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Detection of *Aspergillus* Galactomannan Antigenemia To Determine Biological and Clinical Implications of Beta-Lactam Treatments

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Detection of *Aspergillus* galactomannan (GM) in serum with the Platelia *Aspergillus* enzyme immunoassay (EIA) is useful for diagnosing invasive aspergillosis. From May 2003 to November 2004, 65 patients who did not develop aspergillosis had at least two positive sera while receiving a beta-lactam treatment (GM index [GMI], ≥0.5). Of the 69 treatment episodes scored, 41 consisted of a beta-lactam other than piperacillin-tazobactam ($n = 29$), namely, amoxicillin-clavulanate ($n = 25$), amoxicillin ($n = 10$), ampicillin ($n = 3$), or phenoxymethylpenicillin ($n = 2$). In all cases, antigenemia became negative 24 h to 120 h upon stopping the antibiotic. Monitoring of 35 patients, including 26 with hematological malignancies, revealed three antigenemia kinetic patterns: each was observed with any drug regimen and consisted of a persistent GMI of >2.0 (65.7%), >0.5, and ≤1.5 (25.7%) or a variable GMI (14.3%) from the onset of antibiotic therapy. All available drug batches given to 26 patients cross-reacted with the EIA. Galactomannan titration in batches failed to predict the GM titers in the five patients studied at cumulative doses of ampicillin or amoxicillin-clavulanate, regardless of the time lapse between serum sampling and infusion period. Our results show that beta-lactams other than piperacillin-tazobactam may lead to false presumption of aspergillosis. The resulting kinetic patterns of GM antigenemia are variable, and sampling serum prior to the next beta-lactam dose may not decrease GMI below the threshold. Consequently, testing of suspected antibiotic batches remains the only indicator of possible false EIA positivity.

Invasive aspergillosis (IA) is a threatening opportunistic infection in immunocompromised patients, especially transplant recipients (21). Early detection of IA is crucial for successful treatment, and monitoring of the *Aspergillus* galactomannan (GM) in serum is the only noninvasive biological test with proven usefulness. Detection of this polysaccharide cell wall component is based on the use of a rat monoclonal antibody (MAb), EB-A2, that recognizes the 1→5-β-D-galactofuranose side chains of the *Aspergillus* GM (19, 23). As little as 0.5 to 1 ng of GM per ml of serum can be detected with the double-sandwich enzyme-linked immunosorbent assay Platelia *Aspergillus* (Bio-Rad, Marnes-La-Coquette, France), making this assay 15 to 30 times more sensitive than the former latex agglutination assay (23, 28). The sensitivity and specificity of this enzyme immunoassay (EIA) may vary according to the type of transplant recipient (reviewed in reference 21). Several prospective studies have shown the utility of the GM EIA for the early diagnosis of IA in neutropenic patients (11, 13, 15–17, 20, 25). Meanwhile, the potential usefulness of GM EIA in lung and liver transplant recipients is still unclear (21). Additionally, monitoring of antigenemia has been proposed for predicting the therapeutic outcome of patients with IA (5, 6, 17).

Specificity is a matter of concern, since cross-reactivity of the MAb has been repeatedly described with exoantigens from other fungal genera as well as from *Bifidobacterium* species (10, 19a, 23, 26). Likewise, cross-reactivity with certain fungus-derived antibiotics like piperacillin (PIP) and ampicillin (AMP)-sulbactam has been noticed since 1997 and was explained by the fact that some GM moieties are shared between *Aspergillus* and *Penicillium* species (2). However, the clinical implication of this has only been pointed out very recently and only in relation to the PIP-tazobactam (TZP) treatment (1, 24, 29).

