Impact of a Diagnostic Cerebrospinal Fluid Enterovirus Polymerase Chain Reaction Test on Patient Management

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A SEPTIC MENINGITIS IS A COMMON INFECTION IN THE UNITED STATES, with an estimated 75,000 cases each year. Approximately 80% to 92% of aseptic meningitis cases for which an etiologic agent is identified are caused by enteroviruses (EVs). Occurring mainly in the summer and fall, EV meningitis leads to a large number of hospitalizations of both children and adults. Although new antiviral agents are being developed specifically for EV meningitis, treatment is currently symptomatic, and the course of illness is usually benign. However, EV meningitis raises concern because of the difficulty in distinguishing it from bacterial meningitis based on clinical features alone. To distinguish the 2, hospitalization, empiric prescription of antimicrobial agents, and use of diagnostic testing are common. The ability to rapidly differentiate EV meningitis from bacterial illness has the potential for reduction of such health care services and cost savings. A definitive diagnosis is also prognostically useful in patients with central nervous system infection.

Previously, the diagnosis of EV meningitis required isolation of the virus in cell culture. Although older studies have shown viral culture to be effective in reducing antimicrobial prescription and length of hospital stay, these trials reported rapid turnaround times (2-5 days) that have not been achieved in more recent studies. Hospital stays are shorter now, in general, further eroding the usefulness of viral cultures in patient management decisions. Viral culture is also limited by its relatively low sensitivity (65%-75%), as well as the poor growth of some EV serotypes.

Conversely, the technique of nucleic acid amplification using EV-specific reverse transcriptase polymerase chain reaction (EV-PCR) can provide prompt results and may significantly alter the medical care offered to infected patients. Conventional EV-PCR methods can produce results within 24 hours, and more recently developed colorimetric

Context Enterovirus (EV) infection, the most common cause of aseptic meningitis, can be rapidly diagnosed with an EV-specific reverse transcriptase polymerase chain reaction (EV-PCR) test. However, no studies have examined EV-PCR in a clinical context in which it is routinely used.

Objective To determine the impact of EV-PCR testing on diagnosis and clinical management of suspected aseptic meningitis cases.

Design and Setting Retrospective review of electronic medical records from a 220-bed tertiary care pediatric medical center in San Diego, Calif.

Patients A total of 276 pediatric patients for whom a diagnostic EV-PCR test was performed during the calendar year 1998.

Main Outcome Measures Clinical parameters such as length of stay, medication use, and ancillary test use.

Results One hundred thirty-seven patients (49.6%) had a positive cerebrospinal fluid EV-PCR result. Enterovirus-positive patients with results available before hospital discharge (n=95) had significantly fewer ancillary tests performed (26% vs 72% with at least 1 test performed; P<.001), received intravenous antibiotics for less time (median, 2.0 vs 3.5 days; P<.001), and had shorter hospital stays (median, 42 vs 71.5 hours; P<.001) than EV-negative patients (n=92). A positive EV-PCR result was associated with more rapid hospital discharge (median EV-PCR–to–discharge time, 5.2 hours; P<.001) compared with a negative result (median EV-PCR–to–discharge time, 27.4 hours; P<.001).

Conclusions Our results suggest that a positive EV-PCR result may affect clinical decision making and can promote rapid discharge of patients, and that unnecessary diagnostic and therapeutic interventions can be reduced by use of EV-PCR testing.
assays can be performed in approximately 5 hours. Many studies have described its successful use as a diagnostic tool. In comparisons with viral culture, EV-PCR is more accurate, with a sensitivity and a specificity of virtually 100%. One study has shown that at least two thirds of all cerebrospinal fluid (CSF) specimens from suspected aseptic meningitis patients who had negative results by cell culture had positive results by EV-PCR.

Studies have alluded to the role of EV-PCR testing in achieving cost savings through earlier diagnosis. Unfortunately, these studies are largely hypothetical, extrapolating from a single outbreak, retrospectively performing PCR in bulk on frozen specimens, or attempting to predict clinical management based on modeled scenarios or decision trees. One study has demonstrated a clinical benefit from a new EV-PCR.

