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Assessment of blood enterovirus PCR testing in paediatric populations with fever without source, sepsis-like disease, or suspected meningitis: a prospective, multicentre, observational cohort study

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Summary

Background **Enteroviruses are the most frequent cause of acute meningitis and are seen increasingly in sepsis-like disease and fever without source in the paediatric population. Detection of enterovirus in cerebrospinal fluid (CSF) specimens by PCR is the gold standard diagnostic test. Our aim was to assess a method of detecting enterovirus in blood specimens by PCR.**

Methods **We did a prospective, multicentre, observational study at 35 French paediatric and emergency departments in 16 hospitals. We recruited newborn babies (aged ≤28 days) and infants (aged >28 days to ≤2 years) with fever without source, sepsis-like disease, or suspected meningitis, and children (aged >2 years to ≤16 years) with suspected meningitis, who were admitted to a participating hospital. We used a standardised form to obtain demographic, clinical, and laboratory data, which were anonymised. Enterovirus PCR testing was done in blood and CSF specimens.**

Findings **Between June 1, 2015, and Oct 31, 2015, and between June 1, 2016, and Oct 31, 2016, we enrolled 822 patients, of whom 672 had enterovirus PCR testing done in blood and CSF specimens. Enterovirus was detected in 317 (47%) patients in either blood or CSF, or both (71 newborn babies, 83 infants, and 163 children). Detection of enterovirus was more frequent in blood samples than in CSF specimens of newborn babies (70 [99%] of 71 *vs* 62 [87%] of 71; p=0·011) and infants (76 [92%] of 83 *vs* 62 [75%] of 83; p=0·008), and was less frequent in blood samples than in CSF specimens of children (90 [55%] of 163 *vs* 148 [91%] of 163; p<0·0001). Detection of enterovirus was more frequent in blood samples than in CSF specimens of infants aged 2 years or younger with fever without source (55 [100%] of 55 *vs* 41 [75%] of 55; p=0·0002) or with sepsis-like disease (16 [100%] of 16 *vs* nine [56%] of 16; p=0·008). Detection of enterovirus was less frequent in blood than in CSF of patients with suspected meningitis (165 [67%] of 246 *vs* 222 [90%] of 246; p<0·0001).**

Interpretation **Testing for enterovirus in blood by PCR should be an integral part of clinical practice guidelines for infants aged 2 years or younger. This testing could decrease the length of hospital stay and reduce exposure to antibiotics for low-risk patients admitted to the emergency department with febrile illness.**

Funding **University Hospital Clermont-Ferrand.**

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Introduction

Enteroviruses are the most frequent cause of paediatric aseptic meningitis and are attributed to more than 75% of viral meningitis cases in which a microorganism is identified.[1,2] Detection of enterovirus by RT-PCR from cerebrospinal fluid (CSF) specimens is recommended for diagnosis of meningitis caused by enterovirus.[3–5] Paediatricians are also confronted frequently with young infants with fever without source or sepsis-like diseases. These febrile illnesses account for 3·4–13·6% of cases seen in emergency departments.[6] Symptoms can result either from severe bacterial infection requiring admission to hospital and empirical antibiotic treatments or, most typically, from benign and spontaneously resolving viral infection; therefore, diagnosis is a challenge. Additional molecular tests are needed to speed up diagnosis of conditions associated with enterovirus infections.[5] Several studies have evaluated testing blood specimens,[7–12] but as yet no assessment has been done in a large cohort of paediatric patients.

The aim of our multicentre study was to assess detection of enterovirus by PCR in blood specimens of newborn babies, infants, and children with fever without source, sepsis-like disease, or suspected meningitis.

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Research in context

Evidence before this study

We searched PubMed up to Feb 7, 2018, for papers reporting paediatric enterovirus diseases and enterovirus PCR testing or molecular detection of viruses in cerebrospinal fluid (CSF) or blood specimens of patients with aseptic meningitis, sepsis and sepsis-like disease, or fever without source. We used the search terms "enterovirus", "nonpolio enterovirus", "meningitis", "viral meningitis", "aseptic meningitis", "enterovirus meningitis", "acute meningitis", "sepsis", "sepsis-like disease", "fever", "fever without source", "genome detection", "enterovirus detection", "enterovirus RT-PCR", "molecular detection", "viremia", "viremic", "virus load", "blood", "plasma", and "cerebrospinal fluid". We also reviewed references from relevant articles not identified in the original search. Our search identified 12 studies in which enterovirus detection was reported in blood and CSF. Most studies were retrospective, the number of patients recruited varied between 11 and 34, and blood samples were not obtained in all patients whose CSF was tested. Two studies of 80 and 122 patients aged 90 days or younger with enterovirus infection were referenced to discuss our enterovirus detection frequency in the blood and CSF of febrile infants. In a study of 75 patients aged 16 years or younger with aseptic meningitis, 76% had enterovirus detected in blood samples by PCR. However, in that study, age groups were not analysed separately. In all these studies, symptom duration before lumbar puncture or venepuncture, and time between CSF and blood collection, were not recorded.

Added value of this study

Our study of 360 patients with laboratory findings of enterovirus infection is, as far as we are aware, the largest prospective, multicentre, observational study to assess PCR testing for enterovirus in both blood and CSF samples from newborn babies (aged ≤28 days) and infants (aged >28 days to ≤2 years) with fever without source, sepsis-like disease, or suspected meningitis, and children (aged >2 years to ≤16 years) with suspected meningitis. To our knowledge, our study is the first to show that sensitivity of enterovirus detection in blood samples is related to patients' age and clinical presentation. Detection of enterovirus was more frequent in blood samples than in CSF specimens of newborn babies and infants with fever without source or sepsis-like diseases, and it was less frequent in blood samples of patients with suspected meningitis. Furthermore, our study showed that enterovirus positivity in blood was related inversely to patient's age with meningitis.

Implications of all the available evidence

At present, blood samples from febrile patients in the paediatric emergency department are not sent routinely for enterovirus PCR testing, and only CSF samples are sent for infants younger than 90 days or for patients with suspected meningitis. Guidelines for biological management of patients aged 2 years or younger with febrile illness in the emergency department need to be reconsidered. When blood is sampled for a complete blood count, an additional blood tube should be obtained for enterovirus PCR testing, which can now be done sufficiently rapidly to have a real effect on infection management. PCR testing of blood samples done in routine practice could result in a more accurate assessment of the actual number of positive cases in patients with suspected meningitis, sepsis-like diseases, and fever without source. A positive enterovirus diagnosis could affect beneficially decisions about a patient's management, by reducing antibiotic or antiviral therapy, avoiding ancillary tests, lowering hospital-related costs, and allowing earlier discharge.

