Performance of blood enterovirus and parechovirus polymerase chain reaction testing in young febrile infants: a prospective multicentre observational study

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ABSTRACT

Objectives To analyse the performance of blood enterovirus and parechovirus PCR testing (ev-PCR) for invasive bacterial infection (IBI) (isolation of a single bacterial pathogen in a blood or cerebrospinal fluid culture) when evaluating well-appearing infants ≤90 days of age with fever without a source (FWS).

Methods We describe the well-appearing infants ≤90 days of age with FWS and normal urine dipstick. We performed a prospective, observational multicentre study at five paediatric emergency departments between October 2020 and September 2023.

Results A total of 656 infants were included, 22 (3.4%) of whom were diagnosed with an IBI (bacteraemia in all of them and associated with meningitis in four). The blood ev-PCR test was positive in 145 (22.1%) infants. One patient with positive blood ev-PCR was diagnosed with an IBI, accounting for 0.7% (95% CI 0.02 to 3.8) compared with 4.1% (95% CI 2.6 to 6.2) in those with a negative test (p=0.04). All four patients with bacterial meningitis had a negative blood ev-PCR result. Infants with a positive blood ev-PCR had a shorter hospital stay (median 3 days, IQR 2–4) compared with 4 days (IQR 2–6) for those with negative blood ev-PCR (p=0.02), as well as shorter duration of antibiotic treatment (median 2 days, IQR 0–4 vs 2.5 days, IQR 0–7, p=0.01).

Conclusions Young febrile infants with a positive blood ev-PCR are at a low risk of having an IBI. Incorporating the blood ev-PCR test into clinical decision-making may help to reduce the duration of antibiotic treatments and length of hospital stay.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Febrile infants with a positive cerebrospinal fluid enterovirus and parechovirus PCR (ev-PCR) are at low risk of having an invasive bacterial infection (IBI) and have shorter durations of antibiotic treatment and hospitalisation admission.
⇒ There is still insufficient evidence to conclude that infants with a positive ev-PCR are at low risk of having an IBI.

WHAT THIS STUDY ADDS

⇒ Well-appearing infants under 3 months of age with a normal urinalysis and a positive blood ev-PCR are at very low risk of having an IBI.
⇒ Infants with a positive ev-PCR have shorter hospital stays and receive shorter courses of antibiotic treatments.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Shortening the time to ev-PCR results is expected to decrease hospitalisation rates and antibiotic treatment.

INTRODUCTION

Infants under 90 days of age with fever pose a diagnostic challenge as they often present with fever without a source (FWS) and represent the paediatric population with the highest risk of invasive bacterial infection (IBI) due to immunological immaturity and lack of vaccination. The prevalence of IBI in this group is estimated to be 2%–4%, mainly attributable to *Escherichia coli* and *Streptococcus agalactiae*.[1 2]

In recent years, new management strategies for these patients have been published, including the Paediatric Emergency Care Applied Research Network clinical prediction rule[3] and step-by-step approach.[4] These strategies incorporate biomarkers such as C reactive protein (CRP) and procalcitonin (PCT), which were not taken into account in more classic approaches (eg, Rochester, Lab-score, etc).[5 6] However, none of them has integrated the use of rapid detection tests for viral infections into the risk stratification for IBI.

In recent years, several investigations focusing on this aspect have emerged, aiming to identify prevalent viruses through molecular diagnostic techniques in different bodily fluids, including respiratory secretions, blood and cerebrospinal fluid (CSF). These viruses include rhinovirus (RV), metapneumovirus, bocavirus, enterovirus (EV) and human parechovirus (HPeV).[7] Regarding EV, there are over 100 recognised types known to cause a high number of infections in children. They are associated with different clinical entities, such as FWS in neonates and infants, respiratory and central nervous system infections and viral sepsis.[8–12] There are very few studies that analyse the usefulness of PCR in blood, and there remains insufficient evidence to conclude that infants with a positive EV PCR test are at low risk of having an IBI.[13] HPeV infections are most frequently (80%) diagnosed in infants under 2

months of age, especially in neonates. While HPeV-1, 2 and 6 are typically associated with mild symptoms, such as gastrointestinal or respiratory symptoms, HPeV-3 is related to more severe conditions, such as meningitis or neonatal sepsis.[9 14 15]

To our knowledge, no large-scale studies have analysed the prevalence of IBI in well-appearing febrile infants under 90 days of age who were seen at the paediatric emergency department (PED) and underwent molecular testing for EV or HPeV positivity in blood and/or CSF.