In April 2003, we started to experience a sudden increase of positive test results in patients with hematological disorders, from <5% (from January to March) to ≥38%. These results proved not to be due to technical deficiencies, which prompted our investigation into its origin, focusing on possible sources of GM from fungus-derived antibiotics as reported at that time (2). To this end, from May 2003 to November 2004, we prospectively studied all patients with two positive test results in terms of risk factors for IA, kinetics of antigenemia, and antibiotic regimens. No increase of IA cases was noticed. The EIA reactivity of available antibiotic batches given to patients was assessed, which eventually was revealed to be not restricted to TZP. The kinetics of GM antigenemia were analyzed according to the beta-lactam treatments. Finally, by comparing the GM titers obtained in vitro with those obtained in vivo, we

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sought to know whether this approach could help predict GM levels in serum and to understand how to circumvent beta-lactam-related positive antigenemia.

(Part of this study has been presented at the RICAI 2004, Paris, France.)

MATERIALS AND METHODS

Patient selection. From May 2003 to November 2004, 98 patients tested positive by GM EIA in our hospital (Pitié-Salpêtrière, Paris, France). For these patients studied prospectively, clinical and biological files were reviewed according to (i) the absence of a history or evidence of IA from the time of serum collection until 2 months after the last serum collected, (ii) the existence of at least two positive sera, and (iii) a documented antibiotic therapy including drug name, dosage, and period of treatment. Proven, probable, and possible IA were defined according to criteria established by the European Organization for Research and Treatment of Cancer-Mycoses Study Group (EORTC) (3). Thirty-five patients with at least three sera sampled during the antibiotic treatment and one or more sera sampled apart from this period were eventually selected to study the kinetics of GM antigenemia. For five of these patients, aliquots of serum drawn for other biological tests before (5 min to 60 min) and after one antibiotic dose were made available as a means to study the influence of the sampling period on the GM levels. In addition, two healthy volunteers (coauthors) received a single infusion and were monitored for GM antigenemia disappearance at the end of infusion and 1, 2, and 4 h later.

Galactomannan assay. Galactomannan EIA was performed on human sera and antibiotic batches according to the recommendations of the manufacturer (Bio-Rad, Marnes-La-Coquette, France). The index for each sample was calculated by dividing its optical density by the mean cutoff value of the threshold control serum provided in the test kit (titrated at 1 ng/ml). Like other European colleagues, for 2 years, we have routinely adopted a cutoff value below 1 ng/ml (8, 25, 27, 29). In this study, indices of ≥0.5 were considered positive per the cutoff value. Positive indices below 1.5, ≥1.5 and below 2.0, and ≥ 2.0 were scored as low, medium and high, respectively. For convenience, the term GM index (GMI) simply refers to EIA test results.

Testing the GM-EIA reactivity with antibiotic batches. A total of 38 batches of antibiotics assigned to 31 patients were available for testing. These drugs were TZP, AMP, amoxicillin (AMX), amoxicillin-clavulanate (AMC), phenoxymethylpenicillin (PEN), and ticarcillin. Drug diluents were used as controls in the GM assay along with previously tested negative and positive patient sera. All absorbance measures were performed in duplicate. Intravenous formulations were tested either at full strength in vials (50 mg of AMX or AMP/ml and 200 mg of PIP/ml) or after subsequent dilution in 50-ml infusion bags (15 mg of AMX or AMP/ml and 60 mg of PIP/ml), as recommended by the manufacturers. For testing of oral formulations of PEN and AMC, suspensions were made in distilled water at a drug concentration equivalent to 100,000 IU of PEN/ml or 50 mg of AMX/ml and tested at full strength and at serial dilutions in distilled water. In order to determine the minimal drug concentration that could yield a positive test, some drug batches were tested at various dilutions until the equivalent peak concentrations of the drug in serum (C_{max}) were reached. The C_{max} of a 4-g infusion of PIP is 600 μg/ml, and the C_{max} of a 1-g infusion of either AMX or AMP is 100 μg/ml. Some of theses batches were given to patients with detailed antigenemia kinetics and tested positive with the GM EIA.

