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Utility of Galactomannan and (1-3)-Beta-D-Glucan Assays in the Diagnosis of Invasive Aspergillosis

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Abstract

Objective: The utility of serum and bronchoalveolar lavage (BAL) galactomannan (GM) and (1-3)-beta-D-glucan (BDG) assays in the diagnosis of Invasive Aspergillosis (IA) remains unclear. Variable false positive and false negative rates complicate physician understanding and proper interpretation of test results, which may contribute to overuse of empiric treatment with antifungals. The purpose of this study is to examine the utility of GM and BDG assays for the diagnosis of IA.

Methods: A retrospective chart review was conducted of Michigan Medicine patients who had at least one GM or BDG assay completed from June 2013 to March 2016 to compare diagnostic evidence of IA as defined by EORTC/MSG guidelines to the results of the GM and BDG assays. Effects of piperacillin-tazobactam and solid organ transplants on test efficacy were also evaluated.

Results: GM serum, GM BAL, BDG serum, and BDG BAL assays had a sensitivity of 47.3%, 88.0%, 80.0%, and 100%, respectively and a specificity of 87.1, 58.3%, 40.0%, and 16.7%, respectively. The specificity of the GM serum assay was 87.1% for all patients, compared to 44% in patients on piperacillin-tazobactam. The overall specificity the four assays was 75.8% in patients with a solid organ transplant, compared to 57.6% in patients without a solid organ transplant.

Conclusions: This study found that GM BAL, BDG serum, and BDG BAL assays to have increased sensitivity than previously reported data but significantly lower specificity, thus suggesting that the negative predictive value of these tests is higher than previously stated. Therefore, they could be used as screening assays; however, the positive predictive value is lower than previously found, so positive results should be used cautiously. Use of piperacillin-tazobactam decreased specificity of GM serum assay, as expected. Patients with solid organ transplants had increased specificity compared to patients without transplants, which differs from previous findings.

Keywords: Invasive Aspergillosis; Galactomannan; Beta-D-glucan; Piperacillin-tazobactam; Solid organ transplant; Efficacy; Aspergillus

Introduction

Invasive Aspergillosis is an opportunistic mycotic infection with a mortality rate of 15-30% depending on when the infection is diagnosed and type of therapy elected [1]. While the requirement for a diagnosis of proven invasive pulmonary aspergillosis remains a lung biopsy for histopathology and culture, these patients are immunocompromised by definition and such an invasive procedure is often not recommended [2]. Empiric treatment, likewise, is not ideal because the antifungals required to treat invasive aspergillosis are expensive with treatment of IA in the hospital reaching almost $2900 per day and antifungals are notoriously toxic [3]. Therefore, diagnosticians increasingly rely on alternative, less invasive testing.

The problem with Aspergillus testing is that while the biopsy has sensitivity and specificity nearing 100% each, the less invasive options are extremely variable in their sensitivities and specificities. The current assays are: Galactomannan (GM) serum, GM bronchoalveolar lavage (BAL), Fungitell Beta-D-Glucan (BDG) serum, and Fungitell BDG BAL, with additional assays in development. GM serum has been cited as having a sensitivity of 68-74% and a specificity of 88-90% [4]. GM BAL has a reported sensitivity of 55-87% and specificity of 70-78% [5]. BDG serum has sensitivity of 67-84% and specificity of 80-90% [6]. BDG BAL has a sensitivity of 71% and specificity of 67% [7].

To complicate the use of non-biopsy testing for Aspergillus, the cross-reactivity of the broad-spectrum antibiotic piperacillin-tazobactam (Zosyn) due to the presence of galactomannan in the formulation can lead to false positive tests [8]. While more recent formulations of piperacillin-tazobactam have been shown to decrease the incidence of these false positives, misdiagnoses still occur [9]. Additionally, solid organ transplants, in particular lung transplants, present a diagnostic challenge because colonization without invasion can lead to false positive Aspergillus test results. 25-30% of all lung transplant patients are colonized with Aspergillus; therefore, leading to over-diagnosis and treatment of IA in this cohort and, as a reaction to this finding, this test result can be incorrectly dismissed as a false positive, leading to delayed diagnosis and treatment in these patients as well [10].

