

# Performance of the galactomannan antigen detection test in the diagnosis of invasive aspergillosis in children with cancer or undergoing haemopoietic stem cell transplantation

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## Abstract

Serum galactomannan (GM) antigen detection is not recommended for defining invasive aspergillosis (IA) in children undergoing aggressive chemotherapy or allogeneic haemopoietic stem cell transplantation (HSCT). The ability of the GM test to identify IA in children was retrospectively evaluated in a cohort of children. Test performance was evaluated on samples that were collected during 195 periods at risk of IA. Proven IA was diagnosed in seven periods, all with positive GM test results (true positives, 4%), and possible IA was diagnosed in 15 periods, all with negative GM test results (false negatives, 8%). The test result was positive with negative microbiological, histological and clinical features in three periods (false positives, 1%), and in 170 periods it was negative with negative microbiological, histological and clinical features (true negatives, 87%). The sensitivity was 0.32 and the specificity was 0.98; the positive predictive value was 0.70 and the negative predictive value was 0.92. The efficiency of the test was 0.91, the positive likelihood ratio was 18.3, and the negative likelihood ratio was 1.4. The probability of missing an IA because of a negative test result was 0.03. Test performance proved to be better during at-risk periods following chemotherapy than in periods following allogeneic HSCT. The GM assay is useful for identifying periods of IA in children undergoing aggressive chemotherapy or allogeneic HSCT.

**Keywords:** Cancer, galactomannan antigen, haemopoietic stem cell transplantation, invasive aspergillosis, leukaemia, paediatrics

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## Introduction

Invasive aspergillosis (IA) is an infrequent, but severe, opportunistic infection in children undergoing antineoplastic chemotherapy or haemopoietic stem cell transplantation (HSCT) [1–13]. Serum galactomannan (GM) antigen detection has been suggested as a possible microbiological assay for defining probable cases of IA in immunocompromised hosts, but it is not recommended for defining IA in children

[14,15], because of the unreliability of the test in this patient population [14,16]. To date, only two paediatric series involving large numbers of patients or serum samples have been reported [17,18].

The aim of the present study was to evaluate the performance of GM antigen detection as a diagnostic tool for identifying IA during periods of risk for this complication in a cohort of children undergoing antineoplastic chemotherapy or HSCT.

## Materials and Methods

We retrospectively evaluated the clinical characteristics of a cohort of children receiving antineoplastic chemotherapy or undergoing HSCT at the Department of Paediatric Haemato-

logy and Oncology of the 'G. Gaslini' Children's Hospital, Genoa, Italy, and tested for the presence of serum GM antigen in the period 1999–2005. In this patient population, the test was routinely performed in all patients who presented at least one of the following clinical conditions associated with an increased risk of IA or a clinical picture suggestive of IA: (i) persisting febrile neutropenia (fever not responding to 5–7 days of broad-spectrum antibiotics in a persistently neutropenic subject); (ii) severe acute (grade  $\geq 2$ ) or chronic extensive graft-versus-host disease (GvHD); (iii) corticosteroid therapy at doses  $\geq 1$  mg/kg per day with prednisone or an equivalent for a period  $\geq 1$  week) clinical features suggestive of IA (sudden onset of clinical and radiological signs suggestive of aspergillosis) [15]. Because risk factors for IA may be present throughout the period of antineoplastic treatment or after allogeneic HSCT [19,20], we adopted a pragmatic approach, and considered the diagnosis of IA in different periods of risk, identified by the availability of at least two consecutive samples for GM testing within 1 week. Periods at risk occurring in the same patient were considered to be different if separated by more than 4 weeks. Periods of risk were then stratified according to the various treatment phases, as follows: (i) aggressive chemotherapy (including standard chemotherapy or megatherapy followed by autologous HSCT); and (ii) post-allogeneic HSCT. During the study period, all cases of invasive mycoses were prospectively collected and classified according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group criteria [14,15].