Statistical analysis. Comparisons of the GMI before and after a given infusion of antibiotic were studied in five patients with available kinetics using an exact pairwise permutation test with StatXact version 6 software (Cytel Software Corporation, Mass.).

RESULTS

Of the 98 patients that prospectively tested positive using the GM EIA, 33 did not meet the selection criteria, either because of the lack of a second serum and/or undocumented antibiotic therapy ($n = 19$) or because the diagnosis of IA (probable or proven) was established before or during the prospective period ($n = 14$). As a result, 65 patients with at least two positive sera were retained. None of them had a history of IA or evidence of IA after 3 months following their last positive serum sample. Positive results were first detected at onset or during a beta-lactam treatment, with GMI values ranging from 0.8 to more than 5.0. In all instances, antigenemia became negative upon stopping the beta-lactam treatment. In 28 patients repeatedly surveyed for antigenemia (three to five sera per patient), serum negativity was obtained within 12 h to 96 h upon the last antibiotic infusion. For the two healthy volunteers who received a single infusion, negativity occurred after 1 hour and 2 hours, respectively (data are specified in Table 3). In the 35 remaining patients with available follow-up (4 to 19 sera per patient), antigenemia disappearance occurred 24 h to 120 h after the beta-lactam treatment was discontinued.

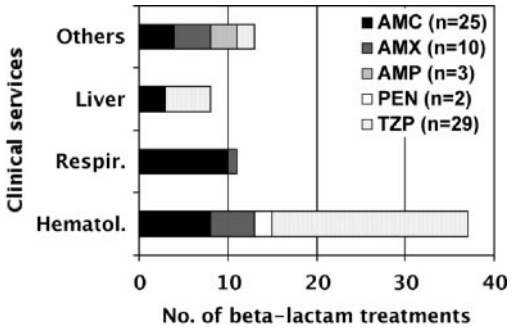

FIG. 1. Distribution of TZP, AMP, AMX, AMC, and PEN treatment episodes related to positive GM antigenemia among clinical departments. Respir., respiratory, Hematol., hematology.

Sixty-nine beta-lactam treatment episodes temporally coincided with positive test results in patient sera (Fig. 1). Only 29 treatment episodes included TZP. The other suspected drugs, accounting for 40 episodes, were AMC ($n = 25$), AMX ($n = 10$), AMP ($n = 3$), and PEN ($n = 2$). As illustrated in Fig. 1, patients with hematological disorders had most of the positive GM results coinciding with TZP treatment (mainly allograft stem cell recipients [22/29 episodes]) compared with other patient populations such as liver transplant recipients (5/29 episodes). Indeed, 22 of the 33 patients of hematology wards with positive antigenemia (66.7%) were treated with TZP. By contrast, positive antigenemia coinciding with AMP, AMX, AMC, or PEN administration was not restricted to patients with hematological disorders (15/40 episodes), since only 12 of them (36.4%) received one of these latter drugs. Three other patient groups had a positive test while receiving a non-TZP treatment (25/40 episodes) (Fig. 1). These groups were as follows: all 11 positive patients (100% of patients; 11 episodes) cared for in the respiratory intensive care unit, 11 of the 13 positive patients (84.6% of patients; 11 episodes) hospitalized in other clinical departments, and 3 of the 8 positive patients (37.5% of patients; 3 episodes) cared for in the liver transplant unit. While intravenous formulations accounted for most situations, oral formulations of AMC and PEN were scored on four occasions.

