Aspergillus galactomannan testing in patients with long-term neutropenia: implications for clinical management

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We carried out a prospective study on galactomannan enzyme immuno assay (GEI) (Platelia™ Aspergillus EIA, Bio-Rad) testing for diagnosis of invasive aspergillosis (IA) in serum and broncho-alveolar lavage (BAL) in 200 patients with hematological malignancies and profound neutropenia. The incidence of proven and probable IA was 6% and 5.5%, respectively. In patients with fever or pneumonia, a single-positive GEI test result (galactomannan index ≥ 0.5) had excellent specificity (100%). Sensitivity was relatively low (40%) at onset of fever, but increased to 94.7% after 6 days of fever. In patients with infiltrates in chest X-ray or computed tomography scan (n = 48), GEI testing in BAL had a favorable diagnostic accuracy as compared with GEI testing in serum (sensitivity 100% versus 71%). Our findings indicate that antifungal therapy should be started immediately at onset of fever in neutropenic patients with positive GEI tests. In patients with fever refractory to broad-spectrum antibiotics (≥ 6 days of fever), the high diagnostic accuracy makes GEI testing a valuable diagnostic tool and questions the common strategy to carry out antifungal treatment irrespective of diagnostic testing in this situation. Our data also show that GEI testing in BAL can be useful for early diagnosis of IA in patients with hematological malignancies and pulmonary infiltrates.

Key words: antigen, Aspergillus, galactomannan, infection, neutropenia

introduction

Invasive aspergillosis (IA) is a major cause of morbidity and mortality in patients with hematologic malignancies undergoing intensive cytotoxic therapy or hematopoietic stem-cell transplantation (HSCT). Due to the increasing use of high-dose chemotherapy and the application of immunosuppressive agents, such as antilymphocyte antibodies, the incidence of IA is currently rising up to 30% in some centers [1–7]. Early diagnosis of IA is generally considered to be mandatory to increase survival. Clinical symptoms and radiological signs of IA, however, are mostly nonspecific and occur late in the course of the disease, making early clinical and radiological diagnosis almost impossible.

The only laboratory test with proven utility for detection of IA, currently commercially available, is the galactomannan enzyme immuno assay (GEI) (Platelia™ Aspergillus EIA, Bio-Rad, Marnes La Coquette, France). The reported sensitivity and specificity for diagnosis of IA have been highly variable ranging from 30%–100%, depending on the patient population, diagnostic procedures and the definitions for IA which were used [8–11]. Of note, there is little knowledge on the diagnostic accuracy of the GEI test in relevant clinical situations: how is the positive predictive value (PPV) and negative predictive value (NPV) of a GEI test result when a patient gets fever during neutropenia and when fever persists after antibiotic therapy? How is the diagnostic accuracy to distinguish between Aspergillus infection and other pathogens when patient has infiltrates in chest X-ray or computed tomography (CT) scan?

Currently, there is a debate in the scientific community on the value of GEI testing in broncho-alveolar lavage (BAL). Recently, promising data were published in recipients of a solid organ transplant with pneumonia [12, 13]. The diagnostic accuracy of GEI testing in BAL of neutropenic patients with hematologic malignancies, however, has not yet been addressed in prospective studies.

Recently, a higher sensitivity of the GEI test was obtained by lowering the cut-off level of the absorbance (A) index [14]. Probably, a further improvement could be obtained by optimizing the frequency of blood sampling. To be able to determine the optimal frequency of blood sampling, it is
desirable to obtain more information on the kinetics of galactomannan (GM) levels in neutropenic patients with IA.

In order to help optimizing the performance and timing of GEI testing, we planned a prospective study on GEI testing in serum and BAL in patients with hematological malignancies and profound neutropenia, who are at high risk for IA.

patients and methods

participants

All patients were recruited and treated at the Department of Hematology, Oncology and Transfusion Medicine in the Charité Campus Benjamin Franklin. Adult patients (≥18 years) with hematological malignancies, receiving chemotherapy likely to induce long-term neutropenia (neutrophil count, <500/mm³ for >10 days) or undergoing HSCT were eligible. Subjects were ineligible for inclusion, if they had evidence or history of probable or proven IA. Patient’s characteristics are detailed in Table 1.

objectives

The objective of this trial was to obtain better knowledge about GEI test results in neutropenic patients in different clinical situations in order to optimize performance and timing of GEI testing.

outcomes

Outcome measurements were the number and kinetics of positive GEI test results during neutropenia. Data were correlated to the presence of probable or proven IA, pneumonia, onset of fever and fever resistant to broad-spectrum antibiotics.

