

Enteroviral meningitis without pleocytosis in children

Ki Wook Yun,^{1,2} Eun Hwa Choi,^{1,3} Doo Sung Cheon,⁴ Jina Lee,⁵ Chang Won Choi,^{1,5} Hee Hwang,⁵ Beyong Il Kim,^{1,5} Kyoung Un Park,⁶ Sung Sup Park,⁶ Hoan Jong Lee^{1,3}

¹Department of Pediatrics, Seoul National University College of Medicine, Seoul, Korea

²Department of Pediatrics, Chung-Ang University College of Medicine, Seoul, Korea

³Department of Pediatrics, Seoul National University Children's Hospital, Seoul, Korea

⁴Division of Enteric and Hepatitis Viruses, Center for Infectious Diseases, National Institutes of Health, Korea Center for Disease Control and Prevention, Osong, Korea

⁵Department of Pediatrics, Seoul National University Bundang Hospital, Seongnam, Korea

⁶Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, Korea

Correspondence to

Professor Eun Hwa Choi, Department of Pediatrics, Seoul National University College of Medicine, 101 Daehak-ro, Jongno-gu, Seoul 110-769, Korea; eunchoi@snu.ac.kr

Accepted 15 June 2012

ABSTRACT

Objectives This study aims to describe the clinical characteristics of enteroviral meningitis in association with the absence of cerebrospinal fluid (CSF) pleocytosis.

Design This was a retrospective analysis of databases of patients diagnosed with enteroviral meningitis by CSF reverse transcription-PCR testing. Presence of CSF non-pleocytosis at each age group was analysed by use of the two criteria. Clinical variables were compared with regard to the presence of CSF pleocytosis. Multiple logistic regression analysis was used to identify factors that were associated with CSF pleocytosis.

Setting Two hospitals in South Korea, between January 2008 and August 2011.

Patients 390 infants and children with enteroviral meningitis.

Interventions None.

Main outcome measures Proportion of enteroviral meningitis without CSF pleocytosis.

Results Among the 390 patients with enteroviral meningitis, 16–18% did not have CSF pleocytosis. In particular, CSF pleocytosis was not present in 68–77% of the neonates with enteroviral meningitis, demonstrating that the proportion of CSF pleocytosis decreased significantly with age ($p < 0.001$). In multivariate models, younger age (adjusted OR 0.981; 95% CI 0.973 to 0.989), lower peripheral white blood cell count (adjusted OR 0.843; 95% CI 0.791 to 0.899), and shorter interval between onset and lumbar puncture (adjusted OR 0.527; 95% CI 0.315 to 0.882) were associated with the absence of CSF pleocytosis in enteroviral meningitis.

Conclusions This study demonstrated high proportion of non-pleocytic enteroviral meningitis in young infants and identified several clinical factors that contributed to the absence of CSF pleocytosis. We suggest that CSF enterovirus PCR testing is likely to detect more cases of enteroviral meningitis, especially in young infants.

INTRODUCTION

Non-polio enteroviruses (EV) are the most commonly identified causes of aseptic meningitis and account for over 80% of cases in which the aetiological agent is identified. The severity of enteroviral infections of the central nervous system (CNS) is variable, but their outcomes are usually favourable in otherwise healthy and immunocompetent patients.¹

The standard method for identification of EVs was the use of neutralising antibodies in virus culture. However, several molecular methods have been recently developed for enteroviral identification. Among them, reverse transcription-PCR (RT-PCR) assays can rapidly detect EVs.^{2–3} As this sensitive molecular method is commonly used in clinical practice, cases of enteroviral meningitis which do not have cerebrospinal fluid (CSF)

What is already known on this topic

- ▶ Non-polio EVs are the most commonly identified causes of aseptic meningitis.
- ▶ Reverse transcription-PCR (RT-PCR) assays can rapidly detect EVs in CSF of patients with enteroviral meningitis.
- ▶ Enteroviral meningitis without CSF pleocytosis have been identified by CSF EV PCR testing, especially in young infants.