The impact of positive EIA test results on the diagnosis of IA and therapeutic decisions for the 33 patients with hematological malignancies is summarized in Table 1. With respect to the EORTC criteria, the only microbiological criterion scored was positive antigenemia, which temporally matched one to two host factors and/or one major clinical criterion or two minor clinical criteria. Accordingly, all 10 episodes of possible IA (related to nine patients) switched to probable IA upon the

TABLE 1. Clinical implications of positive GM antigenemia related to beta-lactam drugs in patients with hematological malignancies

Beta-lactam[b]	Follow-up (no. of patients)[c]	Suspicion of IA[a] (no. of patients)						No. of patients receiving antifungal therapy	
		Before positive antigenemia			After positive antigenemia				
		No	Possible	Probable	No	Possible	Probable	Started[d]	Ongoing
AMC	Yes (4)	3	1	0	0	3	1	3	0
	No (4)	3	1	0	0	3	1	2	1[e]
AMX	Yes (3)	2	1	0	0	2	1	0	1[f]
	No (2)	2	0	0	0	2	0	0	1[e]
TZP	Yes (17)	11	6	0	0	11	6	17[g]	0
	No (5)	3	2	0	0	3	2	1	0

[a] Suspicion of IA according to the EORTC criteria. None of the patients had direct examination or culture or histology that was found to be positive before and/or after the EIA test results.
[b] Thirty-five episodes of beta-lactam treatment were scored among these patients.
[c] Follow-up is defined as patients ($n = 33$) with at least four positive EIA test results.
[d] *Aspergillus*-targeted antifungal therapy prompted by positive EIA test results.
[e] Patients treated empirically with amphotericin B.
[f] Patient treated with amphotericin B for disseminated candidiasis.

appearance of positive antigenemia, and the 24 cases initially not suspected of IA became possible cases. Additionally, *Aspergillus*-targeted antifungal therapy was prompted in 5 of the 13 patients receiving an AMX-based treatment and in 18 of the 22 patients receiving TZP.

A total of 31 available drug batches given to 27 patients with positive antigenemia were tested with the GM EIA (Table 2). Cross-reactivity was seen in all TZP batches ($n = 8$; eight patients) and in intravenous formulations of AMP ($n = 1$; three patients), AMX ($n = 5$; five patients), and AMC ($n = 13$; 10 patients). Oral AMC and PEN given to one patient exhibited weak reactivity (GMI, 0.8) and no reactivity (GMI, 0.25), respectively (Table 2). Regarding three patients with IA (patients excluded), batches of TZP ($n = 5$) and of ticarcillin ($n = 2$) tested negative (data not shown).

To study the kinetics of antigenemia with respect to cumulative doses of antibiotics, we focused on the 35 patients with available follow-up. Sera were sampled twice weekly or more in patients with suspected IA due to persistent antigenemia, fever, or respiratory involvement. Upon analysis, three patterns of antigenemia kinetics emerged, depicted with either beta-lactam (Fig. 2). In the first kinetic pattern (Fig. 2a), GMI values of >2 were detected from the beginning of treatment (22/35 patients; 62.8%). Decline of titers (GMI, >0.5 to <1.5) and then negativity (GMI, <0.5) were detected 24 to 48 h and 48 to 120 h after the last dose, respectively. This pattern was initially observed in patients receiving TZP for at least 4 days ($n = 15$), which was eventually associated with infusions of AMX ($n = 2$) or AMC ($n = 5$). By contrast, the second pattern consisted of GMI values of >0.5 and ≤1.5 throughout the antibiotic regimen, with shorter delays in negativity (24 to 48 h) after the last dose (Fig. 2b). This pattern was observed less frequently (10/35 patients; 28.6%). It was drawn from infusions of either AMC ($n = 2$), AMP ($n = 1$), or TZP ($n = 4$). It also coincided with oral formulations of PEN ($n = 2$) or AMX ($n = 1$). Finally, we occasionally found a hybrid pattern (Fig. 2c) that consisted of variable GMI levels (4/35 patients; 11.4%). This third pattern coincided with either the switch from intra-

TABLE 2. *Aspergillus* galactomannan EIA reactivity with antibiotic batches given to patients with positive antigenemia

