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SERUM AND FAECAL INFLAMMATORY BIOMARKERS FOR DISTINGUISHING BACTERIAL AND VIRAL INFECTION IN YOUNG CHILDREN: A DIAGNOSTIC ACCURACY STUDY

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Abstract: Background: It is hard to distinguish between bacterial and viral infections in young children with acute gastroenteritis (AGE). This uncertainty stimulates the frequent use of antibiotics empirically. An objective and rapid marker would be helpful for antimicrobial stewardship. The serum, haematological and faecal biomarkers were compared in this study. **Methods:** a case-control study in Baghdad, Iraq, enrolled 120 children. 50 individuals had bacterial AGE, 50 individuals had viral AGE and 20 individuals were apparently healthy controls. A complete blood count was performed and neutrophil to lymphocyte ratio (NLR) was calculated. We determined serum C-reactive protein (CRP) and procalcitonin (PCT). Three faecal markers (calprotectin, S100A12 and M2-Pyruvate kinase [M2-PK]) were also measured. The diagnostic performance was evaluated using receiver operating characteristic (ROC) analysis. **Results:** All markers showed higher results than in the viral disease. Procalcitonin was the best single discriminator (AUC 0.969). It was able to achieve 88.0% sensitivity and 96.0% specificity at a cut-off of 0.64 ng/mL. This was followed by c-reactive protein (AUC 0.941). The absolute neutrophil count performed almost as well (AUC 0.924). S100A12 was the best, with an AUC of 0.901, followed by calprotectin (AUC 0.873) as faecal markers. The NLR achieved good discrimination at no additional cost (AUC 0.879). M2-PK and the absolute lymphocyte count were the weakest markers. There was no association of biomarkers with child age or sex. **Conclusions:** Procalcitonin proved to be the best individual marker to distinguish bacterial from viral AGE. Simple neutrophil indices were as useful, at low cost. The addition of faecal neutrophils provided a direct measure of gut inflammation. A multi-tiered, combined panel can help minimize unnecessary antibiotic prescribing for paediatric gastroenteritis.

Keywords: Acute gastroenteritis; procalcitonin; calprotectin; S100A12; neutrophil-to-lymphocyte ratio; M2-Pyruvate kinase; antimicrobial stewardship.

Introduction: The one of the most common and important decisions made in caring for young children is whether a child has a bacterial or a viral infection, and it is one of the most challenging to make confidently. It remains a leading cause of illness and death in children under five years of age worldwide.[1] It is against this backdrop of uncertainty, and mindful of the dangers of missing a serious bacterial infection, that physicians often prescribe antibiotics empirically, with costs of this practice being high; antibiotics have no benefit in viral illness,[2] have been shown to have negative effects in children with proven viral illness (longer hospital stays rather than faster recovery),[3] and collectively contribute to the global increase of antimicrobial resistance, which has been identified as one of the greatest threats to child health worldwide.[1] A rapid, objective way to establish a diagnosis of which febrile child is truly suffering from a bacterial infection would have immediate and widespread value for antimicrobial stewardship.[4]

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There are a number of blood markers that have been investigated in this function. The most commonly used acute-phase protein is C-reactive protein. It increases in hours after an inflammatory stimulus. However, CRP is not specific as it is raised during viral illness also. More specific marker of bacterial infection is procalcitonin (PCT).[3] A big meta-analysis demonstrated that PCT was more accurate than CRP in differentiating between bacterial and viral infection.[3] PCT also increases early and decreases rapidly with resolution of an infection. By comparison, CRP increases more slowly and may be falsely low in the earliest samples. Nowadays, many standard laboratories offer both markers.[3]

