagnosis were sought in these patients. This study was approved by the institutional review board (IRB) of the hospital (IRB No. 1910-028-1068). The informed consent requirement was waived, because this study was retrospective, involved no interaction with patients, and was considered to be of minimal risk.

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Clinical Data and β-D-Glucan Assay

Age, sex, types of hematologic diseases, additional underlying diseases, the indication and the duration of antimold prophylaxis, dates and results of BDG assays, occurrence of IFIs, and in-hospital mortality were reviewed in the electronic medical record. We also searched the records for potential causes for false-positive (FP) BDG assays in the FP episodes [12, 16, 17].

β-D-glucan testing was ordered by attending physicians according to their clinical needs. The Goldstream kit (Gold Mountain River Tech Development, Beijing, China) was used for the test. The cutoff value for the positive BDG assay was 80.0 pg/mL, according to the manufacturer's instructions [8].

Definitions

Positive BDG was defined as 2 or more consecutive BDG results higher than the cutoff. Invasive fungal infections were classified as proven, probable, or possible following the revised diagnostic criteria [11]. Only proven and probable diagnoses were regarded as positive IFIs in this study [9, 10].

According to the positivity of IFI and BDG, all of the episodes were classified into 4 groups [9, 10]. Episodes with IFI having a positive BDG were classified as true positives (TP), and those without a positive BDG were classified as false negatives (FN). Episodes without IFIs but with a positive BDG were classified as FPs, and those without a positive BDG were designated as true negatives (TN). Nonevaluable episodes included cases in which the prophylactic antifungal agents had been switched to other kinds of agents, such as polyenes or echinocandins, due to prolonged neutropenic fever without any evidence of IFI [9, 10].

β-D-glucan assays were classified as diagnostic or for surveillance, based on the situation. When at least 1 BDG assay was performed in the context of neutropenic fever lasting for more than 5 days despite the use of broad-spectrum antibacterial agents, or in the presence of clinical symptoms or abnormal imaging findings indicating IFI, the episode was considered diagnostic [9, 10]. When all of the BDG tests were performed in the absence of clinical evidence of IFI, they were classified as surveillance.

Data Analysis

We calculated the sensitivity, specificity, PPV, and NPV of BDG as follows: the sensitivity was calculated as TP/(TP + FN) × 100%, the specificity was calculated as TN/(TN + FP) × 100%, the PPV was calculated as TP/(TP + FP) × 100%, and the NPV was calculated as TN/(TN+FN) × 100%. These diagnostic values were calculated for both diagnostic and surveillance episodes.

When comparing clinical characteristics between evaluable and nonevaluable or FP episodes and TN episodes, the Student's *t* test or Mann-Whitney *U* test was used to compare continuous variables. The χ^2 test or Fisher's exact test was used to compare categorical variables. These analyses were performed using PASW for Windows (version 25.0; SPSS Inc., Chicago, IL).

Sensitivity Analyses

Sensitivity analyses were performed to account for potential confounders of BDG diagnostic values. First, the sensitivity, specificity, PPV, and NPV were calculated for subgroups consisting of cases having more than 2, 3, and 4 BDG results, respectively, to account for any confounding by the number of BDG tests performed. Second, those values were calculated separately, according to the type of prophylactic drug used, to see whether there were differences related to the choice of antimold agent. Third, we analyzed the subgroup after including only the first episodes in every patients to account effects of including duplicate patients to the diagnostic values. Finally, diagnostic values were calculated after excluding autologous SCT episodes because it has a lower pretest probability of IFI than other conditions.

RESULTS

Study Populations and β-D-Glucan Assays

A total of 203 episodes from 155 patients were identified during the study period. Among them, 117 (57.6%) were male, and the mean age was 52.0 (standard deviation ± 14.4) years (Table 1). The most frequent underlying hematologic diseases were AML (n = 115, 56.7%), multiple myeloma or amyloidosis (n = 30, 14.8%), and lymphoma (n = 26, 12.8%) (Table 1). The most common indications for prophylaxis and the respective antimold agents used were the following, ranked by number of cases: induction chemotherapy for AML or myelodysplastic syndrome with posaconazole, 89 (43.8%); autologous SCT with micafungin, 53 (26.1%); allogeneic SCT with micafungin, 48 (23.6%); and GVHD treatment with posaconazole, 13 (6.4%). Additional frequent underlying diseases included hypertension (n = 28, 13.8%), diabetes mellitus (n = 22, 10.8%), other solid malignancy (n = 18, 8.9%), and chronic liver diseases (n = 7, 3.4%). The median (interquartile range [IQR]) duration of antimold prophylaxis was 20 (17–29) days.

