analesdepediatría

www.analesdepediatria.org

ORIGINAL ARTICLE

# Evaluation of an Xpert EV (Cepheid®) molecular diagnostic technique for enteroviral meningitis☆

**Natalia Alonso Pérez[a,*], Belén Sagastizabal Cardelus[a], Luis Manuel Prieto Tato[a], Sara Guillén Martín[a], Ana González Torralba[b], Isabel García Bermejo[b], José Tomás Ramos Amador[a]**

[a] *Servicio de Pediatría, Hospital Universitario de Getafe, Getafe, Madrid, Spain*
[b] *Servicio de Microbiología, Hospital Universitario de Getafe, Getafe, Madrid, Spain*

Received 25 May 2016; accepted 26 September 2016
Available online 26 August 2017

**KEYWORDS**
Aseptic meningitis;
Enterovirus;
Polymerase chain reaction

**Abstract**
*Introduction:* Polymerase chain reaction (PCR) assays have shown to be useful and quick for the diagnosis of enterovirus in aseptic meningitis. The aim of our study was to analyse the changes in clinical practice after the introduction of a real-time polymerase chain reaction (RT-PCR) technique using the Xpert EV (Cepheid®) assay for the qualitative detection of enterovirus RNA in cerebrospinal fluid specimens from children with suspected viral meningitis.
*Methods:* A retrospective study was performed in children older than 1 year, diagnosed with enterovirus meningitis in a third level hospital from November 2006 to February 2013. The first period, before the availability of Xpert EV (Cepheid®) (Group 1, November 2006–August 2010) was compared with the later period (Group 2, September 2010–February 2013). Clinical characteristics, the mean length of stay, and the cost per inpatient cases, were compared between the 2 periods.
*Results:* Forty-one patients (60.9% male) were included, with a median age of 64 months (interquartile range 28–96). Twenty-six patients (63.4%) were included in Group 2. There were non-statistically significant differences in the epidemiological, disease severity, and laboratory characteristics between both periods of study. A significant difference was observed in the mean length of stay, with it being shorter in Group 2 (48 h vs 40.5 h, $P = .039$), and a significant lower inpatient cost per case (€779.77 vs €656.05, $P < .05$).
*Conclusion:* Xpert EV (Cepheid®) assay was useful for decreasing the length of hospital stay and the costs associated with hospitalization in children with enterovirus meningitis.


☆ Please cite this article as: Alonso Pérez N, Sagastizabal Cardelus B, Prieto Tato LM, Guillén Martín S, González Torralba A, García Bermejo I, et al. Evaluación de una técnica de diagnóstico molecular Xpert EV (Cepheid®) en la meningitis por enterovirus. An Pediatr (Barc). 2017;87:201–205.

* Corresponding author.
*E-mail address:* natalia.alonso.perez@gmail.com (N. Alonso Pérez).

