BRIEF REPORT

False-Positive Galactomannan Platelia *Aspergillus* Test Results for Patients Receiving Piperacillin-Tazobactam

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At the bone marrow transplantation center of the San Martino Hospital (Genoa), we observed an increase in the rate of patients with positive Platelia *Aspergillus* (PA; Bio-Rad) test results, from 10% (38 of 386 patients) in the period from January 1999 through January 2003 to 36% (21 of 59 patients) in the period from February 2003 through May 2003. Positivity was significantly ($P < .001$) associated with the administration of piperacillin-tazobactam (PT) (17 [74%] of 23 patients who received PT had positive results vs. 4 [11%] of 36 who did not receive PT). Multivariate analysis found administration of PT ($\chi^2 = 34.7$; $P < .001$) and underlying disease ($\chi^2 = 21.14$; $P < .001$) to be associated with PA positivity. Of 15 PT batches tested, 12 had positive PA test results.

Despite the availability of new and promising antifungal drugs, the mortality rate among patients with invasive aspergillosis (IA) remains high [1]. This is likely due, at least in part, to the frequent impossibility of obtaining a reliable diagnosis at an early stage of the disease and the consequent delays in starting treatment. In recent years, some progress in early diagnosis has been reported with the combined use of high-resolution CT and serial screening for circulating *Aspergillus* galactomannan, a fungal exoantigen that is part of the fungal cell wall [2–4]. Although contrasting results have been reported, sensitivity and specificity appear to be fairly good, and the test results are sometimes positive before the onset of symptoms or radiological abnormalities. The test was recently approved by the US Food and Drug Administration. At the Bone Marrow Transplantation Center of the San Martino Hospital (Genoa), twice-weekly patient monitoring with the Platelia *Aspergillus* (PA; Bio-Rad) test (performed at the Infectious Disease Unit of the National Institute for Cancer Research [Genoa]) has been common practice since 1999. In April 2003, in the setting of a routine internal quality control assessment, we realized that the proportion of positive samples was increasing, and we decided to undergo a thorough reevaluation process to learn whether the increase was related to an aspergillosis outbreak or to some interaction causing false-positive results.

Methods. The characteristics of the PA test have been described elsewhere [5–9]. We process serum samples according to manufacturer's instructions, and the ELISA results are tabulated as the index between the optical density of the sample tested and the optical density of the threshold positive control. At least 2 subsequent positive test results are required for a patient to be considered positive for IA. The cutoff point we used for defining a positive sample decreased over the years, from 1.5 to 0.7, in accordance with published indications [10–12]. For the purpose of the present quality control, a threshold of 0.7 was used. To evaluate whether or not we were actually facing an increase in the proportion of positive results with respect to our normal values, we calculated the monthly distribution of positive results for the period from January 1999 through May 2003. We then looked at the clinical history of patients with ⩾2 consecutive positive test results in the previous 4 months of observation, and we classified each case as proven, probable, or possible aspergillosis, on the basis of the criteria established by the European Organization for Research and Treatment of Cancer–Mycoses Study Group [13], with and without considering the PA test result. Finally, we looked at major differences in laboratory and clinical procedures, and we performed a multivariate analysis of baseline clinical factors that might have been associated with the chance of having a positive result.

Results. From January 1999 through May 2003, a total of 420 consecutive patients were sequentially monitored twice weekly with the PA test. A total of 4702 serum samples were tested during this period, with a median of 7 specimens per patient (range, 1–64) and 85 specimens per month (range, 35–146). As shown in figure 1, in the period from January 1999 through January 2003, the median positivity rate per month