Treatment (source)	No. of batches ($n = 31$)	Drug concn tested (mg/ml)[b]	GM-EIA results (mean OD)	No. of patients ($n = 27$)
Ampicillin, 1-g vial (Pan Pharma)	1	15	4.80	3
Amoxicillin, 1-g vial (GlaxoSmithKline)	2	15	>5.0	2[d]
	1	15	>5.0	1[d]
	1	15	>5.0	2
	1	15	>5.0	1
Amoxicillin-clavulanate, 1-g–200-mg vial (GlaxoSmithKline)	1	50	>5.0	2
	1	50	>5.0	1
	1	50	>5.0	1
	1	50	>5.0	1
	1	15	3.20	1
	1	15	2.30	1
	1	15	1.20	1
	6	15	1.20–2.50	2
Amoxicillin-clavulanate, 1-g–200-mg tablet (GlaxoSmithKline)	1	50	0.80	1[e]
Phenoxymethylpenicillin, 1-MU tablet (Schwarz Pharma)	1	1[a]	0.25	1[e]
Piperacillin-tazobactam, 4-g–500-mg vial (Wyeth Pharmaceuticals)	10	60	0.90–4.30	8

[a] Concentration of phenoxymethylpenicillin is in megaunits per milliliter.
[b] Dilutions as recommended by the manufacturers.
[c] GM-EIA results expressed as mean OD index from two measures; values of <0.5 were considered negative.
[d] The same patient received three batches.
[e] The same patient received both oral drugs sequentially.

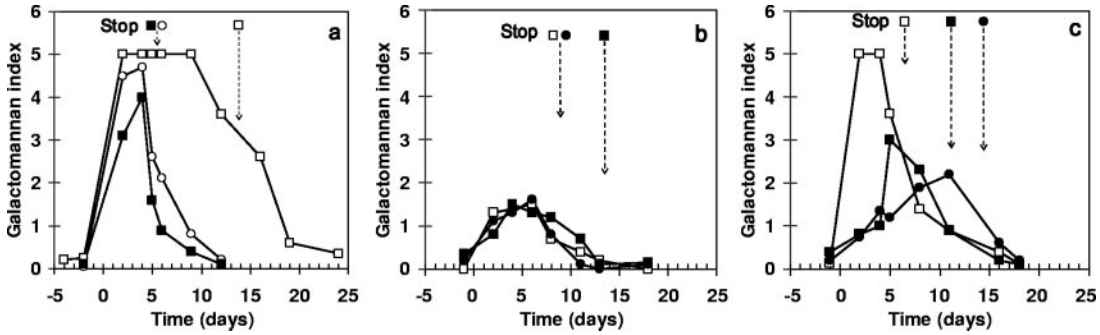

FIG. 2. Representative kinetic patterns of GM in serum of patients receiving AMC (□), AMX (●), AMP (○), or TZP (■) therapy. Broken arrows indicate the time of discontinuation of antibiotic treatment. (a) High GM levels as exhibited in most patients treated with AMC, AMX, or TZP. (b) Low GM levels exhibited in a few patients treated with AMC, AMP, or TZP. (c) Both high and low GM levels exhibited in three patients as a result of a switch from infusion to oral AMC, variable dosage of AMX, and the use of different batches of TZP.

venous to oral AMC treatment ($n = 1$), the accumulation of AMP infusions under a particular regimen ($n = 1$), or the use of several TZP batches ($n = 2$).