Further indications can be seen in simple haematological indices. Another blood test that may be ordered is a complete blood count, which frequently shows an increase in the number of white blood cells. It typically causes an absolute neutrophil count to increase.[6] Viral illness is characterized by lymphocytes.[6] The neutrophil to lymphocyte ratio (NLR) is a combined value of both counts. It doesn't cost much and can be accessed from any basic blood count. A high ratio points towards a bacterial process. A low ratio is more characteristic of a viral disease. The NLR has been found to be useful for the diagnosis of bacterial infection in a febrile patient.[5] Serum markers are markers of inflammation throughout the body. May not detect gut-restricted inflammation. The faecal markers provide a more direct view of the intestinal mucosa. Stool is also readily available without an invasive procedure. This is a good advantage for stool testing in a busy paediatric practice. The most well known faecal marker is calprotectin. It is a protein released by activated neutrophils that binds calcium.[7] It is found in stool and is related to the migration of neutrophils into the bowel wall. Faecal calprotectin is elevated in bacterial gastroenteritis, but not in viral gastroenteritis. It is suggested in children that one can help to differentiate the two. [7]

Other faecal proteins may provide complementary signals. Calgranulin C (S100A12) is another protein secreted by neutrophils. It is very intense when there is active inflammation of the intestine.[8] Faecal S100A12 has been shown to be highly accurate in distinguishing inflammatory bowel disease from non-inflammatory conditions.[9] This indicates that it might also monitor the neutrophilic reaction of bacterial enteritis also. It does not seem to be a major contributor to acute childhood gastroenteritis.

A third marker is a different process. M2-pyruvate kinase (M2-PK) is one of the enzymes involved in cellular energy metabolism. Its level increases in faeces as epithelial cells slough off and not because of neutrophils. Faecal M2-PK is said to be a marker of intestinal inflammation.[10] It could therefore also detect information that is not detected by the neutrophil markers.

The markers that are available have been assessed individually. There are few studies that compare serum and faecal markers in the same patients. Even fewer are concerned with the acute gastroenteritis of young children. Therefore, the relative value of these remains unclears. The faecal markers are also unknown to be used in addition to the routine blood tests.

Materials and Methods: The study was a case control study that consisted of 120 children, 50 with bacterial AGE, 50 with viral AGE and 20 apparently healthy controls in Baghdad, Iraq. Venous blood was drawn into a dipotassium EDTA tube for complete blood count and a plain gel-separator tube for serum, and a fresh stool sample was collected into a preservative-free container, from each child. Serum was separated by centrifugation and serum.

The study had been conducted following the ethical principles that are applicable to research with human subjects. This study is approved by the Ethics Committee of Baghdad University – Baghdad Teaching Hospital, Iraq with Approval Reference No: REC/ARU/2025/0187 on 04/03/2025. The parent of every child enrolled and having specimens taken gave written informed consent prior to enrolment and specimen collection.

Acute gastroenteritis (AGE) was defined as acute onset of a total of three or more loose or watery stools in the previous 24 hours, with or without fever, vomiting and/or abdominal symptoms. The bacterial AGE was diagnosed by the presence of a clinically

important bacterial enteropathogen in stool by culture MacConkey and XLD agar. Viral AGE was diagnosed if a known enteric viral pathogen was detected by a rapid immunochromatographic antigen assay (Rotavirus and Adenovirus Combo Card, CerTest Biotec, Spain) following the manufacturer's instructions in. in children who had clinically compatible AGE but not microbiological evidence of bacterial infection.

After written consent from parents, children 6 months to 5 years of age with acute diarrhea < 14 days duration were included who had a fresh stool collected within 24 hours of presentation. Children were excluded if they had diarrhea >14 days, had any symptoms occurring >48 hours after admission to the hospital, were given systemic antibiotics in the previous 72 hours, provided an inadequate/contaminated specimen, and/or provided a specimen that did not meet collection, transport and storage requirements.

The complete blood count with a five-part differential (white blood cell count, absolute neutrophil count, absolute lymphocyte count, red blood cell count and haemoglobin) was determined using automated haematology analyzer (XN-1000, Sysmex Corporation, Japan) and the neutrophil-to-lymphocyte ratio was calculated. Serum C-reactive protein (CRP) was measured using a latex-enhanced immunoturbidimetric (LEIT) assay performed on a clinical-chemistry analyzer (Cobas c-series, Roche Diagnostics, Germany) and serum procalcitonin (PCT) was measured by an electrochemiluminescence immunoassay (Elecsys BRAHMS PCT, Roche Diagnostics, Germany).