What this study adds

- ▶ 16% of patients with enteroviral meningitis did not have CSF pleocytosis, but this increased to 68% of the neonates.
- ▶ Younger age and lower peripheral WBC count were associated with the absence of CSF pleocytosis in enteroviral meningitis.
- ▶ A shorter interval between onset of symptoms and lumbar puncture was associated with an absence of CSF pleocytosis using nested RT-PCR, but not using GeneXpert assays.

pleocytosis have been confirmed by CSF EV PCR testing, especially in young infants.^{4–6}

In this study, we described clinical features of enteroviral meningitis based on the presence of CSF pleocytosis, and investigate factors influencing CSF pleocytosis in enteroviral meningitis in infants and children.

MATERIALS AND METHODS

Patients

This retrospective study was performed at Seoul National University Children's Hospital (SNUCH) and Seoul National University Bundang Hospital (SNUBH) between January 2008 and August 2011. Three hundred and ninety cases of enteroviral meningitis were identified during the study period. We considered any patient with positive CSF EV PCR results to have enteroviral meningitis. Clinical data were obtained from the medical records of patients and entered into standardised case report forms. Laboratory values for peripheral blood were included only if sampling occurred within 12 h before or after lumbar puncture.

Study definitions

Two criteria for the definition of CSF pleocytosis were used in the analysis of the proportion of

non-pleocytosis in each age group. The first criteria defined CSF pleocytosis as CSF white blood cell (WBC) count of >22 WBCs per mm^3 if the patient's age was <4 weeks, >15 WBCs per mm^3 if the patient's age was 4–7 weeks, or >5 WBCs per mm^3 if the patient's age was ≥ 8 weeks.⁷ The second criteria defined CSF pleocytosis as CSF WBC count of >19 WBCs per mm^3 for neonates aged ≤ 28 days and >9 WBCs per mm^3 for infants aged 29–56 days.⁸ WBC counts in CSF were adjusted by red blood cell (RBC) count in CSF when traumatic (defined as the presence of ≥ 500 RBCs per mm^3 in CSF) lumbar puncture occurred.⁹ The proportion of cases with pleocytosis was calculated using both criteria.^{7 8} However, subsequent analysis for clinical characteristics and risk factors was done based on the first criteria.⁷

Enterovirus PCR testing

Enterovirus PCR assays were performed by in-house semi-nested RT-PCR methods as described previously,¹⁰ except for CSF samples that were collected after June 2008 at SNUCH. GeneXpert assays (Cepheid, Sunnyvale, California, USA)¹¹ were used for the latter samples. The primers and probes were from the same portion of the EV genome that codes for the 5'-nontranslated region in both PCR assays. Primer sequences in semi-nested RT-PCR were 5'-CCC CTG AAT GCG GCT AAT CC-3'(F) and 5'-CAA TTG TCA CCA TAA GCA GCC A-3'(R), in GeneXpert 5'-CCC TGA ATG CGG CTA ATC C-3'(F) and 5'-ATT GTC ACC ATA AGC AGC CA-3'(R). Probe sequence in GeneXpert was 5'-AAA CAC GGA CAC CCA AAG TAG TCG G-3'. The sensitivity for EV detection of semi-nested RT-PCR has been shown to be 85%–100% which is a little lower than that of GeneXpert assay, 98%–100%.^{10–12}

Statistical analysis

SPSS V.19.0 (SPSS, Inc., Chicago, Illinois, USA) was used for data analysis. Statistical comparisons between groups were performed using the nonparametric Mann-Whitney *U* test and Pearson's χ^2 test.

Multivariate logistic regression was used to identify factors that were independently associated with CSF pleocytosis. Variables were included in the multivariate model based on factors considered to be associated with CSF pleocytosis (ie, age, peripheral WBC count, and interval between onset and puncture). Statistical significance was determined as a 2-tailed *p* value of <0.05 .

RESULTS

Clinical characteristics of enteroviral meningitis

Among 390 patients who were diagnosed with enteroviral meningitis, 250 (64%) cases were from SNUBH and 140 (36%) cases from SNUCH. Sixty-four percent ($n=249$) of patients were male and the median patient age was 62.5 months (range, 4 days–226 months). Fever, headache, vomiting and seizure were observed in 379 (97%), 267 (68%), 258 (66%) and 13 (3%) cases, respectively.