The primary objective of this study was to analyse the association of blood enterovirus and parechovirus PCR (ev-PCR) positivity with the presence of IBI in well-appearing infants aged ≤90 days with FWS and normal urine dipstick results. As secondary objectives, we compared the length of hospital stay and duration of antibiotic treatment between hospitalised infants with positive and negative ev-PCR results.

MATERIALS AND METHODS

Study design and data collection

This multicentre, prospective registry was conducted from October 2020 to September 2023. The study, endorsed by the Spanish Paediatric Emergency Research Group, included five Spanish PEDs. The attending paediatricians followed the step-by-step algorithm to guide the management of these infants.[4]

In each participating PED, a collaborating physician-researcher collected epidemiological and clinical data from the medical records of study patients.

Population

Infants were eligible if they were under 90 days of age, well-appearing with FWS, had a normal urine dipstick result and had both a blood ev-PCR and blood culture (BC) obtained.

We opted not to include infants with an abnormal urine dipstick result because in infants with leukocyturia, a significant percentage presents with confirmed urinary tract infections (UTI) and approximately 6% of them have associated bacteraemia.[16] UTI is the most prevalent bacterial infection in these infants, and suspicion of UTI warrants antibiotic treatment and will require hospital admission in most cases.

The exclusion criteria included receiving antibiotic treatment within the previous 48 hours and refusal to participate.

Variables

The primary outcome assessed was the presence of IBI based on ev-PCR results. The recorded variables included age, sex, Paediatric Assessment Triangle (PAT) on arrival at the PED, duration of fever, physical examination findings, results of laboratory tests, diagnosis, need for hospital admission and antibiotic treatment, length of hospital stay and duration of antibiotic treatment. Medical records were reviewed for all patients.

All infants were followed up either by telephone or by reviewing reports of unscheduled visits for a period of 4 weeks after the initial PED visit.

Definitions

FWS: a fever of an axillary or rectal temperature of ≥38°C measured at home or a rectal temperature of ≥38°C measured in the PED, in an infant in whom, after a thorough medical history and physical examination, a specific source of the fever cannot be identified.

Previously healthy infant: an infant born at term, not treated for unexplained hyperbilirubinaemia, not hospitalised longer than the mother, not currently or previously receiving antimicrobial therapy, without prior hospitalisation and without chronic or underlying illness.

Well-appearing: normal findings according to PAT.[17]

Abnormal urine dipstick test: presence of a positive leucocyte esterase and/or nitrite test in urine samples collected using a sterile method (urethral catheterisation or clean-catch technique).

IBI: isolation of a single bacterial pathogen in a blood or CSF culture.[18] Growth of any bacterium classically regarded as a contaminant in cultures obtained from previously healthy immunocompetent infants was not considered to indicate IBI.

Bacterial meningitis: defined as the detection of a bacterial pathogen in CSF with or without associated pleocytosis.

Normal blood test values (step-by-step approach)[4]: absolute neutrophil count (ANC) ≤10 000/mm^3, CRP ≤20 mg/L and PCT <0.5 ng/mL.

Length of stay in hospitalised infants: days from the time an infant was admitted to the hospital ward until the time of discharge.

Duration of antibiotic treatment in hospitalised infants: the number of days the patient received antibiotic therapy (parenteral or oral) during their hospitalisation.