To examine the impact of EV-PCR on the diagnosis and clinical management of patients with suspected EV meningitis, we analyzed, via retrospective chart review, 276 inpatients for whom an EV-PCR was performed at the Children’s Hospital, San Diego, Calif (CHSD). The CHSD established an in-house PCR laboratory in 1996 and as of January 1, 1999, had performed more than 4200 PCR tests (1906 EV-PCR tests). Viral culture is offered at an off-site location, but its usage has waned since introduction of PCR testing. Rather than attempt to predict what decisions would have been made if PCR results were available, this study directly evaluated the impact of EV-PCR on clinical interventions.

METHODS

Data Collection

All patients hospitalized at CHSD between January 1, 1998, and December 31, 1998, for whom an EV-PCR test was ordered were included. We identified 280 patients, 4 of whom were subsequently excluded due to prolonged hospitalizations. Three of these excluded patients had the diagnosis of extreme prematurity, and 1 had acute lymphocytic leukemia. Clinical data for the remaining 276 patients were accessed through the CHSD’s electronic medical record system. Information collected included date and time of admission, date and time EV-PCR test was ordered, date and time EV-PCR results were reported, date and time of discharge, whether ancillary tests were performed (computed tomographic scan, magnetic resonance imaging, chest or abdominal x-ray, electroencephalogram), CSF findings, attending physician, clinical diagnoses, administration and duration of medications, and demographics.

A viral meningitis discharge diagnosis was defined by the following International Classification of Diseases, Ninth Revision (ICD-9) codes: meningitis due to EV (047.0 and 047.1), unspecified viral meningitis (047.8 and 047.9), and unspecified meningitis (322.9). Cerebrospinal fluid pleocytosis was defined, as in previously published studies, as a leukocyte count in excess of the following normal limits: 0.035 × 10^9/L for neonates younger than 1 month old, 0.025 × 10^9/L for infants 1 to 2 months old, and 0.005 × 10^9/L for those older than 2 months.

Analysis of the medications administered was limited to anti-infective medications and central nervous system agents. To analyze seasonal effects on clinical practice, the calendar year was divided into an EV season that included June 1, 1998, through September 30, 1998, corresponding with the seasonal pattern of viral meningitis. To determine whether different physicians used the PCR test similarly, the managing physician was classified as either a hospitalist or an outside physician.

PCR Test

Diagnostic EV-PCR tests were performed by the CHSD’s molecular diagnostics laboratory 3 days per week (Monday, Wednesday, and Friday) in the months of January through May and 6 days per week (Monday through Saturday) for the remainder of the year. The PCR assay uses primers that amplify a segment of the highly conserved 5’ end of the EV genome and was performed as previously described. Preliminary results were reported based on detection of the PCR-amplified product on ethidium bromide–stained agarose gels and confirmed via liquid hybridization of the PCR product with a radioactive phosphorus–labeled EV-specific probe followed by electrophoresis and overnight autoradiography. Standard methods to prevent carriage contamination were used, including positive-pressure PCR tips, dedicated workspace for different stages of the PCR process, and use of uracil-N-glycosylase and deoxyuridine triphosphate in the reaction mixture.

Data Analysis

The data were organized in a database for the purposes of statistical analysis. Additional calculated parameters were PCR-to-discharge time (date and time of discharge minus date and time of PCR result), PCR turnaround time (date and time PCR result was reported minus date and time PCR was ordered), and length of stay (date and time of discharge minus date and time of admission). Comparisons were performed using the Kruskal-Wallis test for comparisons of 3 groups and the Mann-Whitney test for comparisons of 2 groups. The Fisher exact test was used for comparisons of electroencephalogram use because of small sample sizes. All calculations were performed with statistical software (version 9.0, SPSS Inc, Chicago, Ill).