To determine whether the level of antigen present in the antibiotic would predict the level of antigenemia, several batches of AMC, AMX, and AMP were assayed at various dilutions until the theoretical peak concentration of the drug in serum (C_{max}) was reached. These batches were given to patients for whom sera were sequentially sampled in relation to a dose of antibiotic. As summarized in Table 3, for the two healthy volunteers who received a single infusion of AMX or AMC, similar GMI values were found between sera collected at the end of infusion (i.e., 0.5 h or 0.3 h) and the drug diluted at approximately C_{max} (GMI of 1.80 versus 1.75, and 0.80 versus 0.85). Additionally, GM levels in serum became undetectable (GMI, <0.5) within less than 2 hours following the infusion. By contrast, for the five patients with cumulative doses of AMP or AMC, GMI from drug batches could not be related to levels of GMI in sera. In these instances, sera tested positive whatever the sampling period was. In addition, sera sampled at the end of infusion had slightly but significantly higher GM levels than sera sampled at trough (GMI of 1.1 to >5.0 versus 0.8 to >5.0; $P = 0.031$). However, with respect to the GMI values obtained with antibiotics, at least the latter sera were expected to test negative. Indeed, the beta-lactams tested negative when diluted at C_{max}, and their EIA positivity appeared at a 2.5- to 10-fold dilution above the C_{max} (Table 3).

DISCUSSION

This study provides strong evidence that positive (*Aspergillus* GM antigenemia) Platelia *Aspergillus* test results using serum was associated with various beta-lactam treatments. First, antigenemia disappearance always occurred once the beta-lactam treatment was stopped. Second, EIA negativity was obtained within less than 2 hours after a single dose and within 24 h to 120 h upon cumulative doses. Third, cross-reactivity with beta-lactams was demonstrated in a substantial number of cases. Fourth, the kinetics of antigenemia appeared to vary according to the treatment duration and the level of antigen detected in

TABLE 3. Galactomannan levels in sera sampled upon a beta-lactam dose and in related antibiotic batches[a]

Antibiotic	EIA reactivity			Individual[j]			
				GMI in serum		Cumulative amt of beta-lactam (g)[b]	
	No. of batches	Drug dilution regarding C_{max}[c]	GMI	Before infusion	After infusion[d]	At serum sampling	Per day
Amoxicillin	1	1×	1.75	0.08	1.80, 0.70, 0.20	1	None
Amoxicillin-clavulanate	1	2.5×	0.85	0.03	0.80, 0.45, 0.15	1	None
	1	10×	0.90	1.40	1.50	7	3
	6[e]	2.5×	0.50–0.90[e]	0.90[g]	1.30[g]	12	3
				1.10[h]	1.80[h]	26	3
Ampicillin	1[i]	10×	1.60	1.30	1.70	68	6
				0.80	1.10	7	2

[a] GMI values of <0.5 were considered negative.
[b] Total amount of the beta-lactam given to patients at the time of serum sampling.
[c] Last equivalent concentration of the drug tested EIA positive, with the C_{max} of AMP or AMX equal to 100 μg/ml.
[d] Three sera were sampled at the end of infusion and at 1 hour and 2 hours after infusion in two healthy volunteers who received a single dose of beta-lactam; one serum was sampled after infusion in patients undergoing a beta-lactam treatment.
[e] Six batches were common to two patients.
[g] GMI values with sera diluted 1/10; values are >5.0 with undiluted serum.
[h] GMI values with sera diluted 1/5; values are >5.0 with undiluted serum.
[i] The same batch was given to two patients.
[j] Data are for two health volunteers receiving a single beta-lactam dose and five patients under a beta-lactam treatment.

the antibiotic batch. To our knowledge, this is the first extensive study documenting clinical false-positive GM tests with the administration of AMP, AMX, and AMC, except for TZB, as was recently reported (1, 24, 29). Only two very recent single-case reports have incriminated AMC treatment (14, 18).