Sample processing and ev-PCR technique

ev-PCR is used for the detection of EV and HPeV in separate assays. Whole blood samples anticoagulated with EDTA were pretreated by mixing 100 µL of the samples with 100 µL of tissue lysis buffer. Automated nucleic acid purification was conducted using EZ1 Virus Mini Kit V.2.0 cartridges and the Biorobot EZ1 Advanced XL (all from Qiagen; Hilden, Germany), following the manufacturer's instructions. Multiplex real-time PCR amplification and qualitative detection of EV and HPeV RNA was performed using RealCycler EVPA kits (Progenie Molecular, Valencia, Spain) and run on the SmartCycler Dx system (Cepheid, Sunnyvale, California, USA). Molecular typing of positive samples was achieved by VP1 sequencing for EV[19] and VP3/VP1 for HPeV.[20] The EV assay separately detects HPeV. The sample processing method was standardised across the five participating hospitals.

Samples were processed during the morning shift on working days. Results were available within 12–24 hours, except for patients seen between Friday noon and Sunday noon or the day before a public holiday. Thus, while physicians were not blinded to the PCR result, in most cases, the attending paediatrician was not informed of the result when determining antibiotics administration or patient admission.

Statistical analysis

All analyses were conducted using STATA V.17. Numerical variables were described using median and IQR when the data were dispersed. Categorical variables were expressed as counts and percentages. Comparisons between groups were performed using Fisher's exact test for categorical variables and the Mann-Whitney U test for numerical variables, following confirmation by the Kolmogorov-Smirnov goodness-of-fit test that the data did not follow a normal distribution. Statistical significance was set at a p value of <0.05.

Baseline risk factors analysed for length of stay and duration of antibiotic treatment in hospitalised infants included age, duration of fever, maximum temperature, parental reports of irritability, poor feeding difficulties, ANC, CRP, PCT and blood ev-PCR. As an exploratory test, backward stepwise

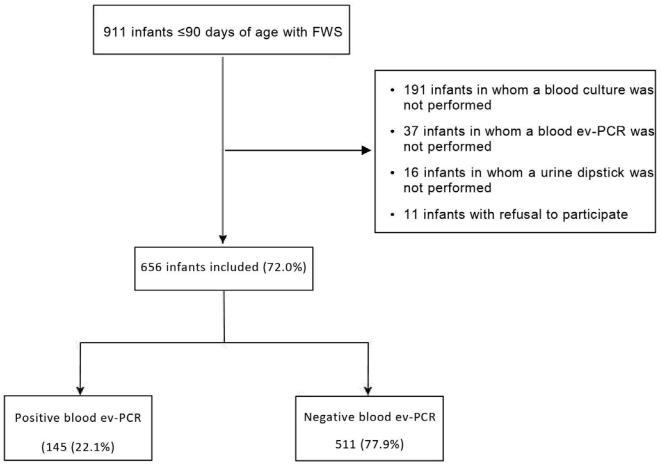

Figure 1 Flow chart of infants included in the study and blood ev-PCR testing results. ev-PCR, enterovirus and parechovirus PCR; FWS, fever without a source.

regression was performed for variables with an inclusion p value of <0.05 and an exclusion p value of >0.1.

The article was drafted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines for observational research.[21]

RESULTS

Between 2020 and 2023, the five institutions recorded a total of 911 episodes involving well-appearing infants under 90 days of life with FWS and normal urine dipstick results; out of these, 656 infants (72.0%) with a median age of 49 days (IQR 27–64 days) were included (figure 1). Blood ev-PCR was positive in 145 infants (22.1%, 95% CI 19.0 to 25.5); 118 were EV positive and 27 were HPeV positive. Table 1 presents the epidemiological and clinical data for the study cohort. Lumbar puncture was performed in 104 (15.9%) infants and CSF ev-PCR was positive in 34 (32.7%, 95% CI 23.8 to 42.6) of them, with pleocytosis in 4 (11.8%); 28 were EV positive (23/28; 82.1% with concomitant isolates in blood) and 6 were HPeV positive (5/6; 83.3% with concomitant isolates in blood). Positive isolates were more prevalent in spring/summer (100/145; 69.0%, 95% CI 60.8 to 76.4) compared with autumn/winter (45/145; 31.0%, 95% CI 23.6 to 39.2).