RESULTS

Summary of Study Populations

Of the 276 patients analyzed, 139 (50.4%) had CSF that was negative by EV-PCR test and 137 (49.6%) had CSF that was positive by EV-PCR test. Among the 137 patients with positive EV-PCR results, 100 (72.9%) were admitted within our empirically defined EV season. One hundred fifty-five (56.2%) of the 276 patients had a discharge diagnosis of viral meningitis and these patients had a median length of stay of 41 hours. Only 15 patients with a discharge diagnosis of viral meningitis dur-
ing this period did not have an EV-PCR test performed on their CSF, indicating that this test was ordered for the vast majority of viral meningitis patients at this institution. In the total sample of 276 patients, males were more common than females, constituting 64.5%. The median age of the patient sample was 5.5 months, with a range of 0 to 201 months. The PCR tests were ordered a median of 5 hours after admission (range, 0-333.60 hours). Overall, positive PCR results were available more rapidly (median, 25.8 hours) than negative results (median, 41.6 hours). During the EV season and thereafter when the test was offered 6 days per week, the median turnaround time was 33.9 hours compared with 45.8 hours earlier in the year.

Clinical Differences Between EV-Negative and EV-Positive Patients

To conduct more meaningful comparisons, we further divided our 276 patients on the basis of the time that the PCR result became available relative to hospital discharge. Ninety-two patients had a negative EV-PCR result available before discharge (EV-negative available), 95 patients had a positive EV-PCR result available before discharge (EV-positive available), and 89 patients were discharged before their PCR result became available relative to hospital discharge. Ninety-two patients on the basis of the time that the PCR result became available relative to hospital discharge. Ninety-two patients had a negative EV-PCR result available before discharge (EV-negative available), 95 patients had a positive EV-PCR result available before discharge (EV-positive available), and 89 patients were discharged before their PCR result became available relative to hospital discharge.

Table 1. Demographic and Clinical Features of 276 Patients Who Received the Enterovirus (EV) Polymerase Chain Reaction (PCR) Test

<table>
<thead>
<tr>
<th>No. (%) of patients</th>
<th>EV-Negative Before Discharge</th>
<th>EV-Positive Before Discharge</th>
<th>EV-PCR Result Not Available Before Discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>92</td>
<td>95</td>
<td>89</td>
</tr>
<tr>
<td>Age, median, mo</td>
<td>3</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>Male, %</td>
<td>68</td>
<td>65</td>
<td>60</td>
</tr>
<tr>
<td>Median cerebrospinal fluid white blood cell count, $\times 10^9/L$</td>
<td>3.8</td>
<td>17.8</td>
<td>1.4</td>
</tr>
<tr>
<td>No. w/ pleocytosis</td>
<td>44/86</td>
<td>74/85</td>
<td>48/79</td>
</tr>
<tr>
<td>Discharge diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral meningitis</td>
<td>19</td>
<td>90</td>
<td>46</td>
</tr>
<tr>
<td>Unspecified infections</td>
<td>28</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Syncope/convulsions</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>3</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Other</td>
<td>39</td>
<td>2</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 2. Comparison of Clinical Parameters of Patient Groups With Enterovirus (EV) Polymerase Chain Reaction (PCR) Test Results Available Before Discharge

<table>
<thead>
<tr>
<th>EV-Negative</th>
<th>EV-Positive</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of stay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>92</td>
<td>95</td>
</tr>
<tr>
<td>Median, h</td>
<td>71.5</td>
<td>42</td>
</tr>
<tr>
<td>Time from PCR test to discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>92</td>
<td>95</td>
</tr>
<tr>
<td>Median, h</td>
<td>27.4</td>
<td>5.2</td>
</tr>
<tr>
<td>Step-down unit stay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>34</td>
<td>18</td>
</tr>
<tr>
<td>Median, d/patient</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>No. (% of patients who received computed tomographic scan or magnetic resonance imaging)</td>
<td>33 (35.9)</td>
<td>9 (9.5)</td>
</tr>
<tr>
<td>No. (% of patients who received a chest or abdominal x-ray film)</td>
<td>51 (55.4)</td>
<td>18 (18.0)</td>
</tr>
<tr>
<td>No. (% of patients who received an electromyogram)</td>
<td>18 (19.6)</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Intravenous antibiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>82</td>
<td>84</td>
</tr>
<tr>
<td>Median, d/patient</td>
<td>5.5</td>
<td>2</td>
</tr>
</tbody>
</table>
viral meningitis. Of these, 90 (58.1%) were EV-positive available, 19 (12.3%) were EV-negative available, and 46 (29.7%) were discharged before the PCR result was available. Comparisons between EV-negative available and EV-positive available patients with a viral meningitis diagnosis are summarized in Table 3. Differences between groups in length of stay, PCR-to-discharge time, number of ancillary tests, and intravenous antibiotic use remained statistically significant when the subset of patients with viral meningitis diagnoses were evaluated.

