**Galactomannan, Beta-D-Glucan and PCR-Based Assays for the Diagnosis of Invasive Fungal Disease in Pediatric Cancer and Hematopoietic Stem Cell Transplantation: A Systematic Review and Meta-Analysis**

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**Key points:** Galactomannan, beta-D-glucan, and polymerase-chain-reaction-based assays to detect invasive fungal disease in pediatric cancer or hematopoietic stem-cell transplantation patients demonstrated high negative predictive values for galactomannan, but overall, variable and generally poor sensitivity, specificity and positive predictive values for all biomarkers.

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ABSTRACT

We systematically reviewed and analyzed the available data for galactomannan (GM), beta-D-glucan (BG), and polymerase-chain reaction (PCR)-based assays to detect invasive fungal disease (IFD) in pediatric cancer or hematopoietic stem cell transplantation (HSCT) patients when used as screening tools during immunosuppression or as diagnostic tests in patients presenting with symptoms such as fever during neutropenia (FN). Out of 1,532 studies screened, 25 studies reported on GM (n=19), BG (n=3) and PCR (n=11). All fungal biomarkers demonstrated highly variable sensitivity, specificity and positive predictive values, and these were generally poor in both clinical settings. GM negative predictive values were high, ranging from 85-100% for screening and 70-100% in the diagnostic setting, but failure to identify non-*Aspergillus* molds limits its usefulness. Future work could focus on the usefulness of combinations of fungal biomarkers in pediatric cancer and HSCT.

BACKGROUND

 Invasive fungal disease (IFD) is an important contributor to morbidity and mortality in pediatric patients who receive intensive chemotherapy or who undergo allogeneic hematopoietic stem cell transplantation (HSCT) [[1-3](#_ENREF_1)]. One important strategy which may improve outcome is the early detection of IFD through the use of biomarkers, including galactomannan (GM), beta-D-glucan (BG), and polymerase-chain reaction (PCR)-based assays [[4](#_ENREF_4)]. These assays can be performed in specimens such as blood, broncho-alveolar lavage (BAL) fluid, cerebrospinal fluid (CSF) or in tissue biopsies. Assays may be used either as a screening tool during high-risk periods, for example, in asymptomatic patients with neutropenia or graft-versus-host disease (GVHD), or as a diagnostic tool in symptomatic patients, for example, in patients with prolonged fever and neutropenia (FN) or in patients in whom IFD is suspected. We hypothesized that the setting of testing (screening versus diagnostic test) would alter the utility of the test through changing the pre-test probability of the infection.

 In adult patients, GM, BG and PCR-based assays are accepted tools to support the diagnosis of IFD, but to date, only GM and BG are incorporated into the criteria of defining opportunistic IFD and invasive aspergillosis (IA) set forth by the European Organisation for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) [[5](#_ENREF_5)]. Importantly, the utility of these tests in children has been questioned [[6-8](#_ENREF_6)], and it is possible that the diagnostic properties of the tests may differ between adult and pediatric populations, which in turn, will have important implications for epidemiological studies as well as for the clinical care of immunocompromised children.

 The objective of this systematic review and meta-analysis was determine whether GM, BG, or fungal PCR are useful tools to detect IFD in pediatric cancer or HSCT patients as screening tools during neutropenia / severe immunosuppression, or as diagnostic tools in patients who present with FN or symptoms suggestive for IFD.

METHODS

 This analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations for reporting [[9](#_ENREF_9)].

Data Sources and Searches

 We used the Ovid SP platform to search MEDLINE, MEDLINE In-Process and Embase and the Wiley platform to search Cochrane Central Register of Controlled Trials for articles indexed up to March 11, 2016. The search strategy included the Medical Subject Heading terms and text words which identified children and adolescents with cancer or undergoing HSCT in which GM, BG or fungal PCR were evaluated as biomarkers to detect IFD. The search is available as Appendix 1. The set was limited to studies published in 1980 or more recently. There was no restriction by language.