Methods

Study design and patients

We did a prospective, multicentre, observational study at 35 paediatric and emergency departments in 16 French hospitals. We restricted enrolment of patients to the seasonal period of increased enterovirus circulation in countries with a temperate climate.[13]

We enrolled newborn babies (aged ≤28 days) and infants (aged >28 days to ≤2 years) with fever without source, sepsis-like disease, or suspected meningitis, and children (aged >2 years to ≤16 years) with suspected meningitis. All participants were admitted to one of the participating hospitals. We required an EDTA blood sample (plasma) obtained by venepuncture for participation. We also did lumbar puncture when clinically indicated.

We defined fever without source as a body temperature of 38°C or higher for less than 7 days in a child whose medical history and physical examination did not reveal the cause of the fever.[14] We defined sepsis as suspected or proven infection and at least two of another four criteria, one of which had to be abnormal temperature (>38·5°C or <36°C) or abnormal white-blood-cell count (elevated [>20 000×10⁹ per L] or depressed [<4000×10⁹ per L] for age), and the other criterion could be either tachycardia or bradycardia, or tachypnoea. Further criteria for sepsis were a platelet count lower than 100 000×10⁹ per L and C-reactive protein greater than 15 mg/L.[15] We defined meningitis as either the presence of age-specific pleocytosis or the presence of at least two of these neurological signs or symptoms: headache, nuchal rigidity, photophobia, bulging fontanelle, irritability, lethargy, nausea, vomiting, or positive Kernig's or Brudzinsky's signs. We defined pleocytosis as a white-blood-cell count in the CSF of more than 19 per µL for newborn babies (aged ≤28 days), ten per µL or more for infants (aged 29–56 days), and five per µL or more for older children (aged >56 days).[16]

Exclusion criteria were refusal of consent from parents, absence of or insufficient blood samples, and bacterial or other viral infections in blood or CSF specimens. We also excluded patients (at a later stage) who were diagnosed

with infections in other biological specimens (eg, urine, nasopharyngeal aspirate, or stool).

The study was approved by the French ethics committee (AU1180), under the Institutional Review Board number 00008526. We obtained verbal consent for use of clinical samples for research from parents or guardians of children aged 8 years or younger and from children older than 8 years.

Procedures

Within 24 h of admission, a doctor completed a standardised questionnaire for every patient, with details of the nature and duration of preadmission symptoms and signs and the results of a physical examination done at the time of admission. Laboratory findings comprised the date and time at which biological specimens were taken, CSF and full blood count characteristics, C-reactive protein assay, and the results of other bacteriological and virological testing of samples recorded by biologists. Symptom duration was the interval between onset of symptoms and venepuncture (and lumbar puncture, if indicated). We estimated the onset of symptoms as either 8 am, 2 pm, 8 pm, or 2 am when symptoms were recorded in the morning, afternoon, evening, and night, respectively. CSF protein concentration was classified as normal if it was 0·9 g/L or lower for newborn babies (aged ≤28 days) and 0·45 g/L or lower for older children (aged >28 days). Investigation for urinary-tract infection in febrile patients was done with urine dipsticks and confirmed by culture of specimens obtained from urethral catheters. A urinary-tract infection was diagnosed as leucocytosis (≥10^4 cells per mL) and clinically significant bacteria (≥10^5 colony-forming units per mL) in urine culture. All samples were submitted for routine bacteriological and virological investigations at every participating hospital, according to local practice. A senior paediatrician and the study team reviewed the final diagnosis at discharge.

We did PCR testing for enterovirus in blood and CSF specimens at microbiology laboratories of five participating hospitals: Centre Hospitalier Universitaire (CHU) de Clermont-Ferrand (Clermont-Ferrand), Cochin Hospital (Assistance Publique-Hôpitaux de Paris, Paris), Grand Hôpital de l'Est Francilien, site de Meaux (Meaux), Centre Hospitalier de Versailles André Mignot (Versailles), and CHU de Rennes (Rennes). We used commercial (Xpert EV, Cepheid, Sunnyvale, CA, USA [only used with CSF samples]; Enterovirus R-GENE, bioMérieux, Marcy l'Etoile, France; and O-DiaENT, Diagenode, Seraing, Belgium) or in-house[17] RT-PCR assays. A diagnosis of enterovirus was established with positive PCR findings in either plasma or CSF, or both. For specimens negative for enterovirus on PCR testing, and if the volume of sample remaining was sufficient, we did a specific RT-PCR parechovirus assay in blood and CSF (Parechovirus R-GENE, bioMérieux). We typed enterovirus-positive specimens at the National Reference Centre for Enteroviruses and Parechoviruses (Clermont-Ferrand, France) by amplification and sequencing of the VP1 capsid protein.[18]

Statistical analysis

We did statistical analyses with Stata 13. Statistical tests were two-sided with a type I error set at an α of 0·05. We presented continuous data as median (IQR) for every age group (newborn babies, infants, and children). We estimated κ coefficients and sensitivity, specificity, and predictive values (with 95% CIs) for blood enterovirus PCR testing and compared these with values in CSF to ascertain validity. We judged κ values according to recommendations: less than 0·2 (negligible), 0·2–0·4 (low or weak consistency), 0·4–0·6 (moderate agreement), 0·6–0·8 (substantial or good agreement), and greater than 0·8 (excellent agreement).[19]

For comparisons between groups, we used χ^2 or Fisher's exact tests for categorical variables, then (when appropriate) we did Marascuilo's procedure. For quantitative parameters, we used Student's *t* test or the Mann-Whitney test when assumptions of the *t* test were not met. We did a regression model for newborn babies and infants to identify factors that were associated independently with viraemia, using a stepwise (backward and forward) approach. We ascertained covariates according to univariate results (entry at p=0·15) and relevant biological and clinical variables—eg, CSF white-blood-cell count, duration of symptoms, tachycardia, hypotonia, irritability, and seizure (adjustment factors). We paid attention to multicollinearity. We expressed results as odds ratios (ORs) and 95% CIs, and we represented findings using forest plots.

We did a sensitivity analysis for multivariate analysis, which we applied to all groups. We also did a sensitivity analysis to assess the effect of any inaccurate dates or times of symptom onset recorded by parents.

Role of the funding source

The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. CA and AL had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Between June 1, 2015, and Oct 31, 2015, and between June 1, 2016, and Oct 31, 2016, we obtained data for 916 eligible patients, of whom 822 were included (figure 1). 169 participants were newborn babies (median age 19 days [IQR 13–25]), 371 were infants (age 68 days [44–135]), and 282 were children (age 6 years [4·8–9·1]). 94 (56%) newborn babies and 229 (62%) infants had fever without source, 36 (21%) newborn babies and 52 (14%) infants had sepsis-like disease, and 39 (23%) newborn babies, 90 (24%) infants, and 282 (100%) children had suspected meningitis.