In addition to the patients with a positive EV-PCR result with a discharge diagnosis of viral meningitis, 10 other patients also had a positive EV-PCR result. These 10 patients included 8 patients with diagnoses of unspecified viral infection (ICD-9 codes 057.9, 079.6, 079.99, and 790.8) and 2 with diagnoses of temperature disturbance in the newborn (ICD-9 code 778.4) and convulsions in the newborn (ICD-9 code 779.0). Twenty-eight (20.1%) of the 139 patients with a negative PCR left the hospital with diagnoses of viral meningitis. Nine of these 28 patients' EV-PCR results were not available at the time of discharge, and 19 were discharged with a viral meningitis diagnosis despite the availability of negative EV-PCR results.

Overall, 250 (90.6%) of 276 patients had CSF cell count data available. To examine the correlation between CSF pleocytosis and EV disease, patients were divided into groupings of neonates and nonneonates based on the fact that neonates younger than 1 month with evidence of EV in their CSF may not have pleocytosis.22-25 In the subset of 187 nonneonates, 145 (77.5%) had pleocytosis and 42 (22.5%) did not. Of these 42 older patients without pleocytosis, 38 (90.5%) had a negative EV-PCR result.

The age and pleocytosis breakdown of all 122 patients with a positive EV-PCR result for whom CSF data were available is shown in the Figure. Only 4 patients older than 1 month had a positive EV-PCR result without pleocytosis, indicating that in nonneonates who lack pleocytosis it is rare to detect EV in the CSF. Neonates showed no apparent correlation between pleocytosis and EV-PCR result, supporting the observation that EV infection can occur in the absence of pleocytosis. We performed an analysis of services use in the subset of patients who were neonates or who had CSF pleocytosis. TABLE 4 compares patients with EV-positive available and EV-negative available results. As with the viral meningitis diagnosis subset, the differences in hospital stay, PCR-to-discharge time, number of ancillary tests, and intravenous antibiotic use between EV-positive and EV-negative groups remained statistically significant.

The Not Available Population
In an attempt to define those patients who did not benefit from EV-PCR, we analyzed the 89 patients who were discharged before their EV-PCR results became available. Since EV-PCR results did not play a role in the management of these patients, many of the PCR tests may have been unwarranted. These 89 patients were significantly older (median age, 1.9 years) than EV-negative available and EV-positive available patients (median age, 3 months; \( P = .01 \); Table 1). Additionally, the median PCR result turnaround time for patients without available results was significantly longer (47.55 hours) than that for patients who received a result before discharge (31.57 hours; \( P < .001 \)). While 73 (82.0%) of the 89 patients without EV-PCR results available had turnaround times of greater than 36 hours, only 68 (36.4%) of the 187 EV-negative and EV-positive available patients waited for results for more than 36 hours.

Overall, 46 (51.7%) of the 89 patients without available PCR results left the hospital with a viral meningitis diagnosis, despite their PCR results not being available at time of discharge. This occurred more frequently during the EV season (31/49 [63.3%]) than in the off-
The management of patients suspected of having viral meningitis is associated with significant health care interventions that would be largely unnecessary if a rapid, definitive diagnosis were available. Several recent studies have alluded to the potential of EV-PCR to streamline the diagnosis and treatment of viral meningitis patients, and thus abrogate much of this avoidable use.7,8,10,18,20,21

The findings of this study suggest that the impact of EV-PCR on the management of patients suspected of viral meningitis may be significant. Patients with a positive CSF EV-PCR result showed significantly lower use of most of the clinical services we measured and a reduction in length of hospital stay of nearly 30 hours. A positive EV-PCR result may help establish a definitive diagnosis and promote immediate discharge of patients. Although negative PCR results took longer to report than positive results in this study, this does not account for the differences observed. The EV-positive patients were discharged a median of 5.2 hours after their result was returned, compared with a median of 27.4 hours for EV-negative patients. A negative EV-PCR result is associated with delayed hospital discharge and further diagnostic evaluation.