**PALABRAS CLAVE**
Meningitis aséptica;
Enterovirus;
Reacción en cadena de la polimerasa

**Evaluación de una técnica de diagnóstico molecular Xpert EV (Cepheid®) en la meningitis por enterovirus**

**Resumen**
*Introducción:* Las técnicas de biología molecular han demostrado ser útiles en la detección del enterovirus en niños con meningitis aséptica. El objetivo de nuestro estudio fue analizar cambios en la práctica clínica tras la introducción de una técnica de RT-PCR a tiempo real, ensayo Xpert EV (Cepheid®), para la detección de enterovirus en muestras de líquido cefalorraquídeo de niños con sospecha de meningitis vírica.
*Métodos:* Estudio retrospectivo de los niños mayores de 1 año diagnosticados de meningitis por enterovirus en un hospital de tercer nivel desde noviembre de 2006 a febrero de 2013. Se comparó el periodo previo a la introducción del ensayo Xpert EV (Cepheid®) (grupo 1: noviembre 2006-agosto de 2010) con el periodo posterior (grupo 2: septiembre 2010-febrero 2013). Se compararon las características clínicas, los tiempos de estancia media y los costes por hospitalización.
*Resultados:* Se incluyeron 41 pacientes con una mediana de edad de 64 meses (rango intercuartílico, 28–96). En el grupo 2 se incluyeron 26 pacientes (63,4%). No hubo diferencias epidemiológicas, de gravedad, ni de laboratorio estadísticamente significativas entre los pacientes valorados en ambos grupos. Se observó una disminución significativa en la duración de estancia media hospitalaria en el grupo 2 (48 h vs 40,5 h, $p = 0{,}039$) y una disminución significativa en el gasto por paciente hospitalizado (779,77 € vs. 656,05 €, $p < 0{,}05$).
*Conclusiones:* La incorporación de la técnica Xpert EV (Cepheid®) permitió disminuir la estancia y el gasto asociado a hospitalización en niños con meningitis por enterovirus.


## Introduction

Enteroviruses are the most frequent cause of aseptic meningitis in the paediatric age group.[1] Cases of meningitis caused by enterovirus are usually benign and only require symptomatic treatment. However, the difficulty of differentiating them from bacterial meningitis in the initial assessment may lead to the unnecessary use of antibiotics, hospital admission and increases in the length of stay.[2–4] Currently, molecular diagnostic tests for the detection of enterovirus in cerebrospinal fluid (CSF) specimens, which have a high sensitivity and specificity and offer quick results, are displacing viral culture,[5,6] which is slower and whose results depend on the viability of the viruses contained in the clinical specimen. Some of these molecular tests are fully automated, as is the case of the Xpert EV assay (Cepheid®), which uses the GeneXpert® Dx system.

The aim of our study was to analyse changes in clinical practice since the introduction of this diagnostic test for the detection of enterovirus in CSF samples in children with suspected viral meningitis.

## Methods

We conducted a retrospective descriptive study of children aged more than 1 year and less than 15 years that were previously healthy and received a diagnosis of meningitis by enterovirus at the Hospital Universitario de Getafe between November 2006 and February 2013. We collected epidemiological, clinical and laboratory data from the electronic health records of patients that tested positive for enterovirus, as well as data on the length of stay and the use of antibiotherapy. We calculated the modified Boyer score based on clinical and laboratory parameters for each patient, as previously described in the literature.[8]

Starting in September 2010, the diagnosis of meningitis due to enterovirus was based on a positive result of the Xpert® EV assay (Cepheid). This technique was available in our Department of Microbiology from that date. Prior to that, the diagnosis was made using conventional molecular techniques, submitting specimens to a reference laboratory and with a mean turnaround time of 7–10 days.

The Xpert® EV assay (Cepheid) was performed according to the directions of the manufacturer, and was available between 8:00 AM and 8:00 PM everyday except Sundays, when it could be requested between 8:00 AM and 1:00 PM.

Cerebrospinal fluid samples were stored in a refrigerator at 4 °C until processing, and subsequently kept at −20 °C. Paediatricians were notified immediately of results.

The Xpert® EV assay (Cepheid) uses the GeneXpert® Dx system, with integrated performance of sample processing, viral RNA extraction, reverse transcription (RT) and nucleic acid amplification, as well as the detection of the target sequence by real-time polymerase chain reaction (PCR), in a single reaction cartridge. This method simplifies microbiological diagnosis and can provide rapid and accurate results in 2.5 h.

We compared patients in the period prior to the introduction of Cepheid's Xpert® EV method, group 1 (November 2006–August 2010) to patients in the period following its introduction, group 2 (September 2010–February 2013).

We calculated the health care costs generated by each patient based on the mean cost of staying in the paediatrics ward (337.55 €/day) in the two periods under study, adding the cost of assessing for the presence of enterovirus in CSF samples with the Xpert EV test (Cepheid®) for patients in the second period (80 €/specimen).

