Serum and urine galactomannan testing for screening in patients with hematological malignancies

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Abstract

Testing for serum galactomannan (GM) has been established as an important method for diagnosing invasive aspergillosis (IA); however, limited data exist regarding the application of urine GM testing. The objective of this study was to evaluate the performance of GM screening of urine specimens and to compare results with serum GM. The study was performed between July 2012 and March 2013 in adult patients with underlying hematological malignancies who were hospitalized at the Medical University of Graz, Austria. Serum and urine screening samples were collected and tested twice weekly (always on the same day). In total, 242 serum samples and a similar number of urine samples were collected from 75 patients. A total of 21/242 (8.7%) serum samples from 13 patients were GM positive. Sensitivity, specificity, positive predictive value, and negative predictive value using a 0.1 optical density index cutoff for urine samples (compared with same-day serum results) were as follows: 47.6%, 86%, 24.4%, and 94.5%, respectively. In 8/10 patients with probable IA, at least one positive GM result was found with this cutoff. After calculating clinical performance of the urine GM test, we found that sensitivity increased to 71.4% and specificity to 88.2%. Spearman–Rho correlation analysis revealed a significant positive correlation between serum and urine samples ($P < 0.001; \rho = 0.252$).
In conclusion, GM detection in urine might be a promising method for IA screening. However, further studies are needed.

**Key words:** invasive aspergillosis, hematological malignancy, galactomannan testing, Serum, urine.

### Introduction

Galactomannan (GM) is a polysaccharide component of the cell wall of *Aspergillus* spp. and other closely related filamentous fungi. GM is hematogenously released by fungal hyphae during invasive growth. GM detection using a Platelia *Aspergillus* antigen test has proven to be useful for diagnosis of invasive aspergillosis (IA). IA accounts for the majority of invasive mould infections (IMIs) in patients with hematologic malignancies [1–5]. GMs have also been shown to be useful for early response assessment and therapy monitoring of patients with IA [6].

In contrast to bronchoalveolar lavage (BAL) and serum GM testing, data are limited on the performance of GM testing with urine samples [7]. Urine specimen testing may provide a number of advantages, including noninvasive and easy sample collection, development of point-of-care (POC) tests including home testing for urine GM, and, in contrast to serum and BAL, more frequent examination of large volumes, which may increase sensitivity of the GM test [8].

To date, however, only a handful of researchers have evaluated the performance of the GM test for urine specimens. As early as 2004, Klont and others discussed promising results [8–12]. The authors summarized a number of small studies that reported on GM detection in urine, indicating that at least a fraction of the circulating GM is cleared renally. More recently, Garcia and colleagues reported on an extremely promising performance of urine GM testing in dogs with IA where GM detection in urine specimens had a sensitivity of 88% and a specificity of 92% for diagnosing IA [13]. Another study confirmed detectable antigen in the urine from children with IA [14]. Very recently, Kieren Marr’s group published a paper about the development of a POC test for a novel immunoglobulin M monoclonal antibody (MAb476) that recognizes GM-like antigens from *Aspergillus* and other moulds in urine specimens [15].

Our aim in this study was to evaluate the performance of the GM test using urine specimens and to compare results with those obtained using serum.

### Methods

The study was performed between July 2012 and April 2013 in adult patients with underlying hematological malignancies hospitalized at the Medical University of Graz, Austria.

Patients aged >18 years at risk for IMI and who were receiving routine GM serum screening were identified and screened using information from clinical rounds, chart reviews, and surveys of electronic documents including microbiological test results. Patients’ medical records were reviewed individually using a standardized data collection template in order to obtain demographic information, clinical data on outcomes of therapy and adverse events, and mycological laboratory test results. Serum and urine samples were collected and tested twice weekly (always on the same day). Serum and urine GM concentrations were determined using the Platelia enzyme immunoassay (EIA; Bio-Rad Laboratories, Marnes-la-Coquette, France). We used a cutoff optical density index (ODI) of 0.5 for serum samples for positivity and evaluated different cutoffs for urine samples; optimal cutoffs have not yet been defined and standardized for the latter [2].