The three faecal markers were measured by commercial sandwich ELISAs after weight-based stool extraction, absorbance read by microplate reader at 450 nm: faecal calprotectin (BÜHLMANN fCAL® ELISA, BÜHLMANN Laboratories, Switzerland) using the CALEX® Cap extraction device, faecal S100A12 (Immundiagnostik, Germany), and faecal M2-pyruvate kinase (ScheBo® M2-PK Stool Test, ScheBo® Biotech, Germany). All of the assays were performed as per the manufacturers' instructions using the provided calibrators and controls.

The data were analyzed using SPSS 26. Normally distributed variables were expressed as mean ± SD and compared by the independent-samples t-test, and non-normally distributed variables as median [interquartile range] and compared by the Mann–Whitney U test; the Kruskal–Wallis and chi-square tests were used for three-group and categorical comparisons. Diagnostic performance was evaluated using receiver operating characteristic (ROC) analysis and the optimum cut-off was determined using the Youden index. A p-value cut-off of < 0.05 was set as the significance level.

Results: Characteristics of the Study Population

The three groups were well matched in terms of age and sex, thus indicating that any differences in biomarker concentration are due to differences in infection status and not due to demographic imbalance. The median ages of children with bacterial infection, viral infection and healthy controls were 2.09 years (IQR 1.23–3.59), 2.24 years (IQR 1.3–3.8) and 2 years (IQR 1.61–3.12), respectively; there was no significant difference among the three groups (p = 0.897, Kruskal–Wallis test). There was no significant difference in sex distribution for the bacterial group (58% male, 42% female) or viral group (58% male, 42% female) compared to controls (45% male, 55% female). Direct comparison of the two infection groups showed no difference in age (p = 0.659) or sex (p = 1.000), and the groups are comparable for the diagnostic comparisons which follow.

Table 1. Demographic characteristics of the three study groups. No statistically significant differences were observed for age or sex.

Characteristic	Bacterial (n = 50)	Viral (n = 50)	Healthy control (n = 20)
Age, years (mean ± SD)	2.39 ± 1.38	2.54 ± 1.43	2.39 ± 1.25
Male, n (%)	29 (58%)	29 (58%)	9 (45%)
Female, n (%)	21 (42.0%)	21 (42.0%)	11 (55.0%)

Haematological Profiles of the Two Infection Groups: The haematology was significantly different in the two infection groups and was the type which was considered to be typical of a bacterial compared to a viral disease. The total white blood cell (WBC) count was substantially higher in bacterial infection (14.40 ± 3.98 ×10⁹/L) than in viral infection (10.70 ± 2.60 ×10⁹/L; Independent t-test (Welch), $p < 0.001$), a large effect (Cohen's d = 1.10). The absolute count of neutrophils was the most marked of the haematological differences between bacterial and viral cases, being more than twice as high in the former (9.37 ± 3.41 ×10⁹/L) than in the latter (4.34 ± 1.28 ×10⁹/L; $p < 0.001$; d = 1.95).

In the opposite direction went the lymphocyte compartment. As is usually the case with viral illness, there was a relative lymphocytosis (increased absolute counts; 4.99 ± 1.40 ×10⁹/L vs 4.11 ± 1.66 ×10⁹/L; p = 0.005). The red-cell indices available were not informative for the above-mentioned differential: both red-cell count (bacterial 4.36 ± 0.50 x 10¹²/L and viral 4.43 ± 0.53 x 10¹²/L; p = 0.482) and haemoglobin (11.96 ± 0.80 g/dL and 11.96 ± 0.96 g/dL; p = 0.985) were essentially the same among the two groups and also within the healthy controls, indicating that the discriminating signal is only within the white-cell lineage. Table 2 shows the complete haematological profile for all three groups.

Table 2. Haematological indices by group. Normally distributed variables are shown as mean ± SD (compared by t-test); values are for the bacterial-versus-viral comparison.