Study Selection

 Eligibility criteria for the studies were defined *a priori* and were as follows: (1) Subjects were children or adolescents with cancer or were HSCT recipients; (2) The manuscript was a fully published primary study; (3) The study evaluated GM, BG or fungal PCR including *Aspergillus* PCR in blood, BAL or CSF since these sources are included in the EORTC/MSG criteria,; (4) The publication included the number of patients with IFD. Reasons for excluding studies were the following: (1) Not a full text publication; (2) Not all included patients were pediatric patients (defined as age < 25 years); (3) Less than 50% of patients had cancer or were undergoing HSCT; (4) The study did not evaluate one of the chosen biomarkers for the detection of IFD; (5) The study did not have a control group (patients without IFD); (6) Less than 5 patients with IFD were evaluated; and (7) *Pneumocystis jirovecii* was the only fungus examined. There was no restriction by study design as long as the eligibility criteria were met.

 Two reviewers (PR and LS) independently evaluated the titles and abstracts of publications identified by the search strategy and any potentially relevant publication was retrieved in full. Each full text paper was evaluated by two reviewers (TL, PR or LS) and final inclusion of a study into the systematic review required agreement by the two reviewers. Agreement of study inclusion between the two reviewers was evaluated using the kappa statistic; agreement was defined as slight (0 to 20%), fair (21 to 40%), moderate (41 to 60%), substantial (61 to 80%) or almost perfect (81 to 100%) [[10](#_ENREF_10)].

Data Abstraction and Methodological Approach

 Two reviewers (TL, PR or LS) abstracted all data in duplicate and any discrepancies were resolved by consensus. The primary variables of interest were sensitivity, specificity, positive predictive value and negative predictive value of the biomarkers to detect IFD.

 The criteria used to declare a biomarker result as positive varied between studies and between biomarkers. We attempted to standardize definitions so that studies were comparable. Data were preferentially reported when a positive GM, BG or PCR test required only one positive result with the threshold being ≥ 0.5 index for GM and ≥ 80 pg/mL for BG. Preferentially, the criteria used to define IFD or IA were according to EORTC/MSG, either in the original or in the revised version [[5](#_ENREF_5), [11](#_ENREF_11)], and an IFD case was defined as proven or probable IFD wherever possible. However, some studies grouped proven, probable and possible IFD together and in these cases, the results were presented in this fashion with a notation of the analyzed IFD classification approach. All analyses were stratified by biomarker and then stratified based upon the setting, namely as screening tests for patients without signs and symptoms of infection throughout therapy, neutropenia or GVHD versus as diagnostic tests evaluating patients with signs and symptoms of infection, such as FN. We also stratified by specimen source, namely blood, BAL or CSF.

 Study-level factors collected included years of study publication and enrollment, country of study conduct, patient age range, patient population (cancer, HSCT or both), study design, whether biomarker was reported to clinicians and clinical indication for testing. We also recorded the frequency of specimen collection in the case of blood testing, antifungal prophylaxis, whether IFD was defined using the EORTC/MSG criteria and the definition of a positive biomarker used in our analysis. The prevalence of proven or probable IFD was also determined for each study.

Assessment of Study Quality

 Study quality was abstracted by two reviewers (TL, PR or LS) and any discrepancies were resolved by consensus. Study quality was assessed using QUADAS-2 for review of diagnostic tests [[12](#_ENREF_12)]. Elements were: patient selection (could selection of patients have introduced bias); index test (could the conduct or interpretation of the test have introduced bias); reference standard (could conduct or interpretation of the reference standard have introduced bias); and flow and timing (were all patients included in the analysis). These elements were rated at low, high and unclear risk of bias.

Statistical Methods

 The true positive, false positive, false negative and true negative rates were abstracted from each study and based upon these results, sensitivity, specificity and predictive values with their 95% confidence intervals (CIs) were calculated. Two analyses were performed, one in which patients with possible IFD were classified as not having IFD and a second analysis in which patients with possible IFD were excluded in the calculation of diagnostic properties of the test. For pooling sensitivity and specificity, bivariate random effects meta-analysis model was employed in order to account for the correlation between these two accuracy measures [[13](#_ENREF_13)]. For synthesis, we excluded studies which did not use EORTC/MSG criteria to define IFD and studies in which possible IFD had to be considered a positive IFD case as reclassification could not be performed with the data provided. For GM synthesis, we excluded studies which used thresholds other than an OD ≥ 0.5 once or twice, or ≥ 0.7 once, and for BG, we excluded studies that used thresholds other than 80 pg/mL. We did not pool predictive values as this approach is not commonly done in meta-analyses as they depend on prevalence of the disease. Publication bias was not investigated given the nature of the outcome, namely diagnostic properties of individual tests. Analyses were conducted using the SAS statistical program (SAS-PC, version 9.4; SAS Institute Inc, Cary, North Carolina) and R (Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The flow of study identification and selection is illustrated in Figure 1. There were 1,532 unique citations identified by the search strategy, of which 55 were retrieved for full-text evaluation. Among these citations, 25 met the eligibility criteria and were included in the systematic review. Reasons for exclusion are described in Figure 1. Agreement of study inclusion between the two reviewers was almost perfect with kappa=92.7% (95% CI 82.7% to 100%).