Two hundred and seventy-seven cases were diagnosed by nested RT-PCR assay, and 113 patients were diagnosed by GeneXpert assay. No significant differences were found in clinical characteristics according to the two PCR methods used ($p>0.05$, data not shown). Enterovirus CSF culture was performed for 273 patients (70% of total cases) and yielded positive results in 104 patients (38% of the tested cases).

The CSF profiles of the study samples were as follows: WBC counts were 0–1530/ mm^3 (median 110/ mm^3); protein levels of 15.9–218.6 mg/dl (median 92.9 mg/dl); CSF/serum glucose

Table 1 Clinical characteristics of 390 patients diagnosed with enteroviral meningitis

	Median	IQR		Range
		25%	75%	
Age (month)	62.5	2.0	93.0	0.13–226
Peripheral WBC (/ mm^3)	10920	8500	14200	3770–25210
ANC (/ mm^3)	8244	4864	11776	1025–21902
CRP (mg/dl)	0.77	0.27	1.86	0–13.3
CSF WBC (/ mm^3)	110.0	20.0	290.0	0–1530.0
CSF Protein (mg/dl)	93.0	46.4	111.5	15.9–218.6
CSF/Serum glucose ratio	0.6	0.5	0.7	0.3–1.1

ANC, absolute neutrophil count; CRP, C-reactive protein; CSF, cerebrospinal fluid; WBC, white blood cell.

ratio of 0.3–1.1 (median 0.6), and traumatic tap was identified in 39 (10%) cases (table 1). The results of bacterial and fungal investigations were negative in all cases.

Clinical findings of enteroviral meningitis by the presence of CSF pleocytosis

The WBC counts in CSF were assessed according to the ages of patients. Patients younger than 4 weeks old showed the lowest mean WBC count of $61.6 \pm 174.7/\text{mm}^3$ ($p<0.001$, figure 1). Table 2 compares the proportion with pleocytosis and non-pleocytosis in different age groups by the two criteria. Of the 390 patients with enteroviral meningitis, 71 (18%) did not have CSF pleocytosis according to the first age-based criteria for pleocytosis. In the analysis by the first criteria, CSF pleocytosis was not present in 77% of infants aged 0–27 days, 44% of infants aged 28–55 days, 30% of infants aged 56 days to 1 year, 10% of children aged 2–4 years, and 6% of children aged 5–18 years, respectively. The proportion of patients without CSF pleocytosis decreased significantly with age ($p<0.001$, figure 2). Similarly, analysis by the second criteria also showed

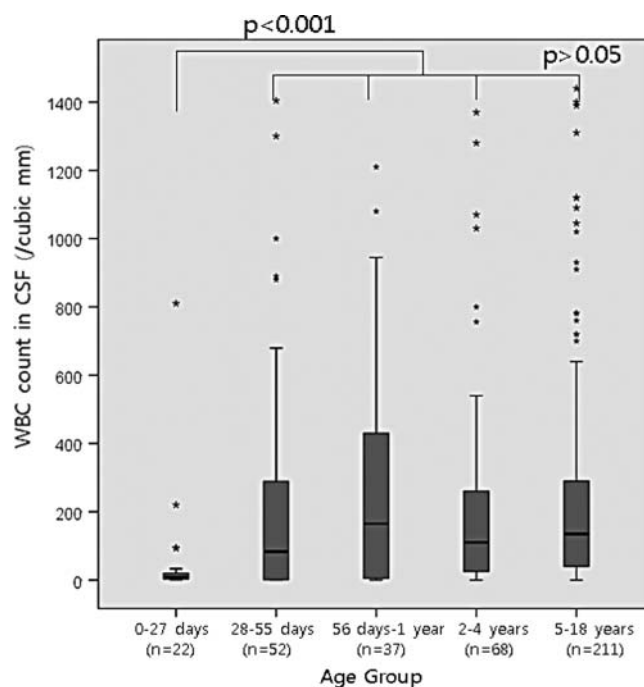


Figure 1 Cerebrospinal fluid (CSF) WBC count according to age. WBC, white blood cell.

