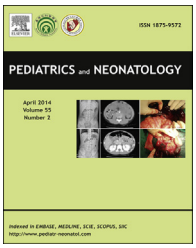
Pediatrics and Neonatology (2014) 55, 120–126

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: http://www.pediatr-neonatol.com



ORIGINAL ARTICLE

The Significance of Serum and Fecal Levels of Interleukin-6 and Interleukin-8 in Hospitalized Children with Acute Rotavirus and Norovirus Gastroenteritis

Shan-Ming Chen [a,b,c], Ching-Pin Lin [d], Jeng-Dau Tsai [a,b], Yu-Hua Chao [a,b], Ji-Nan Sheu [b,c,*]

[a] Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
[b] Department of Pediatrics, Chung Shan Medical University Hospital, Taichung, Taiwan
[c] Department of Pediatrics, School of Medicine, Chung Shan Medical University, Taichung, Taiwan
[d] Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan

Received Jan 14, 2013; received in revised form May 29, 2013; accepted May 30, 2013
Available online 27 July 2013

Key Words
interleukin-6;
interleukin-8;
norovirus;
rotavirus

Background: Rotavirus and norovirus are the most common known causes of viral gastroenteritis in children. This study examined the association between serum interleukin 6 (IL-6) and interleukin 8 (IL-8) levels and disease severity in the acute phase of rotavirus and norovirus gastroenteritis in children, and it also explored the role of fecal cytokine levels in children with viral and bacterial gastroenteritis.

Methods: This prospective study enrolled patients aged 4 months to 14 years admitted with acute gastroenteritis in a tertiary care center. Peripheral blood samples were collected for IL-6 and IL-8 assays within the first 3 days of diarrhea. Stool samples were obtained from the patients in the first 24 hours after admission.

Results: Serum IL-6 and IL-8 were measured in children with viral ($n = 66$) and bacterial ($n = 23$) infections, and in healthy controls ($n = 10$). In the acute phase of gastroenteritis, a moderately positive correlation was found between serum IL-6 levels and disease severity ($r_s = 0.41$, $p < 0.01$). Serum IL-8 levels correlated with the duration of fever ($r_s = 0.28$, $p = 0.03$). Fecal IL-6 levels correlated with the maximum number of daily bowel movements ($r_s = 0.35$,

* Corresponding author. Department of Pediatrics, Chung Shan Medical University Hospital, No. 110, Jianguo North Road, Section 1, Taichung 402, Taiwan.
E-mail address: cshy098@csh.org.tw (J.-N. Sheu).

http://dx.doi.org/10.1016/j.pedneo.2013.05.008

$p < 0.05$). Rotavirus infection induced significantly higher serum IL-8 levels than norovirus infection ($p < 0.05$). Receiver operating characteristic (ROC) curve analysis showed that absolute neutrophil count (ANC), maximum body temperature (BT), and Vesikari score were significant predictors in discriminating rotavirus from norovirus gastroenteritis.
Conclusion: IL-6 and IL-8 are involved in the pathogenesis of acute gastroenteritis in both rotavirus and norovirus. An ANC of less than 9000/mm^3, maximum BT of less than 38.2°C, and Vesikari score of less than 14 at the end of the course are potential predictors of norovirus infection in children compared with rotavirus gastroenteritis.

1. Introduction

Acute gastroenteritis is one of the major causes of morbidity and mortality in children worldwide. It is an infection of the gastrointestinal tract caused by a wide range of enteric pathogens, including bacteria, viruses, and parasites.[1,2] Rotavirus, norovirus, enteric adenovirus, and astrovirus are the most common causes of viral gastroenteritis in children.[1,3–7] As the burden of illness due to bacterial pathogens has decreased with improvements in hygiene and sanitation, viruses have become the leading etiologic agents in severe diarrhea requiring hospital admissions, although most cases are self-limiting.[8] However, it is often difficult to differentiate between viral and bacterial infections by clinical features alone because of similar presenting symptoms.[9,10] Moreover, rotavirus and norovirus are the two most common viral agents causing acute gastroenteritis in children,[2,3] with rotavirus infection accounting for 40% of all outpatient visits for acute gastroenteritis in young children.[8] Recent studies have indicated that norovirus can also cause as severe an illness as rotavirus.[3] More than 90% of the outbreaks of gastroenteritis in which the cause could not previously be identified are now attributed to this virus in the United States.[4] These findings suggest that norovirus is a more important viral agent than other enteric viruses.

