False positive galactomannan results in adult hematological patients treated with piperacillin-tazobactam

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Summary

In this prospective study including 78 adult patients with hematological malignancy (90 episodes) we performed galactomannan (GM) (Platelia *Aspergillus*) screening twice weekly for the diagnosis of invasive aspergillosis. There were five proven and four probable invasive aspergillosis cases. The sensitivity, specificity and positive and negative predictive values were 100, 88, 47 and 100%, respectively. There were eight patients with false positive GM (10.2%). In six patients the false GM reactivity was due to the administration of piperacillin-tazobactam (P-T). A significant association was found between false positive GM ($\geqslant 0.5$) and the administration of P-T ($p < 0.01$). Two other patients with no invasive aspergillosis (2.5%) and false GM reactivity had graft versus host disease (GVHD) and one of them had also mucositis grade IV. The kinetic patterns of false positive GM due to P-T is discussed.

Key words Galactomannan, Piperacillin-tazobactam, False-positives, Aspergillosis, Diagnosis.

Resultados falsos positivos de galactomanano en pacientes hematológicos adultos tratados con piperacilina-tazobactam

Resumen

Se han estudiado prospectivamente dos veces por semana los niveles séricos de galactomanano (GM) (Platelia *Aspergillus*) en 78 pacientes con cáncer hematológico (90 episodios) para el diagnóstico de aspergilosis invasora (AI). Hubo cinco casos de AI probada y cuatro de AI probable. La sensibilidad, especificidad y valor predictivo positivo y negativo fueron de 100, 88, 47 y 100% respectivamente. Hubo ocho pacientes con GM falsos positivos (10,2%). En seis enfermos la falsa reactividad de GM fue debida a la administración de piperacilina-tazobactam (P-T), encontrándose una asociación significativa entre galactomananos falsos positivos y la administración de P-T ($p < 0.01$). Otros dos pacientes sin AI y GM falsos positivos (2,5%) tuvieron como posible causa de falsa positividad la enfermedad injerto contra huésped y uno de ellos además tenía mucositis grado IV. En el trabajo se han analizado los patrones cinéticos con falsa reactividad de GM en relación a P-T.

Palabras clave Galactomanano, Piperacilina-tazobactam, Falsos positivos, Aspergilosis, Diagnóstico

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The commercial sandwich enzyme-linked immunosorbent assay (ELISA) (Platelia *Aspergillus*, Bio-Rad, Marnes La Coquette, France) is widely used worldwide for the diagnosis of invasive aspergillosis (IA) in adult immunocompromised patients [9,20,28,32,42]. Galactomamman (GM) is a polysaccharide from the cell wall of *Aspergillus* spp. usually secreted to the blood in patients with IA. Currently, it is detected by the Platelia *Aspergillus* ELISA test by means of the monoclonal antibody EB-A2 which acts both as a captor and detector [42]. The monoclonal antibody EB-A2 is an immunoglobulin M (IgM) that recognizes the 1-5-β-D galactofuranoside side chains of the *Aspergillus* GM molecule but cross-reaction with several fungal exoantigens from other genera has also been reported [8,24,42].

It is out of doubt the value of screening prospectively for circulating *Aspergillus* GM in adult hematological cancer patients using a stratification scheme defined by Prentice et al. [38] for the diagnosis of IA.

This marker provides an early diagnosis of IA and the implementation of pre-emptive therapeutic strategies [18]. The sensitivity and specificity of GM ELISA appears to be adequate and timely since a positive GM appears before the onset of clinical symptoms or radiological abnormalities detected by high resolution computed tomography scanning (HRCT) [9,18,20,24,32,43]. However, a pitfall in the indirect diagnosis of IA is the occurrence of false positive GM ELISA results which widely varies from 5% to 15% in adults [20,24,28,32,42] to as much as 83% in neonates [43].

