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False-Positive Results by the Platelia *Aspergillus* Galactomannan Antigen Test for Patients Treated with Amoxicillin-Clavulanate▽

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The *Aspergillus* galactomannan test is a valuable tool in the diagnosis of invasive aspergillosis. We hereby report a high rate of false-positive results by the Platelia *Aspergillus* galactomannan antigen test (Bio-Rad Laboratories) for patients treated with amoxicillin-clavulanate.

Invasive aspergillosis (IA) is a fungal infection with a significant morbidity and mortality, especially for neutropenic patients and stem cell transplant recipients. Early detection of IA is difficult due to a lack of sufficiently sensitive and specific diagnostic tools (4).

In recent years, tests have been developed to detect circulating fungal antigen. The *Aspergillus* antigen test detects galactomannan, a polysaccharide cell wall component of *Aspergillus* species.

False-positive results were described previously for some food products and antibiotics (2).

We report a high rate of false-positive test results by the Platelia *Aspergillus* galactomannan antigen test (PAG test) (Bio-Rad Laboratories, Hercules, CA) for patients treated with amoxicillin-clavulanate (AMCL).

Between 25 December 2003 and 10 February 2005, all patients staying more than 3 days on the Medical Intensive Care Unit were tested with the PAG test according to the manufacturer's instructions. At every measurement, the patient's medical treatment regimen was recorded. Statistical analysis was performed by the Centre for Statistics, University Hasselt, Belgium.

A total of 231 serum samples from 94 patients were tested. For 36 patients, there was only 1 measurement, while the remaining patients were tested more than once (range, 1 to 11 measurements).

For two patients, there was microbiological, clinical, or radiological evidence for IA according to the criteria of the European Organization for Research and Treatment of Cancer-Mycosis Study Group (3). These two patients (eight results) were excluded from analysis. Since all included patients were free from IA, all positive results were considered as false-positive results.

Depending on the antibiotic treatment at the moment of sampling, measurements were divided into the following four groups: (i) treatment with AMCL alone or in combination with other antibiotics, (ii) treatment with piperacillin-tazobactam (PIPTAZ) alone or in combination with other antibiotics, (iii) treatment with one or more other antibiotics, and (iv) no antibiotic treatment.

For one measurement, the patient concerned was under treatment with AMCL and PIPTAZ at the same time. Since PIPTAZ is known as a possible interfering factor for the galactomannan test, this observation was excluded (1, 10, 12).

The PAG test gives a numerical outcome that can be dichotomized as negative (<1) or positive (>1.5). This dichotomization is somewhat problematic, since there is a "gray zone" of results (≥ 1 and ≤ 1.5) for which the distinction between negative and positive is ambiguous. Therefore, two different scenarios were considered: one where the gray zone result was considered positive and one where the gray zone result was considered negative.

For each treatment group, we calculated the average test results of all measurements. To exclude the effect of differences in numbers of measurements between patients, we additionally calculated first the averages per patient within one treatment group and then the average of the patient averages (Table 1).

We also calculated false-positive rates for the two above-described scenarios (Table 2).

Results were evaluated as continuous and also as a binary version for the two above-described scenarios. The models adjusted for age and gender as possible confounding factors.

Continuous test results were analyzed by fitting a linear mixed model (i.e., a regression model with random effects). There is a significant difference between the AMCL treatment group and all three other treatment groups as follows: PIPTAZ ($P < 0.0001$), other antibiotics ($P < 0.0001$), and no antibiotics ($P < 0.0001$).

The binary test results were analyzed by fitting a logistic regression model with random intercept. When the gray zone was considered to be a positive result, we obtained a significantly larger false-positive rate for patients under treatment with AMCL than for the three other treatment groups: PIPTAZ ($P = 0.0129$), other antibiotics ($P = 0.0073$), and no antibiotics ($P = 0.0120$). When the gray zone was considered to be a negative result, we also obtained a significantly larger false-positive rate for patients under treatment with AMCL than for patients in the PIPTAZ ($P = 0.0276$) and other antibiotic ($P = 0.0064$) treatment groups.

In none of the three analyses was there a significant differ-

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TABLE 1. Galactomannan test result per treatment group

Treatment group	Avg PAG test score (SD)	
	General	By patient
AMCL	2.34 (2.25)	2.14 (2.06)
PIPTAZ	0.48 (0.59)	0.45 (0.57)
Other antibiotics	0.24 (0.43)	0.30 (0.54)
No antibiotics	0.20 (0.20)	0.16 (0.16)

ence between the false-positive rates for the PIPTAZ group and the group that received other antibiotics.

Our data show that treatment with AMCL results in a proportion of false-positive results by the galactomannan test larger than that seen for the comparison groups.

The reported specificity of the *Aspergillus* antigen test varies between 80 and 99% (5, 6, 8, 9, 11). False-positive reactions obtained with the Pastorex *Aspergillus* latex agglutination test were described for food products and antibiotics, including piperacillin and AMCL (2). False-positive antigenemia caused by food products could be explained by the passage of galactomannan antigen from the gut through the affected intestinal mucosal barrier to the circulation.

False-positive reactions for patients treated with PIPTAZ have been described previously (1, 10, 12). The results of the present study do not necessarily contradict these findings. It might be that the number of observations for the PIPTAZ treatment group was not large enough to illustrate such an effect.

Some results suggest the false-positive reactions were related to specific batches of the antibiotic (1). During the study period, several different batches of AMCL were used in our hospital. Analysis of the results did not show evidence of a time-dependent false-positive rate.

This hypothesis may be supported by the large variation in the incidence of false-positive reactions we noticed in the period after our study.

In one survey, different antibiotics, antifungals, and antivirals showed no positive results. AMCL was not tested. Only recently a patient was described with a positive *Aspergillus* antigen test during AMCL treatment (7). Piperacillin, tazobactam, and amoxicillin are semisynthetic penicillins manufactured from *Penicillium* species. The cell wall of *Penicillium* species contains a type of galactomannan only slightly different from that of *Aspergillus*, which cross-reacts with the PAG test. Most likely, galactomannan originating from *Penicillium* species continues to stay in the prepared antibiotic during the production process. In an in vitro experiment, different concentrations of one batch of AMCL were added to a proven galactomannan-negative sample. Even AMCL concentrations below the normally expected serum levels during AMCL therapy gave positive test results.

In conclusion, treatment with AMCL can give a false-positive PAG test result. This might lead to unnecessary examinations and inappropriate treatment. When judging test results, one should bear in mind not only the clinical context but also the medication regimen at the time of sampling.

TABLE 2. Number of measurements and derived false-positive rates per treatment group

Treatment	No. of measurements				% FP[a] with gray zone result considered:	
	Total	Negative	Gray zone	Positive	Positive	Negative
AMCL	62	23	10	29	63	47
PIPTAZ	25	21	1	3	16	12
Other	109	106	0	3	3	3
None	26	25	1	0	4	0

[a] FP, false positive.

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