We experienced positive tests related to AMP, AMX, and AMC a few weeks after those related to TZP occurred. So far, no modification of the *Aspergillus* GM EIA has been reported by the manufacturer to explain a change in test reactivity. Likewise, no modification of the EIA procedure has been made in our laboratory since the kit was commercialized in 1997. On the other hand, cross-reaction of MAb EB-A2 with other organisms including *Penicillium* species is known and is assumed to be due to the very similar structures of the GMs of *Aspergillus* and *Penicillium* (23, 26). Given that *Penicillium* infections are very rare in humans, a contamination of the penicillin by the *Penicillium* GM or similar moieties able to react with MAb EB-A2 is therefore the most likely hypothesis to explain false positivity in patients treated with PIP-based therapy (1, 29, 30) as well as with the other hemisynthetic penicillins, AMX and AMP. It is reasonable to speculate that a change in the process and/or control of the purification of penicillin G or its derivatives may explain the possible variation of GMI levels between batches as we observed (if not between vials of a given drug, as we experienced once). However, the presence of GM or related moieties able to react with MAb EB-A2 in suspected antibiotics remains to be authenticated, and contamination of the drug with another cross-reactive binding epitope antigen might not be excluded. Nonetheless, whatever the antigen source may be, the occurrence of cross-reactivity may depend on the local provider because of the different worldwide manufacturers and/or suppliers of predrug or final drug. Consistent with this speculation are the different reported rates of false-positive antigenemia between some European countries (1, 24, 29) and other countries (30). Likewise, the absence of EIA reactivity to AMP, AMX, or AMC has been reported in the United States (30), in contrast to a recent report in Europe (18; Z. Racil, I. Kocmanova, and J. Mayer, Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-267, 2004).

Our data emphasize the problem for clinicians to decide whether the diagnosis of IA should be retained because of positive GM antigenemia and whether starting or modifying an antifungal therapy should be appropriate. Given that the "gold-standard" diagnostic tests are not highly sensitive, we cannot definitively rule out the possibility that some patients had IA while transient GM antigenemia coincided with beta-lactam therapy. Indeed, among the 33 patients with hematological disorders, 28 had appropriate risks for IA, and most of them had pneumopathy. Nevertheless, none of computed-tomography scans performed on 19 patients yielded evidence for IA. In addition, none of the patients had mucositis from the onset of antibiotic therapy, a finding that weakens the hypothesis for a passage of dietary GM or *Aspergillus* conidia into the blood (9, 12). Additionally, we cannot exclude that empirical or preemptive antifungal therapy against *Aspergillus* was efficient and thus has led to GM EIA negativity in 22 of these patients (Table 1). *Aspergillus*-targeted therapy was discontinued after 1 to 3 weeks, whether or not the beta-lactam was tested for EIA reactivity, and not necessarily after antigenemia negativity was obtained. On the other hand, among patients who developed IA (patients excluded from the study), three had GMI-positive sera initially suspected to be related to TZP therapy because of the absence of other contemporary biological or clinical factors in favor of IA. However, radiological signs promptly appeared before mycological confirmation by bronchoalveolar lavage fluid or biopsy, and the TZP batches failed to react with the EIA (data not shown). Thus, we do believe that erroneous suspicion of false positivity contributed to delay the diagnosis of IA in these patients. Therefore, we recommend that biologists insist on the usefulness of testing antibiotic batches and carefully interpret GM EIA test results from patient serum and beta-lactam in light of current clinical and biological data.

Available follow-up of 35 patients allowed us to describe the in vivo expression of GMI levels related to beta-lactams. Among the three kinetic patterns found, one predominated, with persistently high levels from the beginning of infusions of AMX or AMC. This kinetic profile was previously seen with most patients treated with TZP and is likely to be the profile reported previously in Western European countries (1, 14, 24, 29) or more recently in the United States (30). Nevertheless, in some cases, we found low GMI levels throughout treatment with either beta-lactam. This suggests that the quantity of antigen present in patient serum would depend not only upon the quantity of antigen present in the antibiotic itself but also upon dosage and duration of treatment. Assuming that fungal GM is the actual cross-reacting antigen, clearance of the GM from the bloodstream, notably via renal excretion, may influence the levels of antigenemia in either situation (4). However, this pathway was not explored in this study, and whether renal failure or dialysis affects the clearance of GM is still not known.