standardized diagnosis and treatment

According to the standard clinical protocol in our institution, all patients received oral amphotericin B solution four times daily (4 × 200 mg/day) plus antibacterial prophylaxis with 500 mg/day levofloxacin orally. Patients were examined daily for clinical signs of IA. Surveillance cultures for fungal organisms (throat, urine and stool) were carried out before neutropenia, once weekly thereafter and after neutropenia. Laboratory tests (e.g. whole blood count and serum creatinine level) were carried out at least every other day. Two separate sets of blood cultures plus a urine culture were obtained in all patients who developed fever. A chest X-ray was carried out 1–3 days before neutropenia and at onset of fever. In all patients with atypical infiltrates in the chest X-ray, high-resolution computed tomography scan (HRCT) and bronchoscopy were carried out. Patients without lung infiltrates but persisting fever despite broad-spectrum antibiotic therapy underwent HRCT within 5 days after the onset of fever.

Patients with neutropenia and fever of unknown origin (FUO) immediately received broad-spectrum antibiotics. All patients without known hypersensitivity to β-lactam antibiotics received piperacillin/tazobactam (3 × 4.5 g/day). Unexplained fever or FUO is defined as a new fever not accompanied by clinical or microbiological evidence of infection: single incident of fever (oral temperature) without any evident cause, temperature 38.3 or 38.0°C lasting for at least 1 h or measured twice within 12 h. Treatment success was reevaluated after 3 days. In case of defervescence, the regimen was continued for 7 days in total. In case of persistent fever, antibacterial therapy was changed. A glycopeptiude drug was added in patients with severe mucositis or suspected central venous catheter infection. Systemic antifungal treatment was given in case of persistent fever after 4–6 days therapy with broad-spectrum antibiotics or when invasive fungal infection (IFI) (possible, probable or proven) occurred.

definitions

Criteria used to define IA were those of the Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer (EORTC) and the Infectious Diseases Mycosis Study Group (MSG) of the National Institute of Allergy [15]. In brief, there are three categories of IFI: proven, probable and possible. The proven category consists of criteria that allow IFI to be diagnosed with certainty and that differentiate between deep-tissue infections and fungemia. For a case of IFI to be considered probable, each of the three elements of host factor, clinical features and mycological evidence has to be present. By contrast, a patient who has at least one criterion from the host factors category but who does not have clinical features or mycological evidence can be classified only as possible. The GEI test was considered to be positive when the A index exceeded 0.5 in serum samples. Fever was defined as a single oral temperature ≥38.3°C or two consecutive oral temperatures ≥38°C.

GEI testing

Blood sampling for GEI testing was carried out every Monday and Thursday during neutropenia. Blood was drawn from the central venous line in all patients before administration of i.v. drugs in the morning. Separate tubes of the central venous line were used for blood sampling and for administration of i.v. drugs. Sampling of BAL was carried out whenever patients underwent diagnostic bronchoscopy. The Platelia™ Aspergillus EIA assay (Bio-Rad) was carried out as described in the package insert of the kit.

statistical analysis

Calculations were carried out using commercially available SPSSWIN (12.0) and PRISM (4.0c) software. Descriptives include absolute and relative frequencies for categorical data, median and range for numerical measurements. Due to the skewed distribution of GM indices, confirmatory comparison between patients recovering from IA and patients dying from IA was carried out using Mann–Whitney’s U test. Diagnostic accuracy of GEI testing with regard to fever, fever resistant to broad-spectrum antibiotics and pneumonia has been quantified in terms of PPV, NPV, sensitivity and specificity.

results

Patient characteristics

Two hundred patients were recruited. Patient characteristics are summarized in Table 1.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n = 200 patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years median (range)</td>
<td>52 (23–74)</td>
</tr>
<tr>
<td>Underlying disease</td>
<td></td>
</tr>
<tr>
<td>AML/MDS</td>
<td>120</td>
</tr>
<tr>
<td>ALL</td>
<td>24</td>
</tr>
<tr>
<td>NHL</td>
<td>56</td>
</tr>
<tr>
<td>Male : female ratio</td>
<td>96 : 104</td>
</tr>
<tr>
<td>Duration of neutropenia (days)</td>
<td>14 (4–51)</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>147</td>
</tr>
<tr>
<td>Autologous transplant</td>
<td>25</td>
</tr>
<tr>
<td>Allogeneic transplant</td>
<td>28</td>
</tr>
</tbody>
</table>

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin’s lymphoma.
incidence of IA
The incidence of IA according to the EORTC–MSG criteria for proven IA, probable IA and possible IA was 6%, 5.5% and 13%, respectively. Not counting the GEI test results as microbiological criterion for definition of IA, the incidence of proven IA, probable IA and possible IA was 6%, 2.5% and 15.5%, respectively.

diagnostic accuracy of GEI testing in serum
We found an overall high diagnostic accuracy of the maximum positive GEI test result of each patient for prediction of IA in our patient population. Details on PPV, NPV, sensitivity and specificity are given in Table 2.