We have expressed quantitative variables as medians and ranges, and compared them by means of the Mann–Whitney *U* test. We have expressed qualitative variables as percentages and compared them by means of Fisher's exact test. We defined statistical significance as a *P*-value of less than .05. The statistical analysis was performed with SPSS version 20.0.

## Results

We included a total of 41 patients for the period under study, 60.9% male, and their median age was 64 months (interquartile range, 28–96 months). Twenty-six patients (63.4%) were included in the second period. Twenty-five patients (60.9%) had received a diagnosis between April and July. The most frequent presenting symptoms were headache (89.45%), vomiting (88.95%) and fever (71.25%)

We did not find statistically significant differences in epidemiological, clinical or laboratory characteristics between patients assessed in the first period and those assessed in the second period, save for the latter presenting less often with nuchal rigidity and/on meningeal signs in the initial evaluation (Table 1). We also found no differences between the two groups of patients when we computed the modified Boyer score (score ≥3, 0% in period 1 vs 7.6% in period 2, $P > .05$). Bacterial growth was not detected in any of the CSF cultures.

The comparative analysis of the two groups found a statistically significant decrease in the median length of stay of patients (48 h in group 1 vs 40.5 h in group 2; $P = .039$).

Only 2 patients (13.3%) in the first period and 2 patients (7.6%) in the second period received broad-spectrum antibiotherapy. We observed a decreasing trend in the use of antibiotherapy in the second period, but it was not statistically significant (4.7 days vs 1 day).

We observed a significant reduction in the costs of hospitalization per patient associated with length of stay: 779.77 € (98.35–1362.85) vs 656.05 € (0–1489.93) ($P < .05$).

## Discussion

In our study, the introduction of the Cepheid Xpert® EV assay was significantly associated with decreases in length of stay and associated health care costs.

**Table 1** Clinical characteristics and laboratory results of children with a diagnosis of meningitis by enterovirus in each period.

| | First period (control group) ($n = 15$) | Second period (enterovirus RT-PCR group) ($n = 26$) | *P* |
|---|---|---|---|
| **Clinical characteristics** | | | |
| *Age (months), median (IQR)* | 63 (28–96) | 65 (28–96) | ns |
| *Male sex, n (%)* | 10 (66.6) | 15 (57.7) | ns |
| *Fever > 38°C, n (%)* | 11 (73.3) | 18 (69.2) | ns |
| *Headache, n (%)* | 13 (86.6) | 24 (92.3) | ns |
| *Vomiting, n (%)* | 14 (93.3) | 22 (84.6) | ns |
| *Abdominal pain, n (%)* | 1 (6.6) | 6 (23.1) | ns |
| *Diarrhoea, n (%)* | 1 (6.6) | 1 (3.8) | ns |
| *Nuchal rigidity, n (%)* | 15 (100) | 19 (73.1) | <.01 |
| *Meningeal signs, n (%)* | 13 (86.6) | 16 (61.5) | .03 |
| *Post-dural puncture syndrome, n (%)* | 7 (46.6%) | 6 (23) | ns |
| *Modified Boyer score ≥ 3, n (0%)* | 0 (0%) | 2 (7.6%) | ns |
| **Laboratory findings** | | | |
| *Complete blood count and chemistry panel* | | | |
| Leucocytes (cells/mm$^3$), median (IQR) | 12 700 (8200–20 300) | 11 500 (5240–21 200) | ns |
| Hb (g/dL), median (IQR) | 12.9 (11.6–14.1) | 12.8 (12–15) | ns |
| CRP (mg/L), median (IQR) | 14.5 (0.5–53.8) | 17.5 (1–136.2) | ns |
| PCT (ng/mL), median (IQR) | 0.1 (0–0.5) ($n = 6$) | 0.2 (0–6.1) ($n = 14$) | ns |
| *CSF chemistry* | | | |
| Leucocytes in CSF, cells/mm$^3$, median (IQR) | 95 (10–415) | 63.5 (7–672) | ns |
| Granulocytes in CSF, %, median (IQR) | 49 (10–97) | 68.5 (7–95) | ns |
| Glucose in CSF (mg/dL), median (IQR) | 63 (49–79) | 58 (43–77) | ns |
| Protein in CSF (mg/dL), median (IQR) | 29 (17–74) | 35 (14–87) | ns |

CSF, cerebrospinal fluid; IQR, interquartile range; ns, not significant.
Enterovirus RT-PCR, real-time polymerase chain reaction (Cepheid's Xpert® EV).

