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FECAL CALPROTECTIN AS A DIAGNOSTIC AND PROGNOSTIC MARKER IN ACUTE INTESTINAL INFECTIONS IN CHILDREN

DISSERTATION SUMMARY

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Thesis materials are available at the Library of Medical University – Varna and online on the University’s webpage (mu-varna.bg).

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ABBREVIATIONS

ORT – oral rehydration therapy

WHO – World Health Organization

FC – fecal calprotectin

CDI – Clostridioides difficile infection

EAEC – enteroaggregative Escherichia coli

EHEC – enterohemorrhagic Escherichia coli

EIEC – enteroinvasive Escherichia coli

EPEC – enteropathogenic Escherichia coli

ETEC – enterotoxigenic Escherichia coli

NGS – next-generation sequencing

PCR – polymerase chain reaction

I. INTRODUCTION

Acute infectious diarrhea (ОЧИ), characterized primarily by the presence of diarrheal syndrome, represents a significant global health problem with considerable medical and social impact. Despite the availability of effective preventive strategies, these infections remain associated with high morbidity and mortality, particularly among children under five years of age, the most vulnerable population group. Worldwide, diarrheal diseases remain among the leading causes of childhood morbidity, and in developing regions acute infectious diarrhea ranks immediately after pneumonia as a cause of mortality in this age group. In economically developed countries, acute infectious diarrhea less frequently leads to fatal outcomes but remains an important cause of outpatient visits, hospitalizations, and increased burden on healthcare systems. Data from the Republic of Bulgaria indicate that acute infectious diarrhea predominantly affects infants and young children, with recent years showing a return of incidence rates to pre-pandemic levels following a temporary decline during the COVID-19 pandemic.

The etiological spectrum of infectious diarrhea in childhood is broad and includes viral, bacterial, and parasitic pathogens. Viral agents predominate as the leading cause of acute infectious diarrhea in children under five years of age, whereas bacterial infections occur less frequently but are often associated with a more severe clinical course. The combination of diverse etiology and similar clinical presentation creates significant diagnostic challenges, particularly in the early stage of the disease.

In clinical practice, various laboratory methods are used to determine the etiology of acute infectious diarrhea, including culture-based, immunochromatographic, and molecular biological techniques. However, these approaches require time, financial resources, and specialized laboratory infrastructure, and despite their application, a considerable proportion of cases remain without an identified etiological agent. This may lead to delays in appropriate therapeutic management or to the irrational use of antibacterial agents, contributing to the growing problem of antimicrobial resistance.

For this reason, increasing attention has been directed toward the use of non-invasive biomarkers that may support rapid clinical assessment in children with acute infectious diarrhea. Among fecal markers of intestinal inflammation, fecal calprotectin (FC) has emerged as a sensitive indicator of mucosal inflammation. Initially widely used in gastroenterology, it is increasingly being considered a potential diagnostic and prognostic

tool in infectious diseases as well. Accumulating evidence suggests that FC levels reflect the intensity of intestinal inflammation, allow differentiation between different etiological forms of acute infectious diarrhea, and provide an opportunity to assess disease severity and prognosis. In Bulgaria, however, systematic studies evaluating the clinical applicability of FC in acute infectious diarrhea in children are lacking. In this context, the present dissertation analyzes the role of FC as a non-invasive diagnostic and prognostic marker in children with acute infectious diarrhea, aiming to contribute to a more objective clinical assessment and to optimize diagnostic and therapeutic approaches in this vulnerable patient population.

II. AIM AND OBJECTIVES

The aim of the present study was to evaluate the diagnostic and prognostic value of FC as a non-invasive marker in acute infectious diarrhea in children aged 1–5 years, including its role in etiological differentiation, severity stratification, monitoring of therapeutic response, and prediction of the clinical course.

To achieve this aim, the following research objectives were defined:

1. To investigate the etiological structure of acute infectious diarrhea in children aged 1–5 years;
2. To determine and analyze FC levels in children with acute infectious diarrhea;
3. To examine the relationship between FC levels and the etiological agent of acute infectious diarrhea with regard to early differential diagnostic assessment;
4. To assess the correlation between FC levels and the severity of the clinical course of acute infectious diarrhea;
5. To monitor the dynamics of FC during treatment in order to evaluate its value as an indicator of therapeutic effectiveness;
6. To evaluate the prognostic value of FC regarding the clinical course of acute infectious diarrhea and the risk of developing complications.

III. MATERIAL AND METHODS

1. Material/Study population

This prospective study included 137 children aged 1–5 years hospitalized at the First Clinic of Infectious Diseases, University Hospital "St. Marina" – Varna, during the period June 2024 to February 2025. Of these, 107 patients were enrolled in the acute stage of acute infectious diarrhea based on clinical and epidemiological data and according to predefined inclusion criteria. The control group consisted of 30 children of the same age group who attended a follow-up examination one month after discharge following acute infectious diarrhea and were clinically healthy at the time of evaluation.

Inclusion criteria

The study included children aged 1–5 years with a clinical presentation consistent with acute infectious diarrhea in whom the etiology was confirmed by microbiological and virological examination of fecal samples. Only cases with a laboratory-confirmed etiological agent were included in the analysis. Participation was allowed after obtaining informed consent from a parent or legal guardian in accordance with applicable regulatory requirements.

Exclusion criteria

Patients without microbiological or virological confirmation of the etiological agent were excluded, as were children with chronic inflammatory bowel diseases, congenital or acquired immunosuppression, and those who had received antibiotic therapy within the preceding two weeks due to the potential influence of these factors on baseline FC levels. Children younger than 1 year were not included because FC levels in infancy are physiologically elevated and highly variable, which may complicate interpretation of the results and their comparability between etiological groups. Children older than 5 years were also excluded, as children under five years of age represent the main risk group for acute infectious diarrhea in international and national epidemiological analyses, with the highest incidence, hospitalization rates, and risk of complications.

The patients were divided into three groups. The first group included 55 children with viral acute infectious diarrhea, and the second group comprised 52 children with

bacterial acute infectious diarrhea. The control group consisted of 30 children from the same age group who attended a follow-up examination and were clinically healthy one month after discharge from the clinic. The control group served as a reference for comparison of FC levels and included children without clinical or laboratory evidence of acute or chronic disease at the time of examination. Patients were enrolled consecutively during the study period without a predefined quota for the individual groups. The resulting groups provided a sufficient sample size for comparative statistical analysis.

The demographic characteristics of the study participants, including age distribution and sex structure in each group, are presented in Table 1.

Table 1. Age and sex distribution of patients by etiological group.

Age group	Sex	Viral acute infectious diarrhea (n, %)	Bacterial acute infectious diarrhea (n, %)	Control group (n, %)
1–2 years	Male	11 (20,0%)	5 (9,6%)	4 (13,3%)
	Female	9 (16,4%)	4 (7,7%)	5 (16,7%)
2–3 years	Male	8 (14,5%)	7 (13,5%)	3 (10,0%)
	Female	8 (14,5%)	5 (9,6%)	4 (13,3%)
3–4 years	Male	7 (12,7%)	9 (17,3%)	4 (13,3%)
	Female	4 (7,3%)	7 (13,5%)	4 (13,3%)
4–5 years	Male	5 (9,1%)	7 (13,5%)	3 (10,0%)
	Female	3 (5,5%)	8 (15,4%)	3 (10,0%)
Total	Male	31 (56,4%)	28 (53,8%)	14 (46,7%)
	Female	24 (43,6%)	24 (46,2%)	16 (53,3%)
	Total	55 (51,4%)	52 (48,6%)	30 (100%)

2. Methods

2.1. Definitions Used

The following definitions were applied in the analysis. Diarrheal syndrome was defined as the presence of ≥3 loose or watery stools within a 24-hour period. The duration of diarrheal syndrome was defined as the number of days from the onset of diarrhea until resolution of symptoms. Fever was defined as a body temperature ≥38.0 °C, and its duration

was calculated as the number of days until sustained normalization of body temperature. Vomiting was recorded when at least one episode occurred within a 24-hour period, with both the number of episodes and their duration documented. Complications were defined as clinically significant conditions requiring therapeutic escalation, prolongation of hospitalization, or additional medical intervention.

2.2. Clinical and Epidemiological Methods

The overall epidemiological assessment of acute infectious diarrhea during the study period was based on data obtained from the hospital medical databases. Clinical methods included medical history taking from the parents or legal guardians of the patients and review of medical records. The collected information included the onset and duration of symptoms, previous treatment, immunization status, and the presence of comorbidities. These data were used to assess compliance with the inclusion and exclusion criteria and to provide a clinical characterization of the study groups.

Upon admission, all patients underwent a clinical examination, followed by assessment of disease severity using the modified Vesikari score. According to the scale, a score of up to 8 points was classified as mild disease, values between 9 and 10 points as moderate disease, and a score above 11 points as severe disease (Table 2).

Table 2. Modified Vesikari Severity Score (MVSS) for acute gastroenteritis

Component and score	Vesikari score	Modified Vesikari score
Duration of diarrhea		
1	1–4 days	1–4 days
2	5 days	5 days
3	≥6 days	≥6 days
Maximum number of diarrheal stools within 24 hours		
1	1–3 stools	1–3 stools
2	4–5 stools	4–5 stools
3	≥6 stools	≥6 stools
Duration of vomiting		
1	1 day	1 day
2	2 days	2 days
3	≥3 days	≥3 days

Component and score	Vesikari score	Modified Vesikari score
Maximum number of vomiting episodes within 24 hours		
1	1 episode	1 episode
2	2–4 episodes	2–4 episodes
3	≥5 episodes	≥5 episodes
Maximum recorded body temperature		
1	37.1–38.4 °C	37.1–38.4 °C
2	38.5–38.9 °C	38.5–38.9 °C
3	≥39.0 °C	≥39.0 °C
Dehydration		
2	Loss of 1–5% of body weight	≥2 signs of moderate dehydration
3	Loss of ≥6% of body weight	≥2 signs of severe dehydration
Treatment		
1	Oral or intravenous rehydration	Oral or intravenous rehydration
2	Hospitalization	Hospitalization
Maximum possible score	20	20
Follow-up period for assessment of scale components	24–32 months	Symptoms at admission and hospitalization within 6 days after admission

For prognostic analysis, an additional classification of the clinical course was introduced based on the duration of diarrhea and the occurrence of complications. In viral acute infectious diarrhea, a duration of up to 3 days without complications was considered a favorable course, whereas a duration of ≥4 days and/or the occurrence of complications was classified as a prolonged course. In bacterial acute infectious diarrhea, the threshold was set at 5 days, with longer symptom duration and/or complications interpreted as a prolonged clinical course. For statistical analysis, the clinical course was coded as a binary variable (0 – shorter course; 1 – prolonged and/or complicated course). For each patient, the duration of symptoms, body temperature, frequency of diarrheal stools, presence of vomiting, degree of dehydration, and clinical signs of complications were recorded.

2.3. FC Testing

All fecal samples were collected prospectively from consecutively hospitalized patients with a clinical diagnosis of acute infectious diarrhea. Sampling was performed within the first 24 hours after admission using sterile containers. In children using diapers, samples were obtained immediately after defecation while avoiding contact with the absorbent material in order to minimize pre-analytical errors and contamination.

Samples were stored at 2–8 °C until the results of microbiological and virological testing became available. Quantitative determination of FC was performed only in patients with laboratory-confirmed viral or bacterial etiology. Samples from cases without etiological confirmation were not analyzed. Testing was carried out within 3–5 days after collection in accordance with the manufacturer's instructions and established pre-analytical requirements.

Quantitative measurement of FC was performed at the Clinical Laboratory "Lina" Ltd., Varna, using an immunoturbidimetric method with latex-enhanced agglutination. FC in the sample induces agglutination of latex particles coated with specific antibodies, and the intensity of the resulting immune complexes is measured photometrically and is proportional to the concentration of the marker. Analysis was performed using an automated biochemical analyzer A15 (Biosystems) and a diagnostic kit REF-12330 (Biosystems). The detection limit is 7.93 mcg/g, and the linear measurement range extends up to 2000 mcg/g. Repeatability and reproducibility are 2.3% and 4.0%, respectively, at concentrations around 115 mcg/g, ensuring analytical reliability within the clinically relevant range.

2.4. Microbiological Techniques

To determine the etiology of acute infectious diarrhea, microbiological testing was performed to identify the most common bacterial and viral pathogens. Fecal samples were analyzed for the presence of *Salmonella spp., Shigella spp., Yersinia enterocolitica, Vibrio cholerae, Escherichia coli, Campylobacter spp., Clostridioides difficile,* as well as Rotavirus, Norovirus, Adenovirus, and Astrovirus.

Bacterial pathogens were identified using conventional bacteriological culture of fecal specimens on selective media under thermostatic incubation conditions for *Salmonella spp., Shigella spp*., enteropathogenic *E. coli, Yersinia enterocolitica*, and *Vibrio cholerae*. Species identification of bacterial isolates was performed using manual biochemical tests,

semi-automated Crystal systems (BD), and automated Phoenix systems (BD). Serotyping of Salmonella spp. and Shigella spp. isolates was carried out by agglutination reaction, while antimicrobial susceptibility testing was performed using the Bauer–Kirby disk diffusion method. The results were interpreted according to EUCAST standards (2016).

Detection of viral pathogens (Rotavirus, Norovirus GI/GII, Astrovirus, and Adenovirus), as well as bacterial pathogens through fecal antigen detection (*Clostridioides difficile* and *Campylobacter spp.*), was performed using an immunochromatographic method (CerTest Biotec S.L.).

All microbiological and virological investigations were conducted in the Microbiology Laboratory of University Hospital "St. Marina" – Varna in accordance with the principles of good laboratory practice.

2.5. Clinical Laboratory Methods

Laboratory investigations were performed in the Clinical Laboratory of University Hospital "St. Marina" – Varna in accordance with established procedures and standards for clinical laboratory diagnostics.

The acute-phase protein C-reactive protein (CRP) was measured in serum samples using an immunoturbidimetric method routinely applied in clinical laboratory practice. The reference range for CRP was 0–5 mg/L.

The erythrocyte sedimentation rate (ESR) was determined using an automated analytical system based on measurement of erythrocyte aggregation properties and mathematical modeling of the results. ESR values were expressed in mm/h, and for the age group 1–5 years the reference range was 2–37 mm/h.

A complete blood count with differential was performed using standard automated hematological methods applied in routine clinical laboratory practice. The analysis included the total leukocyte count measured at hospital admission. Interpretation of the results was performed according to age-specific reference ranges used in the Clinical Laboratory, as follows: 1–2 years – 6.00–15.00 × 10^9/L; 2–3 years – 5.40–13.80 × 10^9/L; 3–4 years – 5.40–13.80 × 10^9/L; 4–5 years – 5.10–12.90 × 10^9/L. This approach allowed an accurate assessment of the leukocyte response in the context of age-related physiological variations.

2.6. Statistical Methods

Statistical data processing was performed on a personal computer running macOS. Microsoft Office (Excel) was used for initial data processing and dataset structuring. Statistical analyses were conducted using IBM SPSS Statistics, version 28.0 (IBM Corp., Armonk, NY, USA). Both parametric and non-parametric methods were applied depending on the type of variables and the distribution of the data. The level of statistical significance was set at $p < 0.05$.

Descriptive analysis included calculation of absolute and relative frequencies (n, %), as well as measures of central tendency and variability, including mean, median, standard deviation (SD), minimum and maximum values, and interquartile range (IQR). The data were presented in tables and graphs, including box plot diagrams. In the control group, a percentile-based approach was applied, with the 95th percentile of the FC distribution used as the upper reference limit.

Comparison of categorical variables was performed using the chi-square (χ^2) test applied to contingency tables after categorizing FC values as normal or pathologically elevated according to the defined reference limit.

Normality of distribution for quantitative variables was assessed using the Shapiro–Wilk test, and the results of this analysis determined the choice of subsequent comparative statistical methods. For normally distributed data, the parametric independent samples t-test was used to compare mean values between two groups. When deviation from normality was observed, the non-parametric Mann–Whitney test was applied for comparison of two independent groups. For comparison of a continuous variable among more than two independent groups, the non-parametric Kruskal–Wallis test was used, including analyses across etiological subgroups and comparisons between groups with different clinical severity.

