D 2114

Aspergillus fumigatus detection by PCR in Bronchoalveolar Lavage Fluid

M.P. HAYETTE, P. BOLLAND, B. EVRARD and P. DE MOL,*
Univ. Hosp. Liege, Belgium

Université de Liège
Marie-Pierre Hayette
Microbiology department; University Hospital
4000 Liege.Belgium. mphayette@ulg.ac.be

Introduction

Aspergillus fumigatus is an opportunistic nosocomial pathogen which causes severe to fatal infection in neutropenic patients. The risk of invasive aspergillosis (IA) increases with the duration of neutropenia and can reach 70% after 5 weeks of neutropenia (1).

The diagnostic of IA is one of the most difficult anf the gold standard is still based on culture and/or histology results which sensitivity is very low. Recently, molecular biology methods were developped to diagnose aspergillosis in urines, serum, blood and BAL.

We used a nested PCR-based amplification of *A. fumigatus* DNA in BAL fluids that targets the genes-encoding alkaline proteases of the fungus to evaluate the usefullness of PCR to diagnose IA.

Abstract

The usefullness of a nested PCR for detection of *Aspergillus fumigatus* DNA was evaluated in bronchoalveolar lavage (BAL) fluid during a period of two years (1996-1998). The aim of the study was to assess the role of PCR in diagnosing invasive pulmonarry aspergillosis (IPA). **Methods**: a nested PCR-based amplplification of fragments of genes-encoding alkaline proteases from Aspergillus fumigatus was used to testt 167 BAL samples. All samples were checked for the absence of amplification inhibitors. Madical, radiological, microbiological records and autopsy findings were reviewed for assessing invasive aspergillosis. All successive patients investigated by BAL were included in the study. They were distributed in three groups: A: proven or probable aspergillosis (n=11),; B, colonization (n=é); C: no evidence of IPA (n=154). PCR results were compared to culture detection as gold standard and to clinical data. **Results**: BAL fluids from 10 patients of group A were PCR positive. One case was falsely negative. Among group B, one case was PCR positive, and the second one PCR negative but had negative BAL cultures (only culture positive sputum). No false positive was detected among group C. Comparing to culture, sensitivity was 91%, specificity, 100%, positive predictive value, 100%, and negative predictive value, 99%. **Conclusions:** Aspergillus fumigatus PCR in BAL fluid was an accurate test to diagnose culture negative patients with IPA and to confirm culture positive samples; however it does't make difference between infection and colonization. 2. It is an appropriate test to exclude *Aspergillus* infection in patients at risk of invasive illness.

Bibliography

•1.Ribaud P., Esperou-Bourdeau H., Devergie A., Gluckman E. Aspergillose invasive et allogreffe de moelle. Path. Biol., 1994, 42, 652-655.
•2.Tang C., Holden D., Aufauvre-Brown, Cohen J. The detection of Aspergillus spp. by the polymerase chain reaction and its evaluation in bronchoalveolar lavage fluid. Am. Rev. Resp. Dis., 1993, 148, 1313-1317.

Methods

•**Clinical data**: all patients undergoing bronchoscopy at the university hospital of Liege between 1996 and 1998 were included in the study and distributed in three groups:
- **•A: proven or probable aspergillosis (n=11)**
- **•B:colonization (n=2)**
- **•C: no evidence of aspergillosis (n=154)**

•**BAL fluid specimens**: all lavages collected by the microbiological laboratory were included in the study and stored at -20°C before processing. Microbiological examination was directly performed on every sample and results recorded.

•**DNA extraction**: adapted from Tang et coll. (2).
- •BAL fluid (250µl) + extraction buffer (250µl) containing proteinase K (100 µg): 65°C for 60 mn
- •extraction once with phenol:chloroform:isoamylalcool (25:24:1) and then with chloroform:isoamylalcool (24:1).
- •Precipitation of DNA by ethanol

•**Nested PCR**: The target DNA corresponds to genes-encoding alkaline proteases from *A. fumigatus (2).*

External primers: alp11 and alp12 (2).
Internal primers: alp13 (5'-CTGGCATACAACGCCGCT-3')
alp 14 (5'-TTGTTGATCGCAACC-3')
Product length after amplification: 527 base pairs.
Mix, (50µl volume): 10mM TRIS-Cl at pH 8,3, 50 mM KCl and 1.5 mM MgCl2 with 100 pmol of both primers and 1.25 U Taq polymerase (Takara Taq, Japan).
Thermal cycling conditions: 30 cycles at 94°C for 30 sec., 63°C for 45sec., and 72°C for 2 min.
Products of PCR : analyzed on 1.5% agarose gels, stained with ethidium bromide and visuallized by UV transillumination.

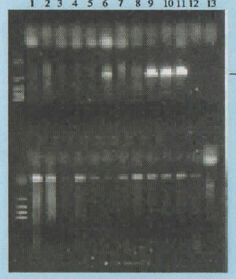

Figure 1. Amplification products on ethidium stained agarose gel. Above: lanes 1-5, 7,8: negative BAL. Lanes 6, 9: patient 7 from group A. Lane :10,11: *A. fumigatus* DNA. Lanes 12, 13: negative sample (water). Below: beta-globine amplification of BAL samples to assess the lack of amplification inhibitors.

Results

GROUP A: API or probable API (n=11)

case	UNDERLYING DISEASE	IPA	CULTURE positive	HISTOLOGY positive	PCR positive	SURVIVAL
1	Hepatic transplantation	yes	yes	yes	yes	no
2	COPB corticoids dependent	yes	yes	yes	yes	no
3	Hematologic malignancy	yes	yes	no histology	yes	no
4	Cardiac transplantation	yes	yes	yes	yes	no
5	Arthritis	yes	no	yes	yes	no
6	COPB corticoids dependent	yes	yes	yes	yes	no
7	Gastric carcinoma	yes	yes	yes	yes	no
8	COPB corticoids dependent	Probable	yes	yes	no	no
9	Bone marrow transplantation	yes	yes	no histology	yes	no
10	Hematologic malignancy	yes	yes	yes	yes	no
11	Hematologic malignancy	yes	yes	no histology	yes	no

GROUP B: colonization (n=2)

case	UNDERLYING DISEASE	IPA	CULTURE positive	HISTOLOGY positive	PCR positive	SURVIVAL
1	Mitral valvulopathy	no	yes	no histology	yes	yes
2	COPB (no corticoids)	no	yes	no histology	no	yes

COPB: Chronic obstructive pulmonary bronchitis.

GROUP C: no evidence of aspergillosis (n=154)

n	UNDERLYING DISEASE	IPA	CULTURE positive (n)	HISTOLOGY positive (n)	PCR positive (n)
154	Respiratory disease	0	0	0	0

Sensitivity and specificity of PCR

	PCR-	PCR+	
IPA	1	10	11
NO IPA	155	1	156
	156	11	**167**

Sensitivity: 91%
Specificity: 100%
Predictive values
negative 99%
positive 100%

Conclusions

1.*A. fumigatus* PCR in BAL fluid was an accurate test to diagnose culture negative patients with IPA and to confirm culture positive samples.
However it doesn't make difference between infection and colonization.

2. This PCR is an appropriate method to exclude *Aspergillus* infection in patients at risk of invasive illness.