

Positive Result of the *Aspergillus* Galactomannan Antigen Assay Using Bronchoalveolar Lavage Fluid from a Patient with an Invasive Infection Due to *Lichtheimia ramosa*[▽]

Positive galactomannan antigen (GM) test results with serum by the Platelia *Aspergillus* assay (Bio-Rad, Marnes-La-Coquette, France) have been reported to occur during invasive fungal infections caused by *Penicillium marneffei* (8), *Cryptococcus neoformans* (2), *Geotrichum capitatum* (3), or *Histoplasma capsulatum* (1, 6, 11). We report on a case of a positive GM result in bronchoalveolar lavage fluid (BAL fluid) in the clinical setting of a possibly invasive infection caused by *Lichtheimia ramosa*. The patient was a 10-year-old girl with acute myeloblastic leukemia in second relapse who presented with fever (101°F), cough, and dyspnea. A chest X ray showed diffuse reticular changes, and a blood count revealed severe neutropenia (200 leukocytes/ μ l). The patient began empirical treatment with ceftazidime and amikacin. Vancomycin was later added on the basis of Gram stain results from a positive blood culture. A computed tomography (CT) scan was performed 48 h after admission. A nodular lesion in the right upper lobe compatible with invasive aspergillosis was observed. Voriconazole therapy was then initiated. Worsening of the patient's clinical condition prompted the addition of meropenem and caspofungin to the antibiotic regimen. Fiberoptic bronchoscopy was performed (14 days after admission), and BAL fluid in saline solution was submitted to the Microbiology Service for microscopic examination, culture, and GM testing. Nonseptate hyphae with irregular branching were seen by calcofluor white staining of a direct smear. *Lichtheimia ramosa* (deposited in the Spanish Type Culture Collection) was isolated on Sabouraud glucose medium within 48 h of plating. Microbial identification was confirmed by direct sequencing of the internal transcribed spacer 2 (ITS2) region of the ribosomal DNA gene (9). The antifungal susceptibility profile as determined by a conventional microdilution method was as follows: the isolate was susceptible to amphotericin B (0.25 μ g/ml) and posaconazole (0.5 μ g/ml) and resistant to the echinocandins (>8 μ g/ml). The BAL fluid (two different aliquots) tested positive by the GM assay (index value, 2.9). The positive results were reproduced in a second analysis of samples. *Aspergillus* species DNA was not detected in BAL fluid by real-time PCR (FXG:RESP Asp⁺ test kit and Cepheid Smart-Cycler; Myconostica Ltd., Manchester, United Kingdom). The patient was admitted to the intensive care unit and died shortly thereafter. Serial serum samples obtained at hospital admission until 16 h prior to death ($n = 3$) tested negative by the GM assay (index values of 0.103, 0.104, and 0.107).

A mycelial extract antigen from the isolated organism was prepared (4) and tested at a 1:100 dilution in phosphate-buffered saline by the GM assay, yielding a positive result (index value, 1.23). Extracellular polysaccharide antigens produced by *Zygomycetes*, such as *Rhizopus oryzae*, *Lichtheimia corymbifera*, and *Cunninghamella bertholletiae* (but not by *Lichtheimia ramosa*), have been shown previously to test negative (index values ≤ 0.5) in the Platelia assay when assayed at a 1:1,000 dilution (1, 10).

Lichtheimia ramosa is an infrequent cause of zygomycosis (7). Although invasive infection was not demonstrated by means of histopathology studies (necropsy was not autho-

rized), it is unlikely that the recovery of *Lichtheimia ramosa* in the current case reflected an environmental contamination.

The possibility of false-positive results with the GM assay occurring in the setting of invasive zygomycosis has not been investigated in depth. Hsu et al. (5) found a weakly positive result with BAL fluid (GM index < 1.0) from a patient with invasive mucormycosis. To the best of our knowledge, this is the first report of a false-positive GM result with the Platelia assay in the setting of a possibly invasive zygomycosis due to *Lichtheimia ramosa* in a patient with no evidence of *Aspergillus* infection. Interestingly, the BAL fluid, but not the serial sera obtained within the hospitalization period of the patient, tested positive. It is uncertain whether early implementation of mold-active antifungal therapy (48 h after admission) may have caused negative results in serum specimens. The clinical course and the CT scan profile of invasive *Lichtheimia ramosa* infection may resemble those seen in the setting of invasive Aspergillosis. Our finding has a major therapeutic implication, as antifungal agents such as voriconazole and caspofungin, deemed appropriate for the treatment of aspergillosis, have been proven clinically inefficacious in the treatment of zygomycosis (7).