Because the same study could evaluate multiple biomarkers, there were a total of 25 publications presenting 33 separate analyses of the chosen biomarkers. All studies evaluated blood specimens and one of these studies also evaluated the diagnostic properties of BAL GM. Table 1 summarizes the characteristics of the studies evaluating biomarkers in the blood and Appendix 2 includes the detailed data abstracted from each study. Most studies of biomarkers assessed in the blood included both cancer and HSCT patients (18/32, 56%).

There were 18 studies enrolling a total of 1,421 patients (184 with proven/probable IFD) that evaluated GM in blood in the pediatric cancer and HSCT setting (Table 2) [[14-31](#_ENREF_14)]. Ten studies used GM as a screening test during neutropenic periods or during GVHD. Conversely, 8 studies assessed GM as a diagnostic tool in children with symptoms potentially suggestive of IFD, such as prolonged FN. Overall, the prevalence of IFD ranged from 0 to 30.8% in individual studies. Antifungal prophylaxis was heterogeneous and often not standardized or specified (n=12). Sixteen studies used EORTC/MSG to define IFD or IA and 12 studies could be evaluated using a GM index of 0.5 once or twice or 0.7 once as the threshold.

Results of GM evaluation are shown as Table 2 and ordered by prevalence of proven or probable IFD or IA. Diagnostic properties are shown both for when possible IFD was included as a negative case and when patients with possible IFD were excluded from the analysis (where possible). Among the 10 studies in which GM in blood was used to screen for IFD among 100 patients with neutropenia or post HSCT, the following ranges were observed: specificity 50-100%, sensitivity 0-100%, positive predictive value 0-100% and negative predictive value 85-100%. Table 2 also shows the results of GM testing performed as a diagnostic test in 84 patients from 8 studies with the following ranges: specificity 35-100%, positive predictive value 0-100%, sensitivity 14-100% and negative predictive value 70-100%.

The only study evaluating GM in BAL reported sensitivity, specificity, positive and negative predictive value for proven/probable IA of 82%, 88%, 82%, and 87%, respectively (data not shown) [[16](#_ENREF_16)].

Table 3 shows the results of the only 3 studies in which BG testing was evaluated in pediatric patients [[19](#_ENREF_19), [29](#_ENREF_29), [32](#_ENREF_32)]. These studies included a total of 226 children, 38 of which were diagnosed with proven/probable IFD and reported the following ranges: specificity 29-82%, sensitivity 50-83%, positive predictive value 17-49%, and negative predictive value 84-96%.

 Table 4 illustrates the 11 studies which evaluated multi-fungal PCR (n=5) or *Aspergillus* PCR (n=6) in the blood [[15](#_ENREF_15), [19](#_ENREF_19), [21](#_ENREF_21), [25](#_ENREF_25), [26](#_ENREF_26), [33-38](#_ENREF_33)]. These studies included a total of 686 children (86 with proven/probable IFD). Among the 3 studies which applied PCR in the screening setting, the following ranges were observed: specificity 43-85%, sensitivity 11-80%, positive predictive value 20-50%, and negative predictive value 60-96%. When PCR-based assays were used as diagnostic tool during febrile episodes, the following were seen: sensitivity 0-100%, specificity 36-83%, positive predictive value 0-71%, and negative predictive value 88-100%. Notably, in the only study in which anti-mold prophylaxis was applied to all patients, the positive predictive value of the PCR was 0% [[33](#_ENREF_33)].