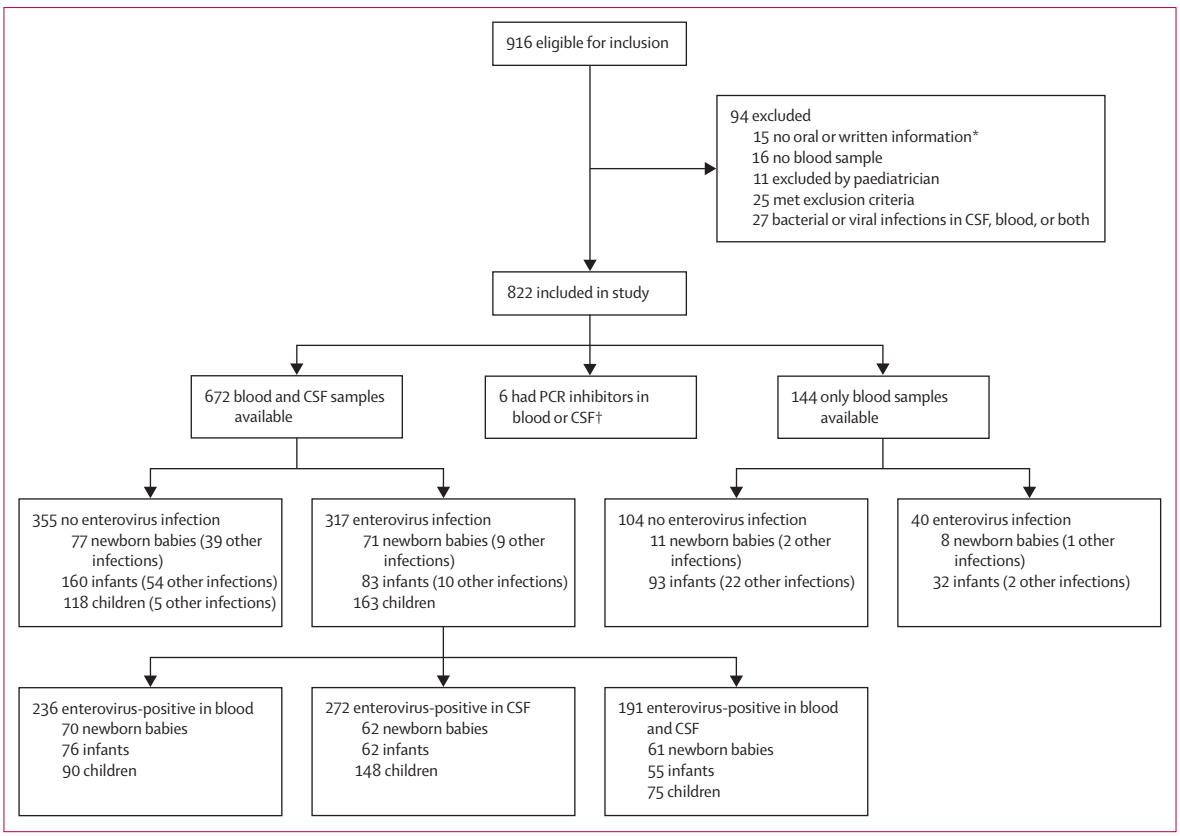

Figure 1: **Flow of enrolled patients**
CSF=cerebrospinal fluid. *Parents did not agree to participation of their child after reading the information leaflet. †PCR inhibitors were present either in blood (n=3) or in CSF (n=3) samples. Of those with PCR inhibitors in blood, two had negative CSF (one infant and one child) and one had positive CSF (newborn baby). Of those with PCR inhibitors in CSF, one had negative blood (infant) and two had positive blood (newborn baby and infant). Three patients had enterovirus infection. All six patients were excluded from the analysis.

Blood samples were obtained by venepuncture from 822 patients (169 newborn babies, 371 infants, and 282 children), and CSF specimens were obtained by lumbar puncture from 675 patients (149 newborn babies, 244 infants, and 282 children). Enterovirus was detected in CSF or blood, or both samples, in 360 (44%) of 822 patients, comprising 81 (48%) newborn babies, 116 (31%) infants, and 163 (58%) children. Bacterial or viral infections other than enterovirus were diagnosed during the clinical course in 144 (18%) of 822 patients. Bacterial infections were detected in 83 patients. Urinary-tract infections were detected in 76 patients (*Escherichia coli*, n=62; *Enterococcus* spp, five; *Klebsiella* spp, four; *Proteus mirabilis*, two; *Citrobacter koseri*, one; *Staphylococcus haemolyticus*, one; and *Staphylococcus aureus*, one) and gastrointestinal infections in seven (*Salmonella* spp, four; *Campylobacter* spp, three). Viruses other than enterovirus were detected in 61 patients. Human parechovirus infection was detected in blood, CSF, or both, of 35 patients, 17 patients had respiratory-tract infection detected in nasopharyngeal swabs (rhinovirus, ten; respiratory syncytial virus, four; coronavirus, one; parainfluenzae virus, one; bocavirus, one), four patients had gastroenteritis viruses in stool samples (rotavirus, two; adenovirus, two), and other viruses were detected in five patients (cytomegalovirus, two; human herpesvirus 6, three). 22 (6%) of 360 patients had co-infections consisting of enterovirus and bacterial infections (mostly febrile urinary-tract infections). No pathogen was identified in 340 (41%) of 822 patients.

See **Online** for appendix

317 patients with enterovirus infection had both blood and CSF specimens available for PCR testing (table 1; appendix p 1). PCR was positive for enterovirus in blood samples from 236 (74%) patients and was the only PCR-positive specimen in 45 (14%) patients. PCR was positive for enterovirus in CSF samples from 272 (86%) patients and was the only positive specimen in 81 (26%). Both specimens tested positive in 191 (60%) patients.

Detection of enterovirus in blood and CSF samples differed by age (table 1; appendix p 1). Enterovirus was detected more frequently in blood samples than in CSF specimens from newborn babies (70 [99%] of 71 *vs* 62 [87%] of 71; p=0·011) and infants (76 [92%] of 83 *vs* 62 [75%] of 83; p=0·008). Enterovirus was detected less frequently in blood samples than in CSF specimens from children (90 [55%] of 163 *vs* 148 [91%] of 163; p<0·0001).