Parameter	Bacterial	Viral	Healthy control	p (B vs V)
WBC (×10⁹/L)	14.40 ± 3.98	10.70 ± 2.60	7.94 ± 1.49	< 0.001
RBC (×10¹²/L)	4.36 ± 0.50	4.43 ± 0.53	4.33 ± 0.42	0.482
Hemoglobin (g/dL)	11.96 ± 0.80	11.96 ± 0.96	11.89 ± 0.79	0.985
Neutrophils (×10⁹/L)	9.37 ± 3.41	4.34 ± 1.28	3.53 ± 0.82	< 0.001
Lymphocytes (×10⁹/L)	4.11 ± 1.66	4.99 ± 1.40	4.41 ± 0.74	0.005

Serum Inflammatory Markers: CRP and Procalcitonin

The Neutrophil lymphocyte ratio was more than twice as high in bacterial infection (median 2.17 [1.56–3.76]) as in viral infection (median 0.88 [0.60–1.18]; Mann–Whitney U, $p < 0.001$), a large effect (d = 1.01). The ratio thus combines two standard cell counts into a single low cost index that indicates a balance between the neutrophil response to bacterial infection and the lymphocyte response to viral infection. The levels of both acute-phase serum markers were significantly higher during bacterial than during viral infection. C-reactive protein was almost five-fold higher in the bacterial group (median 41.68 [27.52–57.99] mg/L) than in the viral group (median 8.80 [6.36–14.86] mg/L; Mann–Whitney U, $p < 0.001$; d = 1.74). In bacterial infections, the median procalcitonin level was 0.95 [0.76–1.48] ng/mL versus 0.25 [0.20–0.37] ng/mL in viral infections ($p < 0.001$). The least overlap in the measured values was found for procalcitonin, which is now known to be the foremost marker in the diagnostic analysis (Section 4.7). The markers were also both close to the bottom of their ranges in the healthy controls, indicating that the elevations are associated with infections. The NLR along with the two markers of serum are summarised in table 3.

Table 3. Neutrophil-to-lymphocyte ratio and serum inflammatory markers by group, shown as median [IQR] (compared by the Mann–Whitney U test).

Parameter	Bacterial	Viral	Healthy control	p (B vs V)	d
NLR	2.17 [1.56–3.76]	0.88 [0.60–1.18]	0.72 [0.68–1.02]	< 0.001	1.01
CRP (mg/L)	41.68 [27.52–57.99]	8.80 [6.36–14.86]	1.55 [1.35–2.10]	< 0.001	1.74
PCT (ng/mL)	0.95 [0.76–1.48]	0.25 [0.20–0.37]	0.08 [0.08–0.11]	< 0.001	1.04

Faecal Markers in Intestinal Infection: The three faecal markers of intestinal inflammation, as with the systemic markers, were always elevated in the bacterial infection group compared with the viral infection group, but there was variation in the magnitude of separation between them. Levels of faecal calprotectin were significantly higher in the bacterial cases (median 455.96 [276.07–641.61] µg/g) than the viral cases (median 210.50 [147.55–263.74] µg/g; $p < 0.001$; $d = 1.30$), consistent with more neutrophilic infiltration of gut mucosa in the bacterial cases of enteritis. Faecal S100A12, a related calgranulin released by activated neutrophils, gave the largest faecal separation (median 94.66 [71.48–150.02] mg/kg versus 43.45 [34.13–58.66] mg/kg; $p < 0.001$; $d = 1.24$).

However, the median value of M2-pyruvate kinase, a marker of epithelial turnover, was significantly different between the bacterial and viral infection groups (38.72 [31.06–48.22] U/mL versus 26.33 [19.42–39.94] U/mL, respectively; $p < 0.001$), and had the smallest effect size (0.71) and maximum overlap (41.8%) between the groups. The two calgranulins of neutrophil origin (calprotectin and S100A12) thus provided the most distinctive information in the faecal panel, with M2-PK providing a lower level of information that complemented that of the other two. The distributions of faecal markers are summarised in Table 4:

Table 4. Faecal inflammatory markers by group, shown as median [IQR] (compared by the Mann–Whitney U test).