To assess changes in FC levels over time within the same patients, a paired samples t-test was applied. The results were presented as t-values, p-values, mean differences between measurements, standard errors, and 95% confidence intervals.

To evaluate the diagnostic and prognostic value of FC, receiver operating characteristic (ROC) analysis was performed. The area under the curve (AUC), sensitivity, specificity, and optimal threshold (cut-off) values were calculated.

To assess the independent prognostic value of FC after adjustment for other inflammatory markers, a multivariable logistic regression analysis was conducted. The clinical course was modeled as a dependent binary variable, while FC, CRP, leukocyte count, and ESR were included as independent variables. The results were reported as odds ratios (ORs), 95% confidence intervals, p-values, overall model significance (likelihood ratio test), and pseudo-R^2.

The study was approved by the Ethics Committee of Medical University – Varna (Protocol No. 91/27.02.2020).

IV. Results and Discussion

1. Demographic, Epidemiological, and Nosological Distribution of Patients and Controls

During the study period, a total of 313 children aged 1–5 years with a diagnosis of acute infectious diarrhea were hospitalized at the First Clinic of Infectious Diseases, University Hospital "St. Marina" – Varna. A laboratory-confirmed infectious pathogen was identified in 52.1% of cases (n = 163), whereas in 47.9% the etiology remained undetermined (Figure 1). From the subgroup with a confirmed etiological agent, and according to the criteria described above, 55 children with viral acute infectious diarrhea and 52 children with bacterial acute infectious diarrhea were included in the present study.

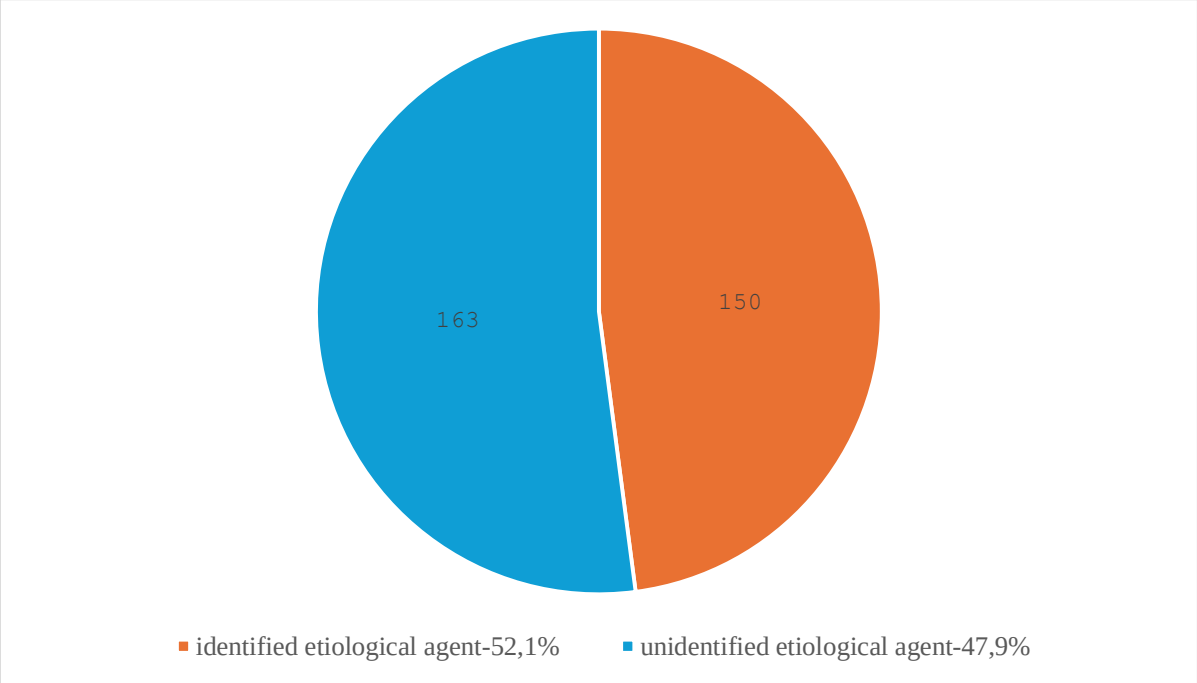

Figure 1. Proportion of patients with acute infectious diarrhea with identified and unidentified etiology

The relatively high proportion of acute infectious diarrhea cases with unidentified etiology in the present study reflects one of the major challenges in the diagnosis of

infectious diarrhea in pediatric populations. These findings are consistent with published international data, which indicate that when conventional laboratory methods are used, the etiological agent remains unidentified in approximately half of the patients. Similar observations have also been reported in Bulgarian clinical studies, where the main limitations are associated with delayed healthcare seeking, prior empirical antibiotic therapy, and the limited use of modern molecular diagnostic approaches.

In the present study, etiological diagnosis was performed using routinely applied culture-based and immunochromatographic methods. Although culture-based diagnostics remain the gold standard for bacterial acute infectious diarrhea, they are associated with delayed turnaround times, the need for specialized laboratory infrastructure, and reduced sensitivity in patients who have received prior antibiotic therapy. Immunochromatographic assays offer rapid results and practical applicability in routine clinical settings; however, their diagnostic performance is limited by lower analytical sensitivity and a restricted pathogen detection spectrum, which likely contributes to the proportion of cases with unidentified etiology.Modern molecular diagnostic techniques have been shown to substantially increase the rate of etiological identification in acute infectious diarrhea. Nevertheless, their routine implementation remains limited due to high costs, interpretative complexity, and the inability to provide microbiological typing or antimicrobial susceptibility testing. In this context, the relatively high proportion of cases with unidentified etiology highlights the need for additional universal biomarkers capable of reflecting the presence and intensity of intestinal inflammation.

Analysis of the etiology of viral acute infectious diarrhea showed that rotavirus gastroenteritis accounted for the largest proportion of confirmed cases, representing 63,64% (n = 35/55). This was followed by adenovirus-associated enteritis, which constituted 27,27% (n = 15/55). In contrast, infections caused by Norovirus and Astrovirus were identified considerably less frequently, accounting for 5,45% (n = 3/55) and 3,64% (n = 2/55) of cases, respectively (Figure 2).

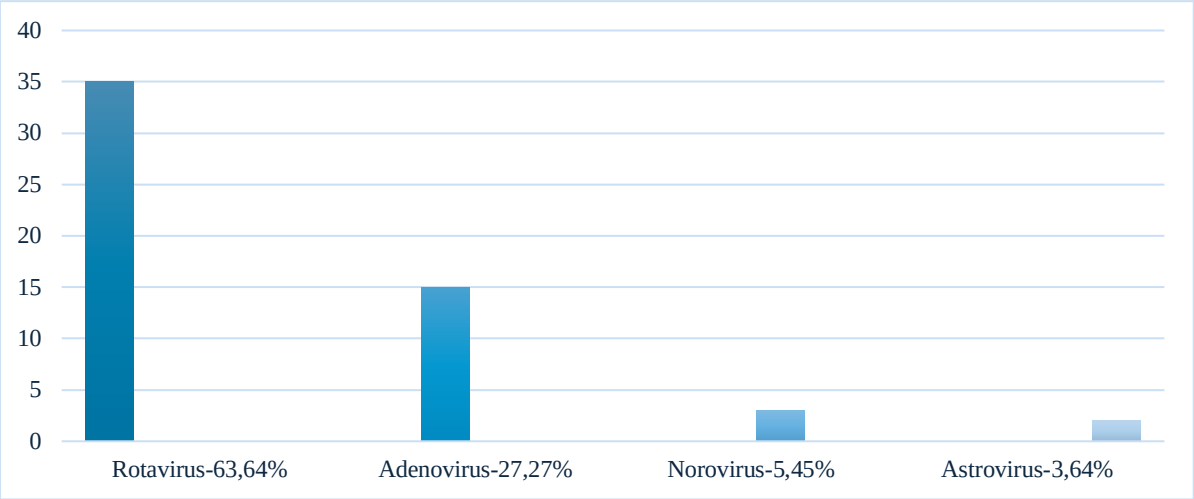

Figure 2. Distribution of positive fecal samples by viral pathogen

The obtained results are consistent with established international and national data indicating that rotaviruses remain the leading etiological agents of viral gastroenteritis in early childhood.

Among patients hospitalized with rotavirus gastroenteritis, active immunization against rotavirus had not been performed in the majority of cases (91.4%). Of the total 35 patients, only two had completed the full vaccination schedule (two doses), while one patient had received a single dose of the vaccine (Figure 3).

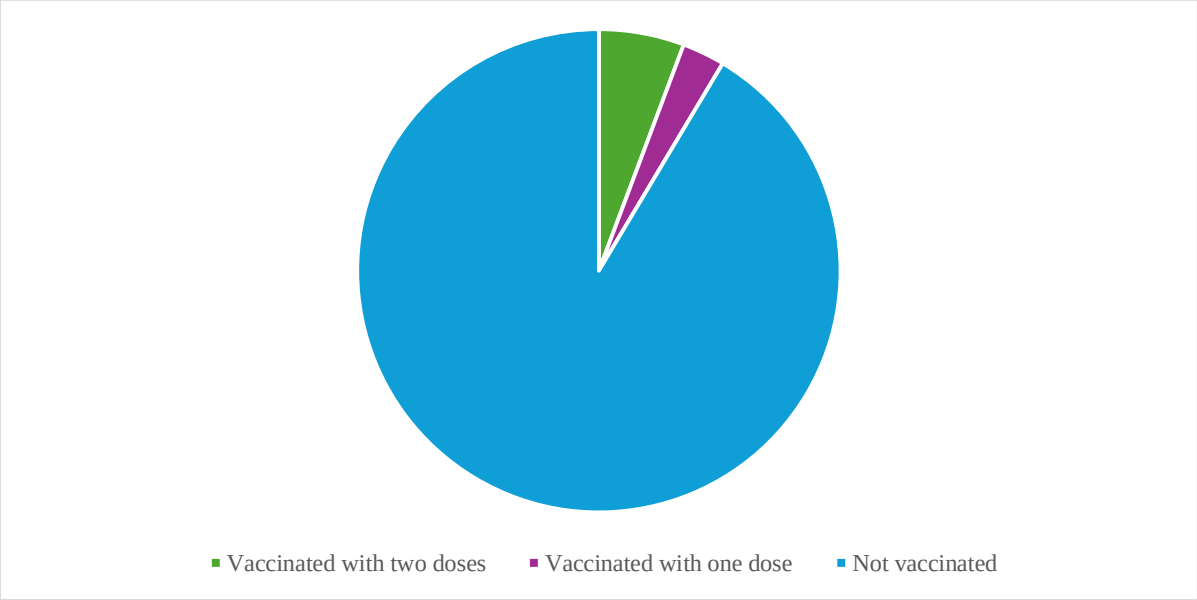

Figure 3. Distribution of patients with rotavirus gastroenteritis by immunization status

In the Republic of Bulgaria, rotavirus vaccination is provided free of charge for infants from six weeks of age and is recommended but not mandatory, with vaccination coverage remaining approximately 39–40%. Available data indicate that vaccination is associated with a significant reduction in hospitalizations due to rotavirus gastroenteritis. In the present study, the majority of hospitalized children with rotavirus gastroenteritis were unvaccinated, further supporting the role of immunization status as a factor associated with more severe clinical outcomes.

Among the 52 children with bacterial acute infectious diarrhea, the following pathogens were isolated: *Salmonella* spp. (40,38%; n = 21), *Campylobacter* spp. (28,85%; n = 15), *Clostridioides difficile* (15,38%; n = 8), *Shigella* spp. (9,62%; n = 5), and *Yersinia enterocolitica* (5,77%; n = 3), with *Salmonella* spp. being the most frequently identified etiological agent (Figure 4).

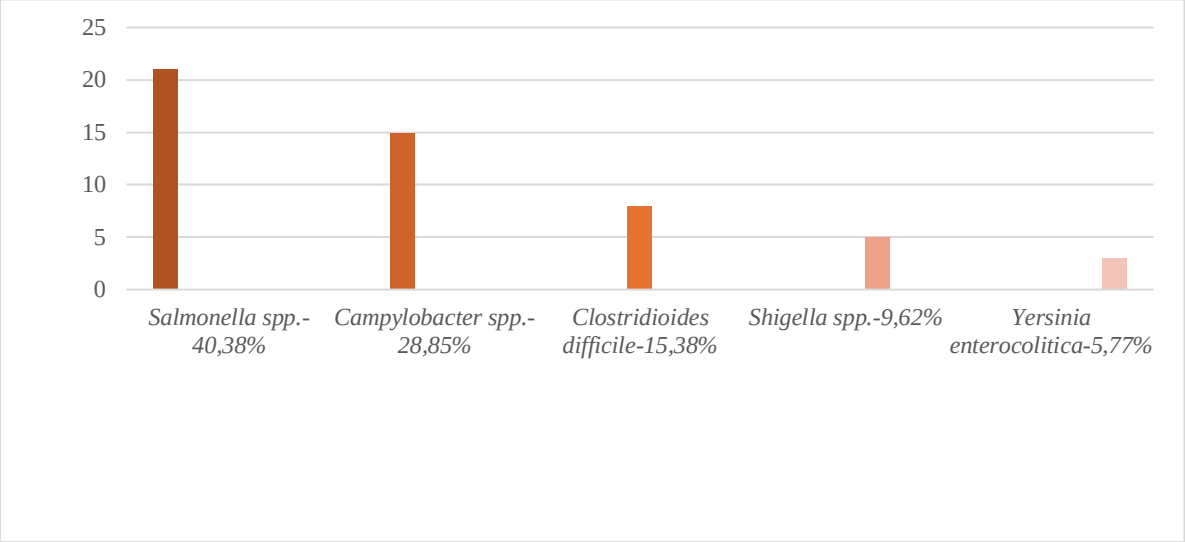

Figure 4. Distribution of positive fecal samples in children with bacterial acute infectious diarrhea by etiological pathogen

This etiological distribution is typical for economically developed countries and is fully consistent with European epidemiological data, in which *Salmonella* spp. and *Campylobacter* spp. predominate among bacterial causes of acute infectious diarrhea in children. In the present study, *Clostridioides difficile* ranked third in frequency among bacterial acute infectious diarrhea cases, reflecting the increasing clinical relevance of this infection in the pediatric population, particularly in the context of community-acquired

cases. The relatively low frequency of shigellosis and yersiniosis reflects both the national epidemiological situation and their limited circulation within the studied population.

In the etiological structure of bacterial acute infectious diarrhea in the present study, no diarrheagenic *E. coli* or parasitic pathogens were identified. This likely reflects the limitations of the routinely applied culture-based diagnostics and the absence of systematic parasitological testing, which do not allow reliable detection of specific pathogenic *E. coli* pathotypes or protozoal infections. Consequently, some cases may have remained etiologically unresolved due to these methodological limitations, representing a limitation of the study.

The age distribution of patients with viral acute infectious diarrhea showed the highest frequency in the 1–2 years age group (36,36%; n = 20), followed by children aged 2–3 years (29,09%; n = 16), with rotavirus infections predominating in both groups. After the age of three years, a gradual decline in case frequency was observed (20,00% in the 3–4 years age group and 14,55% in the 4–5 years age group) (Table 3).

Table 3. Age distribution of patients by etiology of viral acute infectious diarrhea

Age group	**Rotavirus (n, %)**	**Adenovirus (n, %)**	**Norovirus (n, %)**	**Astrovirus (n, %)**	**Total (n, %)**
1–2 years	12 (21,82%)	6 (10,91%)	2 (3,64%)	0 (0,00%)	20 (36,36%)
2–3 years	10 (18,18%)	5 (9,09%)	1 (1,82%)	0 (0,00%)	16 (29,09%)
3–4 years	8 (14,55%)	3 (5,45%)	0 (0,00%)	0 (0,00%)	11 (20,00%)
4–5 years	5 (9,09%)	1 (1,82%)	0 (0,00%)	2 (3,64%)	8 (14,55%)
Total	35 (63,64%)	15 (27,27%)	3 (5,45%)	2 (3,64%)	55 (100,00%)

Among patients with bacterial acute infectious diarrhea, a different age distribution pattern was observed. The highest frequency was recorded among children aged 3–4 years (30,77%; n = 16/52), whereas the lowest number of cases was observed in the 1–2 years age group (17,31%; n = 9/52). *Salmonella* spp. was the predominant etiological agent (40,38%; n = 21/52), followed by *Campylobacter* spp. (28,85%; n = 15/52) (Table 4).

