Impact of real-time polymerase chain reaction in the diagnosis of acute meningitis in children

Microbiology Unit, Clinic Hospital of Valencia, Spain

Abstract

Real-time PCR was compared with traditional cell and bacterial CSF and blood cultures in the diagnosis of acute meningitis in paediatric patients. Sensitivity was higher for molecular methods -especially when meningitis was caused by enterovirus-, permitting in more cases the microbiological confirmation of the aetiology.

Materials & Methods

198 CSF samples and blood culture samples from children aged 10 days to 14 years old (average: 2.44), collected from June 2006 to March 2008, were tested in the Microbiology Unit of Clinic Hospital of Valencia (Spain).

All samples were cultured for bacterial and viral detection following the laboratory standard procedure. Chocolate agar, Sabouraud-chloramphenicol agar and Brain Heart Infusion broths were used for bacterial CSF culture; and for viral detection the CSF samples were inoculated in RD, VERO and MRC-5 cell cultures. Blood culture samples were introduced in the BD BACTEC™ 9120 Blood Culture System (Becton Dickinson) and subcultured when positive.

Nucleic acid from CSF samples was extracted using a BioRobot EZ1 Workstation with EZ1 Viral Mini Kit v2.0 (Qiagen) and with EZ1 DNA Blood 200 µl Kit (Qiagen) for RNA and DNA respectively. Multiplex Real-Time PCR was used to test each sample for Neisseria meningitidis and Streptococcus pneumoniae using Realcycler MENE-01 (Ingenie Molecular) in the Smart Cycler II (Cepheid). Enteroviruses were tested using Real-Time RT-PCR with a enterovirus ASR kit and a Smart Cycler II or Xpert EV kit and a GeneXpert® thermocycler (Cepheid).

Introduction & Purpose

Meningitis is one of the most important infectious syndromes in medical emergencies in hospitals, due to its high morbidity and mortality.

Meningitis caused by viruses normally present milder symptoms and are the most common, however bacterial meningitis causes greater mortality. Both aetiologies must be quickly distinguished in order to manage patients properly.

Patients with enteroviral meningitis are hospitalized and treated with antibiotics until bacterial culture of blood and cerebrospinal fluid (CSF) are negative after 48 hours of incubation. CSF viral culture is poorly sensitive, long-lasting (at least 3 days) and high cost.

Real-Time PCR can accelerate the availability of results in acute meningitis, but a comparison with traditional cell and bacterial culture must be done.

Results

Sixty-three patients (31.8%) were positive, 48 (76.2%) of them were positive for enterovirus, while 15 (23.8%) had a bacterial aetiology.

Discussion & Conclusions

Enteroviral meningitis diagnosis requires certainly the use of molecular techniques due to the low sensitivity of traditional cell culture. Indeed, cell culture in the present work showed 20.8% sensitivity versus 91.6% for the real-time PCR, coinciding with data published by other authors (1,2).

Real-time PCR for the detection of bacterial meningitis has been tested on fewer occasions. Here it has permitted the confirmation of a higher number of cases of neurococcical and meningococcical meningitis than the bacterial culture, like it occurs in other published works (3), although other aetiologies should be taken into account.

Real-time PCR has shown to be a powerful and rapid diagnostic tool in the diagnosis of acute meningitis, differentiating between bacterial and viral aetiologies in 4 to 5 hours, and helping to reduce antibiotic treatment as well as hospitalization time (4).

References