Table 2 Comparison of the presence of pleocytosis in each age group by the two criteria

	No. of patients (%)							
	Criteria 1*				Criteria 2†			
	0–27 days	28–55 days	56 days–18 year	p Value	0–28 days	29–56 days	57 days–18 year	p Value
With pleocytosis (%)	5 (23)	29 (56)	285 (90)	<0.001	8 (32)	30 (59)	284 (90)	<0.001
Without pleocytosis (%)	17 (77)	23 (44)	31 (10)		17 (68)	21 (41)	30 (10)	

p Value was calculated by χ^2 analysis to compare the proportion without pleocytosis in each age group.

*Bonadio *et al.*⁷

†Kestenbaum *et al.*⁸

the overall proportion of CSF non-pleocytosis was 16% (62/390) (68% of neonates and 41% of infants aged 29–56 days did not have CSF pleocytosis).

Clinical characteristics, according to the presence of CSF pleocytosis, are shown in table 3. Patients in the non-pleocytosis group were younger than those in the pleocytosis group ($p<0.001$). The mean counts of total WBCs and neutrophils in peripheral blood of the non-pleocytosis group were 8280/mm³ and 4986/mm³, respectively, which were significantly lower than in the pleocytosis group ($p<0.001$, table 3). Patients who underwent lumbar puncture within 24 h of onset of symptoms were less likely to have pleocytosis ($p=0.015$). Patients for whom the GeneXpert assay was used as the EV PCR method were also less likely to have pleocytosis ($p=0.010$).

Factors associated with CSF pleocytosis in enteroviral meningitis

Peripheral WBC count, absolute neutrophil count (ANC), onset-lumbar puncture interval and age were associated with CSF pleocytosis on univariate analysis (table 3). In the multivariate model, younger age, lower peripheral WBC count, lower ANC and shorter interval between onset and lumbar puncture were independently associated with the absence of CSF pleocytosis (table 3). When clinical parameters associated with the absence of CSF pleocytosis were analysed according to the PCR method used only younger age and lower peripheral WBC count remained associated with CSF non-pleocytosis in both groups (table 4). Shorter interval between onset and lumbar puncture showed significant effect on CSF non-pleocytosis only in the nested RT-PCR group.

A subgroup analysis was done in a group of infants aged 90 days or younger ($n=103$). Only age (aOR 0.974; 95% CI 0.953 to 0.966) and peripheral WBC count (aOR 0.825; 95% CI 0.749 to 0.909) remained associated with CSF pleocytosis.

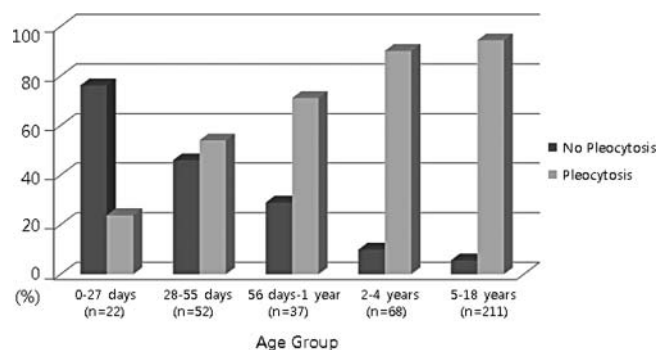


Figure 2 Proportion of enteroviral meningitis based on the presence or absence of cerebrospinal fluid (CSF) pleocytosis in each age group.

DISCUSSION

This study analysed 390 cases of enteroviral meningitis diagnosed by RT-PCR to describe clinical features of enteroviral meningitis based on the presence of CSF pleocytosis, and investigate factors influencing the absence or presence of CSF pleocytosis in children with enteroviral meningitis. We found that 16%–18% of patients diagnosed with enteroviral meningitis did not have pleocytosis. The proportion of enteroviral meningitis without CSF pleocytosis decreased with age and lower peripheral WBC count. The absence of CSF pleocytosis was associated with early lumbar puncture using nested RT-PCR but not the GeneXpert assay.