A total of 629 BDG assays were performed, over a total of 203 episodes (median, 3 [IQR, 2–4] per episode). The median numbers of interval days between the commencement of antimold prophylaxis and the first BDG assay, and between BDG assays, were 6 (IQR, 3–11) and 4 (IQR, 3–7) days, respectively.

There were 62 (30.5%) nonevaluable episodes, whose prophylactic antifungal agents were switched to another class. When compared with evaluable and nonevaluable episodes, AML, induction chemotherapy, and posaconazole prophylaxis was more common in nonevaluable episodes (Supplementary Table 1).

Breakthrough Invasive Fungal Infection Cases

Among the 141 evaluable episodes, there were 8 (5.7%) episodes having positive IFIs, including 5 probable diagnoses of invasive aspergillosis, 2 proven cases of candidemia, and 1 proven case of trichosporosis (Table 2). Among them, only 4 cases (3 invasive aspergillosis and 1 candidemia) had positive BDG. There was no mucormycosis or cryptococcosis during the study period.

Table 1. Baseline Characteristics of All Analyzed Episodes[a]

Characteristic	Analyzed Episodes (n = 203) (%)
Male	117 (57.6)
Age (±SD)	52.0 (±14.4)
Hematologic Diseases	
AML	115 (56.7)
MM or amyloidosis	30 (14.8)
Lymphoma	26 (12.8)
MDS	12 (5.9)
ALL	10 (4.9)
Others	10 (4.9)
Indication of the Prophylaxis	
Induction chemotherapy for AML or MDS	89 (43.8)
Autologous SCT	53 (26.1)
Allogeneic SCT	48 (23.6)
Treatment for GVHD	13 (6.4)
Other Underlying Diseases	
Hypertension	28 (13.8)
DM	22 (10.8)
Solid malignancy	18 (8.9)
Chronic liver disease	6 (3.0)
Chronic kidney disease	3 (1.5)
Antimold Prophylaxis	
Agents used	
Posaconazole	101 (49.8)
Micafungin	102 (50.2)
Duration of antimold prophylaxis, days, median (IQR)	20 (17–29)
BDG Tests	
The number of BDG tests, median (IQR)	3 (2–4)
Duration of the prophylaxis before the first BDG, median (IQR)	6 (3–11)
The interval between BDG tests, days, median (IQR)	4 (3–7)
In-hospital mortality	20 (9.9)

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; BDG, β-D-glucan; DM, diabetes mellitus; GVHD, graft-versus-host disease; IQR, interquartile range; MDS, myelodysplastic syndrome; MM, multiple myeloma; SCT, stem cell transplantation; SD, standard deviation.

[a]Data are presented as n (%), if not otherwise specified.

The duration of the prophylaxis before the occurrence of IFIs was 3–35 days in those cases.

β-D-Glucan Diagnostic Values

There were 4 TP, 21 FP, 4 FN, and 112 TN (Table 3). The sensitivity, specificity, PPV, and NPV were calculated as 50.0%, 84.2%, 16.1%, and 96.5%, respectively. There were 108 (76.6%) BDG assays performed for diagnosis and 33 (23.4%) for surveillance. Among the episodes associated with diagnostic BDG assays, there were 4 TP, 11 FP, 4 FN, and 89 TN, yielding sensitivity, specificity, PPV, and NPV values of 50.0%, 89.0%, 26.7%, and 95.7%, respectively. In the surveillance episodes, there were no positive IFIs, with 10 FP and 23 TN, yielding specificity, PPV, and NPV values of 69.7%, 0%, and 100%, respectively.

Potential Causes of False-Positive β-D-Glucan Results

Among 21 FP BDG assays, frequent potential causes of false positivity were as follows: administration of piperacillin/tazobactam (n = 16, 76.2%), intravenous immunoglobulin (n = 10, 47.6%), administration of meropenem (n = 6, 28.6%), and intravenous albumin (n = 6, 27.3%) (Supplementary Table 2). When we compared frequencies of those potential causes between FP and TN episodes, only intravenous albumin administration was significantly more common in FP episodes.

Sensitivity Analyses

There were 109, 78, and 53 evaluable episodes having more than 2, 3, and 4 BDG results, respectively. The respective prevalences of IFI were 6.4%, 9.0%, and 11.3%, yielding respective PPVs of 16.0%, 16.7%, and 17.6% (Table 4). Among 78 and 63 evaluable cases with micafungin or posaconazole use, PPVs were 0% and 20.0%, respectively, because there were no TP cases in the micafungin subgroup. Positive predictive values were 18.8% and 16.0% in subgroups after excluding duplicated patients or autologous SCT episodes, respectively (Supplementary Table 3).