However, due to the retrospective nature of the study, it cannot be concluded that positive EV-PCR results were the sole cause of the significant reductions we observed. Other factors undoubtedly played a role in the management of each patient. Rather, it should be emphasized that the utility of EV-PCR appears to lie in helping the clinician to identify the subset of patients that do not need further medical intervention and that EV-PCR allows this to be done more quickly than clinical judgment alone. Most patients with negative EV-PCR results had conditions other than viral meningitis that may have led to longer hospital stays and more diagnostic testing even without a PCR result. The differences in use observed between EV-positive and EV-negative patients remained significant when we evaluated the subsets of patients with a viral meningitis diagnosis or those with pleocytosis, supporting our conclusion that EV-PCR affects the management of patients with suspected viral meningitis. Once positive EV-PCR results were returned, patients were discharged from the hospital quickly. This potential for immediate discharge coupled with the relatively short length of stay of viral meningitis patients in general, necessitates an EV-PCR system that provides results quickly. In our experience, offering EV-PCR 6 days per week represents the optimal balance of rapid turnaround and use of laboratory resources. Our data suggest that EV-PCR must be ordered early in the evaluation process to have a maximum impact on patient management. In many patients who had computed tomographic scans or magnetic resonance imaging performed, the decision to undergo these expensive imaging studies was made before PCR results were even returned. If included earlier in the diagnostic workup, the value of a positive EV-PCR result may increase, especially in reducing unnecessary diagnostic tests.

Based on our analysis of patients’ critical care usage, EV-PCR results may also be valuable in decreasing use of intermediate care units by contributing to a more rapid transfer to routine care once a definitive diagnosis is established. Positive EV-PCR results were associated with a median reduction in time spent in an intermediate care unit of 1 day per patient. In the neonatal intensive care unit and pediatric intensive care unit, however, EV-PCR results appear to have no impact on patient management, most likely due to the overshadowing severity of illness.

This study suggests that EV-PCR results can be clinically valuable and affect patient management in certain situations. Yet, 89 patients in this study were
discharged before their results became available, suggesting no role for EV-PCR in their management. In characterizing these patients, we found that they were significantly older (median age, 1.9 years vs 3 months). It is probable that physicians are more comfortable discharging older patients with presumed viral meningitis based on clinical diagnosis alone. The EV-PCR may be more valuable in younger children, for whom clinical differences between viral and bacterial disease are less pronounced.

Another distinguishing feature of these 89 patients discharged without available results was the longer median turnaround from the time the EV-PCR was ordered for these patients than for the general population of San Diego County, even this large population is not caused by EVs. Although EV-PCR has been proven to be extremely sensitive, the possibility remains that false-negative results can occur.

Among the limitations of this study is that we did not attempt to perform a detailed economic analysis. However, conclusions about cost savings can be inferred from the units of service approach used in this study. In addition, because of the retrospective design, we cannot clearly separate the impact of the PCR result from the clinical presentation on patient management. Furthermore, this study may not be generalizable to all institutions since it took place over the course of a single year in a single hospital. Although CHSD serves a patient population representative of the general population of San Diego County, even this large population is not representative of some areas of the United States.

In our institution, EV-PCR has largely replaced viral culture for the diagnosis of viral meningitis. The rapid results are valued by clinicians and this study suggests that EV-PCR may decrease use of services as well. Further studies of the impact of PCR testing on patient management are warranted.

Acknowledgment: We wish to express our gratitude to Teresa Mueller, Rowena Espina, and Charles Woo for their hard work and technical expertise.

REFERENCES