Nucleotide sequence accession number. The *Lichtheimia ramosa* isolate was deposited in the Spanish Type Culture Collection under accession number 20762.

REFERENCES

1. Cummings, J. R., G. R. Jamison, J. W. Boudreaux, M. J. Howles, T. J. Walsh, and R. T. Hayden. 2007. Cross-reactivity of non-*Aspergillus* fungal species in the *Aspergillus* galactomannan enzyme immunoassay. *Diagn. Microbiol. Infect. Dis.* **59**:113–115.
2. Dalle, F., P. E. Charles, K. Blanc, D. Caillot, P. Chavanet, F. Dromer, and A. Bonnin. 2005. *Cryptococcus neoformans* galactoxylomannan contains an epitope(s) that is cross-reactive with *Aspergillus* galactomannan. *J. Clin. Microbiol.* **43**:2929–2931.
3. Giacchino, M., N. Chiapello, S. Bezzio, F. Fagioli, P. Saracco, A. Alfarano, V. Martini, G. Cimino, P. Martino, and C. Girmenia. 2006. *Aspergillus* galactomannan enzyme-linked immunosorbent assay cross-reactivity caused by invasive *Geotrichum capitatum*. *J. Clin. Microbiol.* **44**:3432–3434.
4. Hao, W., Y. X. Pan, Y. Q. Ding, S. Xiao, K. Yin, Y. D. Wang, L. W. Qiu, Q. L. Zhang, P. C. Woo, S. K. Lau, K. Y. Yuen, and X. Y. Che. 2008. Well-characterized monoclonal antibodies against cell wall antigen of *Aspergillus* species improve immunoassay. *Clin. Vaccine Immunol.* **15**:194–202.
5. Hsu, L. Y., Y. Ding, J. Phua, L. P. Koh, D. S. Chan, K. L. Khoo, and P. A. Tambyah. 2010. Galactomannan testing of bronchoalveolar lavage fluid is useful for diagnosis of invasive pulmonary aspergillosis in hematology patients. *BMC Infect. Dis.* **10**:44.
6. Narreddy, S., and P. H. Chandrasekar. 2008. False-positive *Aspergillus* galactomannan (GM) assay in histoplasmosis. *J. Infect.* **56**:80–81.
7. Ribes, J. A., C. L. Vanover-Sams, and D. J. Baker. 2000. Zygomycetes in human disease. *Clin. Microbiol. Rev.* **13**:236–301.
8. Rimek, D., T. Zimmermann, M. Hartmann, C. Prariyachatigul, and R. Kappe. 1999. Disseminated *Penicillium marneffei* infection in an HIV-positive female from Thailand in Germany. *Mycoses* **42**(Suppl. 2):25–28.
9. Schwarz, P., S. Bretagne, J. C. Gantier, D. Garcia-Hermoso, O. Lortholary, F. Dromer, and E. E. Dannaoui. 2006. Molecular identification of zygomycetes from culture and experimentally infected tissues. *J. Clin. Microbiol.* **44**:340–349.
10. Swanink, C. M., J. F. Meis, A. J. Rijs, J. P. Donnelly, and P. E. Verweij. 1997.

Specificity of a sandwich enzyme-linked immunosorbent assay for detecting *Aspergillus* galactomannan. *J. Clin. Microbiol.* **35**:257–260.

11. **Wheat, L. J., E. Hackett, M. Durkin, P. Connolly, R. Petraitiene, T. J. Walsh,**

K. Knox, and C. Hage. 2007. Histoplasmosis-associated cross-reactivity in the BioRad Platelia *Aspergillus* enzyme immunoassay. *Clin. Vaccine Immunol.* **14**:638–640.

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^v Published ahead of print on 16 June 2010.