DISCUSSION

The present study showed limited PPV and high NPV for the BDG assay in hematologic patients given antimold prophylaxis. Positive predictive value was especially low when it was performed for surveillance, and it was not high even when performed for diagnostic purposes, underscoring the necessity for prudent interpretation of the test. However, NPVs were commonly high for all assays performed, indicating the BDG assay's higher value as a rule-out test than as a rule-in test when used during antimold prophylaxis.

There are reports that the BDG assay can be useful in diagnosing IFIs. A meta-analysis from 6 cohort studies comprising 1771 hematologic patients, 215 (12.5%) of whom were diagnosed with proven or probable IFIs, demonstrated the diagnostic value of the BDG assay for IFIs [8]. The sensitivity, specificity, PPV, and NPV for diagnosis of IFIs were calculated as 49.6%, 98.9%, 83.5%, and 94.6%, respectively. However, more than 50% of the patients in the meta-analysis did not receive antimold prophylaxis, leading to a higher prevalence of IFIs than that seen in the present study.

Diagnostic values, especially PPV, must be re-evaluated in settings of much lower prevalence because they could be considerably different. Our findings might provide evidence-based direction for the use of the BDG assay in hematologic patients, especially in the era of broad-spectrum antifungal prophylaxis. Expectedly, among 8 breakthrough IFI cases in this study, only 4 showed positive BDG. Moreover, it was falsely positive in 21 episodes, yielding 16.1% of PPV. However, when evaluating neutropenic fever, in the absence of positive cultures or radiological findings, a negative BDG with a high NPV may be useful in helping exclude IFI.

Table 2. Clinical Characteristics of Breakthrough Invasive Fungal Infection Episodes

Characteristics	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8
Types of IFI	IA	IA	IA	IA	Candidemia	Candidemia	Trichosporosis	IA
Age (years)	28	59	63	41	61	43	26	28
Sex	Male	Male	Male	Male	Female	Male	Male	Male
Episode	AML, induction	AML, GVHD	MDS, GVHD	Lymphoma, alloSCT	AML, induction	AML, GVHD	AA, alloSCT	AML, induction
Days of prophylaxis until the diagnosis of IFIs	25	4	35	13	3	29	13	23
Positive BDG in serum	No	Yes	Yes	No	No	Yes	No	Yes
Fungal culture	Not done	Not done	Sputum, no growth	Sputum, no growth	Blood, growth	Blood, growth	Blood, growth	Not done
Timing of CT scan (before or after BDG)	Before	Before	Before	After	Before	Not performed	After	After
Image findings	Cavitary lung nodule in CT	Cavitary lung nodule in CT	Multiple lung nodules in CT	Multiple cavitary nodules in CT	-	-	Multiple lung nodules in CT	Cavitary lung nodule in CT
Length of hospitalization	77	24	98	53	75	30	95	41
Outcome	Alive	Alive	Alive	Alive	Alive	Dead	Alive	Alive

Abbreviations: AA, aplastic anemia; alloSCT, allogeneic stem cell transplantation; AML, acute myelogenous leukemia; BDG, β-D-glucan; CT, computed tomography; GVHD, graft-versus-host disease; IA, invasive aspergillosis; IFI, invasive fungal infection; MDS, myelodysplastic syndrome.

Sensitivity analyses were performed to account for potential confounding effects on BDG diagnostic values by the number of tests performed, the types of antimold agents, inclusion of duplicate patients, or inclusion of autologous SCT (Table 4 and Supplementary Table 3). Although the numbers of episodes included in those respective subgroups were small, the results were similar, indicating the limited effect of those factors on our conclusions.

These results are similar to some reported for the serum galactomannan test. Although the test is used to diagnose invasive aspergillosis, its PPV is reported to be as low as 11.8% for micafungin [9] and 3.2% under posaconazole prophylaxis [10], when there was no clinical evidence of IFI. Decreased IFI prevalence due to the introduction of antimold prophylaxis may contribute to these low PPVs [15].

Potential causes for FP BDG results include administration of beta-lactam antibiotics, intravenous immunoglobulin or albumin, other infections, and hemodialysis with cellulose membranes [12, 16, 17]. In particular, there was a report that many antimicrobials including piperacillin/tazobactam or meropenem, which are frequently used in neutropenic patients, could yield positive BDG tests [17]. Such antimicrobial agents were used in most of the FP episodes in this study, which is ordinary phenomenon in high-risk hematologic patients. We could not distinguish the exact cause of false positivity between the above-mentioned factors and intrinsic false positivity because of the low prevalence of IFIs; however, what we have found in this study is abundancy of FP episodes in those hematologic patients. Because we could not review how blood samples were collected (via Hickman catheter or by venipuncture), further evaluations are warranted if it could affect BDG false positivity.