	Total enterovirus infections (n)	Positive blood detection	Positive CSF detection	p value	Blood+/CSF–	Blood+/CSF+	Blood–/CSF+	Time between onset of symptoms and venepuncture (h)	Time between onset of symptoms and lumbar puncture (h)	p value
All patients										
Total	317	236 (74%)	272 (86%)	0·001	45 (14%)	191 (60%)	81 (26%)	20 (11–36)	18 (9–32)	0·025
Newborn babies	71	70 (99%)	62 (87%)	0·011	9 (13%)	61 (86%)	1 (1%)	12 (6–25)	9 (5–22)	0·984
Infants	83	76 (92%)	62 (75%)	0·008	21 (25%)	55 (66%)	7 (9%)	16 (8–29)	14 (8–25)	0·131
Children	163	90 (55%)	148 (91%)	<0·0001	15 (9%)	75 (46%)	73 (45%)	25 (15–49)*	24 (16–48)*	0·066
Fever without source										
Total	55	55 (100%)	41 (75%)	0·0002	14 (25%)	41 (75%)	0	13 (7–27)	12 (7–25)	0·011
Newborn babies	31	31 (100%)	27 (87%)	0·046	4 (13%)	27 (87%)	0	12 (7–23)	10 (5–22)	0·209
Infants	24	24 (100%)	14 (58%)	0·002	10 (42%)	14 (58%)	0	16 (9–33)	15 (10–29)†	0·012
Sepsis-like disease										
Total	16	16 (100%)	9 (56%)	0·008	7 (44%)	9 (56%)	0	6 (4–15)	6 (4–9)	0·623
Newborn babies	9	9 (100%)	7 (78%)	0·157	2 (22%)	7 (78%)	0	6 (4–13)	6 (4–7)	0·553
Infants	7	7 (100%)	2 (29%)	0·025	5 (71%)	2 (29%)	0	8 (3–26)	8 (4–9)	0·866
Suspected meningitis										
Total	246	165 (67%)	222 (90%)	<0·0001	24 (10%)	141 (57%)	81 (33%)	23 (13–43)	21 (12–41)	0·266
Newborn babies	31	30 (97%)	28 (90%)	0·317	3 (10%)	27 (87%)	1 (3%)	14 (5–36)	9 (6–25)	0·106
Infants	52	45 (87%)	46 (89%)	0·782	6 (12%)	39 (75%)	7 (13%)	20 (8–32)	15 (8–25)	0·774
Children	163	90 (55%)	148 (91%)	<0·0001	15 (9%)	75 (46%)	73 (45%)	25 (15–49)*	24 (16–48)*	0·066

Data are n (%) or median (IQR), unless otherwise indicated. CSF=cerebrospinal fluid. +=enterovirus PCR positive. –=enterovirus PCR negative. *Time between onset of symptoms and venepuncture or lumbar puncture was significant between children and infants and between children and newborn babies (p<0·0001). †Time between onset of symptoms and lumbar puncture was significant between infants and newborn babies (p=0·045).

Table 1: **Comparative detection of enterovirus in blood and CSF specimens**

	Patients (n)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (%)	κ (95% CI)
Fever without source	165	100% (91–100)	91% (85–96)	78% (65–89)	100% (97–100)	93·3%	0·83 (0·74–0·93)
Sepsis-like disease	55	100% (66–100)	89% (76–96)	64% (35–87)	100% (91–100)	90·9%	0·73 (0·51–0·95)
Suspected meningitis	406	64% (57–70)	87% (81–92)	86% (79–91)	66% (60–72)	74·1%	0·49 (0·41–0·57)
Newborn babies	38	96% (82–100)	70% (35–93)	90% (74–98)	88% (47–99)	89·5%	0·71 (0·45–0·97)
Infants	87	85% (71–94)	85% (71–94)	87% (73–95)	83% (69–93)	85·1%	0·70 (0·55–0·85)
Children	281	51% (42–59)	89% (82–94)	83% (74–90)	62% (55–69)	68·7%	0·39 (0·29–0·48)

Performance was assessed in 626 patients with both CSF and blood specimens available. We excluded 46 infants (aged >3 months to <2 years) with fever without source and sepsis, since CSF enterovirus PCR is not the gold standard diagnosis test of these disease conditions. Performance of blood PCR testing was calculated with respect to the gold standard of CSF enterovirus PCR, in all patients with meningitis and in the youngest infants (aged ≤3 months) with fever without source and sepsis-like disease, for whom collection of CSF samples was integrated in routine practice. Concordance was studied by κ values and accuracy. CSF=cerebrospinal fluid. PPV=positive predictive value. NPV=negative predictive value.

Table 2: **Performance of enterovirus blood PCR diagnosis**

Detection of enterovirus varied by clinical presentation (table 1). Enterovirus was detected more frequently in blood samples than in CSF specimens of newborn babies and infants with fever without source (55 [100%] of 55 *vs* 41 [75%] of 55; p=0·0002) or sepsis-like disease (16 [100%] of 16 *vs* nine [56%] of 16; p=0·008). Detection of enterovirus was similar in blood and CSF specimens from newborn babies and infants with suspected meningitis (p=0·317 and p=0·782, respectively). Detection of enterovirus was less frequent in blood than in CSF samples from children with suspected meningitis (90 [55%] of 163 *vs* 148 [91%] of 163; p<0·0001). This lower frequency was associated with a longer time between onset of symptoms and venepuncture (median 25 h [IQR 15–49]) than in infants (20 h [8–32]) and newborn babies (14 h [5–36]; p<0·0001).

In 144 patients (125 infants and 19 newborn babies), enterovirus PCR testing was done only in blood samples (figure 1). 124 (86%) of these patients were admitted to the emergency department with fever without source. PCR testing was positive for enterovirus in 40 (28%) patients (eight [42%] of 19 newborn babies and 32 [26%] of 125 infants).

The sensitivity, specificity, and accuracy of enterovirus PCR testing in blood samples were calculated with respect to the results of enterovirus PCR testing in CSF for the three clinical presentations (table 2). In this analysis, we excluded 46 infants (aged >3 months to