Interleukin-6 (IL-6), a pleiotropic cytokine produced by lymphoid and nonlymphoid cells, has a broad range of functions including immune responses, acute-phase reactions, and hematopoiesis.[11–13] Interleukin-8 (IL-8), a member of the chemotactic cytokine family, plays an important role in recruiting inflammatory cells such as neutrophils and lymphocytes to an inflammatory site.[14] Their local and systemic effects have been described in hosts with mucosal infections.[15] However, previous studies on IL-6 and IL-8 in the pathogenesis of gastroenteritis have mainly focused on rotavirus and bacterial infections.[9,10,16,17]

The aim of this study was to examine the association of serum and fecal levels of IL-6 and IL-8 and clinical features in children with acute gastroenteritis, and also to explore the significance of these cytokines during the acute phase of norovirus infection.

2. Methods

This prospective survey enrolled patients aged 4 months to 14 years who were admitted to Chung Shan Medical University Hospital between March 2009 and February 2010 for gastroenteritis. The inclusion criterion was acute gastroenteritis, which was defined as the passage of loose or watery stools three or more times in a 24-hour period while excluding noninfectious causes such as medications (e.g., antibiotics, laxatives) or procedures (e.g., enemas, endoscopy).[18] We carefully reviewed each patient's medical history and excluded those with other bowel disorders (e.g., irritable bowel syndrome, Crohn's disease, or allergic colitis) and diarrhea lasting more than 7 days.

The clinical symptoms of gastroenteritis were recorded, including body temperature (BT) and maximum number of vomiting and diarrhea episodes within a 24-hour period. Fever was defined as a body temperature of more than 38°C (100.4°F). The severity of illness was assessed using the 20-point Vesikari scoring system,[19,20] which is based on BT, the severity of diarrhea, vomiting, dehydration, and treatment. Rehydration therapy followed by early reintroduction of age-appropriate feeding was the standard treatment. Age-matched healthy controls were recruited from the general pediatric clinics. The hospital's Institutional Review Board approved this study, and the parents or guardians of all the participants provided informed consent.

2.1. Laboratory analysis

Stool samples were collected from the patients in the first 24 hours after admission, and stored at −80°C until viral testing. All stool samples were also sent for bacterial cultures and microscopically examined for ova and parasites. To isolate bacterial pathogens, xylose lysine deoxycholate (XLD) agar, blood agar plate (BAP), and *Clostridium difficile* Selective Agar (CDSA; Becton Dickinson, Sparks, MD, USA) were inoculated. Rotavirus, norovirus, adenovirus, and astrovirus were detected by commercial enzyme immunoassay (EIA) kits (Ridascreen; R-Biopharm AG, Darmstadt, Germany) according to the manufacturer's instructions. Serum C-reactive protein (CRP) levels were determined by nephelometry on a BN ProSpec analyzer (Dade Behring, Marburg, Germany), while serum white blood cell (WBC) counts were determined using an automated hematology analyzer XE-5000 (Sysmex Corporation, Kobe, Japan). The absolute neutrophil counts (ANCs) were calculated by the following formula: ANC/mm^3 = WBC count × (% bands + % neutrophils) × 0.01.

2.2. Measurement of serum and fecal cytokines

Blood samples for IL-6 and IL-8 assays were collected on admission from each patient within the first 3 days of diarrhea onset. All blood samples were centrifuged at 1000*g* at 4°C for 15 minutes and stored at −80°C until assay. The fecal cytokines were measured according to an extraction method described previously.[21,22] The stool samples were weighed, diluted 1:2 in phosphate-buffered saline (PBS; pH 7.2) containing phenylmethylsulfonyl fluoride (1 mg/mL; Wako Pure Chemical Industries Ltd, Osaka, Japan) and soy trypsin inhibitor (1 mg/mL; Wako Pure Chemical Industries Ltd). The mixtures were centrifuged at 10,000*g* at 4°C for 15 minutes, and then the supernatants were passed through a 0.45 μm filter (Minisart N; Sartorius, Gottingen, Germany) and stored at −80°C until assay. IL-6 and IL-8 levels were measured with a Quantikine Human Interleukin Immuno-assay (R&D Systems, Minneapolis, MN, USA) using the principle of quantitative sandwich enzyme immuno-assay with monoclonal antibodies specific for IL-6 and IL-8. Concentrations of IL-6 and IL-8 were calculated using regression analysis with standard curves and expressed as picograms per milliliter (pg/mL). All samples were assayed in duplicate and the average was used in the statistical analyses. The minimum detectable concentration of IL-6 was < 0.7 pg/mL, whereas that of IL-8 ranged from 1.5 to 7.5 pg/mL.