Table 1 Epidemiological and clinical factors

	Blood ev-PCR+ (n=145)	Blood ev-PCR− (n=511)	Difference (95% CI)
Sex (male)—n (%)	76 (52.4)	313 (61.3)	−8.8 (−18.0 to 0.3)
Not previously healthy—n (%)	18 (12.4)	67 (13.1)	−0.7 (−6.8 to 5.4)
Age (days), median (IQR)	34 (18.5–59)	51 (31–67)	−17 (−25.1 to -8.9)
Age (days)—n (%)			
≤21	49 (33.8)	77 (15.1)	18.7 (10.4 to 27.0)
22–60	59 (40.7)	261 (51.1)	−10.4 (−19.5 to −1.3)
≥60	37 (25.5)	173 (33.8)	−8.3 (−16.5 to −0.1)
Duration of fever (hours), median (IQR)	3 (1–9)	4 (1.5–12)	−1 (−2.4 to 0.4)
Irritability reported by parents—n (%)	32 (22.1)	91 (17.8)	4.3 (−3.3 to 11.8)
Poor feeding—n (%)	10 (6.9)	41 (8.0)	−1.1 (−5.9 to 3.6)
Maximum temperature (°C), median (IQR)	38.3 (38.2–38.4)	38.2 (38.1–38.3)	0.1 (−0.05 to 0.3)

ev-PCR, enterovirus and parechovirus PCR.

Among the infants included in the cohort, 22 (3.4%, 95% CI 2.1 to 5.0) were diagnosed with an IBI, all of whom presented with bacteraemia. The isolated microorganisms included *S. agalactiae* (10), *Staphyloccocus aureus* (4, one of which was methicillin-resistant *S. aureus*), *E. coli* (3), *Enterococcus* spp (2), *Streptococcus pyogenes* (1), *Streptococcus gallolyticus* (1) and *Salmonella* spp (1). The prevalence of IBI in the whole sample and in infants younger than 60 days of life is shown in table 2. A positive blood ev-PCR was associated with IBI with a sensitivity of 95.5% (95% CI 78.2 to 99.2) and a negative predictive value of 95.9% (95% CI 93.8 to 97.3). A well-appearing infant girl aged 54 days with a positive blood ev-PCR and a concomitant IBI had normal blood tests (ANC 2200/mm^3, CRP 3.6 mg/L and PCT 0.1 ng/mL) and bacteraemia caused by *S. pyogenes*. She was discharged home. On receiving the positive BC result, she was contacted by telephone for admission and intravenous antibiotics administration. She had a favourable clinical outcome without acute complications or sequelae. Four patients had bacteraemia associated with bacterial meningitis caused by the same microorganism: *S. agalactiae* (three cases) and *S. gallolyticus* (one case). All four infants had a negative ev-PCR result.

Overall, 200 infants (30.5%) were admitted to the ward, of whom 145 (72.5%) received antibiotic treatment. Table 3 compares admission and antibiotic treatment rates as well as length of hospital stay and antibiotic treatment duration according to blood ev-PCR result. Table 4 shows the factors associated with length of hospital stay and duration of antibiotic treatment in hospitalised infants, derived from the multivariate analysis.

DISCUSSION

The results of this large prospective multicentre study indicate that a positive blood ev-PCR is associated with a reduced risk of developing an IBI. Incorporating this test into the PED management of these infants may be helpful and avoid unnecessary antibiotic treatment and hospital admissions.