In general, across all serum biomarkers, predictive values were similar when possible IFD was classified as not having IFD or when patients with possible IFD were excluded from the analysis. In addition, predictive values did not clearly differ based upon use in the screening setting or as a diagnostic test during FN and test characteristics did not systematically improve as disease prevalence increased (Tables 2-4). Table 5 illustrates the pooled sensitivity and specificity by biomarker stratified by clinical setting. For GM evaluated in the screening setting and for diagnostic testing, 5 studies each were appropriate for synthesis based on using EORTC/MSG criteria, use of proven or probable IFD as a case and using appropriate GM thresholds for positivity. For each setting, the pooled sensitivity and specificity (95% CI) for GM were 68% (51-81) and 91% (86-94), and 89% (79-95) and 85% (51-97), respectively. Combining the results of both settings demonstrated a pooled sensitivity of 81% (69-89) and pooled specificity of 88% (75-95) (Table 5). For BG and for PCR-based assays in the screening setting, no synthesis was possible due to the low number of appropriate studies (two and one study, respectively). For the six appropriate studies evaluating PCR-based assays as diagnostic test, the pooled sensitivity and specificity was 76% (62-86) and 58% (42-72) (Table 5).

DISCUSSION

 Our systematic review and meta-analysis of the fungal biomarkers GM and BG and of PCR-based assays in pediatric cancer and HSCT patients demonstrates that sensitivity, specificity and positive predictive value of these tests were highly variable and generally not robust in both the screening setting and as a diagnostic test in symptomatic patients. However, serum GM negative predictive values were high. Across biomarkers, positive and negative predictive values were similar when patients with possible IFD were classified as not having IFD or when patients with possible IFD were excluded from the analysis. In addition, predictive values did not clearly differ based upon use in the screening or diagnostic testing setting. Only one study which met the inclusion criteria evaluated GM in BAL, and none evaluated fungal biomarkers in the CSF.

The great variability in the diagnostic properties of the fungal biomarkers may have been the result of multiple factors. First, there was significant heterogeneity in the study design and study population (for example, the inclusion of children with hematological malignancies and solid tumors and children undergoing allogeneic HSCT; children with and without neutropenia), and a considerable number of studies included only a small number of patients with proven/probable IFD. In addition, the aggressiveness in the use of other diagnostic tools for IFD such as chest CT scan or BAL most likely differed between the study centers and consequently affected the results. For example, in centers not using these tools routinely, patients with true IFD will unlikely meet the criteria for probable or proven IFD, and thus, a true positive GM will be classified as a false positive result. Also, most of the PCR-based assays tested were non-standardized in-house assays which differed in their methodology including specimen source, DNA extraction methods, amplification target sequences or amplification.

 When pediatric and adult studies were compared, the performance of the biomarkers seems to be similar (Table 5) [[39-41](#_ENREF_39)]. This is important to note as children and adults differ in their underlying malignancies and treatment schedules as well as in immunity and immune reconstitution after chemotherapy [[42](#_ENREF_42)], all of which may influence the performance of diagnostic tests as demonstrated in animal studies [[43](#_ENREF_43)].

In order to determine the value of a test, it is important to consider how biomarkers may be used clinically. As a diagnostic tool, a high positive predictive value is important which may prompt additional diagnostics including imaging studies or the initiation of antifungal therapy, whereas in the screening setting, a high negative predictive value is needed. Unfortunately, for all the biomarkers evaluated in our study, positive predictive values were highly variable and generally poor and unlikely to be clinically useful. The only consistently favorable property was a high negative predictive value with blood GM. However, given that GM can only identify *Aspergillus* species but not other molds, the high negative predictive value is of limited clinical value since clinicians may not be willing to withhold antifungal therapy because of the risk of non-*Aspergillus* fungal infections.

On the other hand, there are arguments which support the use of biomarkers. Whereas the positive predictive values are not reassuring and preclude a recommendation for routine utilization, the upper limits of the positive predictive values reported in some studies may be enough of an increase from the pre-test probability for some clinicians to consider utilizing them. Unfortunately, none of the studies systematically assessed biomarkers in the setting of possible IFD, which may be a more difficult scenario clinically for decision making, but which might help to assess the local epidemiology of IFD.

 The strength of our systematic review is its comprehensive evaluation of all pediatric studies assessing GM, BG and PCR-based assays for the early detection of IFD. The review also included data from studies not published in English, which is important due to the paucity of data in the pediatric population. Importantly, we present data in different clinical settings separately, namely in the screening and diagnostic testing settings, and show positive and negative predictive values relative to each study’s IFD prevalence (i.e. pre-test probability).