GEI testing at time points of given clinical situations
An overview of the diagnostic accuracy in frequent clinical situations is given in Table 3.

GEI testing at onset of fever
In 126 patients, GEI testing was carried out at onset of fever. There were 118 negative and 8 positive results. All eight patients with positive results were diagnosed as probable or proven IA later on. Twelve of the 118 patients with negative results, however, were diagnosed as probable or proven IFI later on.

GEI testing after 3 days fever resistant to broad-spectrum antibiotics
Seventy-six patients had persistent fever after 3 days of therapy with broad-spectrum antibiotics. In 18 of those patients, GEI test results were positive and in 58 patients results were negative after 3 days of fever. All patients with positive results were diagnosed as probable or proven IA. Of the 58 patients with negative test results, 3 patients developed IA afterwards.

GEI testing after 6 days fever resistant to broad-spectrum antibiotics
Fifty-five patients had persistent fever after 6 days of therapy with broad-spectrum antibiotics. In 21 of those patients, there were positive GEI test results and in 34 patients results were negative after 6 days of fever. All patients with positive results were diagnosed as probable or proven IA. Of the 34 patients with negative test results, 2 patients became IA later on.

GEI testing at diagnosis of pulmonary infiltrates in chest X-ray or HRCT scan
Forty-eight patients had pulmonary infiltrates in chest X-ray or HRCT scan during neutropenia. Eighteen of those patients had positive GEI test results in serum at diagnosis of pulmonary infiltrates and 30 had negative results. All 18 patients with positive test results were diagnosed as probable or proven IA. Of the 30 patients with negative GEI results, 5 developed IA.

correlation of GM indices and kinetics to the clinical course of IA
We found considerable differences of maximum GM indices and their kinetics between different patients. There was, however, a correlation of GM indices in peripheral blood and patients clinical course in patients with probable or proven IA. Median maximum GM indices were 1.7 (range 0.6–3.9) and 9.5 (range 2.9–22.8) in patients who recovered and died from IA, respectively \( (P = 0.002, \text{Figure } 1) \). All patients who died due to IA had GM indices >2.5, whereas only 3 of the 18 patients who recovered had GM indices >2.5 and none of them had indices >4.5.

In most patients with IA, the serum GM indices began to rise at onset of fever (Figure 2). Given notable differences between individuals, the increase of GM indices tended to be faster in patients who finally died from infections than in recovering patients (Figure 2). The response to therapy was correlated to

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### Table 2. PPV, NPV, sensitivity and specificity to predict proven or probable IA of the maximum positive GEI test result of each patient

<table>
<thead>
<tr>
<th>Condition</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exclusion of GEI for definition of IA</td>
<td>82.1%</td>
<td>60.7%</td>
<td>100%</td>
<td>97.2%</td>
</tr>
<tr>
<td>Incidence of proven/probable IA</td>
<td>23 patients (11.5%)</td>
<td>17 patients (8.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>82.1%</td>
<td>60.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPV</td>
<td>100%</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>97.2%</td>
<td>93.8%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Separate analyses were carried out using the EORTC–MSG criteria to define IA and excluding the GEI results for definition of IA.

### Table 3. Diagnostic accuracy of the GEI test for prediction of probable or proven IA (according to the EORTC–MSG criteria)

<table>
<thead>
<tr>
<th>Clinical situation</th>
<th>Number of patients</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of fever</td>
<td>126</td>
<td>100</td>
<td>89.8</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>After 3 days of fever</td>
<td>76</td>
<td>100</td>
<td>95</td>
<td>84.2</td>
<td>100</td>
</tr>
<tr>
<td>After 6 days of fever</td>
<td>55</td>
<td>100</td>
<td>97.3</td>
<td>94.7</td>
<td>100</td>
</tr>
<tr>
<td>At diagnosis of pneumonia</td>
<td>48</td>
<td>100</td>
<td>81.8</td>
<td>71.4</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are shown for a single-positive GEI test result at onset of a specified clinical situation.

GEI, galactomannan enzyme immuno assay; IA, invasive aspergillosis; EORTC, European Organization for Research and Treatment of Cancer; MSG, Mycosis Study Group; PPV, positive predictive value; NPV, negative predictive value.
decreasing GM in peripheral blood, regardless of the type of antifungal agent given.