2.3. Statistical methods

The results were expressed as median and interquartile range (IQR). The nonparametric Mann-Whitney U test was used to compare continuous variables between the two groups, while the Kruskal-Wallis test followed by Dunn's multiple comparison test was used for multiple groups. The Chi-square test or Fisher's exact test was used for categorical variables as appropriate. To assess the diagnostic accuracy of the laboratory tests and level of serum cytokines, receiver operating characteristic (ROC) curves were plotted and the areas under the curves (AUCs) were calculated for comparison. Optimal cutoff values were obtained by the Youden index, defined as the maximum value based on the equation: sensitivity + specificity − 1. The correlation between serum cytokines and severity score was evaluated using Spearman's rank correlation analysis. All statistical analyses were performed using the statistical software SPSS (version 12.0; SPSS Inc., Chicago, IL, USA). A p value less than 0.05 was considered statistically significant.

3. Results

A total of 122 children with acute gastroenteritis participated in the study. A single enteric pathogen was detected in 89 patients. Viral pathogens identified included rotavirus ($n = 33$), norovirus ($n = 27$), adenovirus ($n = 5$), and astrovirus ($n = 1$). Isolated bacterial pathogens included *Salmonella* spp. ($n = 20$), *Campylobacter* spp. ($n = 1$), and *Aeromonas* spp. ($n = 2$). Mixed infections were observed in two cases (one norovirus−adenovirus and one rotavirus−norovirus, respectively).

The clinical characteristics and routine laboratory test results between the viral and bacterial infection groups and results of the serum cytokine assays were compared (Table 1). There were statistically significantly higher serum levels of IL-6 and IL-8 in both the virus and bacteria groups compared to the control group ($p < 0.05$). Dunn's multiple comparison test showed that serum IL-6 but not IL-8 levels in the bacteria group were higher than those in the virus group.

Table 1 Demographic data and clinical characteristics of children with acute gastroenteritis and healthy controls.

	Viral ($n = 66$)	Bacterial ($n = 23$)	Controls ($n = 10$)	p
Sex (boy/girl)	29/37	8/15	5/5	0.85[†]
Age (y)[*]	2.0 (1.2−3.4)	1.7 (0.8−2.4)	3.5 (1.7−5.1)	0.08[‡]
Maximum BT (°C)[*]	38.5 (37.6−39.0)	39.1 (39.0−39.8)	36.7 (36.5−37.0)	<0.01[§]
Vesikari score[*]	13 (12−14.25)	16 (15−17)	—	<0.01[‖]
Stool occult blood ≥ 2++	26 (39.3%)	17 (73.9%)	0	<0.01[¶]
Stool pus cell ≥ 5/HPF	5 (8.1%)	8 (34.8%)	0	<0.01[¶]
WBC (/mm^3)[*]	9700 (7530−14,330)	9220 (7070−12,180)	7535 (6678−8708)	0.05[‡]
ANC (/mm^3)[*]	6784 (3862−11541)	4518 (3386−5840)	3097 (2643−5028)	<0.01[#]
CRP (mg/dL)[*]	0.33 (0.30−1.29)	8.40 (2.95−12.60)	0.30 (0.27−0.30)	<0.01[§]
Serum IL-6 (pg/mL)[*]	5.30 (2.10−9.67)	15.99 (10.73−66.58)	0.02 (0−0.48)	<0.01[§]
Serum IL-8 (pg/mL)[*]	14.93 (0.7−37.54)	13.57 (2.72−56.09)	0 (0−0.34)	<0.01[§]

ANC = absolute neutrophil count; BT = body temperature; CRP = C-reactive protein; HPF = high-power field; WBC = white blood cell count.

[*] Data are presented as median [interquartile range (IQR)].

[†] Chi-square test for differences among viral, bacterial, and controls.

[‡] Kruskal-Wallis test for differences among viral, bacterial, and controls.

[§] Kruskal-Wallis test; $p < 0.05$ by Dunn's multiple comparison test within groups, viral versus bacterial, viral versus controls, and bacterial versus controls.

[‖] Mann-Whitney U test for differences between viral and bacterial group.

[¶] Chi-square test for differences between viral and bacterial group.