GM detection was performed with the Platelia Aspergillus test (Bio-Rad, Paris, France), following the manufacturer's recommendations. Results were expressed as an index of positivity. The test result was defined as positive when the GM index cut-off (GM-I = sample optical density/cut-off serum optical density) was  $>0.7$  with a single test (static cut-off), or between 0.5 and 0.7 with at least two consecutive serum samples (dynamic cut-off), according to the definitions adopted during the study period [21].

#### General management of patients

None of the patients received primary antifungal chemoprophylaxis against *Aspergillus*. The combination of a  $\beta$ -lactam and an aminoglycoside or monotherapy with a  $\beta$ -lactam active against *Pseudomonas* (including piperacillin–tazobactam) was used to treat infectious complications on the basis of clinical features and microbiological documentation. Weekly chest computed tomography scans were performed in persistently febrile neutropenic patients or in the presence of clinical signs of respiratory tract infection. Empirical antifungal therapy, mainly with liposomal amphotericin B, was administered to high-risk patients with persistent febrile neutropenia [22].

#### Statistical analysis

In this exploratory study, all cases of invasive disease (e.g. pneumonia) with absence of histological or microbiological documentation and negative GM test results, i.e. cases of 'possible' IA, were arbitrarily classified as 'false negatives', postulating the worst performance of the test. Histologically documented cases with identification of filamentous fungi never growing in culture and positive GM test results were classified as 'probable' IA, and cases of invasive disease without histological documentation and with a positive GM test result as the only 'mycological criterion' were excluded from the analysis. Finally, the clinical records of patients with a positive GM test result in the absence of clinical or radiological findings suggestive of IA were retrospectively evaluated in order to identify the presence of drugs, food or other conditions, such as intestinal GvHD, that might have affected the test results. Because of the possibility that the positive GM test result could, in any case, indicate an occult IA, the follow-up of these patients was prolonged until December 2007. As piperacillin–tazobactam has been reported to be the most frequent cause of false-positive GM test results, the false-positive rate of the test was calculated with inclusion and exclusion of periods with false-positive results attributable to piperacillin–tazobactam [17]. All of the analyses in which the test performance was evaluated were performed after exclusion of the periods with false-positive test results attributable to piperacillin–tazobactam. These included sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), efficiency (i.e. the correct classification rate), and likelihood ratio (i.e. the probability that the test results might increase or decrease the pre-test probability of the disease) [23–25]. We also calculated the probability of missing a diagnosis because of a negative GM result by applying the following equation:

$$\frac{\text{Prevalence} \times (1 - \text{sensitivity})}{\text{Prevalence} \times (1 - \text{sensitivity}) + [(1 - \text{prevalence}) \times \text{specificity}]}$$

Because this parameter, like the PPV and NPV, may vary with the prevalence of the disease [23,26], we also evaluated the PPV, NPV and probability of missing a diagnosis using the sensitivity and specificity that we obtained with our data and the lowest and highest prevalence of IA reported in children [1,3,5,8,10–13,17,27–29]. Statistical analyses were performed using InStat version 3.0a for Macintosh (GraphPad Software, San Diego, CA, USA), and the Dag\_Stat98.xls spreadsheet running on Microsoft Excel 2004 for Macintosh (Microsoft Corp., Seattle, WA, USA).