Table 4. Age distribution of patients by etiology of bacterial acute infectious diarrhea

Age group	***Salmonella* spp. (n, %)**	***Campylobacter* spp. (n, %)**	***Clostridioides difficile* (n, %)**	***Shigella* spp. (n, %)**	***Yersinia enterocolitica* (n, %)**	**Total (n, %)**
1–2 years	3 (5,77%)	2 (3,85%)	3 (5,77%)	1 (1,92%)	0 (0,00%)	9 (17,31%)
2–3 years	5 (9,62%)	4 (7,69%)	2 (3,85%)	1 (1,92%)	0 (0,00%)	12 (23,08%)
3–4 years	6 (11,54%)	5 (9,62%)	2 (3,85%)	2 (3,85%)	1 (1,92%)	16 (30,77%)
4–5 years	7 (13,46%)	4 (7,69%)	1 (1,92%)	1 (1,92%)	2 (3,85%)	15 (28,85%)
Total	21 (40,38%)	15 (28,85%)	8 (15,38%)	5 (9,62%)	3 (5,77%)	52 (100,00%)

The age-related analysis demonstrated a pronounced concentration of viral acute infectious diarrhea in early childhood, particularly in children under three years of age, which is consistent with the increased susceptibility to viral gastroenteritis during this period. In contrast, bacterial acute infectious diarrhea showed a tendency toward higher frequency among older children, likely associated with the expansion of dietary patterns and increased exposure to foodborne pathogens such as *Salmonella* spp. and *Campylobacter* spp.

The sex distribution of patients with viral acute infectious diarrhea and bacterial acute infectious diarrhea is presented in Tables 3 and 4. A slight predominance of males was observed in both viral acute infectious diarrhea (56,36%) and bacterial acute infectious diarrhea (57,69%), with no statistically significant differences between the groups or within the individual etiological categories ($p > 0,05$) (Tables 5 and 6).

Table 5. Sex distribution of children with viral acute infectious diarrhea by etiological agent

Pathogen	Boys (n, %)	Girls (n, %)	Total (n, %)
Rotavirus	19 (34.55%)	16 (29.09%)	35 (63.64%)
Adenovirus	9 (16.36%)	6 (10.91%)	15 (27.27%)
Norovirus	2 (3.64%)	1 (1.82%)	3 (5.45%)
Astrovirus	1 (1.82%)	1 (1.82%)	2 (3.64%)
Total	31 (56.36%)	24 (43.64%)	55 (100.00%)

Table 6. Sex distribution of children with bacterial acute infectious diarrhea by etiological agent

Etiological agent	Boys (n, %)	Girls (n, %)	Total (n, %)
Salmonella spp.	13 (25.00%)	11 (21.15%)	24 (46.15%)
Campylobacter spp.	8 (15.38%)	7 (13.46%)	15 (28.85%)
Clostridioides difficile	5 (9.62%)	3 (5.77%)	8 (15.38%)
Shigella spp.	2 (3.85%)	2 (3.85%)	4 (7.69%)
Yersinia enterocolitica	2 (3.85%)	1 (1.92%)	3 (5.77%)
Total	30 (57.69%)	22 (42.31%)	52 (100.00%)

The slight predominance of male patients observed in both viral acute infectious diarrhea and bacterial acute infectious diarrhea is consistent with findings from international studies reporting a higher incidence of acute diarrheal diseases among boys. However, in the present study this difference did not reach statistical significance ($p > 0.05$), and therefore

no convincing association between sex and the occurrence of acute infectious diarrhea in the studied population can be established.

As part of the epidemiological assessment, attendance in organized childcare settings was also analyzed as a potential risk factor. It was found that 83 (77.57%) of the children included in the study attended nurseries or kindergartens (Fig. 5).

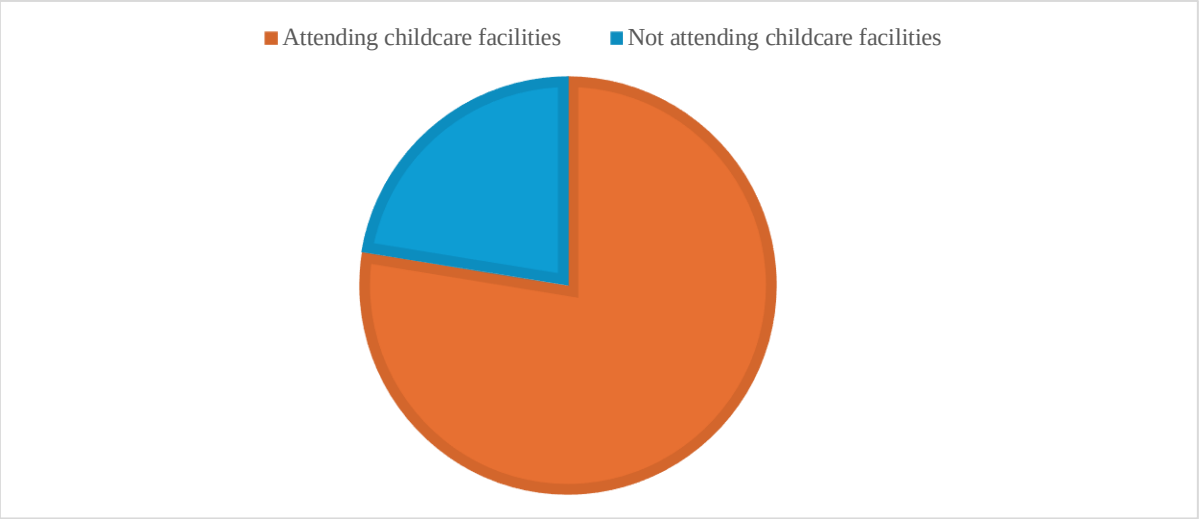

Fig. 5. Attendance at organized childcare facilities among the studied children

The high proportion of children attending organized childcare facilities confirms the role of this environment as an important factor in the transmission of acute infectious diarrhea. This association is largely attributable to immature hygiene habits, frequent close contact between children, and the presence of asymptomatic carriers, all of which facilitate the spread of enteropathogens. The obtained results highlight the need for strengthened preventive and control measures in childcare institutions, as well as for timely diagnosis and early clinical stratification.

In summary, the epidemiological profile of the studied cohort reflects a pattern typical for hospitalized children aged 1–5 years with acute infectious diarrhea. Etiological identification was achieved in slightly more than half of the cases. Among viral acute infectious diarrhea, rotavirus gastroenteritis predominated, whereas among bacterial acute infectious diarrhea the leading etiological agents were *Salmonella* spp. and *Campylobacter* spp., with clear age-related characteristics and a slight predominance of males. The high proportion of cases with unidentified etiology highlights the limitations of routine diagnostic approaches and underscores the need for additional objective indicators for early assessment

of inflammatory activity, disease severity, and the probable clinical course. In this context, FC may be considered a potential integrative biomarker that complements etiological diagnostics and supports clinical stratification and follow-up of patients with acute infectious diarrhea.

2. Distribution and clinical characteristics of patients by disease course and severity in acute infectious diarrhea

The clinical course of acute infectious diarrhea was analyzed based on the manifestations of the general toxico-infectious, upper dyspeptic, and diarrheal syndromes in order to enable subsequent interpretation of FC levels according to disease severity. In all patients, the onset was acute and accompanied by a pronounced general toxico-infectious syndrome. Fever ≥39.0 °C was observed more frequently in bacterial acute infectious diarrhea (51.9%) than in viral acute infectious diarrhea (18.2%), and the difference in the distribution of temperature categories was statistically significant ($p < 0.001$) (Table 7).

Table 7. Distribution of patients by body temperature at admission

Body temperature (°C)	**Viral acute infectious diarrhea (n=55), n (%)**	**Bacterial acute infectious diarrhea (n=52), n (%)**
37.1–38.4	25 (45.5%)	10 (19.2%)
38.5–38.9	20 (36.4%)	15 (28.9%)
≥39.0	10 (18.2%)	27 (51.9%)
p value	p < 0.001	

Vomiting was observed significantly more frequently in viral acute infectious diarrhea (81.8%) compared with bacterial acute infectious diarrhea (38.5%) ($p < 0.001$). In viral acute infectious diarrhea, vomiting was more pronounced, with multiple episodes (≥5 within 24 hours) occurring more often in this group (22.2% vs. 10.0%; $p = 0.009$). The duration of symptoms did not differ significantly between the groups ($p = 0.16$) (Table 8).

Table 8. Characteristics of vomiting in children with viral and bacterial acute infectious diarrhea

Parameter	Viral acute infectious diarrhea (n=45)	Bacterial acute infectious diarrhea (n=20)	p
Duration of vomiting			
1 day	18 (40.0%)	13 (65.0%)	p > 0.05
2 days	17 (37.8%)	5 (25.0%)	
≥3 days	10 (22.2%)	2 (10.0%)	
Maximum number of vomiting episodes within 24 hours			
1 episode	10 (22.2%)	12 (60.0%)	p < 0.05
2–4 episodes	25 (55.6%)	6 (30.0%)	
≥5 episodes	10 (22.2%)	2 (10.0%)	

Diarrhea was present in all patients; however, in bacterial acute infectious diarrhea it was characterized by a more prolonged course and a higher proportion of cases lasting ≥5 days. Pathological stool components, including mucus and/or blood, were detected significantly more frequently in bacterial acute infectious diarrhea (80.8%) than in viral acute infectious diarrhea (18.2%) ($p < 0.001$). Abdominal pain (90.4% vs. 65.5%; $p = 0.002$) and tenesmus (19.2% vs. 3.6%; $p = 0.01$) were also observed more frequently in bacterial acute infectious diarrhea, whereas the maximum number of bowel movements within 24 hours did not differ substantially between the groups (Table 9).

Table 9. Characteristics of diarrhea in children with viral and bacterial acute infectious diarrhea

Parameter	Viral acute infectious diarrhea (n=55), n (%)	Bacterial acute infectious diarrhea (n=52), n (%)	p
Duration of diarrhea			
1–4 days	35 (63.6%)	12 (23.1%)	p < 0.001
5 days	13 (23.6%)	25 (48.1%)	
≥6 days	7 (12.7%)	15 (28.8%)	
Maximum number of diarrheal stools within 24 hours			
1–3 stools	14 (25.5%)	12 (23.1%)	p > 0.05
4–5 stools	20 (36.4%)	18 (34.6%)	
≥6 stools	21 (38.2%)	22 (42.3%)	
Presence of pathological stool components			
With pathological components (mucus, blood)	10 (18.2%)	42 (80.8%)	p < 0.001
Without pathological components (mucus, blood)	45 (81.8%)	10 (19.2%)	
Abdominal pain			
Yes	36 (65.5%)	47 (90.4%)	p = 0.002
No	19 (34.5%)	5 (9.6%)	
Tenesmus			

Parameter	Viral acute infectious diarrhea (n=55), n (%)	Bacterial acute infectious diarrhea (n=52), n (%)	p
Yes	2 (3.6%)	10 (19.2%)	p = 0.01
No	53 (96.4%)	42 (80.8%)	

The clinical presentation in all children included in the study was characterized by an acute onset accompanied by pronounced systemic symptoms, a typical feature of acute infectious diarrhea in childhood, in which the systemic response to the infectious agent often precedes or accompanies gastrointestinal manifestations. Higher body temperature values observed in patients with bacterial acute infectious diarrhea were statistically significant and are consistent with findings in the literature indicating a more pronounced systemic inflammatory response in infections caused by bacterial pathogens, related to their invasive properties and toxin production.

Vomiting was observed significantly more frequently and with greater intensity in viral acute infectious diarrhea, which is consistent with the clinical profile of viral gastroenteritis described in the literature. In contrast, bacterial acute infectious diarrhea was characterized by a more prolonged course of diarrhea and was significantly more often accompanied by pathological stool findings, including mucus and/or blood, as well as abdominal pain and tenesmus—features typical of infections caused by invasive bacterial pathogens. The frequency of bowel movements did not differ substantially between the groups. The observed clinical differences between viral and bacterial acute infectious diarrhea may assist in early etiological differentiation.

The degree of dehydration was assessed based on clinical signs and was used as a criterion for stratification according to disease severity. Patients with first-degree dehydration, who are typically managed on an outpatient basis, were not included in the hospitalized cohort. In both groups, moderate dehydration (grade II) predominated, being observed in 76.4% of children with viral acute infectious diarrhea and in 73.1% of those with bacterial acute infectious diarrhea (Table 10).

Table 10. Distribution of patients by degree of dehydration

Degree of dehydration	Viral acute infectious diarrhea (n=55), n (%)	Bacterial acute infectious diarrhea (n=52), n (%)	p
Grade II (moderate, 5–9%)	42 (76.4%)	38 (73.1%)	p > 0.05
Grade III (severe, ≥10%)	13 (23.6%)	14 (26.9%)	

Disease severity was assessed using the modified Vesikari scale, and patients were categorized into subgroups with moderate and severe clinical courses. In the bacterial acute infectious diarrhea group, 46.2% of the children had a moderate form and 53.8% had a severe form, whereas in viral acute infectious diarrhea the respective proportions were 72.7% with a moderate course and 27.3% with a severe course. The proportion of severe cases was significantly higher in bacterial acute infectious diarrhea (p = 0.005) (Table 11).

Table 11. Distribution of patients by severity of acute infectious diarrhea

Group	Moderate course, n (%)	Severe course, n (%)	p
Viral acute infectious diarrhea	40 (72.7%)	15 (27.3%)	0.005
Bacterial acute infectious diarrhea	24 (46.2%)	28 (53.8%)	

Severe forms of acute infectious diarrhea were observed more frequently in bacterial acute infectious diarrhea, reflecting a more pronounced inflammatory response and a higher risk of complications in bacterial infections. In contrast, moderate forms predominated in viral acute infectious diarrhea, consistent with the self-limiting nature of most viral gastroenteritis cases.

Additional analysis by etiological agents within viral acute infectious diarrhea demonstrated a predominance of rotavirus etiology in both moderate and severe cases, with a similar pattern observed in adenoviral enteritis (Fig. 6).