[#] Kruskal-Wallis test; $p < 0.05$ by Dunn's multiple comparison test within groups, viral versus controls.

Table 2 Results of ROC curve analysis to differentiate between viral and bacterial gastroenteritis.

Test variable	AUC	SE*	Sig.†	95% CI for AUC
WBC (/mm^3)	0.442	0.065	0.41	0.315—0.569
ANC (/mm^3)	0.345	0.059	0.03	0.230—0.501
CRP (mg/dL)	0.939	0.025	<0.01	0.894—0.985
Vesikari score	0.848	0.040	<0.01	0.769—0.927
Maximum BT (°C)	0.786	0.048	<0.01	0.691—0.881
Serum IL-6 (pg/mL)	0.833	0.044	<0.01	0.746—0.920
Serum IL-8 (pg/mL)	0.534	0.071	0.53	0.395—0.672

ANC = absolute neutrophil count; AUC = area under the curve; BT = body temperature; CI = confidence interval; CRP = C-reactive protein; SE = standard error; Sig. = significant; WBC = white blood cell count.
* Under the nonparametric assumption.
† Null hypothesis: true area = 0.5.

ROC curve analysis was performed to assess the diagnostic performance of the laboratory tests, serum cytokines, and severity scores in differentiating between bacterial and viral infections (Table 2). The AUCs for CRP, Vesikari score, maximum BT, and IL-6 reached statistical significance and acceptable discrimination ability (AUC $\geq$ 0.7) between bacterial and viral gastroenteritis. Using the maximum value of the Youden index, the optimal cut-off values were: CRP 2.0 mg/dL (sensitivity 91.3%, specificity 81.8%), Vesikari score 15 (sensitivity 82.6%, specificity 75.8%), maximum BT 38.9 °C (sensitivity 82.6%, specificity 62.1%), and IL-6 8.2 pg/mL (sensitivity 87.0%, specificity 66.7%). Correlation studies between serum cytokines and Vesikari score demonstrated a statistically significant positive correlation between Vesikari score and serum IL-6 level (r_s = 0.41, $p < 0.01$) but not IL-8 level (r_s = 0.18, p = 0.09). Serum IL-8 showed a positive correlation with fever duration (days) (r_s = 0.28, p = 0.03).

Comparisons between rotavirus and norovirus revealed significant differences in Vesikari score, maximum BT, ANC, and CRP levels (Table 3). We compared the serum cytokine levels from the 33 children with rotavirus infections, 27 children with norovirus infections, 23 children with bacterial infections, and healthy controls (Figure 1). Dunn's multiple comparison test showed that serum IL-6 levels in the bacteria group (15.99, IQR: 10.73—66.58) were statistically significantly higher than those in the rotavirus (7.72, IQR: 3.19—12.46) and norovirus (3.7, IQR: 1.39—6.21) groups. However, there was no significant difference in the level of serum IL-6 between the rotavirus and norovirus groups, whereas IL-8 levels differed significantly ($p < 0.05$).

The results of ROC curve analysis for discriminating rotavirus from norovirus gastroenteritis are shown in Table 4. The AUCs for ANC, Vesikari score, maximum BT, and IL-8 revealed an acceptable discrimination ability. The optimal cut-off values were as follows: ANC 9000/mm^3 (sensitivity 54.5%, specificity 81.5%), Vesikari score 14 (sensitivity 72.7%, specificity 74.1%), maximum BT 38.2 °C (sensitivity 87.9%, specificity 66.7%), and IL-8 13.2 pg/mL (sensitivity 75.8%, specificity 66.7%).

Fecal cytokine assays were conducted for 20 children with viral infections (8 rotavirus, 12 norovirus), 13 children with bacterial infections (11 *Salmonella*, 2 *Aeromonas*), and six healthy controls. There were significantly higher fecal IL-6 levels in both the virus and bacteria groups compared to the control group ($p < 0.01$). Dunn's multiple comparison test showed a significant difference in the levels of fecal IL-6 between the viral (1.24, IQR: 0.47—2.67) and bacterial (4.25, IQR: 2.99—9.40) infections ($p < 0.05$). Fecal IL-6 levels were positively correlated with the maximum number of stools in a 24-hour period (r_s = 0.35, $p < 0.05$). No statistically significant difference was observed in fecal IL-6 between rotavirus and norovirus infections (p = 0.13). Fecal IL-8 was detected in only three (15%) cases with viral infections and 10 (76.9%) cases with bacterial infections ($p < 0.01$).