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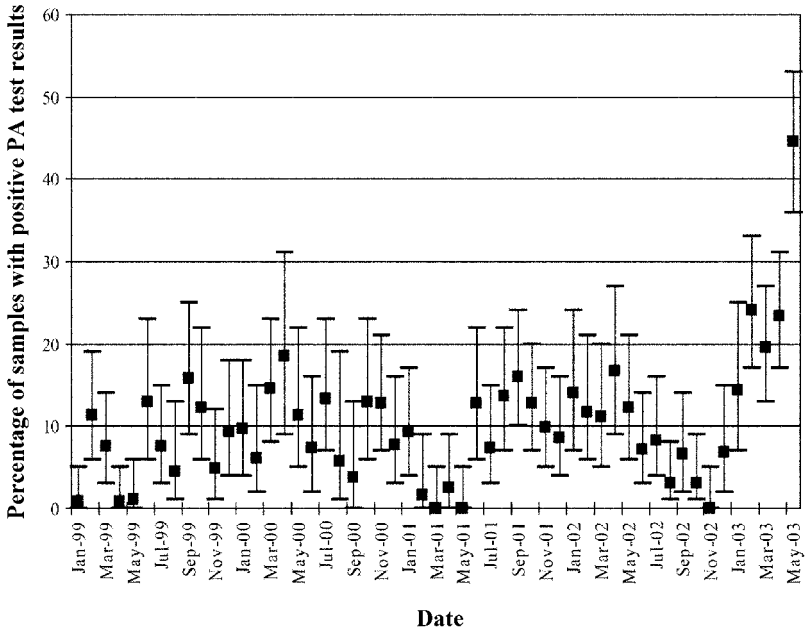

Figure 1. Percentage of samples per month with positive Platelia *Aspergillus* (PA) test results, January 1999–May 2003. All samples were obtained from patients who underwent bone marrow transplantation. Bars indicate 95% CIs.

in terms of blood specimens was 9% (range, 0–18%) and increased to 24% (range, 20–44%) in the period from February 2003 through May 2003. Similarly, the overall proportion of patients classified as positive for IA according to the PA test (i.e., those with ⩾2 samples with positive PA test results obtained within 7 days) increased from 10% (38 of 386 patients) in the first study period to 36% (21 of 59) in the second study period (25 patients overlapped in the 2 periods). Possible explanations for this phenomenon included an outbreak of IA, a technical problem related to the test itself, or an interaction with unknown factors able to cause false-positive results.

To rule out the possibility of an aspergillosis outbreak, we reviewed the clinical histories of the 21 patients with positive PA test results during the period from February 2003 through May 2003, using the standard definitions for invasive fungal infection of the EORTC-MSG consensus group [13]. Overall, no patient had proven aspergillosis, 4 had probable aspergillosis, and 17 had possible aspergillosis. However, not taking into account the results of the PA test, 3 of the 4 cases of probable aspergillosis and all of the cases of possible aspergillosis would have been downgraded to possible aspergillosis or no aspergillosis, respectively. Indeed, the 17 patients with possible aspergillosis had no symptoms whatsoever. This led us to exclude the possibility that the increase in the rate of positive PA test results was actually due to an aspergillosis outbreak.

To rule out the possibility of a technical dysfunction, we reviewed all laboratory procedures. No methodological change had been introduced. In addition, there was no difference in the way that the 2 PA batches that were in use during the study period performed. Finally, the first batch had been in use since January 2003, when the proportion of positive samples was within our normal values (i.e., 0%–18%).

As for clinical factors, patients were treated in the same rooms during the possible outbreak that they had been treated in earlier, and no change had been made in their parenteral or enteral nutrition or immunosuppressive regimens. Similarly, no change in the usual distribution of pathogens that cause infection was documented, and, in particular, no pathogen known to cause false-positive PA test results was isolated [14–16]. However, an important clinical factor was detected. Beginning in February 2003, because of an increasing incidence of piperacillin-sensitive *Enterococcus faecalis* bacteremia, the usual empirical antibiotic regimen (ceftazidime and amikacin) was gradually replaced by a new regimen (piperacillin-tazobactam and amikacin) to improve the antistreptococcal coverage. The proportion of patients positive for IA according to the PA test (i.e., those with ⩾2 positive PA test results within 7 days) was 74% (17 of 23) among those receiving piperacillin-tazobactam, compared with 11% (4 of 36) among those not receiving piperacillin-tazobactam ($P < .001$). For those receiving piperacillin-tazobactam, blood samples were obtained during treatment or within 24 h after discontinuation of treatment. A multivariate analysis of baseline factors potentially able to affect PA test results was performed. The probability of having a positive result in the 59 patients monitored in the period from February 2003 through May 2003 was modeled by means of multivariate logistic regression as a function of several variables, including the underlying disease (acute leukemia, chronic leukemia, my-

eloma and lymphoma, aplastic anemia, or other), type of transplantation (HLA identical, mismatched/unrelated, autologous, or other), status of the underlying disease (first remission, second or third remission, or relapse), use of total body irradiation (yes or no), use of thymoglobulins (yes or no), type of graft-versus-host disease prophylaxis (methotrexate and cyclophosphamide or other), age (continuous variable), sex, type of empirical antibiotic therapy, type of stem cells used (peripheral or BMT), and donor and/or recipient CMV status. The only 2 variables significantly associated with the odds of a positive test result were underlying disease ($\chi^2 = 21.14$; $P < .001$) and type of empirical antibiotic therapy ($\chi^2 = 34.7$; $P < .001$).