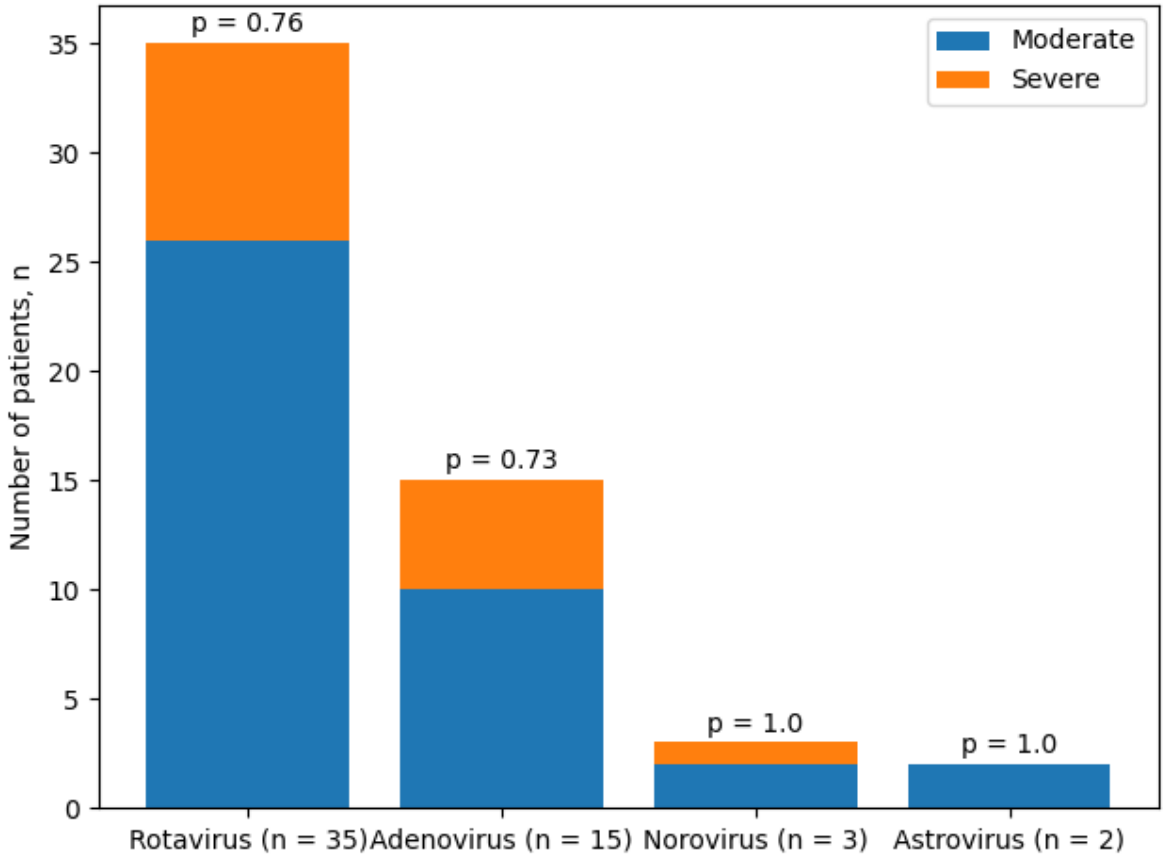

Fig.6. Distribution of patients with viral acute infectious diarrhea by etiology and disease severity (Vesikari scale)

The analysis showed that severe clinical forms were more frequent in bacterial acute infectious diarrhea (53,8%), highlighting the higher clinical risk associated with bacterial etiology of acute infectious diarrhea. The largest absolute number of severe cases was observed in *Salmonella* infections, whereas in campylobacteriosis and CDI a relatively even distribution between moderate and severe forms was noted. In shigellosis, a clear predominance of severe forms was observed, consistent with the invasive potential of *Shigella* spp. (fig.7). In yersiniosis, a tendency toward a more severe clinical course was also observed; however, the limited number of cases does not allow definitive conclusions to be drawn.

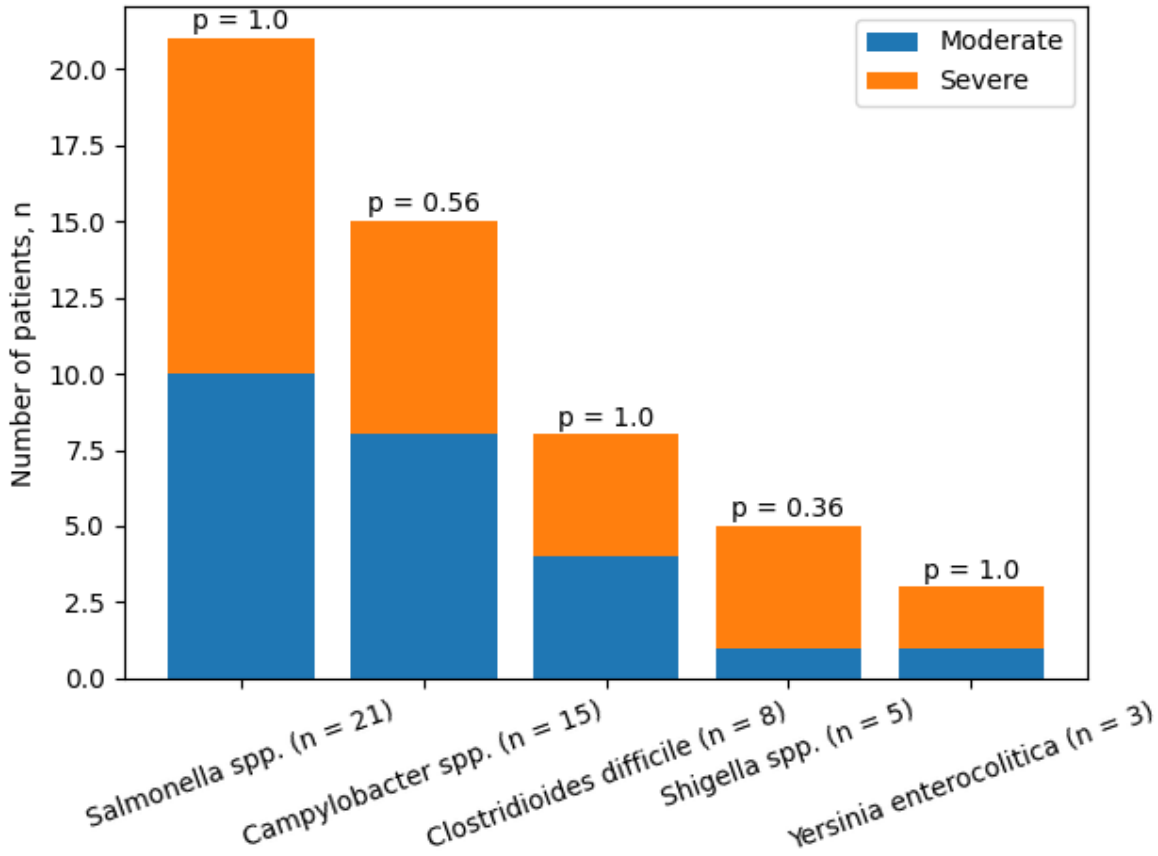

Fig. 7. Distribution of patients with bacterial acute infectious diarrhea by etiology and disease severity (Vesikari scale)

In conclusion, the clinical assessment of disease severity in children with acute infectious diarrhea, based on the analysis of symptoms, degree of dehydration, and the Vesikari scale, demonstrated a higher proportion of severe forms in bacterial acute infectious diarrhea compared with viral acute infectious diarrhea. This stratification provides the basis for the subsequent analysis of FC levels in patients with viral acute infectious diarrhea, bacterial acute infectious diarrhea, and in the control group, as well as for evaluating its diagnostic and prognostic value as a marker of intestinal inflammation.

3. Comparative analysis of FC levels in patients with viral acute infectious diarrhea, bacterial acute infectious diarrhea, and a control group

Considering the observed differences in clinical severity between viral acute infectious diarrhea and bacterial acute infectious diarrhea, the distribution of FC values was analyzed in children with viral acute infectious diarrhea, bacterial acute infectious diarrhea, and in the control group in order to evaluate the diagnostic and prognostic value of this marker in acute infectious diarrhea. The distribution of FC values across the study groups is presented in Fig. 8.

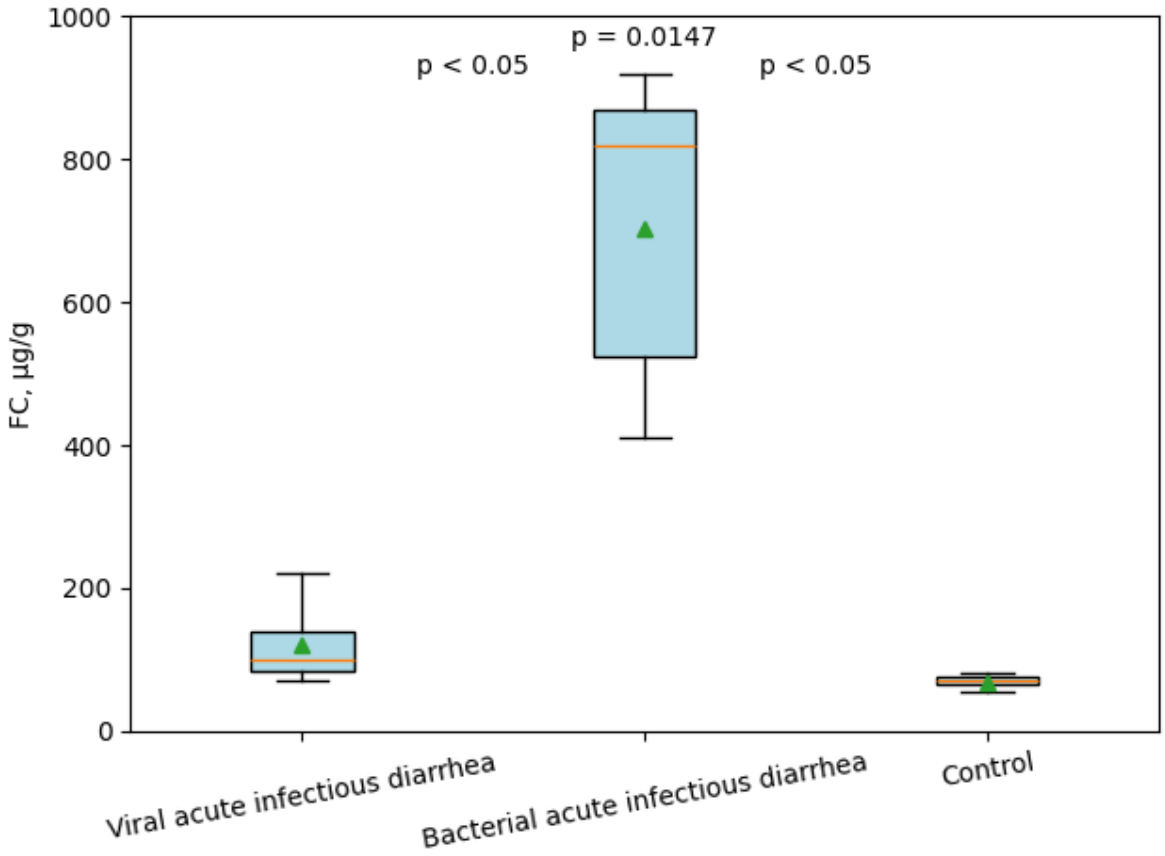

Fig. 8. Distribution of FC values in patients with viral acute infectious diarrhea, bacterial acute infectious diarrhea, and in the control group. The box represents the interquartile range (IQR), the horizontal line indicates the median, X marks the mean value, and the whiskers represent the minimum and maximum values excluding outliers. The p-values for the between-group comparisons are indicated above the brackets.

The mean FC levels in children with bacterial acute infectious diarrhea were significantly higher compared with those observed in viral acute infectious diarrhea (712.65 µg/g vs 108.18 µg/g). A similar pattern was observed for the median values, which were 827.04 µg/g in bacterial acute infectious diarrhea and 78.86 µg/g in viral acute infectious diarrhea. These findings demonstrate a markedly higher expression of FC in bacterial acute infectious diarrhea. The results are consistent with findings from international studies reporting significantly higher FC levels in bacterial acute infectious diarrhea compared with viral gastroenteritis, supporting the diagnostic value of this marker in distinguishing between viral and bacterial etiology.

The control group was characterized by the lowest range of FC values. In 80% (24/30) of the participants, the recorded levels did not exceed 80 µg/g, while in the remaining 20% (6/30) the values were above this threshold. The minimum FC value in the control group was 56.52 µg/g and the maximum was 85.21 µg/g, indicating a narrow range of variability. No statistically significant differences in FC levels were observed according to sex or age subgroups within the control group, which is consistent with the expected physiological characteristics in healthy children in early childhood.

To determine the upper reference limit of FC in the present study, a descriptive statistical analysis was performed using data from the control group. The upper reference value was defined as the 95th percentile, which corresponded to 84.19 µg/g. In the control population, the mean FC level was 70.94 µg/g, the median was 69.21 µg/g, and the interquartile range was 10.04 µg/g, indicating low variability and a narrow distribution of values. The upper reference limit established in this way provides a clinically applicable framework for the interpretation of FC results and serves as a basis for distinguishing between normal and pathologically elevated FC levels in children with acute infectious diarrhea.

FC values were categorized as normal (≤84.19 µg/g) and pathologically elevated (>84.19 µg/g). When comparing children with viral acute infectious diarrhea and the control group, a statistically significant difference in the frequency distribution was observed (χ^2 = 5.95; p = 0.0147), with pathologically elevated values occurring more frequently in viral acute infectious diarrhea. A statistically significant difference was also found when comparing bacterial acute infectious diarrhea with the control group (χ^2 = 69.61; p < 0.001), with a markedly higher frequency of pathological values in bacterial acute infectious

diarrhea. The comparison between viral acute infectious diarrhea and bacterial acute infectious diarrhea also revealed a statistically significant difference ($\chi^2 = 50.54$; $p < 0.001$), with pathologically elevated FC values being significantly more frequent in bacterial acute infectious diarrhea. These results confirm the diagnostic value of FC for distinguishing between viral and bacterial etiology of acute infectious diarrhea.

The analysis of FC in children with acute infectious diarrhea demonstrated clear and statistically significant differences between viral acute infectious diarrhea, bacterial acute infectious diarrhea, and the control group. In bacterial acute infectious diarrhea, markedly higher concentrations and greater variability were observed, whereas in viral acute infectious diarrhea a moderate but more frequent pathological increase was noted compared with healthy controls. These findings confirm FC as a sensitive and clinically applicable non-invasive marker for assessing intestinal inflammation and for distinguishing the etiology of acute infectious diarrhea in children, and provide the rationale for the next stage of the analysis—an etiology-oriented evaluation of FC values.

4. FC levels in patients with different etiologies of viral acute infectious diarrhea and bacterial acute infectious diarrhea

The etiological analysis begins with an evaluation of FC levels in patients with viral acute infectious diarrhea, stratified according to the identified pathogen. The results for the individual etiological agents are presented in Table 12.

Table 12. Mean FC levels in patients with viral acute infectious diarrhea according to the etiological agent

Etiological agent	FC (µg/g)
Rotavirus (n = 35)	111.5 ± 50.53
Adenovirus (n = 15)	84.42 ± 25.89
Norovirus (n = 3)	111.32 ± 60.55
Astrovirus (n = 2)	82.47 ± 7.95

The analysis of FC levels in children with viral acute infectious diarrhea caused by different viral agents revealed moderate etiological differences. The highest mean FC concentrations were observed in rotavirus (111.5 ± 50.53 µg/g) and norovirus gastroenteritis (111.32 ± 60.55 µg/g), suggesting a more pronounced local inflammatory response of the intestinal mucosa in these infections. These findings are consistent with international reports demonstrating a wider range and higher FC levels in rotavirus- and norovirus-associated gastroenteritis. In the norovirus subgroup, despite the limited number of cases, considerable variability in FC values was observed. Lower FC levels were recorded in adenovirus (84.42 ± 25.89 µg/g) and astrovirus infections (82.47 ± 7.95 µg/g). For these etiological agents, the available literature provides limited systematic data on FC levels; therefore, the interpretation relies mainly on the known pathophysiological mechanisms and clinical course, which suggest a more moderate and non-invasive inflammatory response. It should also be noted that the small number of cases does not allow definitive conclusions to be drawn.

The differences in FC levels among the viral etiological groups were assessed using the Kruskal–Wallis test ($H = 7.79$; $p = 0.0505$). Owing to the borderline p-value and the limited size of some subgroups, no statistically significant differences were identified among the individual viral pathogens. These findings support the usefulness of FC as a marker of intestinal inflammation and its severity in viral acute infectious diarrhea, but indicate that it cannot be used as a standalone criterion for reliable etiological differentiation among viral agents.

FC levels in children with bacterial acute infectious diarrhea, stratified by etiological agent, are presented in Table 13 as mean values with standard deviations.