Many studies published in recent years have shown the benefits of using PCR methods in the diagnosis of meningitis caused by enterovirus. Although publications on the subject started to appear in the 1990s, it was in year 2000 that 2 studies conducted in the United States directly evaluated their use and found a reduction in length of stay, antibiotic use and health care costs following the introduction of these techniques.[3,9]

In the past few years, the increased availability and accessibility of rapid, and in some cases automated, RT-PCR techniques have facilitated their widespread use. Data published in Europe and the United States demonstrate their benefits in terms of reduced costs and lengths of hospitalization.[7,10–13]

Two recent publications have assessed the use of such RT-PCR methods (Cepheid Xpert® EV) in Spain, with very good results.[14,15] In the study by Menasalvas Ruiz et al.,[14] as many as 25% of patients were discharged from the emergency department following a positive test result, and the median length of stay was 3.5 days (4.5 days in newborns vs 2.3 days in children aged more than 2 years). However, this study did not assess the impact of the introduction of the technique with a control group. The study conducted by Carrasco Fernández et al.,[15] which compared 37 children with aseptic meningitis selected prospectively after the introduction of Cepheid's Xpert® EV assay with retrospective data for 29 children with a diagnosis of aseptic meningitis, found that the introduction of this technique was associated with a significant reduction in antibiotic use and duration of antibiotherapy, but did not find significant differences in length of stay.

The analysis of health care costs that we present is an analysis of direct costs relative to the length of hospitalization. One of the limitations of our study is that we did not perform a detailed economic analysis and our results are based on the costs of hospitalization averaged through time. This type of analysis, while very simple, has been used by other authors in the past.[10,16] It may not adequately represent the actual savings associated with the use of these methods, although it does reflect the reduction of costs associated with hospitalization, which are probably more relevant in cases of aseptic meningitis. Studies conducted previously in other countries have suggested that the use of these techniques may be associated with reductions in length of stay and economic costs using a similar method for cost analysis.[10,15,17]

Length of stay in these patients has been associated with test turnaround times.[18] One limitation in our study was that the technique was not always available, so that the stay of patients admitted at night or on weekends may have extended until results became available. Another possible limitation was that although there are no priori interpersonal differences in the way the staff of the paediatrics department discharge patients, there could have been variability in the applied discharge criteria, a factor that may have influenced the results we obtained. In spite of these circumstances, we were able to find evidence of the benefits derived from using this technique.

Our study also has other limitations characteristic for retrospective studies. The data used to calculate the length of stay were entered in the electronic records at the times of admission and discharge, so we believe that these data, although collected retrospectively, are reliable.

In conclusion, the introduction of the Xpert® EV assay developed by Cepheid has allowed a significant decrease in the length of stay and associated costs in children with meningitis by enterovirus in Spain.

## Conflict of interests

The authors have no conflict of interests to declare.

