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Diagnostic performance of serum interleukin-6 and interleukin-10 levels and clinical predictors in children with rotavirus and norovirus gastroenteritis

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ABSTRACT

Objectives: Rotavirus and norovirus are the two most common causes of acute viral gastroenteritis in children. This study aimed to explore the association of serum interleukin-6 (IL-6) and interleukin-10 (IL-10) levels and the clinical features in children with rotavirus and norovirus gastroenteritis.
Methods: This prospective study enrolled 168 acute gastroenteritis patients admitted to a tertiary care center. Peripheral blood samples were collected for IL-6 and IL-10 assays within the first 72 h of illness. The diagnostic performance of clinical tests was estimated using the receiver operating characteristic (ROC) analysis. Binary logistic regression modeling was performed to examine the predictive variables.
Results: Serum IL-6 and IL-10 were measured in children with rotavirus infection ($n = 30$), norovirus infection ($n = 25$), *Salmonella* infection ($n = 26$), and in 11 healthy controls. There were significant higher degrees of severity of illness and levels of IL-10 in the rotavirus group as compared to the norovirus group. The binary logistic regression analysis revealed that both the ANC and maximum body temperature (BT) were significant clinical predictors for discriminating rotavirus and norovirus gastroenteritis. The ROC curve to evaluate the accuracy of logistic regression model had an AUC of 0.847 (95% CI: 0.741–0.952, $p < 0.001$).
Conclusions: IL-10 shows a significant discriminating ability between rotavirus and norovirus infection. A model incorporating maximum BT and ANC can help pediatricians to distinguish between rotavirus and norovirus in children with a suspected viral gastroenteritis.

1. Introduction

Gastroenteritis signifies an infection of the gastrointestinal tract caused by various etiologies but most commonly by bacteria, viruses, and parasites. In infants and children worldwide, viral pathogens are the most common cause [1,2]. Viral gastroenteritis is highly contagious and is the cause of millions of cases of diarrhea annually. Rotavirus, norovirus, enteric adenovirus, and astrovirus are the known most frequent causes of viral gastroenteritis [1,3–6]. Rotavirus is the leading cause of clinically severe viral gastroenteritis in young children [1,2,7], accounting for about 40% of all diarrhea-related hospital admissions of children younger than five years of age worldwide [8].

Noroviruses are a common cause of diarrhea requiring hospitalization and clinic consults in adults. It is also the second most common cause of diarrhea-associated hospitalization (following rotaviruses) in children aged <5 years [9]. Noroviruses are generally considered to cause relatively mild illness of short duration [1,9,10]. Recent studies have shown norovirus can also cause serious and sometimes fatal infections, especially in young children [2,10–13]. Moreover, outbreaks can occur over a wide variety of settings like hospital wards and day-care centers [9,10]. A previous study also shows that children with norovirus infection have significantly higher incidences of convulsions compared to rotavirus infection (29.7% vs. 5%) [14]. Thus, norovirus infection recognition and early control is particularly important in hospitalized patients. However, it is difficult to distinguish between the two most common viral infections by clinical features alone because of their similar and non-specific presenting symptoms [15].

Cytokines play important roles in the immune pathogenesis of viral infection [16,17]. Interleukin-6 (IL-6) is a pleiotropic cytokine produced by immune and non-immune cells. It plays a role in a broad range of functions, including immune responses, acute-phase reactions, and hematopoiesis [18]. Interleukin-10 (IL-10) is a cytokine mainly produced by monocytes, macrophages, and

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different T-cell subsets and it influences the functions of monocytes and macrophages, including antigen presentation, immuno-mediator release, and phagocytosis [19]. Previous studies suggest that IL-6 and IL-10 are involved in the pathogenesis of rotavirus gastroenteritis [20–23] but other viral infection remains unclear.

The aims of this study were to examine the significance of IL-6 and IL-10 levels during the acute phase of norovirus infection and explore the association of serum cytokine levels and clinical features in children with rotavirus and norovirus gastroenteritis.