Parameter	Bacterial	Viral	Healthy control	p (B vs V)	d
Faecal calprotectin (µg/g)	455.96 [276.07–641.61]	210.50 [147.55–263.74]	73.10 [64.01–91.78]	< 0.001	1.30
Faecal S100A12 (mg/kg)	94.66 [71.48–150.02]	43.45 [34.13–58.66]	13.09 [10.61–14.83]	< 0.001	1.24
Faecal M2-PK (U/mL)	38.72 [31.06–48.22]	26.33 [19.42–39.94]	13.07 [11.76–17.37]	< 0.001	0.71

Diagnostic Performance of Individual Biomarkers

All markers were used individually to separate bacterial from viral infection and the performance of each marker was quantified by ROC curve analysis (Figures 1 and 2; Table 5). The best single discriminator was procalcitonin (with an AUC of 0.969, 95% CI 0.937–0.992). At the optimal cut-off of 0.64 ng/mL, it had a sensitivity of 88.0% and a specificity of 96.0%, correctly identifying 92.0% of children, a positive likelihood ratio of 22.00 and a negative likelihood ratio of 0.12. C-reactive protein was the second strongest marker (AUC 0.941, 95% CI 0.890–0.979), reaching 84.0% sensitivity and 94.0% specificity at a cut-off of 22.79 mg/L. The absolute neutrophil count was also similar (AUC 0.924); in other words, a basic count of a cell type, which is readily available in all laboratories, has a comparable diagnostic value to the assays of serum.

Among the faecal markers, S100A12 was the strongest (AUC 0.901, 95% CI 0.836–0.954), offering high sensitivity (92.0%) at a cut-off of 60.33 mg/kg, followed by calprotectin (AUC 0.873) at a cut-off of 303.79 µg/g. The AUC for the NLR was 0.879 at a cut-off of 1.47, which is an excellent performance using an index that was created by combining two routine cell counts that does not incur any extra costs. The absolute lymphocyte count (AUC 0.658), faecal M2-PK (AUC 0.709) and total WBC count (AUC 0.792) were the least accurate discriminators, although the discrimination was significant when compared with the other groups, but they resulted in less significant individual discrimination. All results from the operating characteristics of the models are included in Table 5 and ranked AUCs with confidence intervals in Figure 2.

Table 5. Diagnostic performance of individual biomarkers for distinguishing bacterial from viral infection, ranked by AUC. Cut-offs are Youden-optimal. The best single marker (procalcitonin) is highlighted. PPV/NPV computed at the observed 1:1 prevalence.

Biomarker	AUC	95% CI	Cut-off	Sens (%)	Spec (%)	PPV / NPV (%)	Acc (%)
PCT (ng/mL)	**0.969**	**0.937–0.992**	**0.64**	**88.0**	**96.0**	**95.7 / 88.9**	**92.0**
CRP (mg/L)	0.941	0.890–0.979	22.79	84.0	94.0	93.3 / 85.5	89.0
Neutrophils (×10⁹/L)	0.924	0.855–0.976	6.40	84.0	96.0	95.5 / 85.7	90.0
Faecal S100A12 (mg/kg)	0.901	0.836–0.954	60.33	92.0	76.0	79.3 / 90.5	84.0
NLR	0.879	0.800–0.943	1.47	80.0	88.0	87.0 / 81.5	84.0
Faecal calprotectin (µg/g)	0.873	0.800–0.934	303.79	70.0	92.0	89.7 / 75.4	81.0
WBC (×10⁹/L)	0.792	0.698–0.871	12.62	70.0	78.0	76.1 / 72.2	74.0
Faecal M2-PK (U/mL)	0.709	0.604–0.810	31.04	76.0	62.0	66.7 / 72.1	69.0
Lymphocytes (×10⁹/L)	0.658	0.547–0.762	4.04	50.0	84.0	75.8 / 62.7	67.0

Sens = sensitivity; Spec = specificity; PPV = positive predictive value; NPV = negative predictive value; Acc = overall accuracy. The bacterial group is treated as the positive class. Cut-off units follow each marker

Figure 1. ROC curves of the individual biomarkers. Procalcitonin, CRP and the neutrophil count occupy the upper-left of the plot, indicating the strongest discrimination.