Table 13. Mean FC levels in children with bacterial acute infectious diarrhea according to the etiological agent

Bacterial pathogen	**FC (µg/g)**
Salmonella spp. (n = 21)	713.14 ± 187.23
Campylobacter spp. (n = 15)	679.23 ± 189.9

Bacterial pathogen	FC (µg/g)
Clostridioides difficile (n = 8)	686.12 ± 205.31
Shigella spp. (n = 5)	821.53 ± 105.23
Yersinia enterocolitica (n = 3)	765.5 ± 140.94

The analysis of FC levels in children with bacterial acute infectious diarrhea, stratified by bacterial pathogen, demonstrated distinct patterns in marker levels. The highest mean FC concentrations were observed in infections caused by *Shigella spp.* (821.53 ± 105.23 µg/g), reflecting an intense inflammatory process in the intestinal mucosa. This finding is consistent with the pathophysiology of shigellosis, which frequently presents with hemocolitis and pronounced neutrophilic infiltration. Relatively high FC levels were also recorded in infections caused by *Yersinia enterocolitica* (765.5 ± 140.94 µg/g). These results may be explained by the invasive nature of the pathogen and its tropism for the ileocecal region, where a strong local inflammatory response is induced. The often prolonged or relapsing clinical course may contribute to persistently elevated FC levels and the considerable variability observed. Comparable FC levels were found in salmonellosis (713.14 ± 187.23 µg/g), reflecting the active invasion of *Salmonella spp.* into enterocytes and the subsequent development of acute exudative inflammation with marked neutrophil migration. In children with campylobacteriosis, FC levels (679.23 ± 189.9 µg/g) showed a similar mean value but considerable variability, likely related to differences in the degree of mucosal injury and inflammatory activity. A similar heterogeneity was observed in infections caused by *Clostridioides difficile* (686.12 ± 205.31 µg/g), reflecting the broad spectrum of clinical and morphological involvement, ranging from mild disease to severe colitis with extensive mucosal damage.

Despite the observed differences in mean values, the Kruskal–Wallis test showed no statistically significant differences in FC levels among the bacterial groups ($H = 1.87$; $p = 0.759$). The p-value exceeds the threshold for statistical significance and therefore does not indicate the presence of meaningful etiological differences. Regardless of the pathogen—*Salmonella spp.*, *Campylobacter spp.*, *Clostridioides difficile*, *Shigella spp.*, or *Yersinia enterocolitica*—the distribution of FC values remained broadly comparable, without a clearly defined pathogen-specific pattern. These findings indicate that FC is a suitable

marker of the presence and intensity of intestinal inflammation in bacterial acute infectious diarrhea; however, its value for reliable etiological differentiation is limited and the results should be interpreted in the context of clinical and microbiological findings.

A limitation of the present analysis is the small size of some etiological subgroups, which reduces the statistical power and warrants cautious interpretation of the results.

5. FC levels in acute infectious diarrhea according to the severity of the clinical course

The relationship between FC levels and the severity of acute infectious diarrhea was analyzed in the context of the third research objective by comparing the following groups: viral acute infectious diarrhea–moderate, viral acute infectious diarrhea–severe, bacterial acute infectious diarrhea–moderate, and bacterial acute infectious diarrhea–severe. Descriptive statistical analysis was performed, including calculation of mean and median values, standard deviation, minimum and maximum values, and interquartile range (IQR). The results are presented in Table 14.

Table 14. Descriptive analysis of FC values (μg/g) in patients with different severity of acute infectious diarrhea

Group	Mean	Median	Standard deviation	Min	Max	Interquartile range (IQR)
Viral acute infectious diarrhea – moderate	78.46	78.60	4.19	70.14	86.77	5.64
Viral acute infectious diarrhea – severe	168.61	177.08	40.54	88.09	233.04	54.59
Bacterial acute infectious diarrhea – moderate	525.27	522.42	63.06	410.81	658.08	82.16
Bacterial acute infectious diarrhea – severe	873.25	874.82	26.38	823.93	918.12	31.55
Control	70.94	69.21	7.88	56.52	85.21	10.04

The descriptive analysis demonstrated clear differences in FC levels across the study groups. The lowest values were observed in the control group (70.94 µg/g; median 69.21 µg/g) and in viral acute infectious diarrhea–moderate (78.46 µg/g; median 78.60 µg/g), both characterized by low variability. In viral acute infectious diarrhea–severe, FC levels were higher (168.61 µg/g; median 177.08 µg/g) and showed greater dispersion. Substantially higher FC levels were found in bacterial acute infectious diarrhea, with mean values of 525.27 µg/g in bacterial acute infectious diarrhea–moderate and the highest levels in bacterial acute infectious diarrhea–severe (873.25 µg/g). The Kruskal–Wallis test demonstrated statistically significant differences among the groups ($H = 120.10$; $p = 5.09 \times 10^{-25}$). The distribution of FC values across the study groups is illustrated in Fig. 9.

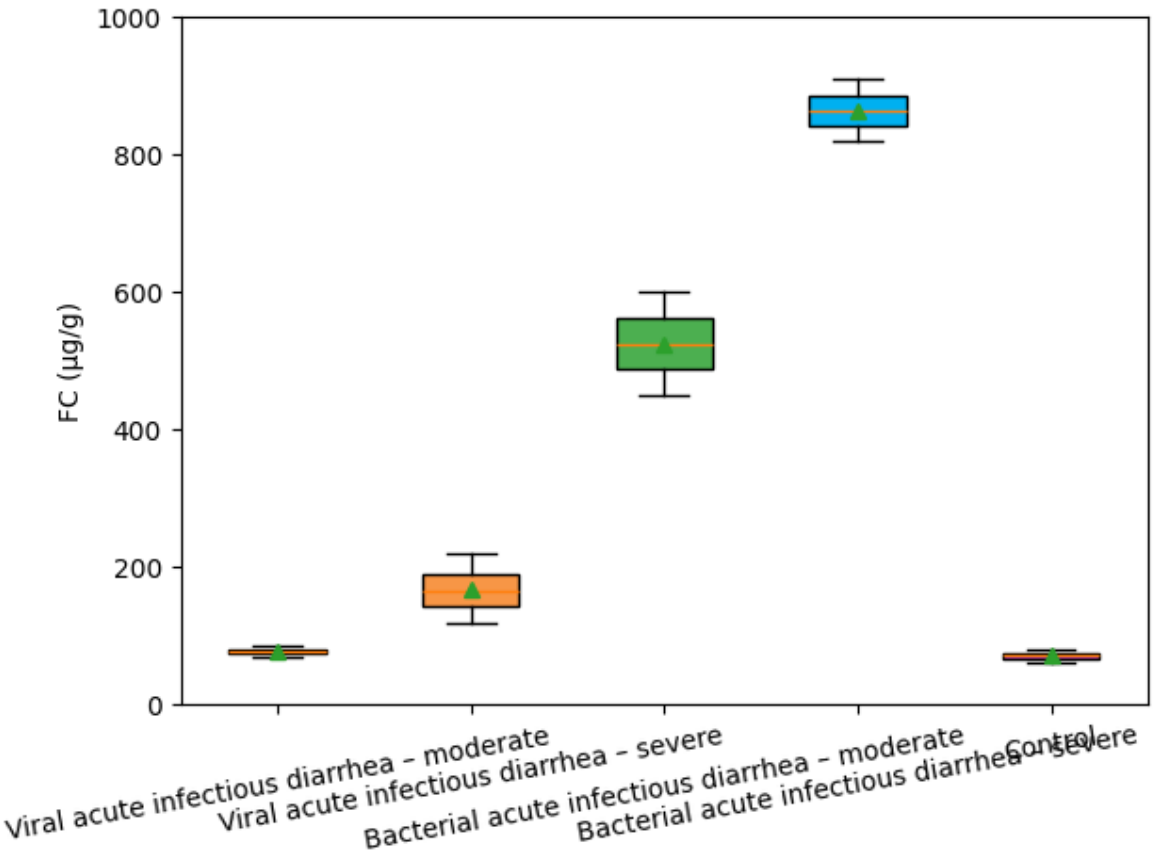

Fig. 9. Distribution of FC values in patients with different severity of acute infectious diarrhea. The box represents the interquartile range (IQR), the horizontal line indicates the median, X marks the mean value, the whiskers represent the minimum and maximum values excluding outliers, and the dots represent individual values outside the IQR.

Previous studies consistently demonstrate an association between FC levels and the severity of acute infectious diarrhea. Several pediatric studies have shown that FC levels increase with increasing clinical severity and are significantly higher in bacterial compared with viral acute infectious diarrhea. These observations are consistent with the present findings, which confirm the relationship between etiology, disease severity, and FC levels. The statistically significant differences identified by the Kruskal–Wallis test indicate that FC levels discriminate between viral and bacterial acute infectious diarrhea and correlate with disease severity. The highest values were observed in bacterial acute infectious diarrhea–severe, followed by bacterial acute infectious diarrhea–moderate and viral acute infectious diarrhea–severe, while the lowest levels were found in the control group and viral acute infectious diarrhea–moderate. In contrast, no statistically significant differences were observed among individual etiological agents within the viral and bacterial groups. These findings support the role of FC as a sensitive marker of intestinal inflammation, reflecting disease severity and highlighting its clinical utility in the assessment of acute infectious diarrhea, particularly in bacterial forms.

The relationship between etiological agent and disease severity in viral acute infectious diarrhea was analyzed by examining the distribution of FC values according to viral agent and clinical severity (Fig. 10).

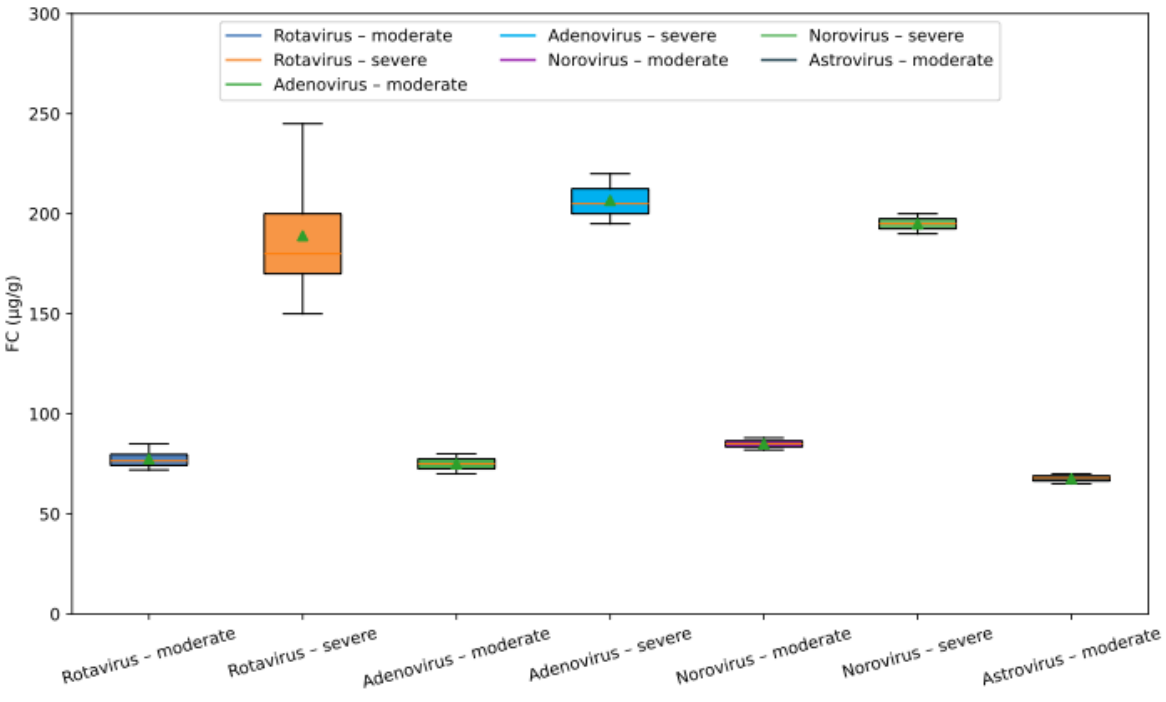

Fig. 10. FC values in children with viral acute infectious diarrhea according to etiology and disease severity. The box represents the interquartile range (IQR), the horizontal line indicates the median, X marks the mean value, the whiskers represent the minimum and maximum values excluding outliers, and the dots represent individual values outside the IQR.

The analysis of FC levels in children with different viral etiologies of acute infectious diarrhea revealed marked variability in the inflammatory response between moderate and severe forms. Although distinct value ranges were observed for each etiology, substantial within-group heterogeneity was evident, particularly in severe cases. In rotavirus infections, FC values ranged from below 75 µg/g in moderate cases to above 240 µg/g in severe disease. A similar pattern was observed in adenovirus enteritis, where values in moderate cases were predominantly below 85 µg/g, while in severe cases they reached and exceeded 220 µg/g. The limited number of cases for norovirus and astrovirus infections precludes definitive conclusions; however, higher FC levels were observed in severe norovirus cases, whereas in astrovirus infections all values remained below 70 µg/g. Overall, the within-group analysis indicates a consistent trend toward higher FC levels in severe forms of viral acute infectious diarrhea.

These findings are consistent with published data showing that rotavirus and adenovirus gastroenteritis are associated with a wide range of FC values and a tendency toward higher levels in more severe disease. The within-group variability observed in the present study, particularly in severe cases, reflects the heterogeneity of the inflammatory response. For norovirus and astrovirus infections, available evidence remains limited; therefore, the present findings contribute to the existing knowledge on FC levels in these etiologies. Overall, these observations support the role of FC as a marker of inflammatory activity and disease severity in viral acute infectious diarrhea in children.

For a more detailed characterization of FC levels in children with bacterial acute infectious diarrhea, FC was analyzed according to the bacterial pathogen and disease severity. Figure 11 shows individual FC values stratified by etiology and severity (moderate and severe). This visual representation illustrates the variability of FC levels within each bacterial group and their association with clinical severity, highlighting the marked within-group heterogeneity of the inflammatory response and complementing the quantitative analysis.

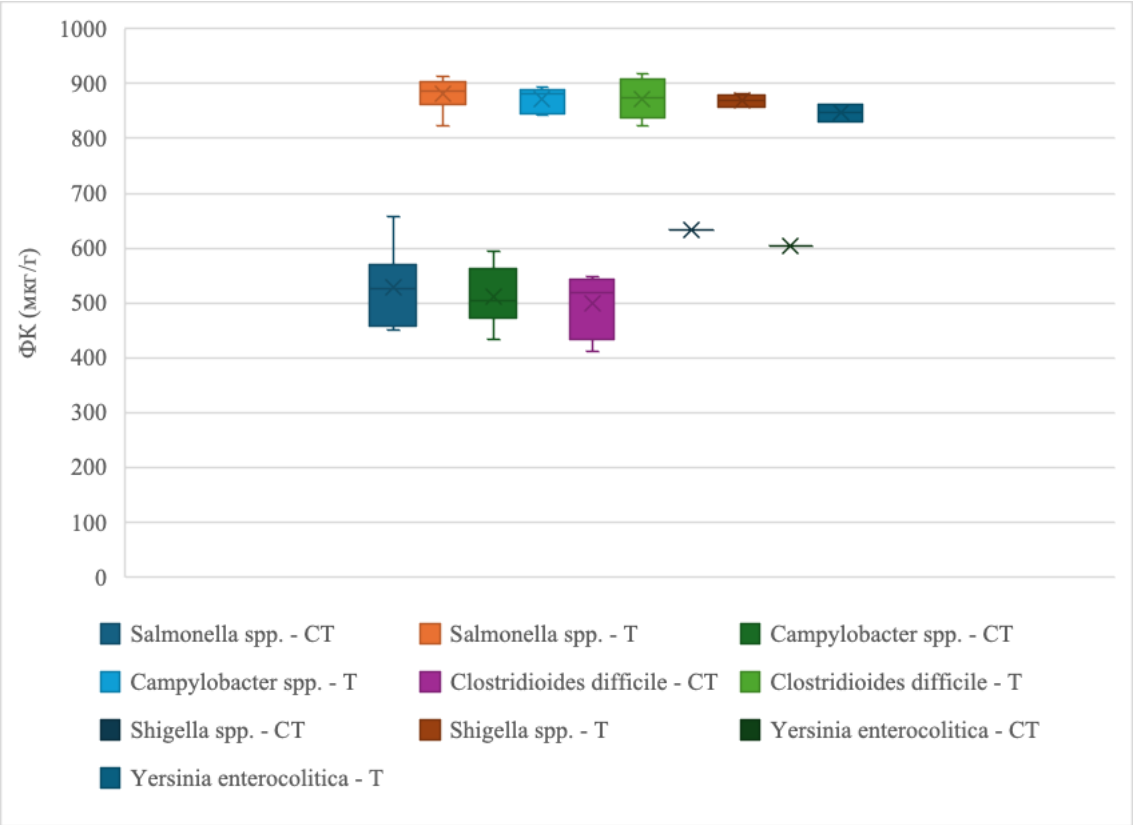

Figure 11. FC levels in children with bacterial acute infectious diarrhea according to etiology and disease severity. The box represents the interquartile range (IQR), the horizontal line indicates the median, X denotes the mean, the whiskers represent the minimum and maximum values excluding outliers, and the points indicate individual values outside the IQR.