4. Discussion

Aside from being considered a multifunctional cytokine with proinflammatory, immunoregulatory, oncogenetic, and hematopoietic effects,[13] an IL-6 response to bacterial infections has been reported including mediated systemic

Table 3 Clinical characteristics of children with rotavirus and norovirus gastroenteritis and healthy controls.

	Rotavirus (n = 33)	Norovirus (n = 27)	Controls (n = 10)	p
Age (years)*	2.5 (1.4—3.7)	1.7 (1.0—2.6)	3.5 (1.7—5.1)	0.10†
Sex (boy/girl)	15/18	12/15	5/5	0.12‡
Vesikari score*	14 (13—16)	12 (11—14)	—	<0.01§
Maximum BT (°C)*	39 (38.4—39.1)	37.8 (37.2—38.7)	—	<0.01§
WBC (/mm^3)*	12,860 (8130—16,320)	9560 (6410—13,060)	7535 (6678—8708)	0.03‖
ANC (/mm^3)*	10,178 (4521—13,798)	5149 (3410—7944)	3097 (2643—5028)	<0.01¶
CRP (mg/dL)*	0.64 (0.30—2.87)	0.32 (0.30—0.66)	0.30 (0.27—0.30)	<0.01‖

ANC = absolute neutrophil count; BT = body temperature; CRP = C-reactive protein; WBC = white blood cell count.
* Data are presented as median [interquartile range (IQR)].
† Kruskal-Wallis test for differences among rotavirus, norovirus, and controls.
‡ Chi-square test for differences among rotavirus, norovirus, and controls.
§ Mann-Whitney U test for differences between rotavirus and norovirus.
‖ Kruskal-Wallis test; $p < 0.05$ by Dunn's multiple comparison test within groups, rotavirus versus controls.
¶ Kruskal-Wallis test; $p < 0.05$ by Dunn's multiple comparison test within groups, rotavirus versus controls and rotavirus versus norovirus.

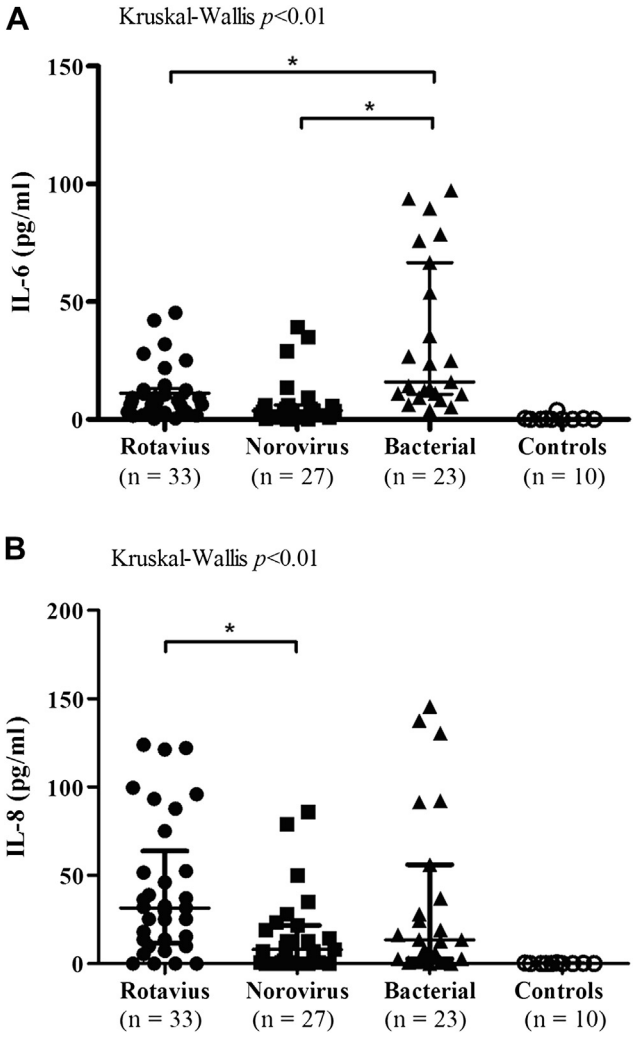


Figure 1 Serum (A) interleukin-6 (IL-6) and (B) interleukin-8 (IL-8) levels in children with rotavirus, norovirus, bacterial gastroenteritis, and in healthy controls. The median with interquartile range (IQR) are shown in each group. *p* value for comparisons by Kruskal-Wallis test; *$p < 0.05$, by Dunn's multiple comparison test between groups.