GEI testing in BAL

In 45 of the 48 patients with pulmonary infiltrates in chest X-ray or HRCT scan, bronchoscopy and GEI testing in BAL was carried out. Twenty-three patients had positive test results and 22 had negative. Six patients with positive GM testing in BAL had a negative fungal culture. When GEI testing was not used to define IA, the PPV, NPV, sensitivity and specificity of GEI testing in BAL to predict probable or proven IA accounted for 73.9%, 100%, 100% and 78.6%, respectively. According to the EORTC–MSG criteria, the PPV, NPV, sensitivity and specificity were 100%, 100%, 100% and 100%, respectively. All patients with probable or proven IA had also positive serum GM results in the course of the disease. GEI test results, however, were higher in BAL than in serum (median 5.3 (range 0.7–23.5) versus 1.4 (range 0.2–10.5), Figure 3, \( P = 0.02 \)). Of note, all patients with probable or proven IA \((n = 23)\) had positive GEI tests in BAL when pneumonia was diagnosed. In contrast, only 18 of those patients (71%) had positive GEI tests in serum at this time point.

**discussion**

In our patient population, we found that maximum GM indices in serum had a high sensitivity and specificity for prediction of IA, which is in line with observations of other groups [8–11]. Analyzing the diagnostic accuracy by only correlating the maximum GM index of each patient to the final diagnosis, however, does not reflect the actual clinical situation. In the clinical practice of patient care, the probability of IA has to be judged on the basis of risk factors, patient’s symptoms, radiology and laboratory testing. Therefore, we carried out separate analyses of GEI tests taken at onset of fever, when fever was refractory to antibiotics and when the patient had infiltrates in the chest X-ray or HRCT scan and focused exclusively on patients at very high risk for IA. Importantly, the sensitivity was relatively low at onset of fever, but became higher when patients had persisting fever after therapy with broad-spectrum antibiotics or when they developed pneumonia. We found that the specificity was excellent in all those critical clinical situations. This is particularly interesting, given that we used a relatively low cut-off level for the GEI test \((GM \text{ index} \leq 0.5)\). Consequently, our results support the view that a lower cut-off level \((e.g. A \text{ index} = 0.5)\) is superior to the relatively high cut-off level \((A \text{ index} = 1.5)\), which was recommended in the past. Our findings are in line with a recent publication on prospective testing of low GM index cut-off levels in adult neutropenic cancer patients [16].

Our findings might have implications for a change of the current standard clinical management of neutropenic patients with hematologic malignancies and infectious complications:

(i) If a GEI test in serum at onset of fever is positive, the patient should immediately receive therapy with antifungal agents and...
(ii) It seems more reasonable not to automatically treat all patients with fever refractory to broad-spectrum antibiotics with antifungal agents (side-effects, drug-drug interactions, high costs and fungal resistance), but rather to decide on a individual basis taking also into account the GEI test results.

In order to optimize the frequency of GEI testing in neutropenic patients, we analyzed the kinetics of GM indices in peripheral blood of patients with IA and found that in patients with severe infections and fatal outcome the increase tended to be rather fast. Since GM indices in most patients began to rise at onset of fever, we consider it to be adequate to increase the frequency of GEI testing at onset of fever to every other day. The impact of such an approach on outcome and on costs, however, was not assessed in our trial.

A difficulty in analyzing our data was to accurately define IA. According to the EORTC-MSG, a positive GEI test result is an important microbiological criterion to define probable IA [15]. In our study, due to the outcome variables, it was problematic to use GEI test results for definition of IA. On the other side, however, not using GEI testing for definition of IA results in exclusion of many patients with a high clinical probability for IA. The effect would be—falsely—a lower specificity of the GEI test not reflecting the true clinical situation. To address these difficulties, we carried out two separate analysis: the first excluding GEI testing for definition of IA and the second using the EORTC–MSG criteria.

Our finding that high maximum GM indices in serum of patients with IA positively correlated with fatal outcome is in line with clinical and experimental observations of other groups [10, 17, 18]. We also could confirm previous findings that described a correlation of response to therapy and decrease of GM indices. We, however, failed to detect any significant impact of the type of antifungal agent on the kinetics of GM indices as previously described by others [19–21].

A current matter of scientific debate is the utility of GM testing in other body fluids, such as urine, cerebrospinal fluid or BAL [12, 22–24]. In our study, we found that GM indices were higher and rose earlier in BAL as compared with serum. As a result, we found that GEI tests were negative in serum while being positive in BAL in a subgroup (>25%) of patients with proven or probable IA at diagnosis of pneumonia. Given that, due to bleeding disorder, fungal histopathology is not feasible in most neutropenic patients, our data support routine GEI testing of BAL in neutropenic patients with pneumonia undergoing bronchoscopy.

In conclusion, we found that the GEI testing in serum or BAL has a high diagnostic accuracy when test results are correlated to clinical data. Our findings can be useful for optimization of performance and timing of GEI testing. Our data also has implications for the clinical management of neutropenic patients with infectious complications, in particular for optimization of therapy with antifungal agents.

Bio-Rad, Marnes La Coquette, France.

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references