However, our results must be interpreted in light of study limitations. First, studies were highly heterogeneous which limits the interpretation of the pooled data. Second, fungal prophylaxis varied or was not specified which is important as studies in adults demonstrated that the administration of anti-mold active compounds decreases the ability of biomarkers to detect IFD [[39](#_ENREF_39), [44](#_ENREF_44), [45](#_ENREF_45)]. Finally, studies that show poor performance of biomarkers may be more difficult to publish, leading to an overestimation of the performance of biomarkers.

 In conclusion, poor positive predictive values were observed for serum GM, BG and PCR-based assays which preclude their application as screening tools during neutropenia or periods of GVHD and as a diagnostic tools in patients presenting with FN or symptoms suggestive for IFD. While the negative predictive value of GM was high, the inability of a negative GM to exclude non-*Aspergillus* molds may also limit usefulness. Future work could address the usefulness of combinations of biomarkers in pediatric cancer and HSCT, and should focus on specific clinical scenarios with higher pre-test probability of IFD such as children with possible IFD.

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Conflict of interest:

TL received research grants from Gilead, is a consultant for Basilea, Gilead, Merck/MSD, and received lecture honoraria from Astellas, Gilead, Merck/MSD, and Pfizer. BF received research grant support from Pfizer, Merck, and Ausun Biopharma. BTF received research grant support from Pfizer, Merck, and Ausun Biopharma. EC is a consultant for Basilea, Gilead, Astellas, Pfizer and received lecture honoraria from Astellas, Gilead and Pfizer. AHG received grants from Gilead and Merck, Sharp & Dohme and Pfizer; is a consultant for Amplyx, Astellas, Basilea, Gilead, Merck, Sharp & Dohme and Schering-Plough, and served at the speakers´ bureau of Astellas, Basilea, Gilead, Merck, Sharp & Dohme, Pfizer, Schering-Plough and Zeneus/Cephalon. WJS is a consultant for Amplyx, Astellas, Cidara and Merck. TEZ received research funding from Merck and Cubist and is a consultant for Merck, Pfizer, Astellas Pharma, and Cubist. All other authors: no conflicts of interest.

References

**Table 1: Characteristics of Studies that Evaluated Fungal Biomarkers in Blood**

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic and Strata** | **No. (%) GM N=18** | **No. (%)** **BDG (N=3)** | **No. (%)****PCR (N=11)** |
| Study Population Diagnosis  |  |  |  |
|  Cancer | 3 (17%) | 1 (33%) | 5 (45%) |
|  HSCT | 3 (17%) | 1 (33%) | 1 (9%) |
|  Both | 12 (67%) | 1 (33%) | 5 (45%) |
| Year Publication ≥ 2010 | 10 (56%) | 2 (67%) | 4 (36%) |
| Indication for Testing |  |  |  |
|  Screening Test | 10 (56%) | 2 (67%) | 3 (27%) |
|  Diagnostic Test | 8 (44%) | 1 (33%) | 8 (73%) |
| Study Design |   |  |  |
|  Prospective cohort | 12 (67%) | 3 (100%) | 7 (64%) |
|  Retrospective cohort | 6 (33%) | 0 | 4 (36%) |
| Assay Results Reported to Clinicians |  |  |  |
|  Yes | 5 (28%) | 0 | 4 (36%) |
|  No | 7 (39%) | 2 (67%) | 5 (45%) |
|  Uncertain | 6 (33%) | 1 (33%) | 2 (18%) |
| Anti-mold Prophylaxis | 0 | 0 | 1 (9%) |
| Invasive Fungal Disease Outcome Analyzed |  |  |  |
|  Proven or probable | 15 (83%) | 3 (100%) | 9 (82%) |
|  Proven, probable or possible | 3 (17%) | 0 | 2 (18%) |
| EORTC/MSG Gold Standard | 16 (89%) | 3 (100%) | 9 (82%) |
| Number of Samples Required for Positivity |  |  |  |
|  One | 11 (61%) | 3 (100%) | 9 (82%) |
|  Two | 5 (28%) | 0 | 2 (18%) |
|  One or two depending on threshold | 2 (11%) | 0 | 0 |
| Prevalence of Proven or Probable IFD |  |  |  |
|  ≥ 5% | 11 (61%) | 3 (100%)  | 8 (73%) |
|  ≥ 10% | 8 (44%) | 3 (100%) | 5 (45%) |
| Low Risk of Bias |  |  |  |
|  Patient selection  | 7 (39%) | 1 (33%) | 3 (27%) |
|  Index test  | 13 (72%) | 1 (33%) | 8 (73%) |
|  Reference standard | 4 (22%) | 2 (67%) | 1 (9%) |
|  Flow and timing | 17 (94%) | 3 (100) | 10 (91%) |