	Viraemia (n=125)*	No viraemia (n=135)†	p value
Demographics			
Newborn babies	61 (49%)	35 (26%)	<0·0001
Infants	64 (51%)	100 (74%)	<0·0001
Age (days)	28 (17–41)	55 (28–100)	<0·0001
Male sex	64 (51%)	70 (52%)	0·916
Admission characteristics			
Fever without source	49 (39%)	93 (69%)	<0·0001
Sepsis-like disease	12 (10%)	20 (15%)	<0·0001
Suspected meningitis	64 (51%)	22 (16%)	<0·0001
Time between onset of symptoms and lumbar puncture (h)	12 (7–24)	17 (7–31)	0·042
Time between onset of symptoms and venepuncture (h)	14 (7–26)	19 (7–31)	0·188
Exposure to a sick contact	58 (46%)	29 (22%)	<0·0001
Clinical symptoms			
Fever	125 (100%)	130 (96%)	0·061
Headache	1 (1%)	1 (1%)	>0·99
Photophobia	1 (1%)	1 (1%)	>0·99
Neck stiffness	3 (2%)	6 (4%)	0·503
Seizures	3 (2%)	13 (10%)	0·015
Rash	12 (10%)	19 (14%)	0·266
Bulging fontanelle	12 (10%)	10 (7%)	0·526
Tachychardia	62 (50%)	46 (34%)	0·011
Nausea or vomiting	12 (10%)	26 (19%)	0·028
Diarrhoea	18 (14%)	26 (19%)	0·296
Abdominal pain	6 (5%)	5 (4%)	0·661
Rhinitis or pharyngitis	29 (23%)	54 (40%)	0·004
Hypotonia	31 (25%)	24 (18%)	0·166
Irritability	85 (68%)	80 (59%)	0·144
Pallor or increased time for skin recolouring	27 (22%)	33 (24%)	0·587
Neurological symptoms	1 (1%)	7 (5%)	0·068
Poor feeding	37 (30%)	53 (39%)	0·102
Blood characteristics			
Glycaemia (mmol/L)	5·2 (4·6–5·8)	5·3 (4·7–5·8)	0·416
White-blood-cell count (×10⁹ per L)	8·8 (6·4–11·5)	10·6 (8·4–16·8)	<0·0001
Polynuclear neutrophils (×10⁹ per L)	3·9 (2·8–6·1)	4·9 (2·6–7·9)	0·059
Lymphocytes (×10⁹ per L)	3·3 (2·1–4·5)	4·6 (2·8–6·3)	<0·0001
Monocytes (×10⁹ per L)	0·9 (0·6–1·3)	1·2 (0·8–2·0)	0·019
Platelets (×10⁹ per L)	345 (274–434)	366 (290–447)	0·695
C-reactive protein >15 mg/L	23 (18%)	51 (38%)	0·001
CSF characteristics			
Pleocytosis	44/116 (38%)	17/125 (14%)	<0·0001
White-blood-cell count (per µL)	5 (1–162)	2 (1–5)	0·0001
Elevated protein	51/120 (43%)	26/131 (20%)	<0·0001
Protein (g/L)	0·6 (0·4–0·8)	0·4 (0·2–0·5)	0·003
Glucose (mmol/L)	3·0 (2·7–3·3)	3·2 (2·9–3·6)	0·0001

Data are n (%) or median (IQR), unless otherwise indicated. Data obtained from 260 patients with both CSF and blood samples available for enterovirus PCR testing. We excluded 112 patients aged ≤2 years with bacterial or viral infections other than enterovirus infections and 19 other patients for whom the time between CSF collection and blood collection was ≥24 h. CSF=cerebrospinal fluid. *Blood PCR-positive samples and CSF PCR-positive or CSF PCR-negative samples. †Blood PCR-negative samples and CSF PCR-negative samples.

***Table 3*: Univariate analysis of characteristics of infants aged 2 years or younger with and without enterovirus viraemia**

<2 years) with fever without source and sepsis because CSF enterovirus PCR is not the gold standard diagnosis test of these disease conditions. In 165 patients with fever without source, the sensitivity and specificity of enterovirus PCR testing in blood samples was 100% (IQR 91–100) and 91% (85–96), respectively. In 55 patients with sepsis-like disease, sensitivity was 100% (66–100) and specificity was 89% (76–96). In 406 patients with suspected meningitis, we recorded 64% (57–70) sensitivity and 87% (81–92) specificity.

Characteristics of patients aged 2 years or younger with and without enterovirus viraemia, who had both blood and CSF specimens available for analysis, were compared regardless of their clinical syndrome (table 3). For this analysis, we excluded 112 patients aged 2 years and younger with bacterial or viral infections other than enterovirus infections and 19 other patients for whom the time between CSF collection and blood collection was 24 h or longer. Tachycardia was the most frequent clinical symptom associated with viraemia, recorded in 62 (50%) of 125 patients with viraemia versus 46 (34%) of 135 without viraemia (p=0·011). Exposure of a patient to a sick contact (parents and siblings with an infectious disease) was associated significantly with viraemia (58 [46%] of 125 with viraemia *vs* 29 [22%] of 135 without viraemia; p<0·0001). Pleocytosis, white-blood-cell count in CSF, and amount of protein in CSF were significantly higher in patients with viraemia than in those without viraemia (p≤0·0001). Few patients had pleocytosis (44 [38%] of 116 with viraemia *vs* 17 [14%] of 125 without viraemia).

A multivariate analysis that adjusted for age, duration of symptoms, hypotonia, irritability, and all factors judged significant in univariate analysis confirmed that patient's age, tachycardia, and exposure to a sick contact were associated significantly with viraemia in patients aged 2 years or younger (figure 2A). In a further multivariate analysis, including patients in all three age groups, the time between onset of symptoms and venepuncture (<24 h, OR 2·96, 95% CI 1·57–5·58; p=0·001; 24 h to <48 h, 2·55, 1·27–5·11; p=0·008) and pleocytosis (7·60, 4·34–13·32; p<0·0001) were also associated with viraemia (figure 2B).

Data for patients in each age group with and without enterovirus infection, who had samples available for PCR testing, were compared for differences in symptoms and laboratory characteristics (table 4). For this analysis, we excluded 144 patients with bacterial or viral infections other than enterovirus infections. Newborn babies infected with enterovirus were significantly younger than were those without infection (median age 18 days [IQR 12–24] with enterovirus infection *vs* 21 days [14–26] without enterovirus infection; p=0·037). Infants infected with enterovirus were significantly younger than those without infection (median age 51 days [IQR 40–86] *vs* 77 days [53–261]; p=0·0001). Children infected with enterovirus were more likely to have headache (p=0·015), stiff neck