Thirty piperacillin-tazobactam vials from 15 different batches taken from the hospital pharmacy were then tested with the PA test. The drug was diluted according to manufacturer's instructions (4.5 g/100mL NaCl 0.9%), and the test was performed on both the diluted and the undiluted solutions (NaCl 0.9%) with the same methodology used for serum specimens. Overall, 12 (80%) of 15 batches (including 2 batches that were being used in the bone marrow transplantation unit during the study period) had positive PA test results, with a median galactomannan index of 4.6 (range, 1.3–5.7), and 3 batches had negative PA test results, with a median index of 0.16 (range, 0.11–0.19). All PA tests performed on the NaCl 0.9% dilutent had negative results.

Discussion. The detection of circulating galactomannan in serum samples obtained from patients who are at high risk for fungal infections is an important advance in the diagnosis of invasive aspergillosis in patients who receive bone marrow transplantation. The sensitivity and specificity of the test varies according to several factors, including the number of samples obtained from each patient, the extent of fungal angioinvasion, and the concomitant presence in the patient of cross-reacting substances or other infections. Indeed, cross-reactions of the monoclonal antibody (MAb) EB-A2, used in this test, have been described with other organisms (such as *Fusarium oxysporum, Rhodotorula rubra* [14], *Trichophyton rubrum, Trichophyton interdigitalis* [15], *Penicillium chrysogenum, Penicillium digitatum, Paecilomices variotii,* and *Alternaria* species [16]) and also with infant milk formulas [17], cyclophosphamide [18], and food [19, 20]. In the clinical field, false-positive results have been especially frequent among premature infants [21]. In 1997, Ansorg et al. [19] studied the in vitro specificity of the latex agglutination test Pastorex *Aspergillus*, which employs the same MAb EB-A2 as the PA test. Among other substances, they also tested piperacillin and amoxicillin and found that both antibiotics sometimes had positive latex test results. It is worth noting that the latex test was detecting only 10 ng/mL of galactomannan in serum vs. 1 ng/mL of galactomannan detected by the ELISA test. In the present study, we found that the administration of piperacillin-tazobactam as empirical antibacterial therapy in recipients of bone marrow transplantation is associated with a high incidence of false-positive PA test results. The test also produced positive results for 12 of 15 batches of piperacillin-tazobactam used at the Bone Marrow Transplantation Center of the Department of Hematology, San Martino Hospital (Genoa), at the time of the study. Piperacillin is a semisynthetic drug derived from molds of the genus *Penicillium,* a filamentous fungus that contains galactomannan in the cell wall [15]. As has already been suggested [19], it may be reasonable to think that galactomannan (or similar moieties bearing the epitope reacting with MAb EB-A2) is carried through the production process of this antibiotic into batches designed for therapeutic use.

In our epidemiological situation, piperacillin-tazobactam is an important drug for the management of febrile neutropenia. For this reason, we have decided to continue to use the drug, although only in the preengraftment period, when the risk of aspergillosis and consequent need for galactomannan monitoring are lower and the risk of severe bacterial infection is higher. With the gradual introduction of this new antibiotic policy, the positivity rate decreased from 45% in May 2003 to 29% in June 2003 and to 5% in July 2003.