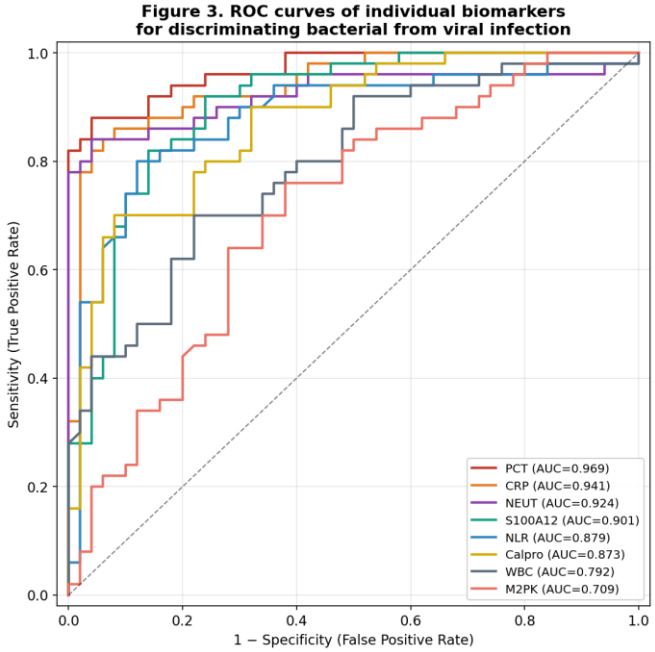
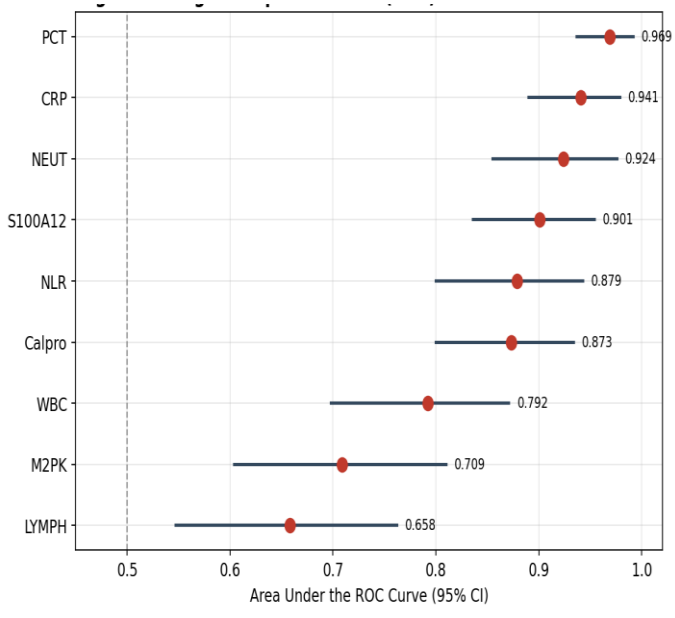


Figure 2. Areas under the ROC curve for each biomarker with 95% confidence intervals. The dashed line at 0.5 marks the value expected of an uninformative test.



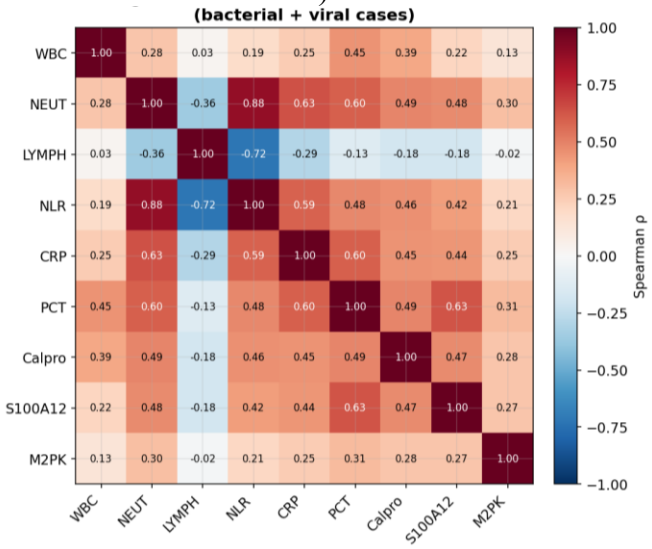
Associations Between Biomarkers and Clinical Characteristics: Markers in each infection group were correlated with age by the Spearman rank coefficient, and compared between the sexes by the Mann–Whitney U test.