Abbreviation:GM – galactomannan; BDG – beta-D-glucan; PCR – polymerase chain reaction; HSCT – hematopoietic stem cell transplantation; EORTC/MSG – European Organisation for Research and Treatment in Cancer/ Mycoses Study Group

**Table 2: Test Characteristics of Galactomannan in Blood in Children and Adolescents with Cancer and Hematopoietic Stem Cell Transplant Recipients**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| First Author | No. Patients  | IFD Definition Analyzed | Prevalence | Specificity | Sensitivity | Positive Predictive | Negative Predictive | Specificity | Sensitivity | Positive Predictive | Negative Predictive |
|  |  |  |  | Possible Included as Negative IFD \* | Possible Excluded |
| *Screening*  |  |  |  |  |  |  |  |  |  |  |  |
| Hayden | 56 | Prov/Prob IA  | 30.4 | 87% (73-96) | 65% (38-86) | 69% (41-89) | 85% (70-94) | 87% (73-96) | 65% (38-86) | 69% (41-89) | 85% (70-94) |
| Rohrlich | 37 | Prov/Prob IA  | 27.0 | 93% (76-99) | 100% (69-100) | 83% (52-98) | 100% (86-100) |  |  |  |  |
| Koltze | 34 | Prov/Prob IFD | 17.6 | 100% (88-100) | 83% (36-100) | 100% (48-100) | 97% (82-100) | 100% (88-100) | 83% (36-100) | 100% (48-100) | 97% (82-100) |
| Badiee | 62 | Prov/Prob IA | 16.1 | 90% (79-97) | 90% (56-100) | 64% (35-87) | 98% (89-100) | 92% (75-99) | 90% (56-100) | 82% (48-98) | 96% (80-100) |
| Gefen | 46 | Prov/Prob/Poss IA | 8.7 | 66% (49-80) | 80% (28-99) | 22% (6-48) | 96% (82-100) |  |  |  |  |
| Tabone | 76 | Prov/Prob IA | 7.9 | 87% (77-94) | 100% (54-100) | 40% (16-68) | 100% (94-100) | 90% (80-96) | 100% (54-100) | 46% (19-75) | 100% (94-100) |
| Bialek | 17 | Prov/Prob/Poss IA | 5.0 | 50% (23-77) | 100% (29-100) | 30% (7-65) | 100% (59-100) |  |  |  |  |
| Hovi | 98 (117 episodes) | Prov/Prob IFD | 2.0 | 90% (84-95) | 50% (1-99) | 8% (0-38) | 99% (95-100) | 93% (86-97) | 50% (1-99) | 14% (0-58) | 99% (94-100) |
| Steinbach | 64 | Prov/Prob IA | 1.6 | 87% (77-94) | 0% (0-98) | 0% (0-37) | 98% (90-100) | 87% (77-94) | 0% (0-98) | 0% (0-37) | 98% (90-100) |
| Fisher | 198 | Prov/Prob IA | 0.5 | 95% (91-98) | 0% (0-98) | 0% (0-31) | 99% (97-100) |  |  |  |  |
| *Diagnostic Testing*  |  |  |  |  |  |  |  |  |  |
| El-Mahallawy | 91 | Prov/Prob IA | 30.8 | 49% (36-62) | 79% (59-92) | 41% (28-55) | 84% (68-94) | 61% (46-75) | 79% (59-92) | 54% (37-69) | 83% (67-94) |
| Choi | 99 | Prov/Prob IA | 23.2 | 82% (70-90) | 91% (72-99) | 66% (47-81) | 96% (87-100) | 82% (70-90) | 91% (72-99) | 66% (47-81) | 96% (87-100) |
| Dinand | 145 (211 episodes) | Prov/Prob IA | 13.8 | 72% (65-78) | 95% (75-100) | 26% (17-38) | 99% (96-100) | 80% (73-85) | 95% (75-100) | 36% (23-50) | 99% (96-100) |
| de Mol | 38 | Prov/Prob IA | 10.9 | 100% (85-100 | 87% (60-98) | 100% (75-100) | 92% (74-99) | 100% (74-100) | 87% (60-98) | 100% (75-100) | 86% (57-98) |
| Armenian | 68 | Prov/Prob/Poss IA  | 4.4 | 98% (88-100) | 14% (3-35) | 75% (19-99) | 70% (58-81) | 98% (88-100) | 100% (29-100) | 75% (19-99) | 100% (92-100) |
| Castagnola | 119 (195 episodes) | Prov/Prob IA | 3.6 | 98% (95-100) | 100% (59-100) | 70% (35-93) | 100% (98-100) | 98% (95-100) | 100% (59-100) | 70% (35-93) | 100% (98-100) |
| Jha | 78 (100 episodes) | Prov/Prob IA | 2.0 | 35% (25-45) | 100% (16-100) | 3% (0-11) | 100% (90-100) | 39% (28-51) | 100% (16-100) | 4% (1-14) | 100% (88-100) |
| Reinwald | 95 (253 episodes) | Prov/Prob IA | 0.0 | 90% (82-96) | NC | 0% (0-37) | 100% (95-100) |   |   |   |   |