In our study, we focused on a very specific subgroup of infants: those who were well-appearing and had normal urine dipstick results. This subgroup presents variability in its clinical management, because infants that are not well-appearing or have abnormal urine dipstick results, typically will receive antibiotic therapy and are admitted to the hospital, regardless of the blood test results. In our sample, we observed that the prevalence of IBI is lower in infants with a positive blood ev-PCR test compared with those with a negative test result (0.7% vs 4.1%). When analysing only the subgroup of infants aged ≤60 days, similar results were obtained (0.8% vs 5.0%). Therefore, incorporating blood ev-PCR into the clinical algorithms would identify a greater number of infants at very low risk of having an IBI. The only infant with an IBI and a positive ev-PCR did not meet the high-risk criteria for having an IBI according to the step-by-step approach (ill-appearing, ≤21 days of age, leukocyturia and altered biomarkers) and initially, a conservative management approach was followed.

The isolation of EV and HPeV predominantly occurs in spring and summer, as described in the literature.[13 14 22–24] However, in our sample, approximately one in three cases occurred during autumn and winter. Therefore, our recommendation, consistent with that previously established by Pintos *et al*,[13] is to perform blood ev-PCR testing throughout the year.

Table 2 Prevalence of bacterial infection according to blood ev-PCR test results

	Blood ev-PCR+	Blood ev-PCR–	Difference (95% CI)	P value
Overall—n (%; 95% CI)	1/145 (0.7; 0.02 to 3.8)	21/511 (4.1; 2.6 to 6.2)	–3.4 (–5.6 to –1.2)	**0.04**
	Blood ev-PCR+	**Blood ev-PCR–**	**Difference (95% CI)**	**P value**
≤60 days old—n (%; 95% CI)	1/108 (0.9; 0.02 to 5.1)	18/338 (5.3; 3.2 to 8.3)	–4.4 (–7.4 to –1.4)	**0.048**

ev-PCR, enterovirus and parechovirus PCR.

The age of infants with a positive blood ev-PCR is lower, with a greater proportion of infants under 21 days of life than in the group with a negative test result. This age is the cut-off established by step-by-step approach to warrant a different management strategy, particularly for well-appearing infants—in which the youngest infants represent the subgroup at the highest risk of having an IBI. This management approach consists of performing a lumbar puncture in addition to blood and urine tests, and hospital admission with intravenous antibiotics regardless of the test results.[4] The incorporation of ev-PCR testing as a routine procedure in the PEDs could significantly aid in the management of these febrile well-appearing infants.

The characteristics of the fever are similar in both groups with no differences found in its duration or in the maximum temperature recorded during the febrile episode. This supports the concept that fever alone is not a reliable discriminator to guide clinical management in these infants, as described in the literature.[25]

Irritability reported by parents of infants with a positive blood ev-PCR has been described in previous studies[13 23] and, although it was also more prevalent in our cohort, no significant differences were observed between both groups. Similarly, there were no differences in other symptoms such as poor feeding or neurological alterations between the two groups (p>0.05).

In our cohort, infants with a positive blood ev-PCR were more frequently admitted to the hospital (46.2% vs 26.0%) and received antibiotic treatment more often (33.1% vs 19.0%) compared with the group with a negative blood ev-PCR. This is likely due to the younger age of these infants—as mentioned above, the step-by-step approach considers them at high risk for having an IBI[4]—and the delayed availability of ev-PCR results in PEDs. However, in children with a positive ev-PCR result, the length of hospital stay and the duration of antibiotic therapy were shorter, likely related to the arrival of the blood ev-PCR result. Similar findings have been reported in the literature, whether the isolation of EV is in blood or CSF.[8 12 13] According to our findings, including the blood ev-PCR is expected to reduce the duration of antibiotic therapy in those patients with a positive result in whom it has been started because of an alteration in blood biomarkers. Although the test only needs around 2 hours to provide the result, nowadays, most PEDs do not currently have access to rapid blood ev-PCR turnover. In this way, the sample was processed in the participating centres on the following working day after it was obtained. For this reason, the use of this test was not useful in reducing the proportion of patients in whom an antibiotic therapy was started. However, shortening this turnaround time may help to incorporate blood ev-PCR testing into the clinical decision-making also in the PED and it could decrease unnecessary hospitalisations and antibiotic treatments.