## References

1. Lee BE, Davies HD. Aseptic meningitis. Curr Opin Infect Dis. 2007;20:272–7.
2. Kulik DM, Uleryk EM, Maguire JL. Does this child have bacterial meningitis? A systematic review of clinical prediction rules for children with suspected bacterial meningitis. J Emerg Med. 2013;45:508–19.
3. Ramers C, Billman G, Hartin M, Ho S, Sawyer MH. Impact of a diagnostic cerebrospinal fluid enterovirus polymerase chain reaction test on patient management. JAMA. 2000;283:2680–5.
4. Robinson CC, Willis M, Meagher A, Gieseker KE, Rotbart H, Glodé MP. Impact of rapid polymerase chain reaction results on management of pediatrics patients with enteroviral meningitis. Pediatr Infect Dis J. 2002;21:283–6.
5. Schlesinger Y, Sawyer MH, Storch GA. Enteroviral meningitis in infancy: potential role for polymerase chain reaction in patient management. Pediatrics. 1994;94:157–62.
6. Romero JR, Kimberlin DW. Molecular diagnosis of viral infections of the central nervous system. Clin Lab Med. 2003;23:843–65.
7. Ninove L, Nougairede A, Gazin C, Zandotti C, Drancourt M, de Lamballerie X, et al. Comparative detection of enterovirus RNA in cerebrospinal fluid: GeneXpert system vs. real-time RT-PCR assay. Clin Microbiol Infect. 2011;17:1890–4.
8. Ramos Lizana J, Vázquez López M, de Cea Crespo JM, Zanotta Alfieri R, González Vergaz A, Carrasco Marina LL, et al. Score para el diagnóstico diferencial entre meningitis bacteriana y viral. An Esp Pediatr. 1996;44:35–9.
9. Nigrovic LE, Chiang VW. Cost analysis of enteroviral polymerase chain reaction in infants with fever and cerebrospinal fluid pleocytosis. Arch Pediatr Adolesc Med. 2000;154:817–21.
10. Huizing KM, Swanink CM, Landstra AM, van Zweet AA, van Setten PA. Rapid enterovirus molecular testing in cerebrospinal fluid reduces length of hospitalization and duration of antibiotic therapy in children with aseptic meningitis. Pediatr Infect Dis J. 2011;30:1107–9.
11. Michos AG, Syriopoulou VP, Hadjichristodoulou C, Daikos GL, Lagona E, Douridas P, et al. Aseptic meningitis in children: analysis of 506 cases. PLoS One. 2007;2:e674.
12. Archimbaud C, Chambon M, Bailly JL, Petit I, Henquell C, Mirand A, et al. Impact of rapid enterovirus molecular diagnosis on the management of infants, children, and adults with aseptic meningitis. J Med Virol. 2009;81:42–8.
13. Nolte FS, Rogers BB, Tang YW, Oberste MS, Robinson CC, Kehl KS, et al. Evaluation of a rapid and completely automated real-time reverse transcriptase PCR assay for diagnosis of enteroviral meningitis. J Clin Microbiol. 2011;49:528–33.
14. Menasalvas Ruiz AI, Salvador García C, Moreno Docón A, Alfayete Miguélez S, Pérez Cánovas C, Sáchez Solís Ml. Enterovirus reverse transcriptase polymerase chain reaction assay in cerebrospinal fluid: an essential tool in meningitis management in childhood. Enferm Infecc Microbiol Clin. 2013;31:71–7.
15. Carrasco Fernández JR, Gómez-Pastrana D, Alados Arboledas JC, Aragón Fernández C, Ortiz Tardío J. Impact of introducing

an enterovirus polymerase chain reaction in the management of aseptic meningitis. An Pediatr (Barc). 2015;82:e26–9.

16. Florén-Zabala L, Chamizo-López FJ, Eisman-Maraver A, Pérez-González C, de Ory-Marchón F, Trallero-Maso G, et al. Aseptic meningitis in an adult population. Etiology and utility of molecular techniques in the clinical management of patients. Enferm Infecc Microbiol Clin. 2012;30:361–6.
17. Robinson C, Willis M, Meagher A, Gieseker K, Rotbart H, Glodé MP. Impact of rapid polymerase chain reaction results on management of pediatric patients with enteroviral meningitis. Pediatr Infect Dis J. 2002;21:283–6.
18. Stellrecht KA, Harding I, Woron AM, Lepw ML, Venezia RA. The impact of an enteroviral RT-PCR assay on the diagnosis of aseptic meningitis and patient management. J Clin Virol. 2002;25:19–26.