2. Patients and methods

2.1. Study design and setting

From March 2008 to January 2009, children aged 4 months to 14 years with acute gastroenteritis who were admitted to a tertiary care center and met the inclusion criteria were prospectively surveyed. The inclusion criteria included diagnosis of acute gastroenteritis, characterized as passage of loose or watery stools occurring three or more times or vomiting occurring twice or more within 24 h while excluding non-infectious causes. Children with diarrhea of more than seven days' duration were excluded.

The clinical features of gastroenteritis were recorded, including body temperature (BT) and the maximum number of vomiting and diarrhea episodes during a 24-h period. Severity of illness was assessed by 20-point scale Vesikari scoring system [24,25], which was based on the severity of diarrhea, vomiting, body temperature, dehydration, and treatment. A score $\geqslant 11$ was considered a severe episode. Fever was defined as oral or ear temperature >38 °C (100.4 °F). Standard treatment was rehydration therapy followed by early re-introduction of age-appropriate feeding. Anti-emetic or anti-diarrheal drugs were not routinely given.

The hospital's institutional review board approved the study and all parents or guardians of the participants provided informed consent.

2.2. Laboratory analysis

Stool samples were collected from the patient within 48 h after admission. All stool samples were frozen and stored at −80 °C until viral testing. Stool samples were sent for bacterial culture, which included *Salmonella* species, *Shigella* species, and *Campylobacter jejuni*, and examined for parasites simultaneously. Detection for rotavirus, adenovirus, norovirus, and astrovirus were performed using commercial enzyme immuno-assay (EIA) kits (Ridascreen®; R-Biopharm AG, Darmstadt, Germany) according to each manufacturer's instructions. According to the product's instruction leaflet, the sensitivity and specificity for detection of viral pathogens were as follows: 95.6% and 99.1%, for rotavirus; 78.6–93.3% and 100%, for norovirus; 100% and 100%, for adenovirus; 76.2% and 100%, for astrovirus, respectively, compared with reverse transcription-polymerase chain reaction (RT-PCR). Blood samples for routine laboratory tests, including white blood cell (WBC) count and differential count, and C-reactive protein (CRP) level were also examined in all study subjects. The WBC count in the peripheral blood was determined by an automated hematology analyzer XE-5000 (Sysmex Corporation, Kobe, Japan). The absolute neutrophil count (ANC) was calculated as follows: ANC (/mm3) = WBC count × (% bands + % neutrophils) × 0.01. Serum CRP levels were measured by nephelometric method on a BN ProSpec analyzer (Dade Behring, Marburg, Germany).

2.3. Measurement of serum cytokines

Blood samples were collected in serum separator tubes for IL-6 and IL-10 assays on the day of admission from each patient within the first 72 h of illness onset and from 11 age-matched healthy control subjects. All blood samples were centrifuged for 15 min at 1000*g* and stored at −80 °C until assay for cytokines. The IL-6 and IL-10 levels were assessed with Quantikine Human Interleukin Immuno-assay (R&D Systems, Minneapolis, MN, USA). Briefly, the quantitative sandwich enzyme immunoassay technique used monoclonal antibodies specific for IL-6 and IL-10. Serum concentrations were calculated by using regression analysis with standard curves and expressed as picograms per milliliter (pg/ml). All samples were measured in duplicate, with averages used in the statistical analyses. The minimum detectable concentrations IL-6 and IL-10 were less than 0.7 pg/ml and 3.9 pg/ml, respectively.

2.4. Statistical methods

All data were recorded and analyzed using the statistical software SPSS (version 12.0, SPSS Inc., Chicago, Illinois, USA). Non-parametric Kruskal–Wallis statistics, followed by a Dunn's multiple comparison test, were used to analyze continuous data. Categorical data were compared using Chi-square test when appropriate. The diagnostic performance of the clinical tests was measured by the area under the curve (AUC) of the receiver operating characteristic (ROC) analysis. The optimal cutoff point was determined by the maximum of Youden index. The Youden index was calculated using the following equation: sensitivity + specificity − 1.

The correlation between serum cytokines and laboratory tests was evaluated using Spearman's rank correlation analysis. Logistic regression analysis using the Enter method was applied to estimate the predictor variables. All clinical predictors were analyzed as continuous data. The Hosmer–Lemeshow test was used to determine the model's goodness-of-fit. A $p < 0.05$ was considered statistically significant.