RT-PCR assays have been increasingly used for the diagnosis of enteroviral infection.^{3 13–15} Previous studies suggest that the use of CSF EV PCR testing decreases lengths of hospital stay and the incidence of unnecessary diagnostic and therapeutic interventions for children.¹⁶ In addition, several authors have demonstrated the potential for significant cost savings if testing is performed during periods of peak enteroviral prevalence.^{17 18} In earlier studies, pleocytosis was mostly detected in CSF of patients with aseptic meningitis, and the WBC count usually ranged between 100 and 1000/mm³ with a predominance of lymphocytes.^{17 19} Predominance of neutrophils within the first 24 h, followed by a shift to lymphocytes was observed in one study.²⁰ The absence of CSF pleocytosis has been reported in pediatric enteroviral CNS infections since RT-PCR method was first applied for the detection of EVs in the early 2000s.^{4–6 21} Although those studies^{4–6 21} included a number of age groups, the proportion of cases without pleocytosis in enteroviral meningitis among infants younger than 3 months was around 30% (30% of infants under 2 months in New Zealand⁴ and Missouri (USA)⁶, 31% in Pennsylvania (USA)⁵, and 28% of infants under 3 months in Korea.²¹) All these studies^{4–6 21} used conventional RT-PCR method for EV detection and applied the first definitions for CSF pleocytosis.⁷ A recent study²² in The Netherlands showed that 40% (23/58) of children younger than 16 years old with enteroviral meningitis did not have CSF pleocytosis and patients without CSF pleocytosis were younger by use of the conventional RT-PCR method and slightly modified criteria for CSF pleocytosis.

The present study demonstrated that a very high proportion of neonates (77%) and young infants aged 4–7 weeks (44%) diagnosed with EV meningitis by nested RT-PCR and GeneXpert assay did not have CSF pleocytosis. This might be due to higher sensitivity of GeneXpert assay for EV detection. In these age groups, lumbar puncture is routinely practiced for the evaluation of patients who present with fever alone, because they rarely have definite signs or symptoms suggesting meningitis. The high proportion of CSF without pleocytosis shown in this study suggests that CSF EV PCR testing is warranted in the evaluation of febrile young infants, even in the absence of CSF pleocytosis.

Table 3 Univariate and multivariate regression analysis of factors associated with CSF pleocytosis in enteroviral meningitis

	Univariate model			Multivariate model	
	Pleocytosis group (n=319)	Non-pleocytosis group (n=71)	p Value†	aOR	95% CI
Age (month)	70.04±47.07	20.83±34.89	<0.001*	0.981§	0.973 to 0.989
LOS (day)	2.93±1.86	3.33±1.85	0.110		
Peripheral WBC count (/mm ³)	12337±3881	8280±3125	<0.001*	0.843¶	0.791 to 0.899
Peripheral ANC (/mm ³)	9240±4365	4986±3204	<0.001*	0.932¶	0.886 to 0.980
CRP (mg/dl)	1.50±1.90	0.99±1.13	0.386		
Onset-puncture interval					
<24 h (n)	144	44	0.015*‡	0.527**	0.315 to 0.882
≥24 h (n)	175	27			
EV PCR method					
Nested RT (n)	236	41	0.010*‡	2.053	0.965 to 4.368
GeneXpert (n)	83	30			

Values for age, LOS, peripheral WBC count, ANC, and CRP were shown by mean value±SD.

*p<0.05

†By Mann-Whitney U test.

‡By Pearson χ^2 test.

§Reflects the increase in the odds of lack of pleocytosis for each 4-week increase in age.

¶Reflects the increase in the odds of lack of pleocytosis for each 500-cell increase in WBC/Neutrophil count.

**Reflects the increase in the odds of lack of pleocytosis for each 24-h increase in time.

ANC, absolute neutrophil count; aOR, adjusted OR; CI, confidence interval; CRP, C-reactive protein; EV, enterovirus; LOS, length of stay in hospital; PCR, polymerase chain reaction; RT, reverse transcription; WBC, white blood cell.