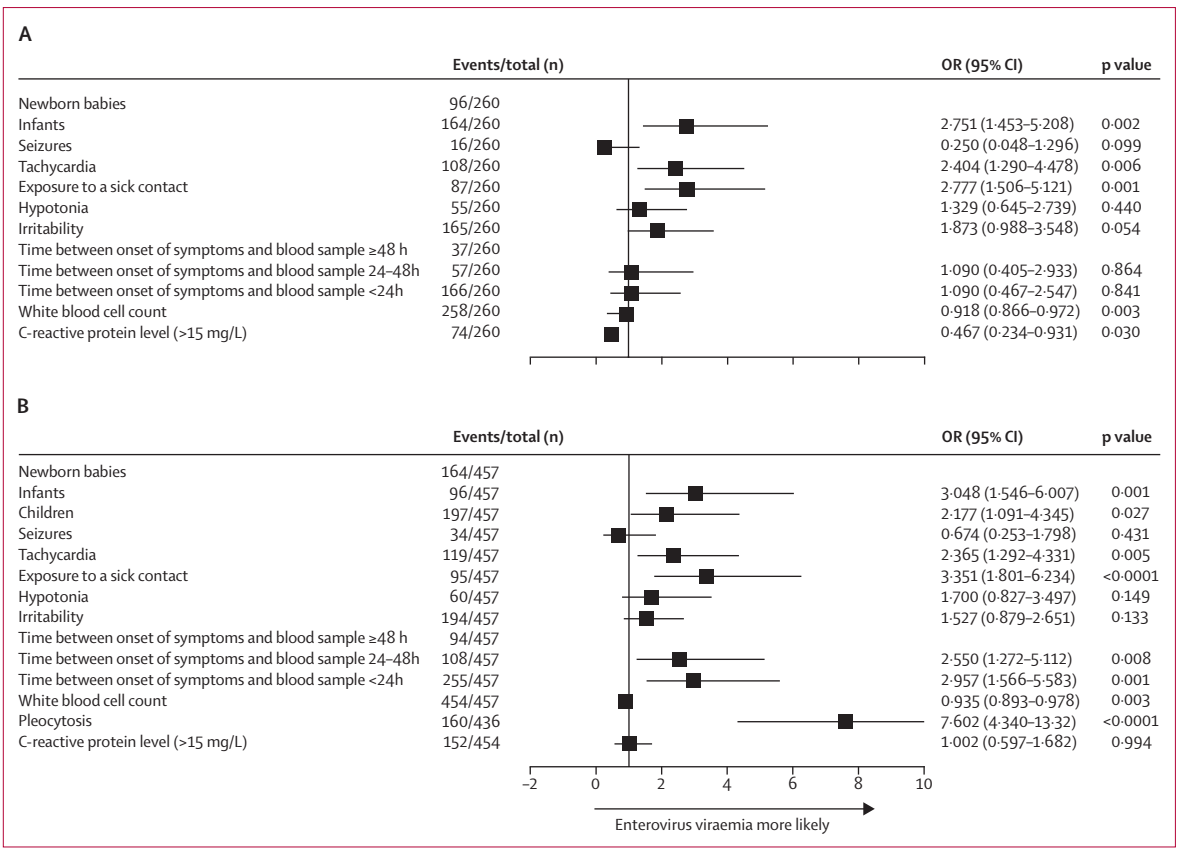

Figure 2: **Forest plot of multivariate analyses of the relation between enterovirus viraemia and clinical and biological characteristics of patients**
Analyses were done in patients (A) aged 2 years or younger and (B) aged 16 years or younger for whom both blood and CSF specimens were available. CSF=cerebrospinal fluid.

(p=0·042), hypotonia (p=0·012), and nausea or vomiting (p<0·0001) than were those without infection. Patients of all ages infected with enterovirus were more likely to have been exposed to a sick contact than were those without infection (p≤0·003). Newborn babies infected with enterovirus were more likely to show symptoms of irritability (p=0·001) and hypotonia (p=0·033) compared with those testing negative for enterovirus. Tachycardia was more frequent in infants infected with enterovirus than in those not infected with enterovirus (p=0·005). All patients recovered from the enterovirus infection.

Pleocytosis was recorded in 192 (66%) of 292 patients infected with enterovirus compared with 59 (24%) of 246 who were not infected (p=0·0001). Pleocytosis increased with patient's age, with 20 (35%) of 58 newborn babies, 33 (47%) of 71 infants, and 139 (85%) of 163 children having pleocytosis. Amounts of protein in CSF were similar in patients infected and not with enterovirus. Blood lymphocyte counts were lower in patients of all ages infected with enterovirus than in those not infected (p≤0·044).

Prospective enterovirus typing was done for 311 (86%) of 360 patients whose CSF or blood specimens, or both, were positive for enterovirus (appendix p 2). Viral strains were assigned to 29 different types, eight within the enterovirus A species (29 patients) and 21 within the enterovirus B species (282 patients). The enterovirus genotypes E9, E25, E7, and CVB5 were more frequent in newborn babies than in infants and children; patients with suspected sepsis were more likely to be infected with E25 genotype than were patients with other clinical presentations; and those with suspected meningitis were more frequently infected with E30, E6, or CVB5 genotypes.

Discussion

Our study shows that, in newborn babies and infants, the sensitivity of enterovirus PCR testing is higher in blood samples than in CSF specimens. This finding substantiates those of previous single-centre studies[7–9] and lends support to use of blood enterovirus testing as a diagnostic adjunct to rapidly identify newborn babies and infants admitted with fever without source, sepsis-like disease, or suspected meningitis whose antibiotic treatment can be discontinued and who are eligible for discharge. Our study also shows that blood enterovirus PCR testing in children with suspected meningitis has no additional benefit compared with PCR testing in CSF.

	Newborn babies (n=118)			Infants (n=283)			Children (n=277)			Interaction p value
	Enterovirus-positive (n=71)	Enterovirus-negative (n=47)	p value	Enterovirus-positive (n=104)	Enterovirus-negative (n=179)	p value	Enterovirus-positive (n=163)	Enterovirus-negative (n=114)	p value	
Demographics										
Age	18 days (12–24)	21 days (14–26)	0·037	51 days (40–86)	77 days (53–261)	0·0001	6·2 years (4·7–8·3)	6·4 years (4·9–10·1)	0·098	..
Male sex	39 (55%)	23 (49%)	0·52	61 (59%)	99 (55%)	0·58	118 (72%)	75 (66%)	0·24	..
Admission characteristics										
Fever without source	36 (51%)	34 (72%)	..	48 (46%)	135 (76%)	..	..	..	..	..
Sepsis-like disease	8 (11%)	9 (19%)	..	8 (8%)	20 (11%)	..	..	..	..	..
Suspected meningitis	27 (38%)	4 (9%)	..	48 (46%)	24 (13%)	..	163 (100%)	114 (100%)	..	..
Time between onset of symptoms and lumbar puncture (h)	10 (7–22)	12 (7–24)	0·189	14 (7–26)	19 (7–36)	0·240	24 (17–48)	29 (14–65)	0·225	..
Time between onset of symptoms and venepuncture (h)	12 (7–29)	19 (7–31)	0·197	17 (7–34)	22 (7–50)	0·103	24 (14–50)	29 (17–65)	0·195	..
Exposure to a sick contact	34 (48%)	10 (21%)	0·003	44 (42%)	39 (22%)	<0·0001	15 (9%)	1 (1%)	0·003	..
Clinical symptoms										
Fever	71 (100%)	44 (94%)	0·061	103 (99%)	177 (99%)	>0·99	155 (95%)	109 (96%)	0·840	0·086
Headache	..	..	..	..	..	..	148 (91%)	92(81%)	0·015	0·719
Photophobia	..	..	..	..	..	..	83 (51%)	48 (42%)	0·148	0·898
Neck stiffness	..	..	..	..	..	..	134 (82%)	82 (72%)	0·042	0·382
Seizures	0	0	>0·99	6 (6%)	16 (9%)	0·337	6 (4%)	13 (11%)	0·012	0·629
Rash	4 (6%)	8 (17%)	0·062	17 (16%)	26 (15%)	0·681	8 (5%)	11 (10%)	0·124	0·743
Bulging fontanelle	1 (2%)	6 (9%)	0·241	6 (6%)	10 (6%)	0·949	1 (1%)	0	1·000	0·502
Hypotonia	19 (27%)	5 (11%)	0·033	20 (19%)	29 (16%)	0·516	12 (7%)	1 (1%)	0·012	0·898
Tachychardia	24 (34%)	10 (21%)	0·141	57 (55%)	67 (37%)	0·005	7 (4%)	6 (5%)	0·708	0·302
Nausea or vomiting	6 (9%)	9 (19%)	0·088	14 (14%)	33 (18%)	0·278	122 (75%)	49 (43%)	<0·0001	<0·0001
Diarrhoea	7 (10%)	10 (21%)	0·084	19 (18%)	37 (21%)	0·625	2 (1%)	9 (8%)	0·009	0·617
Abdominal pain	..	..	..	..	..	..	43 (26%)	28 (25%)	0·733	0·609
Rhinitis or pharyngitis	10 (14%)	13 (28%)	0·068	32 (31%)	83 (46%)	0·010	33 (20%)	26 (23%)	0·608	0·099
Irritability	44 (62%)	14 (30%)	0·001	70 (67%)	111 (62%)	0·371	22 (14%)	18 (16%)	0·593	0·013
Pallor or increased time for skin recolouring	10 (14%)	10 (21%)	0·308	26 (25%)	38 (21%)	0·465	22 (14%)	11 (10%)	0·331	0·188
Poor feeding	20 (28%)	16 (34%)	0·498	34 (33%)	73 (41%)	0·176	34 (21%)	27 (24%)	0·577	0·624