3. Results

Enteric pathogens were detected in stool samples from 123 of the 168 study patients with acute gastroenteritis. Viral pathogens identified were rotavirus ($n = 41$), norovirus ($n = 34$), adenovirus ($n = 6$), and astrovirus ($n = 1$). Other bacterial pathogens, including *Salmonella* spp. ($n = 29$), *Campylobacter* spp. ($n = 2$), and *Aeromonas* spp. ($n = 5$), were isolated in 36 (21.4%) patients. Mixed infections were observed in five (2.9%) cases, including norovirus–adenovirus in two, rotavirus–norovirus in one, adenovirus–*Salmonella* in one, and norovirus–*Aeromonas* in another.

The clinical characteristics and routine laboratory tests among rotavirus, norovirus, and *Salmonella* patients are summarized in Table 1. A Dunn's multiple comparison test showed that there were significant differences in maximum number of diarrhea and vomiting episodes, maximum BT, severity score, and CRP levels between the *Salmonella* and viral groups but the significant differences between rotavirus and norovirus groups were observed only in maximum BT and severity score. More than half of the patients with *Salmonella* infection revealed positive in stool occult blood and stool pus cell test. We conducted the serum cytokine assays from 30 children with rotavirus infection, 25 children with norovirus infection, 26 children with *Salmonella* infection and 11 healthy children. These results showed higher serum IL-6 and IL-10 levels in rotavirus, norovirus, *Salmonella* groups compared to the control group (Fig. 1). A Dunn's multiple comparison test showed that there were significant high serum levels of IL-6 between the

Table 1
Demographic data and clinical characteristics of children with rotavirus, norovirus and *Salmonella* gastroenteritis.

Characteristic data	Rotavirus (n = 41)	Norovirus (n = 34)	Salmonella (n = 29)	p Value
Gender (boy/girl)	18/23	16/18	13/16	0.207[b]
Age (years)[a]	2.9 (1.7–4.7)	1.9 (1.2–4.1)	2.0 (1.2–2.8)	0.055[c]
Diarrhea (maximum/day)[a]	6 (4–9.5)	4 (3–6.25)	8 (6–10)	<0.001[d]
Vomiting (maximum/day)[a]	4 (2–7)	2.5 (1–5.25)	1 (1–2)	<0.001[d]
Fever (>38 °C)	36 (87.8%)	13 (38.2%)	29 (100%)	<0.001[b]
Maximum BT (°C)[a]	38.8 (38.4–39.0)	37.9 (37.2–38.7)	39.1 (38.9–39.6)	<0.001[e]
Vesikari severity score[a]	14 (13–16)	12.5 (11–14)	15 (15–17)	<0.001[e]
⩾11	40 (97.5%)	31 (91.1%)	29 (100%)	0.161[b]
Stool occult blood ⩾2++	16 (39.0%)	8 (23.5%)	18 (62.1%)	0.007[b]
Stool pus cell ⩾5/HPF	4 (9.7%)	0 (0)	15 (51.7%)	<0.001[b]
Blood WBC (/mm3)[a]	9670 (7395–15 840)	10 620 (6418–13 080)	9150 (6770 –11 505)	0.250[c]
ANC (/mm3)[a]	8075 (4225–13 407)	5595 (3555–9558)	4414 (3118–5867)	0.004[f]
CRP (mg/dl)[a]	0.62 (0.30–1.59)	0.31 (0.30–0.71)	6.42 (3.06–12.25)	<0.001[d]

Abbreviations: BT, body temperature; HPF, high-power field; WBC, white blood cell count; ANC, absolute neutrophil count; CRP, C-reactive protein.
[a] Data are presented as median (inter-quartile range).
[b] Chi-square test.
[c] Kruskal–Wallis test for differences among rotavirus, norovirus, and *Salmonella*.
[d] Kruskal–Wallis test, $p < 0.05$ by Dunn's multiple comparison test within groups, rotavirus vs. *Salmonella* and norovirus vs. *Salmonella*.
[e] Kruskal–Wallis test, $p < 0.05$ by Dunn's multiple comparison test within groups, rotavirus vs. *Salmonella*, norovirus vs. *Salmonella*, and rotavirus vs. norovirus.
[f] Kruskal–Wallis test, $p < 0.05$ by Dunn's multiple comparison test within groups, rotavirus vs. *Salmonella*.