The analysis of FC levels in children with bacterial acute infectious diarrhea provides a detailed assessment of the inflammatory response according to the bacterial pathogen and clinical severity. The findings indicate high inflammatory activity and a clear distinction between moderate and severe forms. In salmonellosis, FC levels in moderate cases ranged from 451 to 658 µg/g, whereas in severe cases all values exceeded 823 µg/g, reaching up to 913 µg/g. In campylobacteriosis, FC levels ranged from 435 to 594 µg/g in moderate forms, while in severe cases they were typically above 860 µg/g. In Clostridioides difficile infection (CDI), FC values ranged from 410 to 547 µg/g in moderate cases, whereas in severe forms they exceeded 824 µg/g, with some of the highest values in the cohort observed in this group. Shigellosis and yersiniosis were represented by a limited number of cases, precluding definitive conclusions. Nevertheless, both etiologies showed a consistent trend toward higher FC levels in severe forms. In shigellosis, all recorded values in severe cases exceeded

856 µg/g, while in yersiniosis they exceeded 829 µg/g, indicating a pronounced inflammatory response. Overall, across all bacterial pathogens studied, severe forms of bacterial acute infectious diarrhea were characterized by substantially higher FC levels, clustering within the range of 823–918 µg/g and showing relatively limited variability. These findings support the use of FC as a marker of inflammatory intensity and for clinical severity stratification in bacterial acute infectious diarrhea.

The results of the analysis of FC levels in children with bacterial acute infectious diarrhea were compared with available international data. The observed values in salmonellosis are consistent with published reports showing markedly elevated FC levels in severe cases, often exceeding 700 µg/g, supporting the association between inflammatory intensity and disease severity. In Clostridioides difficile infection (CDI), lower FC levels were observed in moderate cases and clearly higher levels in severe forms, consistent with reports of values reaching up to approximately 1000 µg/g in severe disease. These findings further support the relationship between FC levels and clinical severity in bacterial acute infectious diarrhea.

For campylobacteriosis, shigellosis, and yersiniosis, the available international literature is limited and fragmented, particularly regarding the relationship between FC levels and clinical severity. In the present study, severe forms across all examined etiologies were consistently associated with markedly higher FC levels than moderate cases. The lack of directly comparable data precludes direct comparisons; however, these findings contribute to expanding current evidence on the inflammatory profile of these forms of bacterial acute infectious diarrhea. Overall, the analysis demonstrates a consistent trend toward higher FC levels in severe disease, regardless of the bacterial pathogen, supporting the use of FC as a marker of inflammatory intensity and clinical severity.

Analysis of FC levels according to clinical severity in acute infectious diarrhea shows that the marker reflects the intensity of intestinal inflammation and allows for reliable severity stratification. In viral acute infectious diarrhea, FC levels increase in severe cases, with greater variability and lower absolute values. In bacterial acute infectious diarrhea, FC levels are substantially higher overall, and in severe cases they tend to cluster within a narrower range, highlighting the strong clinical value of the marker.

6. Assessment of changes in fecal calprotectin levels during hospital treatment in children with acute infectious diarrhea

FC is also clinically useful for monitoring changes during hospital treatment. Evaluating changes over time allows assessment of the resolution of intestinal inflammation and the recovery course in children with acute infectious diarrhea.

Following hospitalization, management of patients with acute infectious diarrhea was based on supportive care, including rehydration, correction of fluid, electrolyte, and acid–base imbalances, dietary measures, and support of the intestinal microbiota. Rehydration was administered using glucose–electrolyte solutions, adjusted to the degree of dehydration and ongoing losses.The most common metabolic disturbance was metabolic acidosis, which was corrected with sodium bicarbonate according to the Astrup formula; hypokalemia was treated with potassium chloride based on age and body weight. Early reintroduction of enteral feeding was a key component of management, with recommendations for an easily digestible lactose-free diet or breastfeeding. All patients received a probiotic containing Saccharomyces boulardii at standard pediatric doses. In some children with viral acute infectious diarrhea and severe disease, empirical antibiotic therapy was initiated due to the inability to promptly exclude bacterial etiology. In bacterial acute infectious diarrhea, empirical antibiotic therapy was administered and subsequently adjusted according to microbiological results. This approach ensured relative treatment homogeneity and allowed changes in FC levels to be interpreted as reflecting the course of the inflammatory process during treatment.

Within this relatively standardized treatment approach, FC levels were reassessed during hospitalization following the initial measurement obtained within the first 24 hours after admission. In children with viral acute infectious diarrhea, FC levels were re-measured at a mean of 3.35 ± 1.37 days after admission. Comparison between baseline and follow-up measurements showed a statistically significant decrease in FC levels, reflecting resolution of the inflammatory process. The difference was evaluated using a paired t-test, and the results are presented in Table 15.

Table 15. Descriptive analysis of changes in FC levels in patients with viral acute infectious diarrhea

FC (µg/g)	Mean	Median	Standard deviation	Min	Max	Interquartile range (IQR)
First sample	108.18	78.86	53.33	65.06	243.98	83.54
Second sample	71.50	71.30	20.44	50.02	198.71	17.90

The comparison between baseline and follow-up measurements showed a clear decrease in FC levels over time. The mean value decreased from 108.18 µg/g to 71.50 µg/g, with a similar pattern observed for the median (78.86 µg/g vs 71.30 µg/g), reflecting resolution of the inflammatory process. The higher standard deviation at admission (53.33 µg/g vs 20.44 µg/g) and the wider interquartile range (83.54 µg/g vs 17.90 µg/g) indicate greater variability and more pronounced disease activity in the early phase, followed by a more homogeneous distribution of values during recovery. In 96.36% of patients (n = 53), FC levels normalized below the pathological threshold during follow-up. In two cases (3.64%), values remained above 84.19 µg/g, likely reflecting slower resolution of inflammation or individual variability in mucosal recovery. Comparison with the control group showed that mean FC levels after follow-up in children with viral acute infectious diarrhea (71.50 µg/g) were nearly identical to those in healthy children (70.94 µg/g). This finding confirms normalization of the marker below the pathological threshold and indicates that, in most patients, inflammatory activity returns to levels comparable to the control population, underscoring the value of serial FC measurements in assessing recovery.

Changes in FC levels in viral acute infectious diarrhea were analyzed by etiological agent through comparison of baseline and follow-up measurements, as shown in Figure 12.

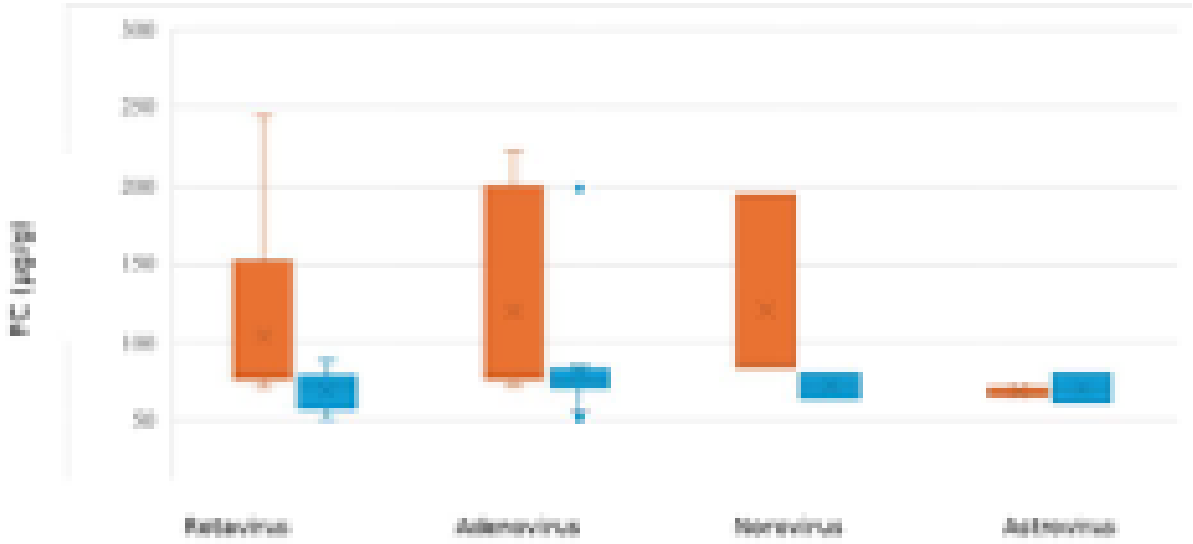

Figure 12. Changes in FC levels in children with viral acute infectious diarrhea according to etiological agent (baseline and follow-up measurements). The box represents the interquartile range (IQR), the horizontal line indicates the median, X denotes the mean, the whiskers represent the minimum and maximum values excluding outliers, and the points indicate individual values outside the IQR.

Across all examined viral etiologies, FC levels decreased at follow-up. In rotavirus infection, baseline values showed considerable variability, with some patients exceeding 200 µg/g, followed by a decline and normalization below the pathological threshold of 84.19 µg/g. In adenoviral infection, baseline FC levels were also elevated, exceeding 200 µg/g, with a more moderate decrease over time, and in some cases values remained above the threshold. In norovirus infection, initial levels exceeded 190 µg/g, with normalization observed at follow-up in all cases. In astrovirus infection, FC levels did not exceed the pathological threshold and remained stable over time. Regardless of etiology, follow-up measurements showed a shift in the median toward lower values and a narrowing of the interquartile range, reflecting resolution of the inflammatory process.

A paired t-test was used to assess the statistical significance of changes in FC levels in children with viral acute infectious diarrhea. A statistically significant difference was observed between baseline and follow-up measurements ($t = 4.997$; $p = 6.47 \times 10^{-6}$), with a mean difference of 36.68 µg/g. The 95% confidence interval ranged from 21.97 to 51.40 µg/g, confirming a marked decline in FC levels during hospitalization and supporting its use for dynamic monitoring in pediatric viral acute infectious diarrhea. The observed decrease in FC reflects both the self-limiting nature of viral infections and the effect of treatment. FC

should therefore be interpreted not as a direct measure of therapeutic efficacy, but as a sensitive indicator of inflammatory regression and early restoration of intestinal barrier function.

The rapid decline in FC levels in viral acute infectious diarrhea reflects an acute, predominantly superficial inflammatory response, characterized by limited neutrophil activation and preserved structural integrity of the intestinal mucosa. In rotavirus and norovirus infections, this is manifested by a moderate, variable, but transient increase in FC levels followed by rapid normalization, whereas in astrovirus infections the absence of significant neutrophil activation corresponds to consistently stable marker levels. In contrast, adenoviral enteritis is associated with more prolonged epithelial injury and delayed resolution of inflammation, reflected in a wider range and a less pronounced decrease in FC during follow-up. In most published studies, FC levels in acute infectious diarrhea are assessed at a single time point, without dynamic evaluation, which limits the ability to capture the temporal evolution of the inflammatory response. The present study addresses this limitation by assessing FC at two consecutive time points during the early hospital course in children with viral acute infectious diarrhea. The observed decline in FC levels is consistent with the underlying pathophysiology of viral infections and highlights the role of FC as a sensitive tool for dynamic assessment of inflammatory activity and recovery of intestinal barrier function.

FC dynamics in patients with bacterial acute infectious diarrhea were assessed by repeat measurement performed at a mean of 2.55 ± 1.21 days after the initial test, in line with clinical practice and the duration of hospitalization. At follow-up, a general decrease in FC levels was observed in most patients. Descriptive statistics of FC levels in children with bacterial acute infectious diarrhea, assessed at baseline and follow-up, are presented in Table 16.

Table 16. Changes in FC levels over time in children with bacterial acute infectious diarrhea

FC (µg/g)	Mean	Median	Standard deviation	Min	Max	Interquartile range (IQR)
First sample	108.18	78.86	53.33	65.06	243.98	83.54
Second sample	71.50	71.30	20.44	50.02	198.71	17.90

During follow-up in children with bacterial acute infectious diarrhea, FC levels decreased, with the mean declining to 436.20 µg/g and the median to 416.15 µg/g. At repeat measurement, the standard deviation was 202.22 µg/g, while the interquartile range narrowed markedly, suggesting that extreme values were present in a small number of patients against an otherwise more homogeneous distribution. The minimum value decreased to 79.1 µg/g, whereas the maximum remained high at 954.96 µg/g, indicating heterogeneous patterns of recovery. FC levels below the pathological threshold of 84.19 µg/g were observed in 7.69% (n = 4) of patients, while in 92.31% (n = 48) the marker remained elevated, although often substantially reduced compared to baseline. The mean FC level at follow-up (436.20 µg/g) remained markedly higher than in the control group (70.94 µg/g), indicating that in most children the inflammatory process had not fully resolved during the early follow-up period. A paired t-test confirmed a statistically significant difference between baseline and follow-up measurements ($t = 11.82$; $p = 3.21 \times 10^{-16}$). These findings demonstrate a substantial reduction in FC levels and reflect a significant change in inflammatory activity during treatment.

Comparison of FC dynamics between viral and bacterial etiologies revealed distinct patterns of inflammatory response. In viral infections, follow-up values approached the reference range, whereas in bacterial cases FC levels remained markedly elevated, indicating a more prolonged and persistent inflammatory process. An increase in FC levels at follow-up was observed in 15.38% (n = 8) of children with bacterial infections, suggesting ongoing or worsening inflammation, an insufficient therapeutic response, or the development of complications, and should be interpreted in the appropriate clinical context. These findings underscore the value of serial FC measurements for the early identification of patients with an unfavorable course and the need for closer monitoring.

In the present study, a comparative analysis of FC dynamics in children with bacterial acute infectious diarrhea according to etiological agent was conducted (Table 17). Owing to greater heterogeneity in clinical and laboratory characteristics, as well as unequal subgroup sizes, the results are presented in tabular rather than graphical form, in contrast to those for viral cases.

Table 17. FC levels in children with bacterial acute infectious diarrhea according to etiological agent (I – baseline measurement; II – follow-up measurement)

Etiological agent	Sample	N	Mean	Median	SD	Min	Max	IQR
Salmonella spp.	I	21	713.14	823.93	187.23	451.40	912.99	355.02
Salmonella spp.	II	21	366.58	375.93	200.57	79.10	636.72	331.88
Campylobacter spp.	I	15	679.23	593.96	189.90	434.73	894.37	367.28
Campylobacter spp.	II	15	375.61	393.27	218.06	81.50	954.96	217.34
Clostridioides difficile	I	8	686.12	685.80	205.31	410.81	918.12	348.54
Clostridioides difficile	II	8	360.39	376.82	61.71	250.05	438.72	71.67
Shigella spp.	I	5	821.53	862.87	105.23	634.01	880.38	16.87
Shigella spp.	II	5	511.97	422.52	231.27	378.58	921.77	73.82
Yersinia enterocolitica	I	3	765.50	829.93	140.94	603.86	862.71	129.42
Yersinia enterocolitica	II	3	452.49	490.54	88.58	351.24	515.68	82.22

Analysis of FC in children with bacterial acute infectious diarrhea revealed etiology-specific differences in inflammatory dynamics. At the group level, FC levels decreased after initiation of therapy for most bacterial pathogens, whereas in a subset of patients they remained elevated or increased, reflecting a heterogeneous inflammatory course and severity.

Serial assessment of FC in children with bacterial acute infectious diarrhea showed a clear decline in FC levels across all examined etiological groups, including salmonellosis, campylobacteriosis, CDI, shigellosis, and yersiniosis. Despite this reduction, FC levels remained markedly above the reference range during the early follow-up period, reflecting persistent intestinal inflammatory activity. Results for less common etiological agents should be interpreted with caution due to the small subgroup sizes, which limit statistical power and preclude definitive etiology-specific conclusions.