Six milliliters of urine were collected in sterile urine tubes from midstream urine on the morning of the days of GM screening. The samples were tested for GM within 24 h at the same time as serum samples. GM testing was performed using native urine after vortexing but without any pretreatment.

The study adhered to the 1996 Declaration of Helsinki, Good Clinical Practice, and the study protocol was approved by the local ethics committee of the Medical University of Graz (protocol number 23–343). Informed consent was obtained from all participating patients. Invasive fungal infection (IFI) was defined according to the consensus definitions provided by the European Organization for Research and Treatment of Cancer Invasive Fungal Infections Cooperative Group and the Mycoses Study Group of the National Institute of Allergy and Infectious Disease [16,17].

All statistical analyses were performed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, IL, USA). Urine GM values were compared with serum GM values obtained on the same day for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) at different cutoffs. The optimal cutoffs for urine samples were defined using the diagnostic odds ratio (DOR) method. All DOR values were displayed with 95% confidence intervals (CIs). The correlation between serum and urine GM results was calculated using Spearman–Rho correlation analysis.
Table 1. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic odds ratio for different galactomannan (GM) cutoffs for urine samples compared with same-day GM serum results.

<table>
<thead>
<tr>
<th>Urine galactomannan cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Diagnostic odds ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 ODI</td>
<td>0.19</td>
<td>0.937</td>
<td>0.222</td>
<td>0.924</td>
<td>3.48 (1.03–11.74)</td>
</tr>
<tr>
<td>0.15 ODI</td>
<td>0.333</td>
<td>0.905</td>
<td>0.25</td>
<td>0.935</td>
<td>4.76 (1.73–13.11)</td>
</tr>
<tr>
<td>0.1 ODI</td>
<td>0.476</td>
<td>0.86</td>
<td>0.244</td>
<td>0.945</td>
<td>5.57 (2.18–14.22)</td>
</tr>
</tbody>
</table>

ODI, optical density index.

Results

A total of 242 serum and urine samples (median, 3 samples; range, 1–11 samples) were collected from 75 patients (46 males, 29 females; median age, 57 years, range, 21–83 years). Acute myeloid leukemia was the underlying disease observed most frequently (39/75 patients) followed by seven cases each of myelodysplastic syndrome, acute lymphocytic leukemia, and multiple myeloma, and seven patients with non-Hodgkin lymphoma. A total of 33/75 patients had undergone recent allogeneic stem cell transplantation and 62/75 were neutropenic at least once during hospitalization. A little less than half of serum and urine samples (100/242, 41%) were obtained during neutropenia (<0.5 × 10⁹/ml). Forty-one of 75 patients (55%) received antifungal prophylaxis (primarily mould active) at some point.

Twenty-one of 242 (8.7%) serum samples from 13 patients were GM positive, 10 of which had probable IA. Sensitivity, specificity, PPV, NPV, and DOR for different cutoffs for urine samples (compared with serum GM results) are included in Table 1.

A cutoff of 0.1 ODI was chosen for urine samples. Using this cutoff, 8/10 patients with probable IA had at least one positive GM result. In four of these eight patients, urine and serum samples became GM positive on the same day; in one patient, the urine sample became positive 3 days before the serum sample. In three other patients, serum samples were positive 1 (in two patients) or 2 weeks (one patient) before urine results were positive.

Overall, 11/21 patients with positive serum GM results had negative urine specimens for GM. Four of these samples were derived from a patient with probable IA and positive serum GM levels, with urine samples becoming positive after a 2-week delay (Fig. 1). These four and another two urine samples (obtained from patients with probable IA) were also clinically considered to be false negatives. In two probable IA cases, initially positive urine samples became negative as patients received appropriate antifungal therapy, while decreasing serum samples remained positive.

Urine samples were negative in three positive serum samples that were considered to be false positives (all obtained from patients who did not meet the criteria of IFI).

