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

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INTRODUCTION

- Invasive aspergillosis (IA):
  - major opportunistic infection in hematological 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-hematology department of Hedi Chaker UH in Sfax-Tunisia, neutropenic (PNL < 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 QIAamp Mini Kit (Qiagen).
  - Real-time PCR system Mx4000 (Stratagene)
- BAL samples: culture Cz, macroscopy and microscopy identification, Molecular identification: PCRun-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

NESTED CASE-CONTROL STUDY

- 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.

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.