(Table 4 continues on next page)

This lower sensitivity, compared with that recorded in newborn babies and infants, was attributable mainly to the long period between onset of symptoms and venepuncture.

To our knowledge, we report here the largest, prospective, multicentre, observational study to show that positive detection of enterovirus in blood is associated with patient's age and clinical presentation. Detection of enterovirus was significantly higher in blood samples than in CSF specimens from newborn babies and infants and varied by clinical presentation, with detection higher in patients admitted with fever without source or sepsis-like diseases than in those with suspected meningitis. A positive result for enterovirus in blood samples from patients with suspected meningitis was related inversely to age, with higher detection in newborn babies (97%), then infants (87%), then children (55%).

Compared with previous reports, enterovirus was detected in blood samples from newborn babies and infants more frequently in our study. In a study of 122 infants (aged ≤90 days) with fever who were infected with enterovirus, PCR yielded equally positive results in blood samples and CSF specimens (77% and 83%, respectively).[7] In another study of 80 infants with suspected sepsis, detection of enterovirus was similar in blood and CSF samples (69% *vs* 78%).[8]

Our study followed guidelines from the UK National Institute for Health and Care Excellence (NICE) for clinical management in emergency departments of newborn babies, infants, and children with febrile illness,[20] which include biological assessments—in

	Newborn babies (n=118)			Infants (n=283)			Children (n=277)			Interaction p value
	Enterovirus-positive (n=71)	Enterovirus-negative (n=47)	p value	Enterovirus-positive (n=104)	Enterovirus-negative (n=179)	p value	Enterovirus-positive (n=163)	Enterovirus-negative (n=114)	p value	
(Continued from previous page)										
Blood characteristics										
Glycaemia (mmol/L)	4·6 (4·2–5·2)	4·6 (4·4–5·4)	0·487	5·5 (5·0–6·1)	5·4 (4·9–6·1)	0·640	5·6 (5·1–6·3)	5·3 (4·7–6·3)	0·677	..
White-blood-cell count (×10⁹ per L)	8·8 (7·0–11·4)	9·7 (8·2–13·8)	0·020	8·8 (6·2–12·3)	11·0 (8·4–15·5)	0·0001	10·9 (8·5–13·4)	11·6 (8·8–15·5)	0·092	..
Polynuclear neutrophil (×10⁹ per L)	4·1 (2·9–6·3)	3·6 (2·0–4·9)	0·066	3·3 (2·4–5·8)	5·2 (2·5–7·9)	0·010	8·5 (6·5–10·8)	8·4 (5·4–12·8)	0·947	..
Lymphocytes (×10⁹ per L)	3·0 (2·1–4·5)	4·7 (3·4–6·6)	0·0005	3·8 (2·1–4·9)	4·3 (2·8–6·2)	0·044	1·4 (0·9–2·0)	1·7 (1·1–2·7)	0·004	..
Monocytes (×10⁹ per L)	0·8 (0·7–1·2)	1·2 (0·9–1·9)	0·092	0·9 (0·6–1·4)	1·2 (0·8–1·8)	0·037	0·7 (0·5–1·0)	0·9 (0·7–1·3)	0·0001	..
Platelet count (×10⁹ per L)	327 (250–420)	377 (273–461)	0·085	390 (309–445)	359 (282–450)	0·389	288 (245–348)	274 (230–339)	0·131	..
Thrombocytopenia	4/70 (6%)	1/47 (2%)	0·647	0/0 (0%)	3/177 (2%)	0·302	1/161 (1%)	3/113 (3%)	0·309	..
C-reactive protein >15 mg/L	9/71 (13%)	15/47 (32%)	0·011	21/104 (20%)	73/179 (41%)	<0·0001	50/161 (31%)	52/113 (46%)	0·012	..
C-reactive protein (mg/L)	8 (3–13)	11 (3–23)	0·171	8 (4–15)	15 (5–44)	0·0004	9 (4–20)	19 (7–62)	0·0001	..
CSF characteristics										
Pleocytosis	20/58 (35%)	4/33 (12%)	0·020	33/71 (47%)	17/101 (17%)	<0·0001	139/163 (85%)	38/112 (34%)	<0·0001	..
White-blood-cell count (per µL)	7 (2–181)	4 (3–14)	0·276	7 (1–177)	2 (1–4)	0·0001	72 (19–218)	2 (1–34)	0·0001	..
Elevated protein	17/61 (28%)	5/36 (14%)	0·112	42/73 (58%)	27/106 (26%)	<0·0001	30/163 (18%)	17/110 (16%)	0·527	..
Protein level (g/L)	0·7 (0·5–1·0)	0·6 (0·5–0·7)	0·148	0·5 (0·3–0·7)	0·3 (0·2–0·5)	0·222	0·3 (0·2–0·4)	0·2 (0·2–0·3)	0·469	..
Glucose (mmol/L)	2·7 (2·5–3·1)	2·9 (2·6–3·4)	0·088	3·1 (2·8–3·5)	3·3 (3·0–3·7)	0·007	3·5 (3·2–3·9)	3·6 (3·2–4·0)	0·451	..

Data are n (%) or median (IQR), unless otherwise indicated. These results were obtained from 678 patients who had samples available for enterovirus PCR testing (excluding 144 patients with bacterial or viral infections other than enterovirus infections). CSF=cerebrospinal fluid.