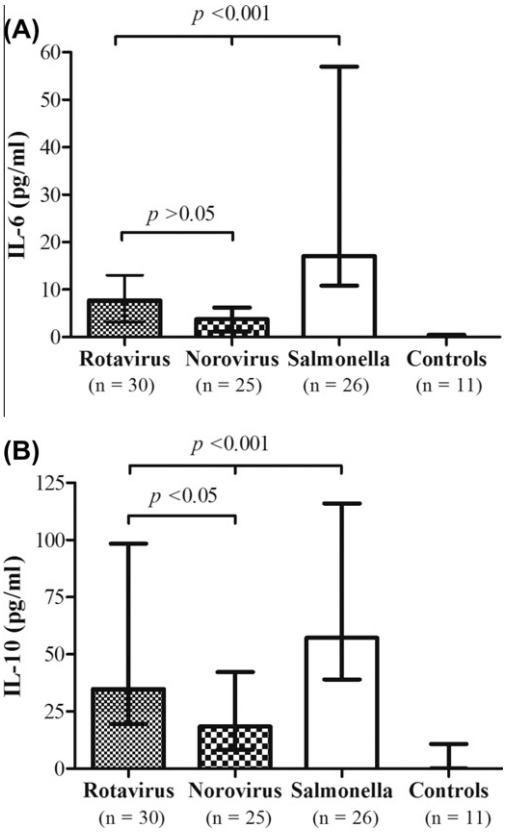


Fig. 1. Serum (A) IL-6 and (B) IL-10 in children with rotavirus, norovirus, *Salmonella* gastroenteritis and in healthy controls. Data are shown as median with inter-quartile range in each group; $p < 0.001$, by Kruskal–Wallis test; Dunn's multiple comparison test within groups, rotavirus vs. norovirus.

Salmonella and viral groups but no significant difference was found between rotavirus and norovirus groups. In contrast, there was no significant difference in serum levels of IL-10 between the *Salmonella* and rotavirus groups; however, the difference between rotavirus and norovirus groups was significant.

The ROC curve analysis was performed to assess the diagnostic performance of routine laboratory tests and serum IL-6 and IL-10 levels in differentiating rotavirus gastroenteritis from norovirus gastroenteritis (Table 2). AUCs in ANC and in serum IL-6 and IL-10 levels revealed statistical significance. Further correlation studies were performed between laboratory tests and serum cytokines between the rotavirus and norovirus groups. There were statistically significant positive correlations between ANC and serum cytokines in both groups (Figs. 2 and 3). There were no significant correlations between serum cytokines and clinical symptoms, including episodes of vomiting or diarrhea per day, and maximum BT.

Table 2
Diagnostic performance of laboratory tests for differentiating between rotavirus and norovirus infections.

Test variables	AUC	SE[a]	Sig[b].	95% CI for AUC
CRP	0.623	0.076	0.120	0.475–0.771
WBC	0.604	0.077	0.187	0.454–0.754
ANC	0.691	0.071	0.016	0.552–0.830
IL-6	0.663	0.075	0.039	0.515–0.811
IL-10	0.710	0.069	0.008	0.574–0.846

Abbreviations: AUC, area under the curve; SE, standard error; Sig, significant; CI, confidence interval; CRP, C-reactive protein; WBC, white blood cell count; ANC, absolute neutrophil count.
[a] Under the non-parametric assumption.
[b] Null hypothesis: true area = 0.5.

The binary logistic regression analysis for possible clinical predictors revealed that maximum BT and ANC were significant for discriminating rotavirus from norovirus gastroenteritis (Table 3). The Hosmer–Lemeshow goodness-of-fit test yielded a value of 4.92 ($p = 0.669$), and the overall correct percentage was 81.8%. Using these significant predictors, the logistic regression model for differentiating rotavirus from norovirus was defined as: $\text{logit}(p) = -58.894 + 1.482 \times \text{maximum BT} + 0.00018 \times \text{ANC}$. The ROC curve was used to assess the predictive accuracy of the logistic regression model and yielded an AUC of 0.847 (95% CI: 0.741–0.952, $p < 0.001$) (Fig. 4). The maximum Youden index ($J = -0.656$) was obtained for the optimal cut-off point with a sensitivity of 83.3% and specificity of 76.0%.