Table 4 Results of ROC curve analyses to differentiate between rotavirus and norovirus gastroenteritis.

Test variable	AUC	SE*	Sig.†	95% CI for AUC
WBC (/mm^3)	0.623	0.072	0.10	0.481—0.765
ANC (/mm^3)	0.709	0.066	<0.01	0.579—0.839
CRP (mg/dL)	0.643	0.071	0.06	0.504—0.782
Vesikari score	0.795	0.057	<0.01	0.683—0.907
Maximum BT (°C)	0.787	0.061	<0.01	0.667—0.907
Serum IL-6 (pg/mL)	0.682	0.071	0.02	0.542—0.822
Serum IL-8 (pg/mL)	0.738	0.065	<0.01	0.610—0.866

ANC = absolute neutrophil count; AUC = area under the curve; BT = body temperature; CI = confidence interval; CRP = C-reactive protein; SE = standard error; Sig. = significant; WBC = white blood cell count.

* Under the nonparametric assumption.

† Null hypothesis: true area = 0.5.

effects such as fever and CRP synthesis.[15] However, early studies focused solely on rotavirus wherein serum IL-6 is a distinguishing marker between bacterial and viral gastroenteritis in children.[9,17] In this study, both serum IL-6 and CRP were able to discriminate significantly between bacterial and viral gastroenteritis, and a significant positive correlation between CRP and serum IL-6 was noted ($r_s = 0.51$, $p < 0.01$). A CRP level ≥ 2.0 mg/dL was able to predict bacterial infection with a sensitivity of 91.3% and specificity of 81.8%, and it appears to be a potential and effective marker in differentiating bacterial from viral gastroenteritis because it is the most commonly used of the acute-phase proteins in children with infections. The level of serum IL-6 was higher in the rotavirus group, which may partly explain the difference in clinical severity between the two viral pathogens.

In this study, IL-6 was detected in the fecal specimens of children with acute gastroenteritis providing evidence of the intestinal mucosal release of IL-6 in both bacterial and viral infections. Fecal IL-6 may be a more direct reflection of intestinal inflammation during infective gastroenteritis than serum IL-6, because the levels of fecal IL-6 positively correlated with the frequency of diarrhea. In addition, the fecal IL-6 concentration was less than that of serum IL-6. Several factors including timing of sample collection, degradation of cytokines and dilution by watery stools may be the reason for the low concentrations of fecal cytokines.[23]

Sheth et al suggested that IL-8 is an important mediator of host response to rotavirus gastroenteritis.[24] In the current study, serum IL-8 levels were significantly higher in the patients with bacterial and viral infections compared to the control group; however, there was no statistically significant difference between the bacterial and viral groups in *post hoc* tests. Similar findings during acute phase responses have also been reported.[9,10] The current study suggests that IL-8 may also play a role in the response to norovirus infection. Our recent study revealed that serum IL-6 has the ability to differentiate between rotavirus and norovirus infections (AUC = 0.66, $p = 0.04$),[25] and similar results were obtained in the current study (AUC = 0.68, $p = 0.02$). However, the AUC value of IL-6 was less than the threshold of acceptable discrimination. These results suggest that in distinguishing rotavirus from norovirus infections, serum IL-8 is more sensitive than IL-6. Further correlation analysis of the relationship between IL-8 and severity score in the patients with rotavirus and norovirus gastroenteritis revealed no significant correlation ($p = 0.25$). One explanation may be that host cytokine responses after viral infections are complex and coordinated by multiple cytokines. Moreover, fecal IL-8 was detected in only three of 20 cases with viral infections and 10 of 13 cases with bacterial infections in the current study, possibly because the cytokine concentrations were below the minimum detectable limit of the ELISA kit. Further detailed studies with a prospective, larger sample size are warranted to validate the findings here and to investigate intestinal cytokine responses in viral gastroenteritis.