Timely diagnosis and treatment is imperative to any potential for cure; this is made exceedingly difficult by the large ranges and variations in sensitivities and specificities of each of the assays, as well as potential clinical factors that affect assay results. Therefore, 3 years of data regarding the sensitivities and specificities of GM and BDG assays at Michigan Medicine will add to the conversation in the literature regarding the utility of these non-invasive tests in clinical decision making.

Methods

After obtaining Institutional Review Board approval (HUM00111625), a retrospective chart review was conducted of 367 Michigan Medicine patients who had at least 1 GM or BDG assay completed between June 2013 and March 2016. Patient demographics, comorbidities, laboratory tests, and treatment were recorded. Patients were included in analysis if they had at least 1 GM or BDG completed either serum or BAL. Not all patients underwent bronchoscopy. If patients had multiple tests completed, each test was analyzed separately and solely in the context of that specific instance. Patients were excluded from analysis if they were transferred out of the Michigan Medicine hospital system and, therefore, were lost to follow-up prior to ultimate diagnosis or if the patient elected to withdraw care prior to determining a diagnosis.

Variables	Sensitivity MM retrospective (%)	Sensitivity previous studies (%)	p	Specificity MM retrospective (%)	Specificity previous studies (%)	p
GM (Serum)	47.3	71	0.001	87.1	89	0.056
GM (BAL)	88	71.5	0.001	58.3	80	0.001
BDG (Serum)	80	75.5	0.029	40	85	0.001
BDG (BAL)	100	71	0.155	16.7	67	0.005

Table 1: Comparative assay sensitivity and specificity.

Variables	Specificity Overall (%)	Specificity for patients on piperacillin-tazobactam (%)	Specificity excluding patients on piperacillin-tazobactam (%)	p
GM (Serum)	87.1	44	93.4	0
GM (BAL)	58.3	57.1	58.4	0.966

Table 2: Comparative Piperacillin-tazobactam specificities in galactomannan assays.

Michigan Medicine sends out Galactomannan serum to Mayo Medical Laboratories, which conducts an EIA on specimens with index<0.5. Galactomannan BAL is sent to Viracor Eurofins, which conducts an EIA on specimens with an index<0.5. BDG serum is sent to Viracor Eurofins, which conducts an assay based on a modification of the Limulus Amebocyte Lysate (LAL) pathway to eliminate factor C to be specific for B-D-glucan. Reference range is: negative<60, indeterminate 60-79, positive>80. BDG BAL is sent to Viracor Eurofins, which conducts microplate titer on the specimen. Reference range is: negative<60, indeterminate 60-79, positive>80.

After looking at the complete clinical picture, reviewers classified each test as true positive, true negative, false positive or false negative. True positive were patients that had a positive test result – either galactomannan or BDG, serum or sputum–and fulfilled 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) diagnostic criteria as determined by reviewers [2]. True negative was negative test result and did not fulfill guidelines. False positive was positive test result and did not fulfill guidelines. False negative was negative test result and did fulfill guidelines. Each patient was reviewed and classified independently by 2 reviewers, who then came together to discuss the disparate outcomes.

The EORTC/MSG guidelines are as follows: proven IA requires histopathologic evidence or culture from a sterile environment. Probable IA requires documentation of immunosuppression (i.e. corticosteroid use, immunosuppressant use, recent prolonged neutropenia, etc.), a positive indirect assay (i.e. GM serum, GM BAL, BDG serum or BDG BAL), and clinical evidence of active infection. Suspected has some of the characteristics present in probable IA, but not all. Not suspected generally consists of clinical evidence of an active infection with a negative assay [2]. Other clinical correlates previously documented to influence assay results were also examined, such as the use of piperacillin-tazobactam and whether the patient had a solid organ transplant, in particular a lung transplant. Statistical significance ($p<0.05$) was determined using chi square analysis and independent samples t-tests.

Results

Assays' sensitivities and specificities

A total of 367 assays were analyzed-125 GM serum, 160 GM BAL, 75 BDG serums and 7 BDG BAL. There were 231 males and 136 females. 84.5% (n=310) of the sample was Caucasian, 6.8% (n=25) African American, 6.3% Asian (n=23), 0.8% Hispanic/Latinx (n=3), 1.6% other/unspecified (n=6). The average age of the patients was 55.5 years with a range of 1.5-90 years. 14 of the patients were pediatric patients and evaluated by Pediatric Infectious Diseases, while Adult Infectious Diseases evaluated the other 253 patients (Table 1).