Herein we report the occurrence of false positive GM ELISA results associated with the use of piperacillin-tazobactam (P-T) treatment and other possible factors in patients with hematological malignancies using a risk stratification scheme. In order to validate the proposal of Prentice et al. [38], our group has studied prospectively a cohort of 78 adult neutropenic patients (90 episodes) exploring the incidence of IFI and IA with the use of GM screening as a diagnostic tool [27]. The incidence of IFI and IA correlated directly and significantly with risk stratification, with the highest incidence (31%) in the high risk group (n = 16), followed by the intermediate high risk (12% incidence) (n = 17) and intermediate low risk group (8% incidence) (n = 37).

Material and methods

Patient selection. From September 2004 to May 2005, a cohort of 78 adult hematological cancer patients treated in the Hospital Doce de Octubre, Madrid, Spain and stratified according to the scheme of Prentice et al. [38], were prospectively analyzed (as a routine screening twice weekly) establishing the GM index (GMI) by using the commercially available sandwich ELISA (Platelia *Aspergillus)* until the risk condition for developing IFI had subsided. All patients were nursed in rooms with HEPA filtration. The clinical assessment of our patients is the standard of care in tertiary hospitals, and has been described by our group elsewhere [32].

Definition of invasive aspergillosis. IA episodes were classified on the basis of the European Organization for Research and Treatment of Cancer / Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infections Diseases Mycoses Study Group (EORTC-IFICG and NIAID-MSG) case definitions [4].

Diagnostic work-up of IFI. In cases of suspicion of IFI, or when the GMI was above 0.5, a diagnostic work-up was started; this included a pulmonary high resolution computed tomography scanning (HRCT) followed, when possible, by bronchoalveolar lavage and/or biopsy for bacterial, mycobacterial, fungal and viral cultures. Direct examination for bacteria and fungi (including *Pneumocystis jiroveci*) was performed for all patients. The presence of *Legionella* antigen in urine was tested.

GM detection. The ELISA was performed as recommended by the manufacturer. Results (GMI) were expressed as the ratio of the optical density (OD) obtained from the patient serum sample and the control (index = OD of the sample / OD of the control). A result was considered a true positive with a GMI above or equal to 0.500 (static index) [17]. The serum was retested in these cases, showing good reproducibility. An index below 0.5 was considered negative.

Mycological studies. When judged necessary, specimens from clinically infected foci were collected and processed as described by Denning et al. [10]. *Aspergillus* species were identified by their macroscopic and microscopic culture characteristics.

Table 1. Characteristics of adult oncohematological patients with evaluation of GM.

Characteristic	Total	Proven IA	Probable IA	No IA*	False positive GM	
					P-T**	Others***
Patients (n)	78	5	4	69	6	2
Age (yr)[a]	52 (16-79)	60 (36-67)	39 (33-44)	51 (16-79)	61 (27-79)	41 (19-63)
Gender (M/F)[b]	42/36	3/2	1/3	38/31	4/2	1/1
Number (%) with underlying diseses[c]						
ALL	9	1	1	7	0	0
AML	14	1	1	12	1	1
CLL	5	0	0	5	0	1
MM	14	1	1	12	2	0
MDS	5	0	0	5	0	0
NHL	24	1	1	22	3	0
HD	6	1	0	5	0	0
SAA	1	0	0	1	0	0
Number of serum samples total (range)	881 (2-55)	68 (5-28)	84 (8-36)	729 (2-55)	93 (2-33)	70 (20-50)
Number of positive samples for GM (range)	62 (1-11)	12 (2-4)	24 (2-11)	26 (1-8)	23 (1-8)	3 (1-2)

*Includes patients with no IA and patients with false positive GM results. **P-T: Piperacillin-tazobactam. ***Two patients had graft versus host disease and one of them had also grade IV mucositis.

[a] Values in parenthesis are ranges. [b] M/F: male/female. [c] ALL: acute lymphocytic leukemia; AML: acute myeloid leukemia; CLL: chronic lymphocytic leukaemia; MM: multiple myeloma; MDS: Myelodisplastic syndrome; NHL: Non-Hodgking`s lymphoma; HD: Hodgkin disease; SAA: severe aplastic anemia.