***Table 4*: Characteristics of each age group of patients with and without enterovirus infection**

particular full blood count, urine testing, and lumbar puncture, when clinically indicated. An additional blood tube was requested in our study for enterovirus PCR testing. The time between collection of the blood sample and collection of the CSF specimen was 2 h or sooner for most patients (472 [70%] of 672; data not shown). Concomitant collection of blood and CSF was important for overall interpretation of virological data. We found that sampling times for CSF and blood after onset of symptoms had major effects on detection of enterovirus: lumbar puncture done at an early stage in the infection course and late blood sampling (>3 days to ≤4 days) can produce negative enterovirus results. In our study, of 317 patients infected with enterovirus who had both blood and CSF samples available, 45 (14%) were not identified by the CSF PCR assay. The time between onset of symptoms and lumbar puncture in these patients was short (median 15 h [IQR 8–23]), with 14 (31%) of these 45 patients having symptom duration of 8 h or less (data not shown). By contrast, in 81 (26%) of 317 patients with a positive CSF PCR assay and negative blood PCR, the time between symptom onset and venepuncture was longer (median 26 h [IQR 16–70]). These data suggest that viraemia occurs early and is of short duration, a finding rarely noted in other reports and only with small patient populations.[21,22]

In some biological diagnostic practices, CSF PCR testing is done only in patients with pleocytosis. In our study, we did lumbar puncture in 675 patients, of whom 371 did not have pleocytosis (data not shown). In these patients, however, enterovirus was detected in CSF (n=70) and blood (n=98). Without detection of enterovirus in blood or CSF samples, these patients without pleocytosis would have been discharged without aetiological diagnosis. Thus, the diagnostic practice to do enterovirus testing solely in patients with pleocytosis can lead to enterovirus infections being missed. Moreover, 144 patients had enterovirus PCR testing done only in blood samples, of whom 40 (28%) had enterovirus infection—mostly newborn babies or infants with fever without source. Ahmad and colleagues detected enterovirus in blood samples from 34 (24%) of 139 neonates with sepsis-like illness.[11]

The diversity of prevailing enterovirus genotypes during the two seasons of the present study and differences in the distribution of genotypes among age

groups and clinical presentations are all factors that should be considered in enterovirus detection in blood samples. The proportion of enterovirus genotypes in patients admitted to hospital reflects global circulation patterns of virus strains across countries and within the general population of one country.[23] Harvala and colleagues[24] reported higher or similar viral loads in CSF compared with plasma in 11 children younger than 3 years with CNS disease. They found low or undetectable CSF viral loads and high plasma viral loads in 14 children with sepsis. A previous study by our group[25] showed that among 156 patients with acute meningitis, enterovirus viral loads in CSF were higher in newborn babies than in infants and adults and that genotypes were associated with different viral loads.

The analysis of clinical and biological characteristics in patients aged 2 years or younger showed that viraemia was detected more frequently in younger infants (median age 28 days [IQR 17–41]) with acute-stage disease, as suggested in earlier retrospective studies of smaller patient populations.[21,22] Clinically, tachycardia was present in 62 (50%) of 125 young patients with viraemia and fever without source, sepsis, or suspected meningitis. Other reports have cited fever, irritability, lethargy, and poor feeding in patients with enterovirus viraemia.[11,21] In our study, patients with enterovirus viraemia were also more likely to have been exposed to a sick contact.

All patients with viraemia in our study were febrile. Amounts of C-reactive protein in serum greater than 15 mg/L were detected more frequently in patients without viraemia. Pleocytosis was noted in 44 (38%) of 116 patients with enterovirus viraemia, mainly those with suspected meningitis (data not shown). Dagan and colleagues[21] reported an inverse relation between viraemia and pleocytosis. The number of lymphocytes and monocytes was significantly lower in patients with viraemia than in those without viraemia. This finding and earlier data[21,26] suggest a detrimental effect of enterovirus infection on populations of mononuclear leucocytes during viraemia. This effect can be caused by virus replication in mononuclear leucocytes, because some enterovirus genotypes can replicate in vitro in human peripheral blood mononuclear cells.[27,28] Accordingly, the concentrations of circulating mononuclear cells at different ages and the ability of enterovirus genotypes to replicate in these cells can affect the sensitivity of blood enterovirus detection.

Concomitant bacterial infections, mostly urinary-tract infections, occurred in a small proportion (22 [6%] of 360) of patients with fever and enterovirus infection. Amounts of C-reactive protein in serum greater than 15 mg/L were noted in five (23%) of these patients (data not shown). The frequency of concomitant enterovirus and bacterial infections was consistent with that in other studies.[7,8,29] No enterovirus and bacterial co-infections were identified in CSF of patients with meningitis and in blood samples of patients with sepsis-like disease.

Our study has three limitations. First, the date and time of symptom onset were recorded less reliably by parents of children than by those of newborn babies and infants. A sensitivity analysis (data not shown) excluding the inaccurate dates and times of symptom onset for 190 (23%) of 822 patients did not affect our results. Second, virology management (including detection of viruses other than enterovirus) and bacteriology management varied between the 16 hospitals taking part. In 340 (41%) of 822 patients, no diagnosis was established, a figure similar to that reported by Ahmad and colleagues (43%).[11] Third, four different RT-PCR assays were used in our multicentre study. It is unlikely that this variability affected the overall results, because methods used are assessed annually by an external quality assessment programme, and all yielded correct results.

In conclusion, detection of enterovirus in blood improved diagnostic yield in newborn babies and infants admitted for fever without source, sepsis-like disease, or suspected meningitis, compared with detection in CSF. The high frequency of detection of enterovirus in blood samples from very young patients with fever without source and sepsis-like disease suggests that enterovirus febrile illnesses are underdiagnosed. It is important to reconsider the guidelines for biological management of patients aged 2 years or younger with febrile illness in emergency departments, to obtain—at the time of blood sampling—an additional tube for enterovirus PCR testing, which can be done sufficiently rapidly to have a real effect on management. A positive enterovirus blood diagnosis could beneficially affect patient management decisions by reducing antibiotic or antiviral therapy, avoiding ancillary tests, lowering hospital-related costs, and allowing earlier discharge. Blood enterovirus genome can also be used as an alternative biomarker in case of contraindications for CSF sampling and failure of lumbar puncture.

Contributors

AL and CA are principal investigators. ASL'H, FM, BP, MD, MNA, FG, FF, FA, SM-J, MAG, GL, MV, FR, AM, HP-L, CH, J-LB are study investigators. CA, AL, and JL designed the study and oversaw conduct of the trial. CA and JL coordinated individual trial sites. AL, CA, and JL reviewed clinical files. ASL'H and FR contributed to data collection and assembled data. CA, JL, J-LB, and CH analysed data and wrote the first draft. ASL'H, FM, BP, MD, MNA, FG, FF, FA, SM-J, MAG, GL, MV, FR, AM, and HP-L critically reviewed the report and contributed to its content. All authors and members of the study team contributed to data collection and approved the final version before submission.

Blood Enterovirus Diagnosis Infection (BLEDI) in paediatric population study team

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Declaration of interests

We declare no competing interests.

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