Of the 125 GM serum assays, 26 were considered true positives (20.8%), 61 were true negatives (48.8%), 9 were false positives (7.2%) and 29 were false negatives (23.2%). This resulted in a sensitivity of 47.3% with a 95% confidence interval (CI) [33.9, 61.1]. The sensitivities in GM serum assays have been previously documented at 71%, 95% CI [68, 74], which is different than the current study at a statistically significant level (p<0.001) [4]. There was a specificity of 87.1%, 95% CI [76.5, 93.6]. The specificities in GM serum assays have been documented at 89%, 95% CI [88, 90], (p=0.056) [4].

Of the 160 GM BAL assays, 2 were excluded from analysis: one patient was lost to follow-up secondary to hospital transfer immediately following testing and one patient refused further care; therefore 158 assays were analyzed. There were 44 true positives (27.8%), 63 true negatives (39.9%), 45 false positives (28.5%), and 6 false negatives (3.8%). This resulted in a sensitivity of 88%, 95% CI [75, 95]. In previously documented GM BAL assays there has been a sensitivity of 71.5%, 95% CI [50, 93] (p<0.001) [5]. There was a specificity of 58.3%, 95% CI of [48.4, 67.6]. In contrast, the previously documented specificity is 80%, 95% CI [73, 87] (p<0.001) [5].

Of the 75 BDG serum assays, 28 were true positives (37.3%), 16 were true negatives (21.3%), 24 were false positives (32%), and 7 were false negatives (9.3%). This resulted in a sensitivity of 80% with a 95% CI [62.5, 90.9]. The previously published sensitivity was 75.5%, 95% CI [67, 84] (p=0.029) [6]. This study had a specificity of 40%, 95% CI [25.3, 56.6]. The previously documented specificity was 85%, 95% CI [80, 90] (p<0.001) [6].

Of the 7 BDG BAL assays, 1 was a true positive (14.3%), 1 was a true negative (14.3%), 5 were false positives (71.4%), and 0 were false negatives. This resulted in a sensitivity of 100% with a 95% CI [5.5, 100]. The historical sensitivity was 71%, 95% CI [42, 90.4], (p=0.155) [7]. The specificity of this study is 16.7% with a 95% CI [0.8, 63.5]. Previously documented specificity was 67%, 95% CI [56.9, 75.3], (p=0.005) [7].

Piperacillin-tazobactam

Next, the effect of piperacillin-tazobactam on the specificity of each assay was analyzed. The GM serum specificity overall was 87.1%, the specificity of GM serum assay of patients on piperacillin-tazobactam was 44% and whereas GM serum specificity of patients not on piperacillin-tazobactam was 93.4% (p=0.000) (Table 2).

The GM BAL specificity overall was 58.3%, the specificity of patients on piperacillin-tazobactam was 57.1% and patients not on piperacillin-tazobactam had specificity of 58.4% (p=0.966).

The BDG serum specificity overall was 40%, the specificity of patients on piperacillin-tazobactam was 0%; however, this is only n=2. Patients not on piperacillin-tazobactam had specificity of 41%. This was not a statistically significant difference and requires additional patients to fully evaluate.

The BDG BAL specificity overall was 16.7%, the specificity of patients on piperacillin-tazobactam was 50%; however, total patients not on piperacillin-tazobactam had specificity of 0%. This was not a statistically significant difference and requires additional patients to fully evaluate.

Solid organ transplant

The relationship between solid organ transplant and overall specificity was analyzed. The false positive rate for patients with a solid organ transplant was 24.2% (n=16), resulting in a specificity of 75.8%, 95% CI [63.4, 85.1]. The false positive rate for patients without a solid organ transplant was 42.4% (n=67), resulting in a specificity of 57.6%, 95% CI [49.5, 65.3], p=0.000.