Urine samples were positive in 31/221 negative serum GM samples; 26 of these were considered potential false positives. Twelve of these 26 samples were obtained from patients with possible IFI; however, 14 were from patients who did not meet the criteria of IFI. In one patient (probable IA), urine GM results were positive 3 days before serum GM (Fig. 2). In two probable IA cases, urine remained GM positive while the patient underwent appropriate antifungal therapy; the serum samples were again negative. In an additional two cases of probable IA, urine became positive after a 1-week delay (compared with serum samples), when serum samples were already negative again with patients receiving appropriate antifungal therapy.

When calculating the clinical performance of the urine GM test with a cutoff of 0.1 (6 false negatives, 26 potential false positives), sensitivity was 71.4%, specificity 88.2%, PPV 36.6%, NPV 97%, and DOR 18.75 (95% CI, 6.68–52.59). If one considers that those 12/26 “false-positive” GM urine test results that occurred in patients with possible IFI may in fact be real positives, clinical specificity would increase to 94% and PPV to 52%.

The Spearman–Rho correlation analysis revealed a significant positive correlation between serum and urine samples (P < 0.001; ρ = 0.252).

Discussion

We evaluated the use of GM testing of urine specimens from patients with underlying hematological malignancies and found that GM detection in urine might be a promising method for IA screening, with a high clinical NPV of 97%.

GM was noted as being present in urine from animals with IA [10,11,13]. In a recent study, Fisher and colleagues evaluated urine GM screening in pediatric hematologic patients. Not only did they report a positive urine GM result (threshold 0.5 ODI) in samples from the only
Figure 1. Patient with probable invasive Aspergillosis and delayed positive urine galactomannan results. Urine galactomannan (cutoff 0.1 U/l) and serum galactomannan results (cutoff 0.5U/l) graphed relative to neutropenia, date of diagnosis of disease, and receipt of antifungals. EIA, enzyme immunoassay; GM, galactomannan; ODI, optical density index.

patient with probable IA observed during the study period, but also a considerable number (about 20%) of false positive urine GM results [14]. Before our study, only a small number of samples from adult patients had been evaluated, and results suggest that the test may be useful, especially if the urine is pretreated using dialysis, centrifugation, or filtration [8]. As in Fisher’s study, we did not process our urine samples before testing. Nonetheless, our results suggest that GM testing of urine may be useful when screening for early IA, and this warrants further investigation. In three of the eight patients with probable IA that had positive serum and urine GM test results, serum specimens were positive first, while in one patient the urine sample was positive prior to the serum sample. Due to the small numbers in our study, these results are difficult to interpret, and larger sample sizes from probable or proven IA cases are needed to further evaluate this issue. Because urine specimen collection does not require invasive procedures, it seems logical to use this type of specimen for antigen detection. Urine is also well suited for POC testing, as shown recently by Dufresne and colleagues who developed a POC test for MAb476, which recognizes GM-like antigens from Aspergillus and other moulds in urine specimens. These researchers established that POC diagnosis of IA based on urinary GM detection is feasible [15].

Diagnostic test performance calculations in the field of IA are limited by the insensitivity of all current diagnostics (including serum GM EIA). Imperfect gold standards are therefore the largest issue. It should therefore be acknowledged that use of the GM EIA serum value as the gold standard may not be appropriate for calculating the performance of the urine assay. The biology of both tests may differ—urine values may become positive because of clearance, while serum levels remain negative and cases with positive urine and negative serum samples may in fact be true positives. When we excluded the “false positive” GM urine test results that occurred in patients with possible IFI, clinical specificity of urine GM increased to 94%.

For the first time, GM screening in urine specimens was evaluated in adult patients with hematological malignancies. Still, the optimal approach for processing urine specimens before GM testing as well as the optimal cutoff have yet to be defined. Also, we do not know how conventional
GM testing of urine specimens compares with the MAb476 POC test. Prospective studies with larger sample sizes, in particular of GM-positive samples, will be necessary before urine testing can be established in clinical routine.

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**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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