False positivity of the Aspergillus galactomannan Platelia ELISA because of piperacillin/tazobactam treatment: does it represent a clinical problem?

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Objectives: False-positive results of the galactomannan (GM) ELISA caused by concurrent administration of piperacillin/tazobactam have been reported in patients with febrile neutropenia.

Patients and methods: This prospective study investigated different sampling times in 30 patients receiving piperacillin/tazobactam for febrile neutropenia.

Results: Prior to the first piperacillin/tazobactam infusion, a median GM index of 0.2 [interquartile range (IQR) 0.1–0.3] was noted; in two patients (7%) the index was 0.5. Immediately after piperacillin/tazobactam infusion, the median index increased to 0.3 (IQR 0.2–0.4, P = 0.002) leading to 21% (7/30) false-positive results, if C 0.5 is assumed as the cut-off level. GM indices before the next piperacillin/tazobactam infusion were not increased (median 0.2, IQR 0.2–0.35, P > 0.05), but 10% (3/30) were still C 0.5. With a cut-off level of > 0.7, no false-positive results were noted at any sampling time point.

Conclusions: We conclude that the clinical relevance of false-positive GM results during piperacillin/tazobactam treatment is small if samples are collected prior to infusion and if a cut-off level of > 0.7 is used.

Keywords: febrile neutropenia, cut-off level, fungal infection

Introduction

Patients with prolonged neutropenia after chemotherapy for haematological malignancies are at particularly high risk of developing an invasive fungal infection (IFI), commonly caused by Aspergillus spp. Unfortunately, IFI frequently presents a diagnostic challenge, especially when mould infections are assumed, because direct detection of the pathogen (i.e. culture of blood or tissue) is often impossible. For this reason, a number of indirect markers have been established in the past, including detection of the galactomannan antigen, a component of the fungal cell wall. The most common method of detection is the Platelia-ELISA,¹ which determines an optical density (OD) index and has been widely investigated in febrile neutropenia. By definition, two consecutive positive results are required to make IFI a probable diagnosis.² The definition of a positive result, however, has long been the subject of debate; whereas originally a galactomannan (GM) OD index of C 1.5 was considered positive, recent studies have lowered the threshold to > 0.7³ or even C 0.5⁴ to increase the sensitivity of the test. On the other hand, there are numerous reports of a high rate of false-positive results in patients with haematological malignancies treated with β-lactam antibiotics⁵–⁷ including piperacillin/tazobactam.⁸–¹⁰ This phenomenon is thought to be due to a high level of GM in the infusion batches.¹¹,¹² Other investigators could not reproduce the rate of false positivity¹³ or claim that appropriate sampling time (i.e. before the piperacillin/tazobactam infusion)¹⁴ and careful adjustment of the cut-off level¹⁰ may prevent false-positive results. Our study investigates the increase in GM indices induced by piperacillin/tazobactam treatment for febrile neutropenia in a prospective fashion, evaluating different sampling time points, comparing the serum results to the respective level in the...
piperacillin/tazobactam batch and determining the rate of false positivity for different cut-off levels.

Patients and methods

After approval from the local Ethics Committee and after written informed consent, patients with haematological malignancies receiving piperacillin/tazobactam monotherapy for febrile neutropenia were included between March and May 2005 in this prospective diagnostic study. Inclusion criteria were 18 years and older, neoplastic disease receiving myelosuppressive chemotherapy and fever in neutropenia (temperature >38°C and leukocytes <1.0 × 10^9 cells/L) as well as informed consent. Exclusion criteria were concurrent probable or proven invasive Aspergillus infection and invasive Aspergillus infection during the last 6 months. All patients received a daily dose of 3 × 4.5 g piperacillin/tazobactam, and in none of our patients was there a dose reduction because of renal impairment necessary. The anti-infective prophylaxis administered before the start of the piperacillin/tazobactam therapy was oral co-trimoxazole (960 mg twice daily) in all patients and oral itraconazole solution in 17 patients. In all patients receiving itraconazole, the serum trough concentration of itraconazole exceeded 500 ng/mL.

In order to examine the kinetics of circulating galactomannan antigen associated with piperacillin/tazobactam, serial blood samples were collected. Time point 0 was immediately before the start of the very first piperacillin/tazobactam infusion at the onset of fever (T0), then immediately after the end of the piperacillin/tazobactam infusion (T1), 8 h after the first infusion (T2), after several days of piperacillin/tazobactam therapy immediately before the morning infusion (T3) and after several days after the end of piperacillin/tazobactam therapy (T4). Furthermore, the concentration of galactomannan antigen was measured in each of the 30 piperacillin/tazobactam infusion solutions that were administered as the first dose.

Serum galactomannan concentrations were determined by the Platelia Aspergillus ELISA (Bio-Rad Laboratories, Munich, Germany). The assay uses the rat monoclonal antibody EB-A2, which is directed against Aspergillus galactomannan. Test variable is the titre of the Aspergillus galactomannan antigen, measured in the OD index (GM index). Whereas initially, the cut-off level was chosen at an index of ≥1.5, more recently, GM indices of >0.7^3 or ≥0.5^4 have been considered positive.

The data were analysed using SPSS (version 14.0 for windows, Munich, Germany) and median and interquartile range (IQR) used as descriptive methods. The Mann–Whitney test, the Wilcoxon test and the Fisher’s exact test were used to test for differences, where appropriate, a two-sided P value less than 0.05 was considered significant.

