Detection of Enterovirus and Parechovirus in Plasma Specimens of Pediatric Patients in Chicago

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Updated Abstract

Human Enteroviruses (EV) and Parechoviruses (HPeV) are well known causes of meningitis and other serious infections in young children. The typical presentation of children infected with these viruses can involve fever, irritability, non-specific rash, vomiting, diarrhea, congestion, rhinorrhea, or sepsis-like symptoms. Data involving detection of these pathogens in plasma is limited.

The aims of this study therefore were to examine the prevalence and clinical characteristics of EV and HPeV infections in plasma specimens of patients < 2 months of age in the U.S. from September to November of 2015 using different PCR platforms.

Plasma specimens from 10 patients with EV-positive cerebrospinal fluid (CSF) were tested as a validation set. Then a convenience sample of 64 plasma specimens was collected during the late summer and fall of 2015 from children who were < 2 months old and who were seen in the Emergency Department or Neonatal Intensive Care Unit at Ann & Robert H. Lurie Children's Hospital of Chicago (LCHOC).

All samples were tested for EV and/or HPeV by real-time RT-PCR, either by ARIES®, GeneXpert®, and/or LightCycler®. Additionally, the CDC confirmed samples as positive or negative via genotype sequencing. Clinical information was gathered by reviewing patient electronic medical record.

Among the 10 plasma specimens from the validation group, 5 were positive for enterovirus in the plasma by sequencing. All 64 young infant plasma specimens were negative for HPeV. Five were confirmed positive for EV by sequencing, indicating a 7.8% prevalence in the patient sample. These data show that enterovirus can be detected in plasma samples from pediatric patients in whom EV was detected in CSF, and from young infants who present with fever, rash, congestion, vomiting, or aseptic meningitis.

Methods

As a proof of concept, 10 plasma samples were collected from patients whose cerebrospinal fluid (CSF) was enterovirus-positive either the same day (N = 9) or within 1 day (N = 1) of the plasma being collected. All samples were obtained between late August and late November of 2015. These samples served as a validation set. A convenience sample of 64 plasma specimens was collected between September 2015 and November 2015 of young infants (< 2 months). Upon reviewing patient electronic medical records, age, gender, other PCR results (if available), clinical signs and symptoms, antibiotics used (if any), hospital length of stay (if applicable), and appropriate laboratory and imaging data were documented.

All plasma samples were extracted using the NucliSENS® easyMAG® instrument (bioMérieux Inc., Durham, NC). RT PCR was performed on all specimens (N = 74) for the detection of enteroviruses and parechoviruses. The validation set (N = 10) was tested for EV using both GeneXpert® (Cepheid, Sunnyvale, CA) and ARIES® (Luminex, Austin, TX). The young infant group (N = 64) was tested for EV using GeneXpert®, and for HPeV using a previously developed assay[1] on the LightCycler® (Roche, Indianapolis, IN). Total nucleic acids were used for GeneXpert® and LightCycler®, and plasma was used for ARIES®. Total nucleic acids for all seventy-four specimens were also sent to the Centers for Disease Control and Prevention (CDC) in Atlanta, GA for confirmation by PCR and for genotyping by sequence analysis. Samples were typed using reverse transcriptase-seminested PCR that targeted regions in the VP1 gene.[2]

ARIES®

GeneXpert®

LightCycler®

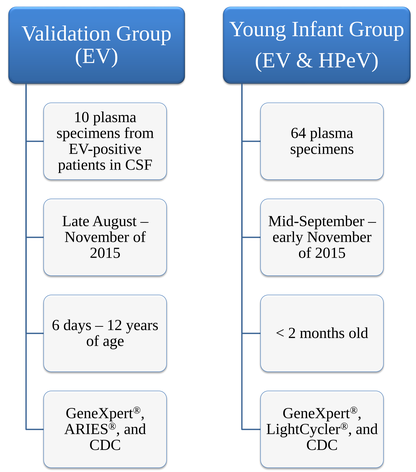

FIGURE 1. Methodology of validation and young infant groups.