Fortunately, the concentrations of biomarkers were not significantly related to age. No other marker was found to have a statistically significant association with age, and any such association was mild and negative, with the total WBC count in the bacterial group being the only such marker, and the association being weak and negative (Spearman ρ = -0.305, p = 0.031), reflecting the mild and negative physiological decline in total WBC count with advancing age in young children. There were no significant associations between any serum or faecal markers and age in either group (all $p > 0.05$), and no significant differences between the different markers in boys and girls in either group. It is important to note that the discriminatory signal in these markers is not related to age of the child or the sex, but to infection type. The age correlations are summarized in Table 6 and fig3.

Table 6. Spearman (ρ) and Mann-Whitney (p) correlations between biomarker concentrations and age within each infection group. Significant correlations ($p < 0.05$) are shown in red. Only WBC in the bacterial group reached significance.

Biomarker	Bacterial ρ	Bacterial p	Viral ρ	Viral p
WBC (×10⁹/L)	-0.305	0.031	+0.109	0.450
Neutrophils (×10⁹/L)	+0.270	0.058	-0.027	0.854
Lymphocytes (×10⁹/L)	+0.044	0.761	+0.094	0.518
NLR	+0.114	0.431	-0.083	0.568
CRP (mg/L)	+0.082	0.570	+0.082	0.570
PCT (ng/mL)	+0.198	0.168	+0.157	0.275
Faecal calprotectin (µg/g)	+0.062	0.667	-0.063	0.665
Faecal S100A12 (mg/kg)	+0.109	0.452	-0.122	0.399
Faecal M2-PK (U/mL)	+0.041	0.779	+0.131	0.365

Figure3. Inter-marker correlation matrix (bacterial and viral cases combined).



Discussion

Procalcitonin: PCT was the best discriminator in our data (AUC 0.969). There was only a small overlap in the distributions of the bacteria and viruses. This is appropriate given its biology. PCT will increase rapidly and specifically following bacterial and endotoxin exposures. It remains low during most of the viral disease. We obtained an optimal cut-off of 0.64 ng/mL. However, some paediatric work reports weaker performance of PCT. The AUC of Cho and his team was surprisingly low in a mixed gastroenteritis cohort (0.66) [12]. This difference may be due to the age and case mix. They included lots of mild illnesses and some adults. A new dimension is added by Jan and co-workers. They concluded that PCT is more a marker of severity than a pathogen-specific test [11]. Higher PCT predicted admission and longer stay in their cohort of patients

attending the ED [11]. This was likely due to a well defined, culture confirmed bacterial group, that is, our better result. The combination of faecal marker was also helpful for the prognosis, and results were best for PCT [13].

C-reactive protein: CRP was the second best marker (AUC 0.941). Levels were almost five-fold higher in bacterial cases. CRP is part of the acute phase protein family. It increases very rapidly after an inflammatory stimulus. However, it does not specify. It also increases in numerous viral infections. This limits its stand-alone value. In their gastroenteritis series, Cho and colleagues reported a lower CRP AUC (approximately 0.85) [12]. This increased figure may simply be due to a tighter division of groups, as in the past. The overall message is the same in all studies. CRP is a good test but not as specific as PCT.

White-cell and neutrophil counts: Only a modest discriminator was the total white-cell count (AUC 0.792). The absolute neutrophil count was much better (AUC 0.924). This is a very practical consideration. Simple differential count was as good as the specialised serum assays. Neutrophils are pushed out of the marrow by bacterial infection. Lymphocytes are more susceptible to viral infection. This division was exactly our data reflected. Cho and colleagues likewise found white-cell counts helpful but secondary to CRP [12]. Neutrophil count is widely available and is inexpensive. This means it is appealing in resource-poor areas like ours.

Neutrophil-to-lymphocyte ratio: Two regular counts are aggregated into a single index by the NLR. It performed well (AUC 0.879) at a cut-off of 1.47. This was provided without any additional laboratory expenses. Our cut-off is similar to those reported elsewhere. Lakshmanan et al. reported a threshold value of NLR at 1.74 for bacterial pneumonia in children [14]. The NLR was less specific and more sensitive than CRP in their study [14]. Our ratio is similar to this balance. The NLR is best considered a quick triage tool. It is particularly recommended when the implementation of PCT is not feasible or expensive, like in most parts of the region.