4. Discussion

Some studies have shown that severe gastroenteritis similar to rotavirus infection can occur in hospitalized patients with norovirus infection [12,13,26]. From a clinical perspective, the manifestations of these two viruses are quite similar and difficult to

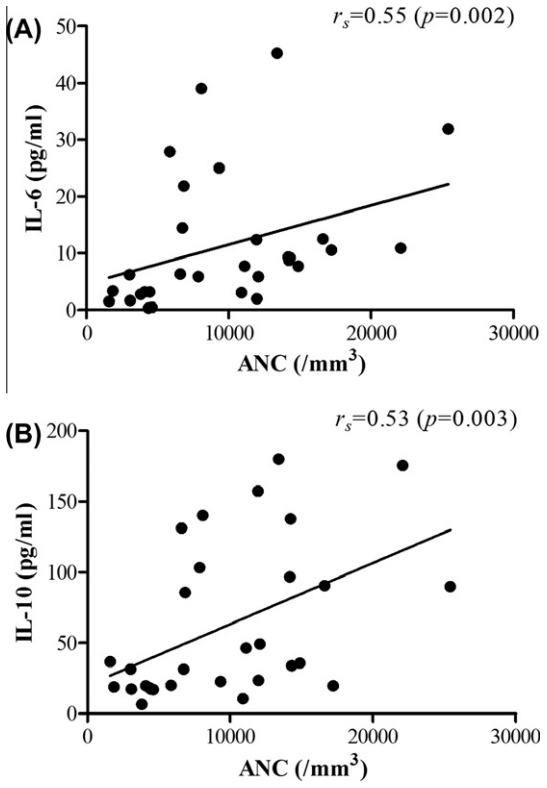


Fig. 2. Associations of ANC with serum (A) IL-6 and (B) IL-10 levels in children with rotavirus gastroenteritis. Spearman's rank correlation coefficient (r_s) is shown.

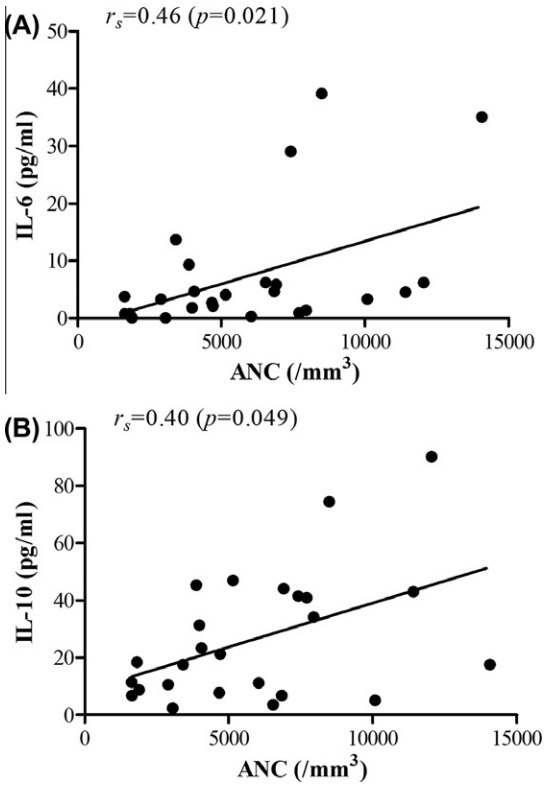


Fig. 3. Associations of ANC with serum (A) IL-6 and (B) IL-10 levels in children with norovirus gastroenteritis. Spearman's rank correlation coefficient (r_s) is shown.

distinguish. O'Ryan et al. reported that rotavirus gastroenteritis is more often associated with fever and a maximum number of diarrhea episodes within a 24-h period [11]. Sakai et al. described a significant difference in maximum BT [12]. In the current study, rotavirus gastroenteritis has a significantly higher maximum BT and high percentage of patients with fever but not the number of diarrhea episodes in a day. This may be due to the difference in study populations. Both outpatients and hospitalized patients with acute gastroenteritis were enrolled in the study by O'Ryan et al. Our study only enrolled hospitalized patients. Thus, the majority of patients included in our study had a higher score in severity than O'Ryan's report. There is a significant difference in the severity score between rotavirus and norovirus infections. However, the Vesikari score has been developed as an outcome measure in clinical study and not for use as a diagnostic tool. There are no significant differences between these two viruses from results of routine laboratory testing, including stool occult blood, stool pus cell, WBC count, and CRP levels. Nevertheless, there is a higher ANC in patients with rotavirus infection compared to norovirus.