We suspected oral formulations of beta-lactams to be responsible for positive test results in two allograft bone marrow transplant recipients. Both patients had received a first beta-lactam treatment (TZP- or oral AMX-based therapy), during which patients became positive, with high GMI values (>1.5). Upon switching to oral PEN at hospital discharge along with *Aspergillus*-targeted prophylaxis, GMI decreased to below 1.5 and was sustained as long as oral PEN treatment lasted. However, these formulations either were not tested or tested negative when diluted. Thus, these results shed doubt on the causative role of these oral drugs towards positive antigenemia. A more likely explanation is a passage of either dietary GM or *Aspergillus* conidia present in the airways or the digestive tract into the blood, possibly because of local damage due to a viral infection, graft-versus-host disease, and related therapies (9, 12). Besides, autoreactive antibodies or paraproteins associated with chronic graft-versus-host disease have been suggested to be responsible for false-positive test results (7).

To avoid the risk of false presumption of IA due to false-positive test results, the restriction of TZP medication in bone marrow transplant patients with febrile neutropenia has been suggested (29). In our center, TZP has been discarded from the antibiotic arsenal for treatment of hematological patients at risk for IA, and we now experience this problem very sporadically. Meanwhile, if confronted with false-positive results related to AMX- or AMP-based therapies, we thought of a solution to circumvent this issue. First, we started to test all new batches centralized at the General Pharmacy to ensure an internal quality control. However, we promptly abandoned this

costly strategy because it did not prevent all departments from using positive vials for patients at risk for IA. Second, we asked for a collection of some volume of drug infusion along with patient serum. Unfortunately, the infusion sampling was often missed, thus constraining us to identify the batch and re-collect the vial(s) for any patient treated with a given beta-lactam.

In a recent in vitro study, Singh et al. hypothesized that EIA negativity should be obtained by sampling serum at trough levels or prior to the administration of a dose of contaminated TZP, presuming that GM (if not an EIA cross-reacting antigen) clearance from blood would match that of the beta-lactam (22). Nevertheless, Walsh et al. showed that GM EIA was still positive in sera of rabbits treated for 7 days with TZP, whatever the sampling period (30). Our results, based on five patients with cumulative infusions of either AMC or AMP, also tend to invalidate the hypothesis of Singh et al. Despite a significant GMI decrease at trough periods, serum samples still tested positive after 2 to 11 days of beta-lactam therapy. In addition, all antibiotic batches tested negative at equivalent C_{max}, which indicates that the in vitro EIA GMI may not predict in vivo GM titers. While a correlation between the concentration of AMX in plasma and GMI has been previously reported, as is very likely for AMP or PIP, the persistence of positive antigenemia is likely to result from different rates of antigen clearance from the blood and possibly the variable concentrations of antigen in different contaminated batches as previously suggested (18, 22).

Overall, this clinically based study provide evidence for a strong association between AMP, AMX, and AMC administration and false-positive test results with the Platelia *Aspergillus* EIA as previously described for TZP. At cumulative doses, which is the common therapeutic situation encountered in immunocompromised patients, our results support that there is no strict parallel between the clearance of GM and the clearance of beta-lactam from blood. Thus, sampling of serum at trough will not abolish the EIA positivity; at best, it would lower it. Moreover, assessing the level of EIA reactivity of the antibiotic batch will not help to predict that of patient serum. Therefore, although demanding and costly, the testing of every batch given to a patient at risk for IA remains the only indicator of possible false EIA positivity. Thus, we recommend this procedure as a quality control for *Penicillium*-derived beta-lactams. Finally, our data suggest that EIA positivity that is presumably due to the fungal GM present in certain batches of these hemisynthetic penicillins may also depend upon various factors related to the patient's status. Whether serum components of unknown origin such as nonproteic antigens, autoantibodies, or paraproteins that are insufficiently neutralized during pretreatment could amplify EIA reactivity is another issue that may deserve research interest.

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