Comparison of FC dynamics between viral and bacterial acute infectious diarrhea reveals distinct inflammatory profiles in both intensity and rate of resolution. In viral cases, follow-up measurements show a rapid and statistically significant decline in FC levels, with central tendency measures shifting toward values comparable to those of the control group, accompanied by reductions in standard deviation and interquartile range. In contrast, bacterial infections are characterized by higher baseline levels and a slower, incomplete, and heterogeneous decline, consistent with invasive pathogenesis and prolonged neutrophil activation.

In summary, FC monitoring provides clinically relevant information on the rate and pattern of recovery in acute infectious diarrhea. The marker should be interpreted as an indirect, non-specific indicator influenced by etiology, disease severity, disease stage, and the timing of assessment. This study captures the early dynamics of the inflammatory process and supports the use of FC as a prognostic marker of disease course.

7. Prognostic value of fecal calprotectin for clinical course in children with acute infectious diarrhea

In addition to its established diagnostic value in acute infectious diarrhea, the potential of FC as a prognostic marker for disease course was also evaluated. For this purpose, clinical course was defined based on the dynamics of the diarrheal syndrome, with duration of diarrhea used as the primary criterion for distinguishing between favorable and unfavorable outcomes. This approach is supported by the central role of diarrhea in the clinical presentation of acute infectious diarrhea, as well as by the pathophysiological relationship between symptom duration and the degree of intestinal inflammation reflected by FC levels.

In children with viral acute infectious diarrhea, the median duration of diarrhea was 3 days (mean 3.43 days; IQR 1.75). A duration of ≤3 days without complications was considered a favorable course, whereas ≥4 days and/or the presence of complications was considered unfavorable. The variable was dichotomized (0 – favorable; 1 – unfavorable).

Table 18. Descriptive statistics of systemic inflammatory markers in patients with viral acute infectious diarrhea at admission

Parameter	**CRP (mg/L)**	**Leukocytes (×10⁹/L)**	**ESR (mm/h)**
Mean (M)	9.79	6.34	12.64
Standard deviation (SD)	7.18	1.41	4.46
Minimum (Min)	0.01	3.35	6.00
Maximum (Max)	29.52	10.17	25.00
First quartile, Q1 (25th percentile)	4.22	5.22	10.00
Second quartile, Q2 (median, 50th percentile)	7.81	6.20	12.00
Third quartile, Q3 (75th percentile)	14.06	7.38	15.00

Descriptive analysis showed a mean CRP level of 9.79 mg/L (±7.18), with a range of 0.01–29.52 mg/L and a median of 7.81 mg/L, indicating that in most children values were within the reference range or only mildly elevated, while higher levels were observed in a smaller proportion of cases. Leukocyte counts showed a relatively homogeneous distribution (mean 6.34 × 10⁹/L; ±1.41; median 6.20 × 10⁹/L; range 3.35–10.17 × 10⁹/L), without evidence of significant leukocytosis. ESR averaged 12.64 mm/h (±4.46), with a median of 12 mm/h and a range of 6–25 mm/h, consistent with a mild to moderate acute-phase response and no evidence of pronounced systemic inflammation. In the context of published data,

these markers have limited diagnostic and prognostic value in viral acute infectious diarrhea. CRP is relatively more informative mainly for distinguishing bacterial from viral etiology, whereas ESR and leukocyte count have limited discriminatory value. Overall, this laboratory profile indicates that conventional systemic markers do not reliably reflect the intensity and dynamics of local intestinal inflammation, supporting the use of mucosa-oriented biomarkers such as FC to complement assessment in children with viral acute infectious diarrhea.

After the descriptive analysis, a univariate comparative analysis was carried out to examine the association between CRP, leukocyte count, and ESR and the predefined clinical course in viral acute infectious diarrhea. Normality was assessed using the Shapiro–Wilk test within each subgroup. CRP was normally distributed in both groups (favorable course: $W = 0.961$; $p = 0.210$; unfavorable: $W = 0.974$; $p = 0.890$), allowing the use of an independent samples t-test. A similar distribution was observed for leukocyte count ($W = 0.967$; $p = 0.323$ and $W = 0.976$; $p = 0.912$). For ESR, a borderline result was observed in the favorable subgroup ($W = 0.943$; $p = 0.052$), although the variable was considered approximately normally distributed. CRP levels were significantly higher in the unfavorable group (12.64 ± 8.33 mg/L vs. 8.47 ± 6.39 mg/L; $p = 0.040$). No significant differences were observed for leukocyte count ($p = 0.309$) or ESR ($p = 0.361$). Overall, these findings indicate that systemic markers have limited prognostic value in viral acute infectious diarrhea and support further evaluation of FC.

Before the comparative analysis of FC, normality of distribution was assessed using the Shapiro–Wilk test. In the subgroup with a favorable course, no deviation from normality was observed ($W = 0.977$; $p = 0.619$), whereas a significant deviation was identified in the unfavorable subgroup ($W = 0.857$; $p = 0.014$), justifying the use of the non-parametric Mann–Whitney test. A statistically significant difference between the two subgroups was demonstrated ($U = 37.0$; $p < 0.0001$), with FC levels significantly higher in patients with an unfavorable clinical course. Descriptive statistics showed higher central values and greater variability in this subgroup, reflecting increased heterogeneity and the presence of elevated baseline FC levels in cases with an unfavorable course (Table 19).

Table 19. FC levels according to clinical course in children with viral acute infectious diarrhea

Group	Number of patients (n)	Mean FC (µg/g)	SD	Median	Min	Max
Favorable course	43	79.53	38.87	77.16	51.00	213.05
Unfavorable course	14	134.66	63.94	81.02	52.82	243.98

To evaluate the prognostic potential of FC in viral acute infectious diarrhea, a ROC analysis was performed to assess the ability of baseline values to discriminate between favorable and unfavorable clinical course. The analysis demonstrated excellent discriminative performance (AUC = 0.94), indicating a high prognostic value of FC for predicting prolonged and complicated disease course (Figure 13). The optimal cut-off value was 151.07 µg/g. At this threshold, sensitivity reached 88.2% and specificity 100%, with no false-positive results observed among patients with a favorable course. The combination of high sensitivity and specificity supports the role of FC as a reliable tool for early risk stratification in children with viral acute infectious diarrhea at initial evaluation.

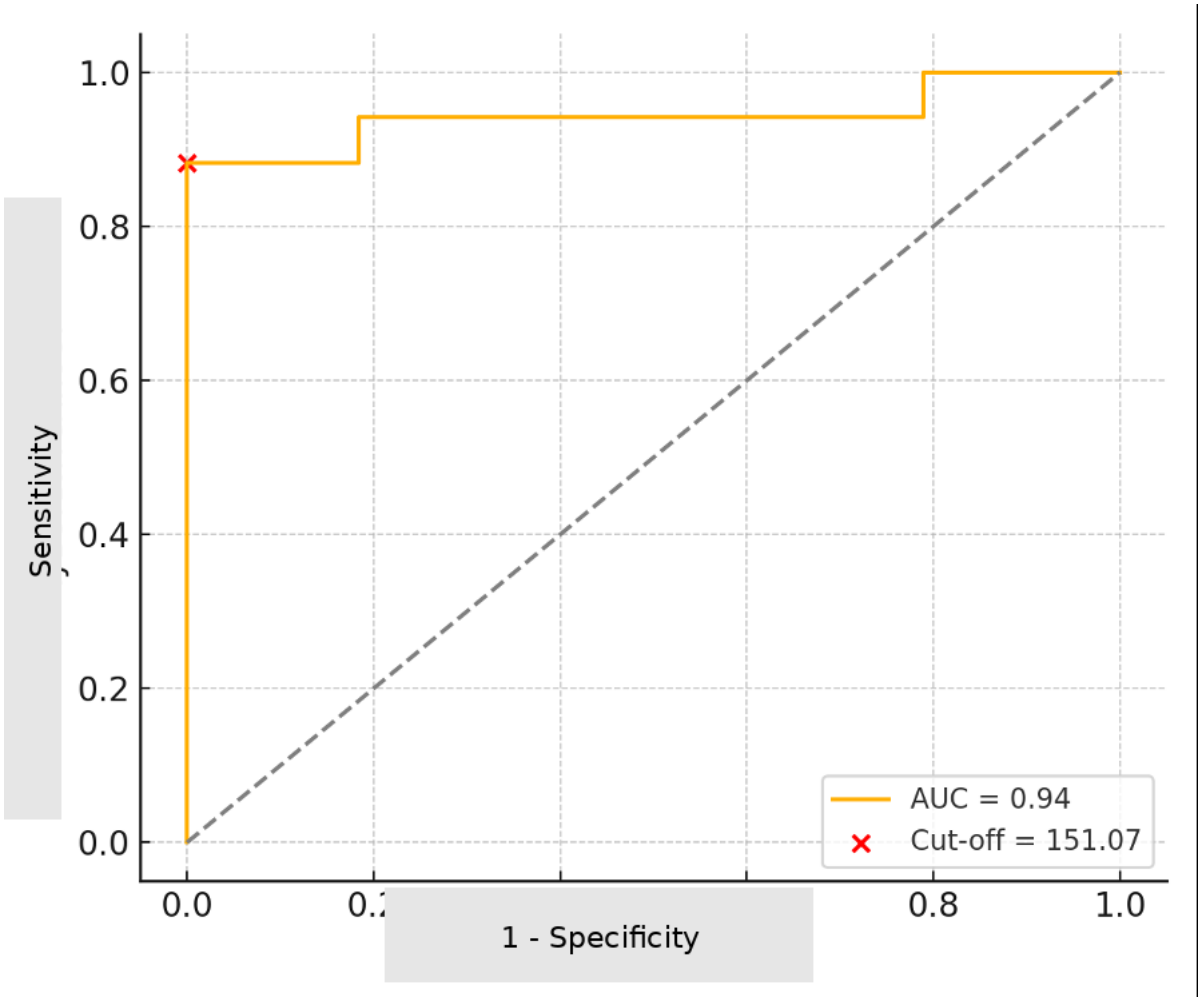

Fig. 13. ROC curve of FC as a prognostic marker for clinical outcome in children with viral acute infectious diarrhea

Following the identification of a significant association between FC and clinical course in the univariate analysis, a multivariable approach was applied to assess the independent prognostic value of FC. A logistic regression model was constructed using a binary dependent variable ("clinical course": 0 – favorable, 1 – unfavorable), with FC (first sample), CRP, leukocyte count, and ESR included as independent variables. Logistic regression enabled the simultaneous evaluation of the effects of individual inflammatory markers while controlling for potential confounders, in order to determine whether the association between FC and clinical course remained significant after adjustment for systemic markers. This model provides a more precise and clinically relevant assessment of prognosis in children with viral acute infectious diarrhea (Table 20).

Table 20. Results of multivariable logistic regression analysis for predicting clinical outcome in children with viral acute infectious diarrhea

Variable	OR	95% CI	p-value
FC (first sample)	1.08	0.98–1.20	0.1291
Leukocytes (×10⁹/L)	0.63	0.15–2.68	0.5293
ESR (mm/h)	1.03	0.71–1.48	0.8898
CRP (mg/L)	1.08	0.74–1.58	0.6843

In the multivariable model, none of the predictors reached statistical significance at the 95% confidence level. However, FC demonstrated the lowest p-value ($p = 0.129$) and the highest odds ratio (OR = 1.08; 95% CI: 0.98–1.20), suggesting a trend toward an association with an unfavorable clinical course, although statistical significance was not achieved. The model demonstrated good calibration (pseudo $R^2 = 0.764$; LLR $p < 0.0001$), reflecting the combined effect of the included variables within the studied sample. No significant associations with clinical course were observed for leukocyte count, ESR, or CRP ($p > 0.5$). Although FC did not retain independent statistical significance, it remained the only marker demonstrating a consistent trend toward association with unfavorable outcome. The lack of statistical significance is likely attributable to the limited sample size and the small number of unfavorable cases.

The results indicate that in children with viral acute infectious diarrhea, systemic inflammatory markers (CRP, leukocyte count, and ESR) have limited prognostic value in relation to clinical course. Although CRP showed a significant difference in the univariate analysis, none of these markers retained an independent association in the multivariable model, reflecting the moderate systemic inflammatory response characteristic of viral acute infectious diarrhea. In contrast, FC demonstrated a consistent association with clinical course. Significant differences in baseline values, excellent discriminative performance (AUC = 0.94), and a trend toward association in the multivariable model identify FC as the most informative laboratory marker for predicting clinical outcome. Higher baseline levels likely reflect more pronounced local inflammation and a more prolonged recovery. FC

provides additional prognostic information and may support early risk stratification and optimization of clinical management in children with viral acute infectious diarrhea.

The analysis of FC as a potential prognostic marker for clinical course in children with bacterial acute infectious diarrhea required a methodological approach tailored to the specific clinical features and recovery dynamics of this condition. In contrast to patients with viral acute infectious diarrhea, in whom the clinical course is typically self-limiting, children with bacterial acute infectious diarrhea exhibited more prolonged symptom duration. In the present analysis, the duration of diarrhea was used as the primary criterion for classifying clinical course as favorable or unfavorable. Given that complete clinical recovery was achieved in all patients, assessment of clinical course was based on the number of days with persistent diarrheal symptoms. Based on the distribution of diarrhea duration in bacterial acute infectious diarrhea, a threshold of 5 days was defined for classification. A duration of ≤5 days without complications was defined as a favorable course, whereas >5 days and/or the presence of complications defined an unfavorable course. For statistical analysis, clinical course was coded as a binary variable (0 – favorable, 1 – unfavorable).

The prognostic value of FC was further evaluated in comparison with CRP, leukocyte count, and ESR to determine its relative informativeness in discriminating clinical course. The analysis was based on the hypothesis that higher baseline values are associated with an unfavorable course. In bacterial acute infectious diarrhea, systemic markers exhibited considerable variability, reflecting a heterogeneous systemic inflammatory profile (Table 21).

Table 21. Descriptive statistics of inflammatory markers in patients with bacterial acute infectious diarrhea at admission

Parameter	**CRP (mg/L)**	**Leukocytes (×10⁹/L)**	**ESR (mm/h)**
Mean (M)	71.62	10.23	31.35
SD	48.61	4.34	14.46
Min	13.12	4.08	9.00

Parameter	CRP (mg/L)	Leukocytes (×10⁹/L)	ESR (mm/h)
Max	180.68	18.74	57.00
Q1 (25th percentile)	34.07	5.96	19.50
Median (Q2, 50th percentile)	57.68	9.73	33.00
Q3 (75th percentile)	83.65	14.25	41.00

The descriptive analysis of systemic inflammatory markers in bacterial acute infectious diarrhea demonstrated marked elevation and considerable variability. The mean CRP level was 71.62 mg/L (±48.61), with a range of 13.12–180.68 mg/L, reflecting an active and heterogeneous systemic inflammatory response. The leukocyte count had a mean value of 10.23 ×10⁹/L (±4.34), with a median of 9.73 ×10⁹/L and an interquartile range of 5.96–14.25 ×10⁹/L (overall range 4.08–18.74 ×10⁹/L), indicating values ranging from upper-normal to markedly elevated levels. The mean ESR was 31.35 mm/h (±14.46), with a median of 33 mm/h and a third quartile of 41 mm/h, confirming the presence of an inflammatory process in most patients. Overall, the laboratory profile was heterogeneous, consistent with the variable clinical course of bacterial acute infectious diarrhea. The combined analysis confirmed that, in children, bacterial acute infectious diarrhea is associated with a pronounced yet variable systemic inflammatory response from the early stage of disease, as reflected by the wide ranges of CRP, leukocyte count, and ESR. This laboratory profile is consistent with the bacterial etiology of acute infectious diarrhea, in which CRP and, to a lesser extent, leukocyte count are typically elevated. Despite their diagnostic value, systemic markers showed limited and inconsistent association with clinical course and symptom dynamics. In this context, the present study extends the evaluation by considering FC not only as a diagnostic marker but also as a potential prognostic marker of clinical course in children with bacterial acute infectious diarrhea.

Following the descriptive analysis, the association between CRP, leukocyte count, ESR, and clinical course in bacterial acute infectious diarrhea was evaluated using a univariate comparative analysis. The normality of distribution was assessed using the Shapiro–Wilk test, which indicated significant deviations from normality for CRP ($W = 0.852$; $p = 0.000012$), leukocyte count ($W = 0.926$; $p = 0.003161$), and ESR ($W = 0.951$; p

= 0.032412). Consequently, the Mann–Whitney U test was applied. No significant differences were observed for CRP and leukocyte count ($p > 0.05$), whereas ESR demonstrated a statistically significant difference ($p < 0.05$).