IL-6 is considered a useful marker of pro-inflammatory cytokine activation while IL-10 is widely known as an immuno-suppressive and anti-inflammatory cytokine [18,19]. It has been shown in several studies that these two cytokines may play a role in the pathogenesis of rotavirus gastroenteritis [20–23]. In this study, we agree with the study of Lin et al., in which they assessed the effectiveness of IL-6 and CRP for differentiation of viral and bacterial infections [23]. However, the serum IL-6 level is higher in the rotavirus group compared to the norovirus group but the result does not reach statistical significance in the post hoc test (Dunn's multiple comparisons test). Our results suggest that both rotavirus and norovirus infection may trigger a weak mucosal and systemic synthesis of proinflammatory cytokines compared to bacterial pathogen. For Th2 responses, the serum IL-10 level is obviously high in *Salmonella* group than that of rotavirus group, but there is no statistical significance among the difference. Therefore, in further larger studies, the monitoring of IL-10 level in the phase of recovery is needed to assess the exact change. Furthermore, significantly higher levels of serum IL-10 were detected in both rotavirus and norovirus group. Significantly higher IL-10 levels in the rotavirus group than in the norovirus group may reflect higher anti-inflammatory responses during acute rotavirus infection. Moreover, the ROC curve analysis is used to assess the diagnostic performance of WBC count, ANC, and CRP, IL-6 and IL-10 levels in differentiating between rotavirus and norovirus infections. IL-10 has an accuracy superior to that of IL-6 (AUC, 0.71 vs. 0.66). Our findings provide biological evidence to support diverse severities in children with rotavirus and norovirus infections. Serum IL-10 shows a significant discriminating ability between the two most common pathogens of viral infections in acute phase of gastroenteritis.

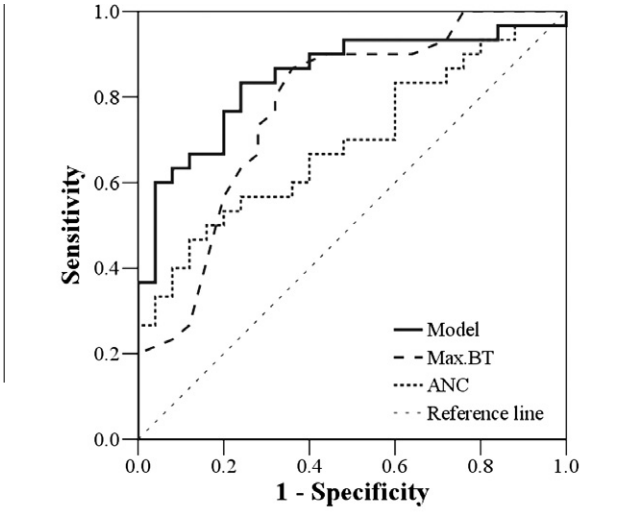
Correlation analyses were conducted in order to evaluate the association of the cytokine levels and routine laboratory tests. There were moderate positive correlations between serum IL-10 levels and ANC in both rotavirus and norovirus group. Previous studies reported that IL-6 supported the differentiation of hematopoietic progenitor cells and had important roles in regulating hematopoiesis [27,28]. The current results may partly be explained by lower IL-6 in the norovirus group but the reason is not entirely clear. Furthermore, the results here show no significant association between either severity score or clinical symptoms and serum cytokine levels in both viral infections. One likely explanation is that multiple cytokines and the complex cytokines responses are involved in the pathogenicity of gastroenteritis after virus infection. Another may be the limited number of study subjects enrolled in the study.