References

1. Herbrecht R, Denning DW, Patterson TF, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med **2002**; 347:408–15.
2. Caillot D, Casasnovas O, Bernard A, et al. Improved management of invasive pulmonary aspergillosis in neutropenic patients using early thoracic computed tomographic scan and surgery. J Clin Oncol **1997**; 15:139–47.
3. Maertens J, Van Eldere J, Verhaegen J, Verbeken E, Verschakelen J, Boogaerts M. Use of circulating galactomannan screening for early diagnosis of invasive aspergillosis in allogeneic stem cell transplant recipients. J Infect Dis **2002**; 186:1297–306.
4. Machetti M, Feasi M, Mordini N, et al. Comparison of an enzyme immunoassay and a latex agglutination system for the diagnosis of invasive aspergillosis in bone marrow transplant recipients. Bone Marrow Transplant **1998**; 21:917–21.
5. Stynen D, Goris A, Sarfati J, Latgé JP. A new sensitive sandwich enzyme-linked immunosorbent assay to detect galactofuran in patients with invasive aspergillosis. J Clin Microbiol **1995**; 33:497–500.
6. Verweij PE, Stynen D, Rijs AJMM, De Pauw BE, Hoogkamp-Korstanje JAA, Meis JFGM. Sandwich enzyme-linked immunosorbent assay compared with Pastorex latex agglutination test for diagnosing invasive aspergillosis in immunocompromised patients. J Clin Microbiol **1995**; 33:1912–4.
7. Rohrlich P, Sarfati J, Mariani P, et al. Prospective sandwich enzyme-linked immunosorbent assay for serum galactomannan: early predictive value and clinical use in invasive aspergillosis. Pediatr Infect Dis J **1996**; 15:232–7.
8. Maertens J, Verhaegen J, Demuynck H, et al. Autopsy-controlled prospective evaluation of serial screening for circulating galactomannan by a sandwich enzyme-linked immunosorbent assay for hematological patients at risk for invasive aspergillosis. J Clin Microbiol **1999**; 37: 3223–8.
9. Viscoli C, Machetti M, Gazzola P, et al. *Aspergillus* galactomannan

antigen in the cerebrospinal fluid of bone marrow transplant recipients with probable cerebral aspergillosis. J Clin Microbiol **2002**; 40:1496–9.
10. Verweij PE, Erjavec Z, Sluiters W, et al. Detection of antigen in sera of patients with invasive aspergillosis: intra and interlaboratory reproducibility. J Clin Microbiol **1998**; 36:1612–6.
11. Sulahian A, Boutboul F, Ribaud P, Leblanc T, Lacroix C, Derouin F. Value of antigen detection using an enzyme immunoassay in the diagnosis and prediction of invasive aspergillosis in two adult and pediatric hematology units during a 4-year prospective study. Cancer **2001**; 91:311–8.
12. Herbrecht R, Letscher-Bru V, Oprea C, et al. *Aspergillus* galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. J Clin Oncol **2002**; 20:1898–906.
13. Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis **2002**; 34:7–14.
14. Kappe R, Schulze-Berge A. New cause for false positive results with the Pastorex *Aspergillus* antigen latex agglutination test. J Clin Microbiol **1993**; 31:2489–90.
15. Stynen D, Sarfati J, Goris A, et al. Rat monoclonal antibodies against *Aspergillus* galactomannan. Infect Immun **1992**; 60:2237–45.
16. Swanink CM, Meis JF, Rijs AJ, Donnelly JP, Verweij PE. Specificity of a sandwich enzyme-linked immunosorbent assay for detecting *Aspergillus* galactomannan. J Clin Microbiol **1997**; 35:257–60.
17. Gangneux JP, Lavarde D, Bretagne S, Guiguen C, Gandemer V. Transient *Aspergillus* antigenaemia: think of milk. Lancet **2002**; 359:1251.
18. Hashiguchi K, Niki Y, Soejima R. Cyclophosphamide induces false-positive results in detection of *Aspergillus* antigen in urine. Chest **1994**; 105:975.
19. Ansorg R, Van den Boom R, Rath PM. Detection of *Aspergillus* galactomannan antigen in foods and antibiotics. Mycoses **1997**; 40:353–7.
20. Letscher-Bru V, Cavalier A, Pernot-Marino E, et al. Recherche d'antigène galactomannane aspergillaire circulant par Platelia *Aspergillus*: antigénémies positivies en l'absence d'infection. J Mycol Méd **1998**; 8: 112–3.
21. Siemann M, Koch-Dorfler M, Gaude M. False-positive results in premature infants with the Platelia *Aspergillus* sandwich enzyme-linked immunosorbent assay. Mycoses **1998**; 41:373–7.