Limitations

This study has several limitations. First, part of the patient enrolment occurred during the COVID-19 pandemic, which altered the usual circulation of viruses, including EV and HPeV. Second, due to the predominance of COVID-19 during part of the study and its subsequent status as one of the most frequent viruses isolated in the environment, blood tests were not performed in febrile well-appearing infants who tested positive for COVID-19. Consequently, many infants were not included in the cohort, with greater loss of patients than initially expected. Third, only four patients were diagnosed with a bacterial meningitis; therefore, this study was underpowered to draw conclusions about differences in the rate of this specific type of IBI. Fourth, in all hospitals included in the study, samples are processed only on working days, leading to delayed results for infants seen during weekends. We did not compare the reduction in the length of hospital stay and the duration of antibiotic treatment between infants seen on working days and those admitted during weekends. Finally, no proper cost-benefit analysis has been carried out, but given that the ev-PCR costs approximately €20, we believe it is very likely that its inclusion would be cost-effective.

CONCLUSION

Young febrile infants with a positive blood ev-PCR are at low risk of having an IBI, despite being younger. Incorporating the blood ev-PCR test into the clinical decision-making may help to reduce the duration of antibiotic treatments and length of hospital stay.

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Contributors JAAC contributed to the study conception and design, material preparation and analysis, wrote the first draft of the manuscript and act as the guarantor. BG and RV conceptualised and designed the study, coordinated and supervised the data collection, and critically reviewed the manuscript. NCA, JAU,

Table 3 Comparison of hospital stay and antibiotic treatment by blood ev-PCR

	Blood ev-PCR+ (n=145)	Blood ev-PCR– (n=511)	Difference (95% CI)	P value
Admission rate—n (%)	67 (46.2)	133 (26.0)	20.2 (11.2 to 29.1)	**<0.001**
Length of stay*—median (IQR)	3 (2–4)	4 (2–6)	–1 (0.1 to 2.1)	**0.02**
Antibiotic therapy received—n (%)	48 (33.1)	97 (19.0)	14.1 (5.7 to 22.5)	**<0.001**
Duration of antibiotic therapy* (days)—median (IQR)	2 (0–4)	2.5 (0–7)	–0.5 (0.3 to 2.5)	**0.01**

*Length of stay and rate and duration of antibiotic therapy were calculated among admitted patients.
ev-PCR, enterovirus and parechovirus PCR.

Table 4 Multivariate analysis to identify independent risk factors for length of stay (n=200) and duration of antibiotic treatment in hospitalised infants (n=145)

Risk factors for length of stay*	Coefficient	95% CI	P value
Age (days)	−0.03	−0.06 to 0.007	**0.01**
CRP (mg/L)	0.02	0.01 to 0.4	**0.007**
PCT (ng/mL)	0.2	0.1 to 0.3	**<0.001**
Blood ev-PCR	−2.8	−3.7 to −1.1	**0.02**
Risk factors for duration of antibiotic treatment†	**Coefficient**	**95% CI**	**P value**
Blood ev-PCR	−3.4	−4.4 to −2.3	**<0.001**

*Variables excluded using the backward stepwise regression method: evolution of fever, irritability reported by parents, poor feeding, maximum temperature and absolute neutrophil count.
†Variables excluded using the backward stepwise regression method: age, evolution of fever, irritability reported by parents, poor feeding, maximum temperature, absolute neutrophil count, CRP and PCT.
CRP, C reactive protein; ev-PCR, enterovirus and parechovirus PCR; PCT, procalcitonin.

MS-BH and SM revised the data collection form, collected data and critically reviewed the manuscript. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects contained.

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Competing interests None declared.

Patient consent for publication Consent obtained from parent(s)/guardian(s).

Ethics approval This study was approved by Hospital Infantil Universitario Niño Jesús (R-0024/20). Participants gave informed consent to participate in the study before taking part.

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