Limit of detection testing was performed on ARIES® to determine analytical sensitivity. A tissue culture of EV-D68 of known concentration ($10^{7.8}$ $CCID_{50}$/mL) from the CDC was used as the standard. This reference virus was 10-fold serially diluted from 10^{-5} to 10^{-8} with EV-negative plasma, and each dilution was tested in triplicates on ARIES®. Analytical specificity testing was performed on ARIES® to investigate potential cross reactivity. Twenty-three viral and thirty-one bacterial targets were tested.

Results

All of the targets for specificity testing were negative. The ARIES® EV assay detected all 3 aliquots at the level of 1 x $10^{1.8}$ $CCID_{50}$/mL. Among the 10 plasma specimens from the validation group, 7/10 were EV-positive using ARIES® and 8/10 were EV-positive using GeneXpert®. Five were confirmed via genotyping by the CDC: two were identified as Echovirus 18 and one each were identified as Coxsackievirus A9, Echovirus 30, and Echovirus 5 (Table 3). The discrepant samples (C7, C16, and C17) were negative for specific rhinovirus testing at the CDC.

TABLE 3. A comparison of Enterovirus plasma detection methods used for the validation set, along with the number of amplification cycles needed to detect a positive signal (the cycle threshold, C_t), and the genotyping results from the CDC.

Patient	ARIES® (C_t values)	GeneXpert® (C_t values)	CDC Genotype Results
C2	30.5	27	Echovirus 5
C5	27.3	21.9	Coxsackievirus A9
C6	35.6	31.6	Echovirus 30
C7	35.6	34.1	Negative
C10	Negative	Negative	Negative
C12	29.5	25.7	Echovirus 18
C15	Negative	Negative	Negative
C16	37.8	34.1	Negative
C17	Negative	36.1	Negative
C18	37.6	32.8	Echovirus 18

The test group consisted of 64 plasma samples from young infants, of which none was confirmed to have an infection with HPeV. The one sample that was positive by the LightCycler® (C_t = 38.03) was sent to the CDC and was negative.

Five of the 64 young infants were positive for enterovirus using GeneXpert®. Four of these five were confirmed as EV-positive at the CDC: one each were identified as Coxsackievirus B5, Coxsackievirus A9, Echovirus 5, and Echovirus 18 (Table 5). One additional sample was EV-positive from the CDC (identified as Coxsackievirus B5) that was negative by GeneXpert® (Table 4). All positive samples by either methodology had compatible clinical symptoms (all had fever, and many had other signs of a viral illness such as rash, diarrhea, or congestion).

The negative data from the young infant group is very consistent (Table 4). Fifty-eight out of 64 young infant plasma samples were negative for EV on both GeneXpert® and at the CDC; both the clinical specificity and negative predictive value (NPV) were 98%. The clinical sensitivity and positive predictive value (PPV) were 80%.

TABLE 4. A comparison of Enterovirus detection methods using plasma specimens from young infants.

		CDC	
		Positive	Negative
GeneXpert	Positive	4	1
	Negative	1	58

The most common symptom of the EV-infected patients in our study was fever (5/5 from validation set and 5/5 from young infant group = 100%). The average temperature for these patients was 38.6 ° C (101 ° F). Other symptoms included rash, congestion, vomiting, fussiness, and aseptic meningitis (5/5 from validation group and 1/5 from young infant group = 60%).

TABLE 5. A comparison of CDC genotyping results with clinical presentation in EV-infected young infants.

Patient	CDC Genotype Results	Clinical Symptoms
104	Echovirus 5	Fever and petechial rash
120	Coxsackievirus B5	Fever and fussiness (Dx = viral meningitis)
122	Coxsackievirus B5	Fever, congestion, fussiness
177	Coxsackievirus A9	Fever, congestion, fussiness
268	Echovirus 18	Fever

Conclusion

In summary, GeneXpert® was able to identify a 7.8% (5/64) prevalence of enterovirus infection in our patient sample. When excluding young infants who were afebrile and lacked symptoms or signs of a viral illness, GeneXpert® was able to identify an enteroviral etiology in 11.4% (5/44) of febrile young infants who otherwise did not have a specific etiologic diagnosis. Two of these five (40%) received 2 days of antibiotics pending bacterial cultures, which were negative. Using plasma to detect EV may allow for quicker diagnosis, sparing young infants from unnecessary hospital admission or antibiotic exposure.

References

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