A recent study showed that the introduction of the rotavirus vaccine dramatically decreased related hospitalizations among US children,[26] and currently norovirus is considered to be the most important causative agent of nonbacterial gastroenteritis in the United States.[14] Previous

studies have shown that afebrile seizures commonly occur in hospitalized children with norovirus gastroenteritis.[27,28] Thus, the early detection of norovirus outbreaks is particularly important in hospitalized patients. Our previous study described the role of maximum BT and ANC as clinical predictors for discriminating rotavirus and norovirus infections by binary logistic regression analysis.[25] In the current study, we applied ROC curve analysis and AUC values of maximum BT and ANC, both of which revealed significant discrimination abilities. Our results have important clinical implications in that they can guide clinicians to detect a norovirus infection if a patient has an ANC of less than 9000/mm,[3] maximum BT of less than 38.2°C, and Vesikari score of less than 14 at the end of the course. Although this cannot be used to detect a norovirus infection, in clinical practice, it may be able to help clinicians reduce the uncertainty of the clinical diagnosis when no bacterial agent is isolated in stool specimens and detection of norovirus by fecal antigens or nucleic acid is unavailable.

The strengths of the current study include the analysis with AUCs of ROC curves of various markers in comparing bacterial infections with viral infections, and in comparing rotavirus and norovirus. Moreover, we conducted correlation analysis to examine the association between serum cytokines and clinical features or disease severity. There are some limitations to this study. First, serum cytokine levels may be affected by measurement variability such as the effects of long-term storage and timing of blood sample collection. A previous study indicated that there were no statistically significant differences in serum IL-6 and IL-8 levels between groups in which blood was drawn early (0—36 h) and late (36—72 h).[10] In this study, all serum samples were collected within 72 hours of diarrhea onset, and all fecal samples were obtained no more than 24 hours after drawing blood. Therefore, confounding by the timing of sample collection may have had a limited effect on our statistical results. Second, children with mild or subclinical illnesses were not included in the current study, and most had severe illness (Vesikari score $\geq$ 11) that required intravenous fluid therapy. The current findings may therefore not be immediately generalized or extrapolated to other pediatric populations with acute gastroenteritis. Third, the lower sensitivity yielded from the EIA kits may be due to the inability to detect samples with low viral loads. Therefore, the patients detected as being positive for rotavirus or norovirus may represent those with higher viral loads. The possibility of overcall of false-positive cases with regards to the cytokine analysis is the major concern of our study. A high specificity tool (low false-positive rate) is preferable to a high sensitivity tool, and the new-generation EIA kit that we used showed significant improvements in both sensitivity and specificity.[29,30] We therefore consider that using an EIA test is feasible, and that our results are reliable.

In conclusion, serum IL-6 levels showed a significant correlation with clinical severity score. Serum IL-8 levels showed a clinical correlation with fever duration and were more sensitive than IL-6 in distinguishing rotavirus from norovirus infections. Lower ANC, maximum BT, and score on the Vesikari scale are potential predictors of norovirus infection in children compared with rotavirus gastroenteritis.

Conflicts of Interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

This research was supported by a research grant from Chung Shan Medical University Hospital, Taiwan (CSH 2009-A-011).