## Results

During the study period, 1812 serum samples for *Aspergillus* GM antigen detection were obtained from 119 patients with a median age of 9.5 years (range, 1 month to 20 years); 14 samples (0.8%) were excluded because they were not followed by another sample within 1 week, and therefore did not identify a period of risk. The remaining 1798 samples identified 210 periods of risk, with a median of one period per patient (range, 1–9). The median duration of these periods was 15 days (range, 2–193 days), with a median of five samples (range, 2–67) per period. Table 1 shows the number of periods of risk by treatment phase of the underlying disease. A proven/probable IA was diagnosed in seven periods (3%): two pneumonias due to *Aspergillus fumigatus*, and five pneumonias due to filamentous fungi never growing in culture, all with positive GM test results. The GM test also yielded positive results in four (2%) periods in the presence of clinical and radiological findings suggestive of pulmonary IA, but without any other microbiological or histological proof. Clinical and radiological features were suggestive of IA in 15 (7%) other periods, but no histological or microbiological proof could be obtained. A total of 40 samples (2.2% of all samples evaluated) with false-positive GM test results were observed in 14 (false-positive rate, 7%) periods occurring in 12 patients with a median age of 9 years (range, 2–17 years). Administration of piperacillin–tazobactam was identified as the cause of the false-positive test result in 11 of these, and their exclusion yielded a false-positive rate of 1.5%. The remaining three (1.4%) periods with a false-positive test result were observed in one patient with severe intestinal GvHD following allogeneic HSCT who was receiving total parenteral nutrition, but no oral feeding and no intravenous piperacillin–tazobactam. These three periods were interspersed with other periods of negative GM test results, for a total of 41 weeks of follow-up. The patient

underwent repeated extensive diagnostic and clinical work-ups, but IA was never documented. Five years after the last positive GM test result, the patient is alive, in good clinical condition, and has no IA. No sign of IA was observed in the other patients with false-positive GM test results during follow-up. Finally, no clinical or radiological signs suggestive of IA were present in 170 (81%) periods with negative GM test results.

The four periods with positive GM test results in the presence of clinical features suggestive of IA but in the absence of other microbiological or histological documentation, and the 11 periods of false-positive GM test results related to piperacillin–tazobactam, were excluded from all further analyses.

### Evaluation of the GM test performance for the diagnosis of IA

The performance of the GM test was therefore evaluated in 195 (93%) of the periods at risk, with seven proven/probable IA cases with positive GM test results (true positives, 4%), 15 possible IA cases with negative GM test results (false negatives, 8%), three periods with positive test results with negative microbiological, histological and clinical features (false positives, 1.5%), and 170 periods with negative test results and negative microbiological, histological and clinical features (true negatives, 87%). Table 2 summarizes the performance of the GM test by the treatment phases of the underlying disease. Overall, the test showed poor sensitivity (0.32), but good specificity (0.98), with similar results being obtained in the various phases of antineoplastic treatment. As regards the predictive values, the overall PPV proved to be acceptable (0.70), but it was poor (0.40) after allogeneic HSCT, and very high after aggressive chemotherapy (1.00). On the other hand, the NPV was always  $\geq 0.90$ , as was the efficiency of the test (Table 2). The overall positive likelihood ratio was 18.3, but it was not evaluable in periods following aggressive chemotherapy, because of the absence of

**TABLE 1.** Underlying diseases and phases of therapy in 210 periods of risk for invasive aspergillosis in 119 immunocompromised children

Underlying disease	No. of patients	Periods at risk	Phases of therapy	
			Aggressive chemotherapy	Allogeneic HSCT
Acute lymphoblastic leukaemia/non-Hodgkin's lymphoma	47	81	47	34
Acute non-lymphoblastic leukaemia	26	56	34	22
Juvenile chronic lymphoblastic leukaemia/myelodysplasia	10	15	1	14
Solid tumour	23	34	28	6
Non-malignant haematological disease	13	24	0	24
Total	119	210	110	100

HSCT, haemopoietic stem cell transplantation.