Results and discussion

Patient characteristics

Thirty patients were included, 19 (63%) of whom suffered from acute myeloid leukaemia, one from acute lymphatic leukaemia (3%), six (20%) from malignant lymphoma and four (13%) from multiple myeloma. The median age of the patients was 65 years (IQR 54–72 years), 24 patients were male. None of the patients had signs of invasive Aspergillus infection and all patients responded to antibacterial therapy. The median duration of piperacillin/tazobactam therapy was 7 days (range 4–19). None of the patients received an antibacterial combination therapy at the time of serum sampling. Prior to piperacillin/tazobactam therapy, two patients had a GM index of 0.5 and all others had clearly negative values (median 0.2 IQR 0.1–0.3).

GM index immediately after infusion in relation to GM index in piperacillin/tazobactam infusion solutions

The median GM OD index measured in the 30 piperacillin/tazobactam infusion solutions was 2.0 (IQR 0.9–7.1). As piperacillin is derived from mould species, it is not surprising to find positive indices and it should be pointed out that this does not equate to a contamination of the batches with Aspergillus spp. Seven samples had a very high GM index (above 10), six samples had an intermediate GM index (4.2–6.2) and 17 samples had a low GM index (0.1–2.1, see x-axis of Figure 1b). Immediately after the first piperacillin/tazobactam infusion (T1), the median GM index in the patients’ serum increased to 0.3 (IQR 0.2–0.4, P = 0.002 compared with T0). This was largely due to an increase in 14 patients rendering 7 patients positive if a cut-off level of ≥0.5 is assumed (Figure 1a). There was a trend towards a higher increase in the patients’ serum if the GM index measured in the respective infusion was higher as well. The group of patients who received a solution with a high concentration of galactomannan antigen (GM index >4) had a maximum increase of 0.4 (median 0.1). In contrast, those who

![Figure 1](image_url)

*Figure 1.* (a) Recorded values of the optical density index of the galactomannan antigen before (T0) and immediately after (T1) the first piperacillin/tazobactam infusion. (b) Association of the galactomannan antigen concentration (measured as optical density index) in the piperacillin/tazobactam infusion solution on the change of the galactomannan antigen optical density index in serum before (T0) and immediately after (T1) the first infusion of the antibiotic.
received an infusion solution with a low GM index had a maximum increase of 0.1 (median 0, Figure 1b), although this correlation failed to reach statistical significance.

Samples 8 h after (T2) first antibiotic administration and during and at end of treatment

The median GM index 8 h after the first infusion (T2) was 0.2 (IQR 0.2–0.35), which is very close to the value prior to the first infusion of piperacillin/tazobactam. Also, the number of patients with a GM index of 0.5 was almost identical (two at time point 0 and three at time point 2). In comparison with the GM index directly after the piperacillin/tazobactam infusion (T1), we found a median decrease by −0.02 8 h after piperacillin/tazobactam administration. At time point T3 (prior to a piperacillin/tazobactam infusion after a median of 9 days of treatment), we again determined a GM index almost identical to those at T0 and T2 (median 0.2, IQR 0.1–0.2). Similarly, several days after the end of piperacillin/tazobactam therapy (T4, median 3 days after the end of piperacillin/tazobactam), the median GM index was 0.2 (IQR 0.1–0.2). Of note, itraconazole prophylaxis did not influence GM indices as there was no difference between patients with itraconazole prophylaxis versus those without prophylaxis in any of the blood samples collected at different time points (data not shown).

Evaluation of different cut-off levels

There was a small but significant increase in GM indices immediately after the piperacillin/tazobactam infusion (T0 versus T1, from 0.2 to 0.3). Consequently, the number of patients with a GM index of 0.5 more than trebled (from two patients at T0 to seven patients at T1) which means that the rate of positivity increased from 7% to 23% (Table 1) if a cut-off level of ≥0.5 is assumed as suggested recently.4 In contrast, if a cut-off level of >0.7 is assumed as suggested in an earlier study,3 no false-positive results were detected at any time point in our patient population (Table 1). In our view, our findings support the proposed cut-off level of >0.7 instead of ≥0.5. However, the advantage of a lower rate of false-positive results with a cut-off level of >0.7 has to be weighed up against the potential disadvantage of lower sensitivity.

Our results show that the increase in GM index caused by piperacillin/tazobactam infusion is only small and an adjustment of the cut-off level to a previously published level can abrogate the increase in false-positive findings. In addition, sampling for GM ELISA just before the next infusion of piperacillin/tazobactam gives almost identical results as prior to piperacillin/tazobactam treatment. We therefore conclude that if these premises are accepted, piperacillin/tazobactam can be administered in febrile neutropenia without causing diagnostic confusion.

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Transparency declarations

None to declare.

References


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**Table 1. Rate of positivity depending on time point and threshold**

<table>
<thead>
<tr>
<th>GM index cut-off</th>
<th>Number of patients testing positive at time pointa [n/N (%)]</th>
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<tbody>
<tr>
<td>&gt;0.5</td>
<td>T0: 2/30 (7%) T1: 7/30 (23%) T2: 3/30 (10%) T3: 2/30 (7%) T4: 1/30 (3%)</td>
</tr>
<tr>
<td>&gt;0.7</td>
<td>0 0 0 0 0</td>
</tr>
</tbody>
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*aTime points are as follows: T0, before first dose of piperacillin/tazobactam; T1, after first dose of piperacillin/tazobactam; T2, before second piperacillin/tazobactam dose; T3, before piperacillin/tazobactam dose after 9 days; T4, after the end of piperacillin/tazobactam therapy.*