Abbreviations: IFD – invasive fungal disease; IA – invasive aspergillosis; Prov – proven; Prob – probable; Poss – possible; NC- not calculable

\* In studies in which possible IFD or IA were included as a case and data were not provided to permit re-calculation, possibles could not be included as a negative control (baseline analysis). However, these studies were excluded from GM synthesis (see Methods)

**Table 3: Test Characteristics of Beta-D-Glucan in Blood in Children and Adolescents with Cancer and Hematopoietic Stem Cell Transplant Recipients**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| First Author | No. Patients  | IFD Definition Analyzed | Prevalence | Specificity | Sensitivity | Positive Predictive | Negative Predictive | Specificity | Sensitivity | Positive Predictive | Negative Predictive |
|  |  |  |  | Possible Included as Negative IFD | Possible Excluded |
| Koltze | 34 | Prov/Prob IFD | 17.6 | 29% (13-49) | 83% (36-100) | 20% (7-41) | 89% (52-100) | 29% (13-49) | 83% (36-100) | 20% (7-41) | 89% (52-100) |
| Zhao | 130 | Prov/Prob IFD | 16.9 | 82% (74-89) | 82% (60-95) | 49% (32-66) | 96% (89-99) |  |  |  |  |
| Badiee | 62 | Prov/Prob IA | 16.1 | 52% (38-66) | 50% (19-81) | 17% (6-35) | 84% (67-95) | 46% (27-67) | 50% (19-81) | 26% (9-51) | 71% (44-90) |

Abbreviations: IFD – invasive fungal disease; IA – invasive aspergillosis; Prov – proven; Prob – probable; Poss – possible