**TABLE 2.** Performance of the galactomannan antigen test during periods of risk for invasive aspergillosis stratified according to various treatment phases (false-positive rate, 1.5%)

Phase of antineoplastic treatment and number (n) of at-risk periods	True positive (%)	False positive (%)	False negative (%)	True negative (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Efficiency (95% CI)	Likelihood ratio	
										Positive (95% CI)	Negative (95% CI)
Overall, n = 195	7 (4)	3 (1)	15 (8)	170 (87)	0.32 (0.14–0.55)	0.98 (0.95–1.00)	0.70 (0.35–0.93)	0.92 (0.87–0.95)	0.91 (0.86–0.94)	18.3 (5.1–65.8)	1.4 (1.1–1.9)
Aggressive chemotherapy, n = 107	5 (5)	0 (0)	10 (9)	92 (86)	0.33 (0.12–0.62)	1.00 (0.96–1.00)	1.00 (0.48–1.00)	0.90 (0.83–0.95)	0.91 (0.83–0.95)	#DIV/0!	1.5 (1.0–2.1)
Allogeneic HSCT, n = 88	2 (2)	3 (3)	5 (6)	78 (89)	0.29 (0.04–0.71)	0.96 (0.90–0.99)	0.40 (0.05–0.85)	0.94 (0.86–0.98)	0.91 (0.83–0.96)	7.7 (1.5–38.7)	1.3 (0.8–2.1)

HSCT, haemopoietic stem cell transplantation; #DIV/0!, division by 0; NPV, negative predictive value; PPV, positive predictive value.

false-positive test results in this subgroup, and it was found to be acceptable (7.7) after allogeneic HSCT. Finally, the probability of missing a diagnosis because of a negative GM test result was 0.03 (with a 4% prevalence of IA). In particular, it was 0.03 after aggressive chemotherapy (5% prevalence of IA) and 0.01 after allogeneic HSCT (2% prevalence of IA).

In a further analysis, we calculated the probability of missing a diagnosis because of a negative GM test result and the predictive values of the test according to the prevalence of IA reported in children with cancer (Table 3). Even in this analysis, the test presented an overall very good NPV (with the lowest value, 0.84, after allogeneic HSCT), although not a good PPV. The risk of missing a case because of a negative GM test result was highest after allogeneic HSCT in the presence of the highest prevalence of IA.

## Discussion

We evaluated the performance of the *Aspergillus* GM test for the diagnosis of IA on 1812 serum samples obtained from 119 children undergoing aggressive chemotherapy or allogeneic HSCT during 210 periods of risk for this complication.

Overall, the performance of the test was found to be good with regard to specificity and NPV, with high values and narrow 95% CIs, but the specificity and PPV were not good, with low values and wide 95% CIs. These results are consistent with other studies in both children and adults [17,18]. A recent, wide meta-analysis of the performance of the GM test in the diagnosis of IA in immunocompromised patients [23] revealed worse results in children than in adults. However, this meta-analysis included few paediatric studies, with low numbers of children enrolled in all but one of them [18]. This may have biased the results, especially considering the low prevalence of IA in children with cancer or undergoing allogeneic HSCT [30]. Our results seem to confirm this hypothesis, even if it must be noted that, in general, the performance of the test was better in periods following aggressive chemotherapy than in those following allogeneic HSCT, even though specificity and NPV were always very good, with high mean values and narrow 95% CIs. We also evaluated the likelihood ratio of the test, i.e. the probability that the test result will increase or reduce the pretest probability of the target disease [25]. Also, the usefulness of this test is affected by the prevalence of the disease. The prevalence of proven IA among our patients (i.e. the pre-test probability) was only 4%. Overall, the positive likelihood ratio was high (18.3), indicating the relevance of the test for the diagnosis of IA, and even though its 95% CI was wide, the fact that the lower limit was 5.1

**TABLE 3. Test performance and probability of missing a diagnosis for various prevalences of invasive aspergillosis (IA) (false-positive rate, 1.5%)**