References

1. Gastañaduy AS, Begue RE. Acute gastroenteritis. *Clin Pediatr (Phila)* 1999;**38**:1—12.
2. Elliott EJ. Acute gastroenteritis in children. *BMJ* 2007;**334**:35—40.
3. Ramani S, Kang G. Viruses causing childhood diarrhoea in the developing world. *Curr Opin Infect Dis* 2009;**22**:477—82.
4. Dennehy PH. Viral gastroenteritis in children. *Pediatr Infect Dis J* 2011;**30**:63—4.
5. Simon J, Kotloff K. New and candidate vaccines for gastrointestinal infections. *Curr Opin Gastroenterol* 2010;**26**:12—6.
6. Bhutta ZA. Acute gastroenteritis in children. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF, editors. *Nelson textbook of pediatrics*. 18th ed. Philadelphia: Saunders; 2007. p. 1605—18.
7. Grimwood K, Forbes DA. Acute and persistent diarrhea. *Pediatr Clin North Am* 2009;**56**:1343—61.
8. Bernstein DI. Rotavirus overview. *Pediatr Infect Dis J* 2009;**28**:S50—3.
9. Lin CH, Hsieh CC, Chen SJ, Wu TC, Chung RL, Tang RB. The diagnostic value of serum interleukins 6 and 8 in children with acute gastroenteritis. *J Pediatr Gastroenterol Nutr* 2006;**43**:25—9.
10. Vaisman N, Leibovitz E, Dagan R, Barak V. The involvement of IL-6 and IL-8 in acute invasive gastroenteritis of children. *Cytokine* 2003;**22**:194—7.
11. Rankin JA. Biological mediators of acute inflammation. *AACN Clin Issues* 2004;**15**:3—17.
12. Eckmann L, Kagnoff MF. Cytokines in host defense against Salmonella. *Microbes Infect* 2001;**3**:1191—200.
13. Song M, Kellum JA. Interleukin-6. *Crit Care Med* 2005;**33**:S463—5.
14. Feghali CA, Wright TM. Cytokines in acute and chronic inflammation. *Front Biosci* 1997;**2**:d12—26.
15. Svanborg C, Godaly G, Hedlund M. Cytokine responses during mucosal infections: role in disease pathogenesis and host defence. *Curr Opin Microbiol* 1999;**2**:99—105.
16. Jiang B, Snipes-Magaldi L, Dennehy P, Keyserling H, Holman RC, Bresee J, et al. Cytokines as mediators for or effectors against rotavirus disease in children. *Clin Diagn Lab Immunol* 2003;**10**:995—1001.
17. Mangiarotti P, Moulin F, Palmer P, Ravilly S, Raymond J, Gendrel D. Interferon-alpha in viral and bacterial gastroenteritis: a comparison with C-reactive protein and interleukin-6. *Acta Paediatr* 1999;**88**:592—4.
18. Chen SM, Ni YH, Chen HL, Chang MH. Microbial etiology of acute gastroenteritis in hospitalized children in Taiwan. *J Formos Med Assoc* 2006;**105**:964—70.
19. Ruuska T, Vesikari T. Rotavirus disease in Finnish children: use of numerical scores for clinical severity of diarrhoeal episodes. *Scand J Infect Dis* 1990;**22**:259—67.
20. Givon-Lavi N, Greenberg D, Dagan R. Comparison between two severity scoring scales commonly used in the evaluation of rotavirus gastroenteritis in children. *Vaccine* 2008;**26**:5798—801.

21. Raqib R, Ljungdahl A, Lindberg AA, Andersson U, Andersson J. Local entrapment of interferon gamma in the recovery from *Shigella dysenteriae* type 1 infection. *Gut* 1996;**38**:328—36.
22. Saiki T, Mitsuyama K, Toyonaga A, Ishida H, Tanikawa K. Detection of pro- and anti-inflammatory cytokines in stools of patients with inflammatory bowel disease. *Scand J Gastroenterol* 1998;**33**:616—22.
23. Ko G, Jiang ZD, Okhuysen PC, DuPont HL. Fecal cytokines and markers of intestinal inflammation in international travelers with diarrhea due to noroviruses. *J Med Virol* 2006; **78**:825—8.
24. Sheth R, Anderson J, Sato T, Oh B, Hempson SJ, Rollo E, et al. Rotavirus stimulates IL-8 secretion from cultured epithelial cells. *Virology* 1996;**221**:251—9.
25. Chen SM, Ku MS, Lee MY, Tsai JD, Sheu JN. Diagnostic performance of serum interleukin-6 and interleukin-10 levels and clinical predictors in children with rotavirus and norovirus gastroenteritis. *Cytokine* 2012;**59**:299—304.
26. Curns AT, Steiner CA, Barrett M, Hunter K, Wilson E, Parashar UD. Reduction in acute gastroenteritis hospitalizations among US children after introduction of rotavirus vaccine: analysis of hospital discharge data from 18 US states. *J Infect Dis* 2010;**201**: 1617—24.
27. Chen SY, Tsai CN, Lai MW, Chen CY, Lin KL, Lin TY, et al. Norovirus infection as a cause of diarrhea-associated benign infantile seizures. *Clin Infect Dis* 2009;**48**:849—55.
28. Chan CM, Chan CW, Ma CK, Chan HB. Norovirus as cause of benign convulsion associated with gastro-enteritis. *J Paediatr Child Health* 2011;**47**:373—7.
29. Gray JJ, Kohli E, Ruggeri FM, Vennema H, Sánchez-Fauquier A, Schreier E, et al. European multicenter evaluation of commercial enzyme immunoassays for detecting norovirus antigen in fecal samples. *Clin Vaccine Immunol* 2007; **14**:1349—55.
30. Siqueira JA, Linhares Ada C, Oliveira Dde S, Soares Lda S, Lucena MS, Wanzeller AL, et al. Evaluation of third-generation RIDASCREEN enzyme immunoassay for the detection of norovirus antigens in stool samples of hospitalized children in Belém, Pará. *Diagn Microbiol Infect Dis* 2011;**71**: 391—5.