Because serum cytokine is not routinely determined by laboratory examination, this study attempted to explore the feasibility and application of predictive logistic model for gastroenteritis caused by the two most common viruses. A binary logistic regres-

Table 3
Logistic regression analysis of clinical predictors in differentiating rotavirus from norovirus infections.

Variable	B	Sig.	OR	95% CI for OR	
				Lower	Upper
Age	−0.153	0.321	0.858	0.634	1.161
Diarrhea (maximum/day)	−0.074	0.469	0.929	0.761	1.134
Vomiting(maximum/day)	0.219	0.143	1.245	0.929	1.667
Maximum BT	1.482	0.002	4.402	1.755	11.041
CRP	0.697	0.057	2.008	0.980	4.116
ANC	0.00018	0.033	1.00018	1.00001	1.00035
Constant	−58.894	0.001			

Nagelkerke *R*-square = 0.564.
Cox and Snell *R*-square = 0.422.
Abbreviations: B, coefficient of rogation; Sig, significant; OR, odds ratio; CI, confidence interval; BT, body temperature; CRP, C-reactive protein; ANC, absolute neutrophil count.



Test variable	AUC (95% CI)	Sig.	Cut-off value	SEN / SPEC
Model	0.847(0.741–0.952)	<0.001	-0.656	83.3% / 76.0%
Maximum BT	0.777(0.651–0.903)	<0.001	38.2°C	86.7% / 64.0%
ANC	0.691(0.552–0.830)	0.016	10488	46.7% / 88.0%

Abbreviations: AUC, area under the curve; Sig., significant; CI, confidence interval; SEN, Sensitivity; SPEC, Specificity; BT, body temperature; ANC, absolute neutrophil count.

Fig. 4. ROC curve analyses for evaluating the diagnostic performance of ANC, maximum BT and logistic regression model in differentiating rotavirus and norovirus infections.

sion analysis performed includes age, maximum number of vomiting/diarrhea episodes, maximum BT, CRP, and ANC as potential predictors. The results indicate that a higher maximum BT and ANC are significant predictors of children with rotavirus infection. The maximum Youden index is obtained for a cut-off point of 38.2 °C in maximum BT and 10,488 in ANC in this model. The ROC curve analysis assessing the accuracy of the predictive model yields high sensitivity (83.3%) and modest specificity (76.0%) at the optimal cut-off point. Chen et al. have reported a high rate of convulsions and longer hospital stays in hospitalized patients with norovirus infection compared to rotavirus infection [14]. Previous studies have noted that the seasonal peak of norovirus infections occurred during the cooler months of the year in Taiwan and was similar to that of rotavirus gastroenteritis [14,29]. To alleviate this health and economic burden, the early identification of norovirus outbreaks is particularly important in hospitalized patients. However, it is not easy to distinguish between the two most common viral infections based on clinical presentations alone in hospitalized patients because their high disease severity. Our findings suggest maximum BT and ANC can help pediatricians to distinguish between rotavirus and norovirus in children with a suspected viral gastroenteritis before laboratory confirmation by viral detection assays.

The current study has some limitations that are worth noting. First, the majority of enrolled patients have severe infection and mild or sub-clinical subjects have been excluded. There may be a selection population bias in this study. Second, additional sources of measurement variability, including the timing of blood collection and effects of long-term storage, may affect serum cytokine levels. Thirdly, the small number of cases with enteric adenovirus and astrovirus does not allow for a better understanding of any simultaneous inter-virus relationship. However, we consider that this may have only a limited effect on our results because both enteric adenovirus and astrovirus infections are much less common in Taiwan according to our previous report and current study [29]. Lastly, the roles of only two serum cytokines as markers of immune response have been investigated. Further studies are warranted to confirm the role of other cytokines or cellular responses following these two viral infections.

In conclusion, IL-10 exhibits a significant high level in the acute phase of rotavirus and norovirus gastroenteritis in children and also shows a significant discriminating ability between the two most common pathogens. A higher maximum BT and ANC are significant predictors of children with rotavirus infection. Furthermore, a model incorporating maximum BT and ANC can help pediatricians to distinguish between rotavirus and norovirus in children with a suspected viral gastroenteritis.

Conflict of interest disclosure

The authors declare that they have no conflict of interest.

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