**Table 4: Test Characteristics of Fungal Polymerase Chain Reaction in Blood in Children and Adolescents with Cancer and Hematopoietic Stem Cell Transplant Recipients**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| First Author | No. Patients  | IFD Definition Analyzed | Prevalence | Specificity | Sensitivity | Positive Predictive | Negative Predictive | Specificity | Sensitivity | Positive Predictive | Negative Predictive |
|  |  |  |  | Possible Included as Negative IFD\* | Possible Excluded |
| *Screening* |  |  |  |  |  |  |  |  |  |  |  |
| Badiee | 62 | Prov/Prob IA | 16.1 | 85% (72-93) | 80% (44-97) | 50% (25-75) | 96% (85-99) | 96% (80-100) | 80% (44-97) | 89% (52-100) | 93% (76-99) |
| Bialek | 17 | Prov/Prob/Poss IA  | 5.0 | 43% (18-71) | 67% (9-99) | 20% (3-56) | 86% (42-100) |  |  |  |  |
| Armenian | 68 | Prov/Prob/Poss IA  | 4.4 | 79% (61-91) | 11% (1-33) | 22% (3-60) | 60% (44-75) |  |  |  |  |
| *Diagnostic Test* |  |  |  |  |  |  |  |  |  |  |
| Lin | 42 (83 episodes) | Fungemia | 40.5 | 72% (51-88) | 100% (80-100) | 71% (49-87) | 100% (81-100) | 72% (51-88) | 100% (80-100) | 71% (49-87) | 100% (81-100) |
| El-Mahallawy | 91 | Prov/Prob IA | 30.8 | 83% (71-91) | 75% (55-89) | 66% (47-81) | 88% (77-95) | 92% (80-98) | 75% (55-89) | 84% (64-95) | 87% (74-94) |
| Dendis | 24 | Prov/Prob IFD | 20.8 | 74% (49-91) | 100% (48-100) | 50% (19-81) | 100% (77-100) | 74% (49-91) | 100% (48-100) | 50% (19-81) | 100% (77-100) |
| Cesaro | 62 | Prov/Prob IA | 12.9 | 37% (24-51) | 88% (47-100) | 17% (7-32) | 95% (76-100) | 37% (24-51) | 88% (47-100) | 17% (7-32) | 95% (76-100) |
| Hummel | 71 | Prov/Prob IA | 7.0 | 74% (61-84) | 80% (28-99) | 19% (5-42) | 98% (89-100) | 81% (64-93) | 80% (28-99) | 40% (12-74) | 96% (81-100) |
| Landlinger | 125 (150 episodes)  | Prov/Prob IFD | 6.7 | 57% (49-65) | 100% (69-100) | 14% (7-25) | 100% (95-100) | 64% (55-73) | 100% (69-100) | 19% (9-31) | 100% (95-100) |
| Mandhaniya | 29 | Prov/Prob IA | 3.4 | 36% (19-56) | 0% (0-98) | 0% (0-19) | 91% (59-100) | 33% (16-55) | 0% (0-98) | 0% (0-21) | 89% (52-100) |
| Reinwald | 95 (253 episodes) | Prov/Prob IA | 0.0 | 54% (43-64) | NC | 0% (0-8) | 100% (93-100) | 53% (39-66) | NC | 0% (0-13) | 100% (88-100) |

Abbreviations: IFD – invasive fungal disease; IA – invasive aspergillosis; Prov – proven; Prob – probable; Poss – possible; NC- not calculable

\* In studies in which possible IFD or IA were included as a case and data were not provided to permit re-calculation, possibles could not be included as a negative control (baseline analysis).

**Table 5: Pooled Sensitivity and Specificity for the Biomarkers Galactomannan, Beta-D-Glucan and PCR-based Assays in Children and Adults**

|  |  |  |
| --- | --- | --- |
|  | **Pediatrics\*** | **Adults** |
|   | Sensitivitiy | Specificity | Sensitivitiy1 | Specificity1 |
| **Galactomannan** |   |   |   |   |   |   |   |   |
|  Screening (n=5) | 68% | (51 to 81) | 91% | (86 to 94) |  |   |  |   |
|  Diagnostic Test (n=5) | 89% | (79 to 95) | 85% | (51 to 97) |  |   |  |   |
|  Total | 81% |  (69 to 89) | 88% | (75 to 95) | 82% | (73 to 90) [[39](#_ENREF_39)] | 81% | (72 to 90) [[39](#_ENREF_39)] |
|   |   |   |   |   |   |   |   |   |
| **Beta-D-Glucan** (n=2) | NA |  | NA |  | 76.8% | (67.1 to 84.3) [[40](#_ENREF_40)] | 85.3% | (79.6 to 89.7) [[40](#_ENREF_40)] |
|   |   |   |   |   |   |   |   |   |
| **PCR**  |   |  |   |   |  |   |  |   |
|  Screening (n=1) | NA |  | NA |  |  |   |  |   |
|  Diagnostic Test (n=6) | 76% | (62 to 86) | 58% | (42 to 72) |  |   |  |   |
|  Total |  |  |  |  | 80.5% | (73 to 86.3) [[41](#_ENREF_41)] | 78.5% | (67.8 to 86.4) [[41](#_ENREF_41)] |

\*NA too few studies to synthesize

**Figure 1**

Potentially relevant references identified (n=1,833)

Citations screened by title/abstract (n=1,532)

Full text references retrieved for detailed evaluation (n=55)

Duplicates removed (n=301)

Citations excluded as did not meet eligibility criteria (n=1,477)

Studies included in systematic review (n=25)

Excluded (n=30)

 Not a full text publication of a primary study (n=1)

 Not pediatric patients (age < 25 years) (n=9)

 Less than 50% of patients had cancer (n=7)

 Less than 5 patients (n=1)

 Number of patients with IFI not described (n=4)

 Does not evaluate one of the fungal biomarker tests (n=7)

 Not retrievable (n=1)

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