Comparison of Use of Cerebrospinal Fluid, Serum, and Throat Swab Specimens in Diagnosis of Enteroviral Acute Neurological Infection by a Rapid RNA Detection PCR Assay

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A PCR assay for detection of enterovirus RNA in multiple specimen types from patients with neurological infections was evaluated. Combined PCR assay of cerebrospinal fluid and serum (systemic specimens) was more sensitive than assaying either specimen alone in children but not in adults. Compared with PCR in systemic specimens, detection of enterovirus RNA in throat swabs showed a sensitivity of 62.5% and a specificity of 75.6%.

Enteroviruses (EVs) of the Picornaviridae family, including the polioviruses, echoviruses, group A and B coxsackieviruses, and more recently numbered EVs (67 distinct serotypes) are important human pathogens (10, 14). Neurological infections with the nonpolio EVs are common and important causes of morbidity in both children and adults (1, 3, 6). Previous reports have clearly established the etiological role of these viruses in aseptic meningitis, encephalitis, and chronic meningoencephalitis, as well as in paralytic myelitis, cerebellar ataxia, Guillain-Barré syndrome, and transverse myelitis (10). However, attempts to isolate EVs from cerebrospinal fluid (CSF), pharyngeal, and stool samples are frequently unsuccessful because of the low viral titer in clinical specimens and because some serotypes grow poorly in cell culture (4). Therefore, PCR techniques for the detection of the enterovirus genome have been introduced (2, 9, 11).

In this report, we used a commercially available PCR assay which utilizes a single enzyme for both reverse transcription (RT) and PCR steps, incorporates uracil-N-glycosylase to prevent carryover contaminations, and detects the EV amplicons in a microwell colorimetric assay within 6 h. Using this PCR assay, we investigated the potential utility of a PCR assay of both CSF and serum specimens (systemic specimens) for rapid diagnosis of acute EV neurological disease in adult and children patients. Moreover, the EV PCR results from throat swabs were compared to those obtained from systemic specimens.

Forty-four children and 17 adults hospitalized for suspected neurological virus infections were included. CSF specimens were obtained by lumbar puncture. Whole-blood samples were obtained by peripheral venous puncture, and the sera were sterilily discarded after centrifugation (10 min, 4°C, 3,500 x g). Throat swab specimens were obtained by using the Virocult system (Medical Wire and Equipment, Corsham, United Kingdom) (7).

CSF and throat swabs specimens were inoculated in duplicate into 24-well plates covered with monolayers of human diploid fibroblasts (MRC-5) and buffalo green monkey kidney (BGMK) cells as previously described (13).

EV RT-PCR assays were performed with the amplicor Roche EV kit (Amplicor PCR Diagnostics, Hoffmann-La-roche, Basel, Switzerland) according to the manufacturer’s instructions. Briefly, after chemical denaturation, the single-stranded PCR products were hybridized to the probe and detected with the avidin-horseradish peroxidase system. Results were scored as positive when the optical density value at 450 nm was >0.34 (7). \(X^2\) testing with Yates correction was used to compare the results of biological assays (\(P\) values of <0.05 were considered significant).

In the group of children, we detected specific EV RNA sequences in 22.7% (10 of 44) of CSF specimens, whereas the rates of EV isolation by cell culture were only 2.3% (1 of 44) in these samples (Table 1). At the same time, detection of EV RNA in serum was positive in 20.45% (9 of 44) of children studied (Table 1). This positive EV RNAemia was associated with a positive EV PCR result for CSF specimens in three patients with aseptic meningitis and in one patient with Guillain-Barré syndrome. Interestingly, a positive EV RNAemia result allowed us to establish the etiological diagnosis of neurological virus infection in one patient with encephalitis and in three patients with aseptic meningitis (Table 1). Combination of EV PCR testing of CSF and serum specimens was more sensitive than a single PCR test of a CSF (14 of 44 versus 10 of 44; \(P = 0.014\)) or of a serum (14 of 44 versus 9 of 44; \(P = 0.007\)) specimen from infants.

In the adult patient group, we detected EV RNA in 43.7% (7 of 16) of CSF specimens tested, whereas no EV strain was recovered from these specimens by cell culture isolation. A positive detection of EV RNA in serum was observed in one patient with aseptic meningitis and in one patient with myelitis (Table 1). The percentage of positive EV RNA detection was not significantly different between the combined EV PCR assay for CSF and serum specimens and the single PCR detection of a CSF (8 of 15 versus 7 of 16; \(P = 0.87\)) or a serum (8 of 15 versus 2 of 16; \(P = 0.075\)) specimen.

Throat specimens were positive by PCR in 31.8% of the children and in 11.8% of the adults studied (Table 1). The overall performances of the PCR test for throat swabs versus the PCR test for systemic specimens are shown in Table 2. Of the 16 throat specimens positive by PCR, only 10 were corre-
stated to a positive EV detection in one of the two systemic specimens (sensitivity of 62.5%); of the 45 throat specimens negative by PCR, 34 were correlated to an absence of EV RNA sequences detectable by PCR in CSF and/or serum (specificity of 75.6%) (Table 1).

Previous reports demonstrated the advantages of the PCR assay used in this work for diagnosis of neurological EV infection over traditional tissue culture isolation from CSF (7, 9, 11). In our prospective study, more diagnoses of an enteroviral neurological syndrome were achieved by PCR-microwell hybridization of CSF than by cell culture isolation (Table 1). The low percentages of enteroviral isolation from CSF specimens could be explained by poorly cultivable enteroviral serotypes or by a small number of infectious particles in CSF samples at the time of CSF puncture (4, 15). In order to investigate the diagnostic value of EV viremia in neurological syndromes, we compared the results of the detection of EV RNA by PCR in CSF and serum specimens taken from children and adult patients (Table 1). The detection of EV RNA either in CSF or in serum proved entroviral infection, whereas a positive PCR detection in throat swabs alone was considered not significant (11). A positive EV PCR assay of serum was observed in 5 of 10 children and in only 1 of 7 adult patients with a positive EV PCR result in the CSF sample. An isolated positive EV PCR detection in serum was observed in four children and in one adult patient suffering from an acute EV infection. Moreover, the results demonstrated that a combined PCR assay of CSF and serum was significantly more sensitive than a single PCR detection of CSF or of serum specimens from children patients, suggesting that this approach improves the diagnosis of neurological enteroviral diseases in infants. Comparatively to the child group, adult patients demonstrated a lower percentage (20.45 versus 12.5%) of positive EV RNAemia (Table 1). Moreover, the EV RNA sequence detection by PCR was more sensitive in CSF than in serum (7 of 16 versus 2 of 16; \( P = 0.056 \)), suggesting the value of PCR analysis of CSF in adult patients. The discrepant results obtained between the adult and children patient groups could be linked to a shorter viremia phase after the onset of neurological symptoms in adults (5, 6, 8).

We evaluated the performance of PCR testing of throat swabs versus PCR testing of systemic (CSF and serum) specimens. The low percentages of sensitivity (62.5%) and specificity (75.6%) clearly demonstrated the low diagnostic value of detection of EV RNA in throat specimens from children and adult patients (Table 2). The sensitivity of PCR of throat swabs did not exceed that of tissue culture and was lower than that obtained by Rotbart et al. in a similar study (12) (Table 1). These discrepancies could be explained by the presence of high levels of RNase activity or by Taq DNA polymerase inhibitors in the tested throat swabs (2).

In summary, the results of the present study suggest the diagnostic value of a rapid combined EV RNA detection method for CSF and serum from children with acute neurological EV infections and suggest that this approach could become a screening method for diagnosis of neurological EV diseases.

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