A previous study⁵ proposed that the lack of pleocytosis in CSF samples that were positive for enteroviral RNA was probably due to the immunologic immaturity of young infants. It is speculated that the chemokine response required for the recruitment of leukocytes to sites of infection had not yet been developed in these patients.⁶ In the present study, peripheral WBC was identified as the factors independently influencing CSF pleocytosis. This finding coincides with the recently published data regarding factors associated with CSF pleocytosis in enteroviral meningitis.^{5 22}

On the other hand, we hypothesised that if the time from onset of enteroviral meningitis to lumbar puncture is fairly short, the immunologic response that recruits WBCs to CSF may not be complete. Although retrospectively collected, we were able to demonstrate correlation between the time from onset of enteroviral meningitis to lumbar puncture and CSF pleocytosis, especially in nested RT-PCR group. The data suggest that the recruitment of WBCs to CSF may not be complete in the early stages of disease, however, the highly sensitive GeneXpert assay reduces this phenomenon. Similarly, recent studies of CSF parameters in neonates who were diagnosed with bacterial meningitis by CSF culture have shown that

neonates are likely to lack CSF pleocytosis in early stage of bacterial meningitis.^{23 24} Although definitive criteria for CSF pleocytosis are not currently available, we applied two standards for CSF pleocytosis suggested in the literature.^{7 8} In analysis using the most frequently applied standard (>22 WBCs per mm³ in neonates)⁷ and another recent one with slightly lower upper normal limit of CSF WBC counts (>19 WBCs per mm³ in neonates),⁸ the proportion of patients with CSF pleocytosis increased significantly with age. When we applied an alternative standard with a slightly higher upper limit (>29 WBCs per mm³ in neonates), the findings were similar (proportion of CSF without pleocytosis 90% in patients<28 days old and 10% in patients ≥56 days).

This study has several limitations. Its retrospective design only allowed us to identify patients for whom the CSF EV PCR test result was positive. Our interpretations of time between symptom onset and lumbar puncture were only estimated. Data may be affected by spectrum bias because the indications for performing lumbar puncture vary with age. Two other PCR methods used for enteroviral detection may play as a confounder, although we adjusted for this confounder in statistical methods. Despite these limitations, we believe our results are valuable and clinically important. This study was performed on a scale large enough to obtain good statistical power and used multidirectional statistical approaches.

Taken together, our findings suggest that individual variation among patients, and the time required to amplify inflammatory responses to recruit WBCs in serum, may contribute to WBC count in the CSF of paediatric patients with enteroviral meningitis. CSF EV PCR testing may be warranted to evaluate enteroviral infection in CNS, especially in young infants during the enteroviral season, even though CSF profiles do not show pleocytosis.

Contributors KWY, EHC and HJL conceived the original research idea and were the lead authors for this paper. EHC is the guarantor of the paper. KWY drafted the study, performed the registration of data, performed statistical analyses, wrote a draft and completed the manuscript. All three authors named above mainly contributed to the development of the final manuscript. DSC, KJP, and SSP contributed to the draft of the study, were the leaders of the microbiological laboratory analyses, critically revised the manuscript and approved the final manuscript. Data in SNUBH were collected by JL, CWG, HH, and BIK critically appraised the manuscript.

Table 4 Multiple logistic regression analysis of factors associated with CSF pleocytosis according to enteroviral PCR method

PCR method	Nested RT-PCR		GeneXpert	
	aOR	95% CI	aOR	95% CI
Age (month)*	0.975	0.963 to 0.986	0.977	0.965 to 0.989
Peripheral WBC count (/mm ³)†	0.829	0.760 to 0.903	0.893	0.815 to 0.979
Peripheral ANC (/mm ³)†	0.942	0.879 to 1.010	0.934	0.862 to 1.012
Onset-puncture interval (day)‡	0.442	0.228 to 0.855	0.685	0.354 to 1.326

*Reflects the increase in the odds of lack of pleocytosis for each 4-week increase in age.

†Reflects the increase in the odds of lack of pleocytosis for each 500-cell increase in WBC/Neutrophil count.

‡Reflects the increase in the odds of lack of pleocytosis for each 24-h increase in time.

ANC, absolute neutrophil count; aOR, adjusted OR; CI, confidence interval.

Funding This study was supported by the 2011 Seoul National University Hospital Research Fund (0420110410), Seoul, Korea.

Ethics approval Approval for the study was obtained from the institutional review board of Seoul National University Hospital, Seoul, Korea.

Provenance and peer review Not commissioned; externally peer reviewed.

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Arch Dis Child published online July 19, 2012
doi: 10.1136/archdischild-2012-301884

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