The assessment of normality for FC also demonstrated a significant deviation from normality ($W = 0.814$; $p < 0.001$), necessitating the use of non-parametric analysis. The Mann–Whitney U test revealed a statistically significant difference between the subgroups ($U = 152.5$; $p = 0.0015$), with higher baseline FC values associated with an unfavorable clinical course. The descriptive statistics presented in Table 22 confirm higher and more variable values in this subgroup, supporting the prognostic potential of FC in bacterial acute infectious diarrhea.

Table 22. Descriptive statistics of FC values in patients with bacterial acute infectious diarrhea according to clinical course

Group	**Number of patients**	**Mean FC (µg/g)**	**SD**	**Median (µg/g)**	**Min (µg/g)**	**Max (µg/g)**
Favorable course	34	657.41	160.92	640.57	410.81	875.04
Unfavorable course	18	838.86	109.72	856.76	824.15	918.12

As shown in Table 22, patients with an unfavorable clinical course exhibited significantly higher baseline FC values compared to those with a favorable course. In the favorable subgroup, the mean value was 657.41 ± 160.92 µg/g (median 640.57 µg/g), whereas in the unfavorable subgroup it reached 838.86 ± 109.72 µg/g (median 856.76 µg/g). The unfavorable subgroup was characterized by a shift toward higher values and relatively reduced variability, reflecting persistently elevated mucosal inflammatory activity. These findings support an association between higher baseline FC values and a more prolonged or complicated clinical course in bacterial acute infectious diarrhea, highlighting FC as a potential marker for early risk stratification.

A ROC analysis was performed to quantitatively assess the ability of FC to discriminate between patients with favorable and unfavorable clinical course in bacterial acute infectious diarrhea (Figure 14).

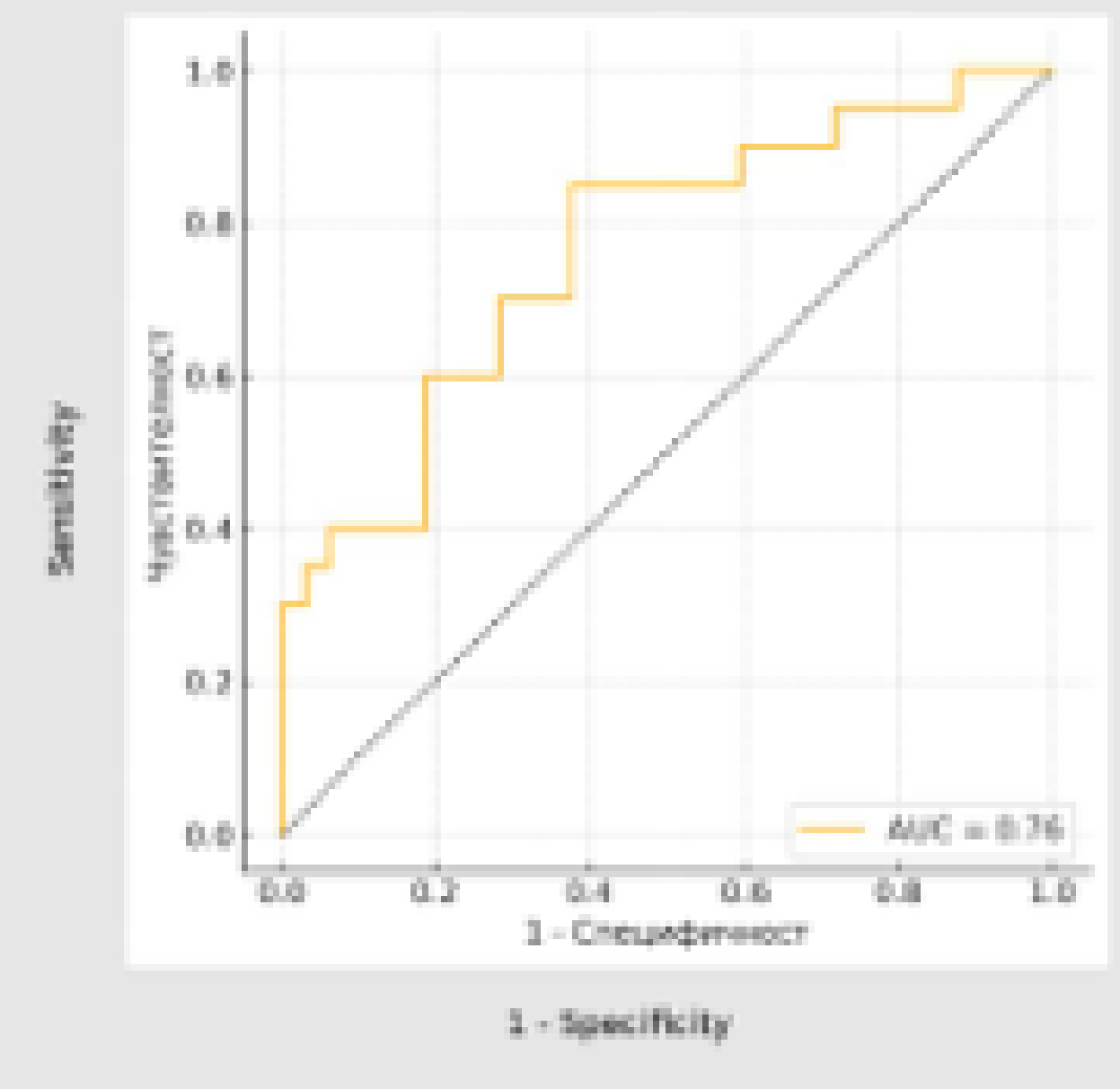

Fig. 14. ROC curve of FC as a prognostic marker for clinical outcome in patients with bacterial acute infectious diarrhea

The ROC analysis demonstrated good prognostic performance of FC in bacterial acute infectious diarrhea (AUC = 0.76). The optimal cut-off value was 658.08 µg/g, at which sensitivity reached 85% and specificity 62.5%. These findings indicate that the majority of patients with an unfavorable clinical course can be identified at the time of initial assessment,

albeit with a moderate rate of false-positive results. The combination of high sensitivity and moderate specificity supports the use of FC as a suitable tool for early risk stratification in children with bacterial acute infectious diarrhea. The identified cut-off provides a quantitatively grounded basis for clinical decision-making and supports the use of FC as a prognostic marker in the context of a variable clinical presentation.

The independent prognostic value of FC in bacterial acute infectious diarrhea was evaluated using multivariable logistic regression analysis. FC (first sample), leukocyte count, ESR, and CRP were included in the model as independent variables, while clinical course was defined as a binary dependent variable (0 = favorable; 1 = unfavorable) (Table 23).

Table 23. Results of multivariable logistic regression analysis for predicting clinical outcome in children with bacterial acute infectious diarrhea

Variable	OR	95% CI	p-value
FC (first sample)	1.00	0.99–1.01	0.8578
Leukocytes (×10⁹/L)	0.96	0.61–1.51	0.8518
ESR (mm/h)	1.15	1.02–1.30	0.0220
CRP (mg/L)	1.02	0.99–1.05	0.1958

The model demonstrated statistically significant overall predictive performance (LLR $p < 0.00001$) with a pseudo R^2 of 0.480, indicating good explanatory power. ESR was the only variable that retained an independent association with clinical course ($p = 0.022$; OR = 1.15; 95% CI: 1.02–1.30), with each 1 mm/h increase associated with an approximately 15% higher likelihood of an unfavorable outcome. FC ($p = 0.858$), leukocyte count ($p = 0.852$), and CRP ($p = 0.196$) did not demonstrate independent effects. The lack of statistical significance for FC contrasts with the findings from the univariate and ROC analyses and likely reflects overlap in prognostic information with systemic markers, as well as the limited sample size. These findings support the use of FC for early risk stratification, while emphasizing the need for a multifactorial interpretation.

The analysis confirmed the prognostic value of FC in acute infectious diarrhea in children. Baseline values measured in the early phase were associated with clinical course in both viral and bacterial acute infectious diarrhea. In viral acute infectious diarrhea, FC demonstrated excellent discriminative performance (AUC = 0.94), with a cut-off of 151.07 µg/g yielding 88.2% sensitivity and 100% specificity. Although the marker did not retain independent significance in the multivariable model, it remained the variable with the strongest association among those analyzed. In bacterial acute infectious diarrhea, FC also showed a significant association with clinical course (AUC = 0.76; cut-off 658.08 µg/g; sensitivity 85%; specificity 62.5%). In the multivariable analysis, only ESR retained an independent effect. Despite this, FC remains a clinically useful tool for early risk stratification, particularly when interpreted in combination with systemic inflammatory markers.

V. CONCLUSION

The present study characterized the epidemiological and nosological profile of acute infectious diarrhea in children aged 1–5 years hospitalized at the First Clinic of Infectious Diseases, University Hospital “St. Marina” – Varna. Etiologically confirmed cases accounted for slightly more than half of the patients, highlighting the limitations of routine diagnostics and the need for complementary biomarkers. Among the identified pathogens, comparable groups of viral acute infectious diarrhea and bacterial acute infectious diarrhea were established. Rotavirus gastroenteritis predominated in viral acute infectious diarrhea, whereas Salmonella spp. and Campylobacter spp. were most common in bacterial acute infectious diarrhea, with less frequent but clinically significant pathogens such as Clostridioides difficile, Shigella spp., and Yersinia enterocolitica also identified. Clinical analysis demonstrated a more severe course in bacterial acute infectious diarrhea, characterized by higher fever, longer duration of diarrhea, more frequent presence of pathological stool components, and a higher proportion of severe cases according to the modified Vesikari scale. In contrast, viral acute infectious diarrhea was predominantly associated with moderate disease severity and prominent upper gastrointestinal symptoms. These differences support the need for objective markers for early risk stratification.

FC demonstrated clearly distinguishable profiles across viral acute infectious diarrhea, bacterial acute infectious diarrhea, and the control group. In bacterial acute infectious diarrhea, levels were significantly higher and more variable, reflecting more intense mucosal inflammation. The marker reliably differentiated bacterial from viral etiology at the population level; however, it did not allow precise etiological discrimination between individual pathogens, as it primarily reflects the intensity of neutrophil-mediated inflammation.

Longitudinal assessment showed rapid normalization of FC in viral acute infectious diarrhea and persistently elevated levels in bacterial acute infectious diarrhea during the

early hospital phase. Serial measurements provide objective insight into recovery dynamics and facilitate the identification of cases with an unfavorable clinical course.

With regard to prognosis, baseline FC levels were associated with the clinical course. In viral acute infectious diarrhea, FC demonstrated excellent discriminative ability and high prognostic performance. In bacterial acute infectious diarrhea, its prognostic value was moderate, while erythrocyte sedimentation rate retained an independent association in multivariable analysis. These findings further highlight the importance of integrating local and systemic inflammatory markers in clinical decision-making.

In summary, FC is a sensitive and clinically informative marker in acute infectious diarrhea in children. It contributes to etiological differentiation, objective assessment of disease severity, monitoring of inflammatory dynamics, and early risk stratification, supporting its inclusion as a complementary tool in the integrated diagnostic–prognostic algorithm for acute infectious diarrhea.

VI. SUMMARY OF FINDINGS

1. The etiological analysis of acute infectious diarrhea in children aged 1–5 years, treated at the First Clinic of Infectious Diseases, University Hospital "St. Marina" – Varna between June 2024 and February 2025, identified a laboratory-confirmed pathogen in 52.1% of cases. Viral acute infectious diarrhea was predominantly associated with rotavirus infections, whereas bacterial acute infectious diarrhea was mainly caused by *Salmonella* spp. and *Campylobacter* spp.; a clear age-related differentiation between viral and bacterial forms was also observed.

2. In children with acute infectious diarrhea, significantly higher and more variable FC levels were observed compared to the control group of healthy children, in whom an upper reference limit of 84.19 µg/g (95th percentile) was established. These findings confirm FC as an indicator of intestinal inflammation in early childhood.
3. Analysis of FC levels demonstrated a statistically significant association with the etiology of acute infectious diarrhea, with substantially lower levels observed in viral acute infectious diarrhea compared to bacterial acute infectious diarrhea, in the absence of a strictly pathogen-specific pattern.

4. An association was established between baseline FC levels and the severity of clinical presentation in acute infectious diarrhea. In viral acute infectious diarrhea, elevated FC levels were associated with more severe clinical forms, highlighting its prognostic value in viral enteritis. In bacterial acute infectious diarrhea, a relationship between FC levels and clinical severity was also observed, although with more moderate discriminative ability.
5. Longitudinal assessment of FC during hospitalization demonstrated a statistically significant reduction in levels, reflecting the regression of intestinal inflammation. In

viral acute infectious diarrhea, the decline occurred more rapidly, with a tendency toward early normalization, whereas in bacterial acute infectious diarrhea, the reduction was slower, incomplete, and heterogeneous, with persistently elevated levels observed in a substantial proportion of patients.

6. Statistical analyses demonstrated high prognostic accuracy of FC for clinical outcome in viral acute infectious diarrhea and good discriminative ability in bacterial acute infectious diarrhea, enabling early risk stratification in children with acute infectious diarrhea. The identified cut-off values of 151.07 µg/g for viral acute infectious diarrhea and 658.08 µg/g for bacterial acute infectious diarrhea were associated with an increased likelihood of an unfavorable clinical course.

VII. CONTRIBUTIONS OF THE DISSERTATION

ORIGINAL CONTRIBUTIONS

1. An original clinical and epidemiological analysis of hospitalized children aged 1–5 years with acute infectious diarrhea is presented, systematizing data on demographic characteristics, etiological structure, and clinical severity in this age group.
2. For the first time in Bulgaria, a targeted clinical study was conducted on the diagnostic and prognostic value of FC in children with acute infectious diarrhea, stratified by viral and bacterial etiology.
3. A comprehensive analytical approach was introduced for evaluating the diagnostic and prognostic value of FC using ROC analysis and logistic regression, enabling an objective quantitative assessment of clinical risk.
4. For the first time, cut-off values of FC for predicting an unfavorable clinical course in viral and bacterial acute infectious diarrhea in children were established, based on a statistically validated model.
5. The applicability of FC as a tool for early severity stratification in children with acute infectious diarrhea was demonstrated, with potential to optimize the diagnostic and therapeutic approach at the early stage of the disease.

SCIENTIFIC AND APPLIED CONTRIBUTIONS

1. A comparative analysis of FC with conventional systemic inflammatory markers (CRP, erythrocyte sedimentation rate, and leukocyte count) was performed, demonstrating the complementary—and in certain clinical contexts superior—informative value of FC in assessing local intestinal inflammation.
2. The clinical applicability of FC in children with acute infectious diarrhea was substantiated based on its diagnostic, dynamic, and prognostic characteristics.

VIII. PUBLICATIONS AND SCIENTIFIC COMMUNICATIONS RELATED TO THE DISSERTATION

Publications in peer-reviewed scientific journals:

1. Fecal calprotectin in the differential diagnosis of acute intestinal infections
   Vlkova E.D., Gospodinova M.D., Todorov I.T.
   Journal of Infectology, Vol. 10, No. 2, 2018, pp. 117–122
   DOI: 10.22625/2072-6732-2018-10-2-117-122
   Type of publication: review article
2. Functions and potential of lipocalin-2 as a fecal biomarker in acute gastrointestinal infections
   Lyutsova E.D., Gospodinova M.D., Bocheva Y.D.
   Clinical Laboratory Diagnostics, Vol. 66, No. 6, 2021, pp. 371–373
   DOI: 10.51620/0869-2084-2021-66-6-371-373
   Type of publication: review article

Scientific communications:

- Lyutsova E., Stoyanova K., Gospodinova M.
  Missed pathogens: challenges and opportunities in the routine diagnosis of acute infectious diarrhea in childhood
  7th National Conference on Epidemiology with International Participation
  September 26–28, 2025, Medical University – Pleven
  Poster presentation – First Prize