Data source	Sensitivity (95% CI)	Specificity (95% CI)	Range of prevalence of invasive aspergillosis (as from [1,7,8,10,11,14,19,21,22,24,28-30]) (%)	PPV (95% CI)	NPV (95% CI)	Probability of missing a diagnosis
GCH overall <sup>a</sup>	0.32	0.98	1	0.16	0.99	0.01
Aggressive chemotherapy	0.33	1.00	20	0.82	0.85	0.12
Allogeneic HSCT	0.29	0.96	4	#DIV/0!	0.99	0.01
			4	#DIV/0!	0.97	0.03
Meta-analysis, aggressive chemotherapy (95% CI) (28) <sup>b</sup>	0.70 (0.62-0.77)	0.92 (0.88-0.99)	20	0.60	0.84	0.12
Meta-analysis, allogeneic HSCT (95% CI) (28) <sup>b</sup>	0.82 (0.70-0.90)	0.86 (0.83-0.88)	5	0.31 (0.28-0.35)	0.98 (0.97-0.99)	-
Meta-analysis, paediatric data (95% CI) (28) <sup>b</sup>	0.89 (0.51-1.00)	0.85 (0.85-0.89)	20	0.69 (0.65-0.72)	0.91 (0.89-0.92)	-
Paediatric allogeneic HSCT (95% CI) (30) <sup>b</sup>	0.29 (-0.04-0.62)	0.73 (0.46-0.99)	0	0.32 (-0.23-0.86)	0.99 (0.94-1.00)	-

GCH, Gaslini Children's Hospital; HSCT, haemopoietic stem cell transplant; #DIV/0!, division by 0; NPV, negative predictive value; PPV, positive predictive value.  
<sup>a</sup>PPV and NPV were calculated using prevalence data reported in the literature.  
<sup>b</sup>PPV and NPV were calculated according to the prevalence of IA reported in the specific study.

indicates that a positive test result increases by at least five times the pre-test probability that IA is present [25]. However, again, in periods following HSCT the test showed the worst performance, as a 7.7 likelihood ratio is considered to give a moderate shift from pre-test to post-test probability, with a wide 95% CI with the lower limit approaching the value of 1 [25]. Taking into account that the low prevalence of IA in children may influence studies on the performance of the GM test, we also evaluated the performance of our results using the prevalence of IA reported in the most recent paediatric literature, but the results did not change significantly. Finally, we also calculated the probability of missing an episode of IA because of a negative GM test result, considering both the prevalence that we observed in our study and the prevalence reported in the paediatric literature. This probability was low in patients receiving aggressive chemotherapy, whereas it increased in the presence of the highest prevalence of IA, especially after allogeneic HSCT. This is a peculiar aspect of our study that has never been analysed in this setting [17,23], and our results cannot therefore be compared with others, but may only be used for future comparisons.

One last comment concerns the false-positive results. After exclusion of periods with false-positive results clearly attributable to piperacillin-tazobactam [17,23], some 'unexplained' false positives were still observed in risk periods following allogeneic HSCT. This aspect has already been reported and attributed, at least partially, to the presence of GvHD [16,31,32]. Moreover, dietary contamination by GM (especially formulated milk) has been indicated as another possible cause of false-positive results in children [16,31,33,34]. This aspect should be taken into account in evaluating any individual patient, even though oral feeding is not common in children during the phases of neutropenia, because of the frequent occurrence of severe mucositis, or when severe intestinal GvHD is present. In our series, repeated periods of false-positive results were observed only in one allogeneic HSCT recipient with severe intestinal GvHD receiving total parenteral nutrition and no oral feeding at the time of the false-positive results [35]. This case must be considered to be a true false-positive result, as the patient never developed IA during a 5-year follow-up period, and it confirms the possible role of intestinal GvHD as a cause of false-positive GM antigen test results after allogeneic HSCT, but not the possible role of oral feeding.

In conclusion, the present study, together with other large paediatric studies [17,18], suggests that the GM test may be useful for the diagnosis of IA in children receiving aggressive antineoplastic chemotherapy or undergoing allogeneic HSCT,

and that the GM test should be included in the diagnostic criteria for IA in these patient populations.

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## Transparency Declaration

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