Faecal calprotectin: Serum markers are indicators of overall inflammation. What they may miss is an intestinal inflammation that does not involve the rest of the body. This is something which is directly addressed by faecal markers. Activated neutrophils in the bowel wall release Calprotectin. It was definitely higher in the cases with bacteria (AUC 0.873). This is consistent with the known biology of invasive bacterial enteritis. Salmonella and similar organisms cause a strong neutrophil response. Rotavirus do not cause this. Discrimination was also found in children with faecal calprotectin in Chinese children, as reported by Guo et al. [13]. Czub et al. were less optimistic [15]. In the hospitalised group, there was no definitive superiority of calprotectin over CRP [15]. The difference might be due to the variety and level of disease.

Faecal S100A12: Another protein of neutrophils is S100A12 (calgranulin C). This was the highest area under the curve (AUC 0.901) for faecal marker. It also provided the greatest faecal separation between the groups. S100A12 is a highly sensitive biomarker of active neutrophilic inflammation. Cenni and colleagues demonstrated that it is a good indicator of disease activity in paediatric inflammatory bowel disease [16]. Heida and colleagues have set reference values for children and adolescents demonstrating that low levels are found among healthy children [17]. In this regard our results expand the marker to acute bacterial enteritis. The agreement between S100A12 and calprotectin is predicted. Both are derived from the same source of neutrophils. This is why it is not helpful to have them together.

Faecal M2-pyruvate kinase: The process of M2-PK is different. Is an enzyme of epithelial turnover, rather than a product of neutrophils. It was the lowest performing faecal marker (AUC 0.709). It was also found to have the highest overlap between the groups. This is in line with previous studies. In acute childhood diarrhoea, Chub and colleagues identified that M2-PK was not very helpful [15]. However, M2-PK can detect data not detected by the neutrophil markers. It should be used as a secondary marker not as a primary marker.

Putting the panel together: When the neutrophils derived markers are viewed collectively, they are tightly grouped. All of the above, including neutrophils, NLR, CRP, PCT and calprotectin, indicate a bacterial response. This is the reason why only small improvements can be expected from a combination of highly correlated markers. It's better to follow a step-by-step plan. A low neutrophil level can be used as a screening test. If resources are available, then PCT can call this out. In patients with suspected gut-limited infection a faecal neutrophil marker may be helpful. This is a strategy that is appropriate for the regional context. Viral AGE is the most common type of AGE in ME, and in Iraq specifically [18,19,20]. Empiric use of antibiotics still continues to be practiced. A simple, objective rule out will decrease over-prescribing. It would also help to curb antimicrobial resistance. Limitations are our single-centre design and our relatively small sample size. The clinic separation into the clean group might not apply to other, unselected clinic groups. Information on time of illness and severity of symptoms was also not available. Larger, multi-centre studies throughout the region are appropriate next steps. They should validate the cut-offs and implement the tiered approach in practice.

Conclusion: All the markers were above in bacterial disease. The value of their diagnosis varied, however. The single most interesting test was procalcitonin. It was characterized by both high sensitivity and specificity and low overlap among the groups. The absolute neutrophil count was next, with c-reactive protein close behind. This indicates that the accuracy of a simple blood differential is close to that of specialised assays. One of the points that should be noted is the neutrophil-to-lymphocyte ratio. Provided good discrimination for no extra charge. This makes it well suited to resource-limited environments, which would be a majority of the region. The faecal markers for neutrophils, S100A12 and calprotectin, provided a direct measurement of gut inflammation. M2-pyruvate kinase was found to be less effective, and more of a complement. The informative markers are all neutrophil driven and hence they are highly correlated. So adding them together will make little difference. It makes more sense to have a tiered approach. A cheap index can be used to screen, procalcitonin can be used to confirm and a faecal marker can clarify gut-only disease. This would help minimize the use of non-essential antibiotics and aid in stewardship of antibiotics in paediatric gastroenteritis. These cut-offs should be confirmed in larger multi-centre studies throughout the Middle East.

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