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Distinct systemic and central nervous system disease patterns in enterovirus and parechovirus infected children

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Summary *Objectives:* Enteroviruses (EV) and human parechoviruses (HPeV) infections are increasingly identified in neonates and young children with sepsis, meningitis and encephalitis. We investigated EV and HPeV viral loads in plasma and cerebrospinal fluid (CSF) among those presenting with sepsis or central nervous system (CNS) disease to gain understanding of the nature of these infections.
Methods: Detections frequencies and viral loads of EV and HPeV RNA were compared in plasma and CSF obtained from infected children originally identified on sepsis or CNS screening.
Results: Two distinct infection profiles were identified; 11 subjects with CNS disease, showed higher or similar viral loads in CSF than in plasma (median plasma:CSF ratio 0.5), whereas 14 children with sepsis showed low or undetectable viral loads in CSF and high viral loads in plasma (mean ratio 5700). HPeV type 3 and one EV serotype (coxsackievirus B2) were primarily associated with the latter presentation.
Conclusions: Simple detection of EV or HPeV RNA in CSF is not predictive of CNS disease, especially in the absence of clinical markers (*i.e.* pleocytosis). Screening of plasma can identify EV and HPeV RNA in a substantial proportion of sepsis cases, some of which will be missed if CSF samples alone are screened.

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Introduction

Human enteroviruses (EV, genus *Enterovirus*) and parechoviruses (HPeV, genus *Parechovirus*) belong to the family *Picornaviridae*, which are a large group of non-enveloped viruses with positive-sense RNA genomes. Although EV and HPeV are genetically distinct,[1] they cause clinically indistinguishable infections in neonates.[2] Most present with fever, irritability, rash and poor feeding; signs which are also typical in life-threatening bacterial sepsis most commonly caused by *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitidis*. In addition, some EV and HPeV infected infants show features of central nervous system (CNS) disease including seizures, focal neurological signs, a cerebrospinal fluid (CSF) pleocytosis and abnormalities on brain imaging.[3] While over 30 different EV (sero)types regularly cause infections in young children, only HPeV type 3 from among the 16 types has been identified as a significant cause of neonatal infections.[4–6]

Laboratory detection of EV and HPeV is now based on molecular methods such as reverse transcriptase polymerase chain reaction (RT-PCR) that is faster and substantially more sensitive than traditional viral cell culture. Most viral diagnostic laboratories in the UK currently perform EV and HPeV RT-PCR on all CSF samples (15 out of the 20 clinical virology centres based on the Clinical Virology Network Audit, April 2013), while screening other sample types (stool, respiratory and blood samples) is variable and generally infrequent. Although it has been shown previously for EV that the combination of both CSF and blood specimens improve the diagnostic yield of enterovirus infections in young infants,[7,8] similar data is lacking for HPeV. Furthermore, plasma samples taken from infants with suspected neonatal sepsis for PCR-based bacterial screening are almost invariably omitted from EV and HPeV screening.

EV and HPeV RNA is detectable in CSF samples in many cases of neonatal sepsis and are generally reported as meningitis, although most of these infants lack CSF pleocytosis and other diagnostic features of CNS disease including focal neurological signs and tense fontanelle.[2–6] This led to our hypothesis that a substantial proportion of neonatal EV and HPeV infections with detectable virus in CSF are primarily systemic infections where virus in blood dominates. The main aim of this study was to determine the relationship between clinical presentations and viral load ratio in blood and CSF samples and other laboratory markers in a series of EV and HPeV infected infants.

Materials and methods

Clinical samples

A total of 128 EDTA blood samples (plasma) submitted for bacterial sepsis screening by PCR (*Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae*) from children under the age of 3 years between January and December 2012 were included in this study. All 128 blood samples tested negative for *N. meningitidis*, *S. pneumoniae* and *H. influenzae* by PCR, and blood cultures obtained from these children resulted no growth. All samples were taken within 24 h from presentation at the Royal Hospital for Sick Children, and submitted to routine virological investigations at the Specialist Virology Centre of Royal Infirmary Edinburgh. All samples were archived according the protocol approved by the NHS Lothian Bioresource Archive Ethics Committee (10/S1402/33). Stored data included age band, recorded clinical information including reason for request and results from neuroimaging if done, referral source, month of sample collection and results of routine virological (including results from CSF biochemistry) testing of the sample. Additionally, 10 plasma samples were identified for EV screening from individuals whom CSF sample had been tested positive for EV RNA in 2011.

A second group of 46 EV infected subjects were identified as those with EV RNA detected in CSF sample referred for routine virological testing between January and December 2011 (total $n = 1207$). From these, a total of 10 stored EDTA samples were identified (all previously untested) and screened for EV.

RT-PCR

EDTA blood samples were screened for EV and HPeV RNA by multiplexed real-time quantitative RT-PCR and RNA standards were used for viral load measurements of EV and HPeV-positive samples.[9]

EV and HPeV typing

Extracted RNA was amplified by a combined RT and first round PCR using the Superscript III (Invitrogen, UK) followed by a second amplification reaction with nested primers specific for species A or B sequences as previously described; if negative repeated with general VP4 primers.[9] Positive HPeV samples were amplified in the VP3/VP1 region.[5] All HPeV-positive samples were successfully typed but only 15/22 of the EV-positive samples were typed, a problem originating from the small volumes of EDTA samples left (<5 μl) or low viral load in CSF samples (100 copies/ml).

Clinical definitions

Sepsis was defined as the combination of irritability, fever (or hypothermia), impaired feeding, lethargy, and with or without signs of haemodynamic abnormalities (tachycardia or bradycardia and low blood pressure). CNS disease (meningitis or encephalitis) was defined by neurological signs or symptoms (bulging fontanel, nuchal rigidity, seizures, altered consciousness, hemi pareses or apnoea), abnormal findings on neuroimaging without another explanation (cerebral ultrasound or MRI) or pleocytosis in CSF (white cell count [WCC] $> 19 \times 10^9$/l in those under one month of age and $>9 \times 10^9$/l in those over one month of age).[10] CSF protein counts were also measured (normal range 0.25—0.95 g/l in those under one month of age and 0.15—0.75 g/l in those over one month of age).