More specifically, the relationship between lung transplant and specificity was analyzed. The lung transplant patients had a false positive rate of 23.6% (n=13), which is a specificity of 76.4%, 95% CI [62.7, 86.3] and non-organ transplants were at 42.4% (n=67), specificity of 57.6%, 95% CI [49.5, 65.3] (p<0.02).

Discussion

It was found that, within the Michigan Medicine system, the GM BAL, BDG serum, and BDG BAL assays to have increased sensitivity than previously reported but significantly lower specificity. GM serum, on the other hand, was found to have decreased sensitivity in comparison to prior citations. This may suggest that negative results in GM BAL, BDG serum and BDG BAL may be a helpful screening tool that would aid in avoiding the use of toxic antifungal treatments in patients with relatively low suspicion for IA. However, positive results of all four assays may not be as helpful as previously cited in the literature. Consideration of further testing, whether that be ordering an additional non-invasive test for further corroboration or revisiting the idea of biopsy, as well as continually revisiting the full clinical picture, should be steps taken by the primary and consulting teams when considering how to act on a positive result from one of these assays.

In this cohort, use of piperacillin-tazobactam was found to be associated with decreased specificity with GM serum. The relationship between piperacillin-tazobactam and GM assays has been described previously in the literature, particularly for generic compounds; however, there has been some debate as to whether this continues to be a source of false positives [8,9,11]. This study suggests that this relationship does continue to confound test results and next steps should include confirming a positive GM serum assay with GM BAL or serum BDG.

Interestingly, patients with solid organ transplants had increased specificity compared to patients without organ transplants, suggesting that positive results in solid organ transplant patients are more reliable than previously assumed.

Conclusion

Sensitivities and specificities for non-biopsy testing for invasive pulmonary aspergillosis are highly variable between institutions. There is inconsistency and uncertainty behind their results at baseline but additionally, there are the potential effects of concomitant use of piperacillin-tazobactam on assay results; it was previously documented to decrease specificity, then reported to not have effect on specificity due to new assay techniques, and now in this study to show effects on some assays but not others. The effects of solid organ transplant on assay results are equally as inconsistent and it would not be surprising if there were yet to be identified factors at play as well. This creates a very confusing picture for a physician attempting to interpret one of these assays, particularly due to the high risk of mortality if antifungal therapy is not started as soon as possible.

It is important for researchers to continue evaluating the sensitivities and specificities of these assays, as well as the factors that alter their accuracy, to provide as exact information as possible for clinicians to take into context when making diagnoses. The development of new assays with higher, more consistent sensitivities and specificities should be another goal for improving the treatment of IA. It is of the utmost importance that the patient's entire clinical picture and clinical course be taken into account when considering IA. While lung biopsy is diagnostic for IA, it is imperative to remember that less invasive tests are currently not reliable enough on their own to make this diagnosis.

Strengths and Limitations

The strength of this study is the sample size for the GM serum, GM BAL, and BDG serum of at least 75 subjects each–this decreases the margin of error found in many studies with smaller sample sizes. Conversely, a major limitation is the sample size of n=7 for the BDG BAL. This is in part due to fact that the Adult Infectious Disease division at Michigan Medicine deems the test to be too unreliable to warrant obtaining it.

Another major limitation is that this is a retrospective study; therefore, investigators were forced to rely on notes describing clinical encounters. These patients are complex by nature, which made the process of unraveling the exact sequence/timing of events challenging for some patients. Similarly, BDG serum and BAL assays can be utilized to diagnose other invasive fungal infections including *Candida* spp., *Acremonium* spp, *Coccidioides immitis*, *Fusarium* spp., *Histoplasma capsulatum*, *Trichosporon* spp., *Sporothrix schenckii*, *Saccharomyces cerevisiae*, and *Pneumocystis jiroveci* [6,7]. Therefore, this test is frequently utilized if there is concern for any type of fungal infection, not just invasive aspergillosis and it is retrospectively difficult to determine if Aspergillus was the primary organism of concern. Additionally, the manufacturer of the piperacillin-tazobactam could have been determined/potentially changed in these patients in order to determine whether the formulation was affecting the result of the assays, as has been noted in other studies [8].

A major strength of this study is having 2 investigators work together to classify patients as proven/probable/suspected/not suspected IA in order to double check the accuracy of the classification based on the EORTC/MSG guidelines.

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