The present study has some limitations. First, this study was conducted retrospectively and the number of episodes was limited, necessitating further evaluation in a larger, prospective cohort. Second, the BDG assays were not performed regularly and their number varied among episodes. Although we obtained similar results across subgroups represented by different numbers

Table 3. Diagnostic Values of β-D-Glucan

Characteristics	Overall (n = 141)	Diagnostic (n = 108)	Surveillance (n = 33)
TP, n (%)	4 (2.8)	4 (3.7)	0 (0.0)
FN, n (%)	4 (2.8)	4 (3.7)	0 (0.0)
TN, n (%)	112 (79.4)	89 (82.4)	23 (69.7)
FP, n (%)	21 (14.9)	11 (10.2)	10 (30.3)
Sensitivity, % (95% CI)	50.0 (15.7–84.3)	50.0 (15.7–84.3)	-
Specificity, % (95% CI)	84.2 (76.9–90.0)	89.0 (81.2–94.4)	69.7 (51.3–84.4)
PPV, % (95% CI)	16.1 (8.0–29.8)	26.7 (13.0–46.9)	0.0
NPV, % (95% CI)	96.5 (93.3–98.2)	95.7 (91.7–97.8)	100.0

Abbreviations: CI, confidence interval; FN, false negative; FP, false positive; NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive.

Table 4. Diagnostic Values of β-D-Glucan for Subgroups Defined by Test Numbers and Antimold Agents

		Subgroups According to the Numbers of BDG Tests			Subgroups According to Antimold Agents	
Characteristics	Overall (n = 141)	≥2 (n = 109)	≥3 (n = 78)	≥4 (n = 53)	Micafungin (n = 78)	Posaconazole (n = 63)
TP, n (%)	4 (2.8)	4 (3.7)	4 (5.1)	3 (5.7)	0 (0.0)	4 (6.3)
FN, n (%)	4 (2.8)	3 (2.8)	3 (3.8)	3 (5.7)	2 (2.6)	2 (3.2)
TN, n (%)	112 (79.4)	81 (74.3)	51 (65.4)	33 (62.3)	71 (91.0)	41 (65.1)
FP, n (%)	21 (14.9)	21 (19.3)	20 (25.6)	14 (26.4)	5 (6.4)	16 (25.4)
Sensitivity, % (95% CI)	50.0 (15.7–84.3)	57.1 (18.4–90.1)	57.1 (18.4–90.1)	50.0 (11.8–88.2)	0.0 (0.0–84.2)	66.7 (22.3–95.7)
Specificity, % (95% CI)	84.2 (76.9–90.0)	79.4 (70.3–86.8)	71.8 (59.9–81.9)	70.2 (55.1–82.7)	93.4 (85.3–97.8)	71.9 (58.5–83.0)
PPV, % (95% CI)	16.1 (8.0–29.8)	16.0 (8.3–28.6)	16.7 (8.7–29.6)	17.6 (7.9–34.8)	0.0 (NA)	20.0 (11.0–33.5)
NPV, % (95% CI)	96.5 (93.3–98.2)	96.4 (92.0–98.5)	94.4 (87.7–97.6)	91.7 (82.9–96.2)	97.3 (97.1–97.4)	95.3 (86.8–98.5)

Abbreviations: BDG, β-D-glucan; CI, confidence interval; FN, false negative; FP, false positive; NA, not applicable; NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive.

of BDG tests, the predictive value of BDG testing should be reassessed in a patient population with a generally higher number of BDG tests. Third, BDG tests itself was used as a mycological evidence in the diagnosis of IFI, which may contribute to the overestimation of its performance. However, PPV of BDG was limited in this study despite such a predisposition. Fourth, therapeutic drug monitoring for posaconazole had not been performed in this study. Finally, we used the Goldstream kit for the assay rather than widely studied Fungitell (Associates of Cape Cod, Falmouth, MA) [12, 13, 18]. Although similar performance of the 2 tests has been reported [19], further validation of the Goldstream kit is needed.

CONCLUSIONS

In conclusion, in hematologic patients who are taking prophylactic posaconazole or micafungin, BDG test is not helpful in diagnosing IFIs and should be used to exclude IFI rather than to diagnose it.

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.

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