Interest of Real-Time PCR for the Diagnosis of Invasive Aspergillosis

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INTRODUCTION

- Invasive aspergillosis (IA) :
  - major opportunistic infection in heamatology patients.
  - early diagnosis is critical to a good outcome, but is difficult to achieve with current methods.

AIM

- Comparison of real-time PCR assay and a PCR-ELISA assay in both serum and bronchoalveolar lavage (BAL) samples for IA diagnosis.

MATERIALS & METHODS

NESTED CASE-CONTROL DESIGN

COHORT STUDY

➢ Patients hospitalized in onco-heamatology departement of Hedi Chaker UH in Sfax-Tunisia, neutropenic (PNN < 500/µl) and feverish (T°>38.5°C).

➢ prospective clinical and biological practice :

- sera samples twice weekly
- broncho-alveolar lavage (BAL) if status of patients permit

➢ Patients with IA were diagnosed on the basis of EORTC/MSG criteria (2002, 2008).

NESTED CASE-CONTROL STUDY

➢ Using a nested case-control design, patients with proven or probable IA were compared to at-risk patients who were included in the cohort study and who had no evidence of invasive fungal infection during the follow-up. They were matched to the group cases with respect to age, sex, and follow-up duration.

➢ All samples (sera and LBA) of patients classified as IA and their controls were analyzed with the three techniques:

- Galactomannan Ag (GM), Platelia *Aspergillus* kit (Biorad).
- PCR-ELISA, (Roche Diagnostics) after extraction with QIA amp Mini Kit (Qiagene).
- Real-time PCR system Mx4000 (Stratagene)
- BAL samples: culture Cz, macroscopy and microscopy identification, Molecular identification: PCR-sequencing: (ITS1), 5.8S, and ITS2 region rRNA sequence analysis

STATISTICAL ANALYSIS

➢ Description of the diagnostic contribution of each test in IA patients.

➢ Determination of diagnostic index for each technique by comparing IA patients (proven and probable) with controls.

RESULTS

COHORT

- 163 patients were included, 47 with IA: IA proven:1, IA probable: 31, IA possible: 15.
- Lethality: 70.9% and 40% for IA probable and possible, respectively.
- ITS sequencing proved that all isolates collected from BAL were *Aspergillus flavus*.

NESTED CASE-CONTROL STUDY

- 47control cases were matched to the group cases with respect to age, sex, and follow-up duration.
- Analysis of 459 sera and 42 LBA.

Diagnostic performance of PCR-ELISA and real-time PCR monitoring in blood samples for the diagnosis of IA in at-risk patients with hematological malignancies according to the EORTC 2008 or 2008 criteria.

	EORTC 2002 criteria		EORTC 2008 criteria	
	PCR-ELISA	Real-time PCR	PCR-ELISA	Real-time PCR
Sensitivity	96.9	93.8	86.4	72.7
[95% CI]	[90.2-96.9]	[86.6-93.8]	[80.3-86-4]	[66.3-72.7]
Specificity	100	100	100	100
[95% CI]	[95.5-100]	[95.1-100]	[94.3-100]	[94.0-100]
LR+	Inf	Inf	Inf	Inf
[95% CI]	[19.890-inf]	[17.681-inf]	[14.177-inf]	[10.971-inf]
LR-	0.031	0.063	0.136	0.273
[95% CI]	[0.031-0.103]	[0.063-0.141]	[0.136-0.209]	[0.273-0.359]
DOR	Inf	Inf	Inf	Inf
[95% CI]	[194.02-inf]	[125.11-inf]	[67.93-inf]	[30.57-inf]

Diagnostic performance of galactomannan Ag (GMA), PCR-ELISA and real-time PCR in bronchoalveolar fluid samples for the diagnosis of IA in (n=42) at-risk patients with hematological malignancies.

	GMA	PCR-ELISA	Real-time PCR	Direct Examination	Culture
Sensitivity	85.7	71.4	64.3	21.4	35.7
[95% CI]	[67.4-94.3]	[53.6-77.2]	[46.4-70.1]	[9.8-21.4]	[21.4-35.7]
Specificity	92.9	96.4	96.4	100	100
[95% CI]	[83.7-97.2]	[87.5-99.3]	[87.5-99.3]	[94.2-100]	[60.1-100]
LR+	12	20	18	Inf	Inf
[95% CI]	[4.144-33.322]	[4.282-115.970]	[3.711-106.123]	[1.698-Inf]	[3.007-Inf]
LR-	0.154	0.296	0.370	0.786	0.643
[95% CI]	[0.058-0.389]	[0.229-0.531]	[0.301-0.612]	[0.786-0.957]	[0.643-0.846]

DISCUSSION

- *A. flavus*: ethiologic agent of IA in our region.
- Real-time PCR, although slightly less sensitive, is much more workable than PCR-ELISA in the clinical laboratory setting.
- PCR assays for *Aspergillus* DNA detection in sera proved efficient for IA diagnosis, especially when associated with GMA detection, in a prospective screening strategy in patients at high-risk for IA.