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Alimentary Tract

Exploring novel biomarkers in pediatric ulcerative colitis: The role of Lipocalin-2, MMP-9, and MMP-9/LCN-2 complex

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ABSTRACT

Background and aims: Lipocalin-2 (LCN-2), Matrix Metalloproteinase-9 (MMP-9), and the MMP-9/LCN-2 complex are emerging biomarkers for ulcerative colitis (UC). While extensively studied in adults, data in children are limited. This study aimed to evaluate their serum levels in children with newly diagnosed UC, compare them with healthy controls, and assess correlations with disease severity and extent.
Methods: In this prospective case-control study, 32 children with UC (6–18 years) and 38 healthy controls were enrolled. Baseline clinical (Pediatric Ulcerative Colitis Activity Index/ PUCAI), laboratory (albumin, hemoglobin, Erythrocyte Sedimentation Rate, C-reactive protein/CRP, fecal calprotectin), and endoscopic (extent, Ulcerative Colitis Endoscopic Index of Severity/UCEIS) data were collected. Serum LCN-2, MMP-9, and MMP-9/LCN-2 complex levels were measured by ELISA.
Results: Serum LCN-2, MMP-9, and MMP-9/LCN-2 levels were significantly higher in UC patients than controls. ROC analysis indicated LCN-2 had the best diagnostic performance. Higher LCN-2 and MMP-9 levels were observed in children with more severe endoscopic disease (UCEIS > 4) or pancolitis. LCN-2 levels inversely correlated with albumin, whereas MMP-9 positively correlated with CRP and UCEIS.
Conclusions: LCN-2 and MMP-9 are promising biomarkers in pediatric UC, reflecting disease severity and extent. Their measurement may have clinical utility in monitoring disease progression and guiding management in children.
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1. Introduction

Ulcerative colitis (UC) is classically defined as a chronic inflammatory disease affecting the rectum and a variable extent of the colon in continuity [1]. In industrialized countries, the incidence of inflammatory bowel diseases (IBD) in pediatric age is constantly increasing [2]. As a chronic condition, with alternating periods of remission and exacerbation, it requires long-term medical care and the need for constant monitoring. To date endoscopy remains the gold-standard for assessing disease activity in IBD, but it is not without drawbacks, including high costs, limited accessibility, and the potential for complications [3].

As a result, there is a pressing need to identify accurate, non-invasive predictive and prognostic biomarkers for disease activity, which has been identified as a knowledge gap in the path towards precision medicine applied to IBD [4]. Although various blood, stool, and urine tests have been developed for this purpose, serum C-reactive protein (CRP), fecal calprotectin (FC), still are the only assays currently available at a large scale incorporated in the diagnostic and monitoring algorithm. However, these biomarkers have significant limitations [5,6].

Lipocalin-2 (LCN-2), also known as neutrophil gelatinase-associated lipocalin (NGAL), siderocalin, or 24p3, is a member of

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the lipocalin superfamily and a pleiotropic mediator of various inflammatory processes. This 25-kDa secretory glycoprotein was initially identified and purified from neutrophil granules and is encoded by a gene located at chromosome locus 9q34.11 [7]. In the gut, the main sources of LCN-2 are intestinal epithelial cells and myeloid cells, and the LCN-2 receptor 24p3R is expressed in intestinal innate and adaptive immune cells. Upon gastrointestinal damage, bacterial infection, or intestinal inflammation, LCN-2 is strongly upregulated, resulting in high mucosal and fecal concentrations of this molecule [7]. Furthermore, LCN-2 has been investigated in its relationship with IBD and as a possible marker for intestinal inflammation that correlates well with disease activity. However, its functional impact on chronic intestinal inflammation remains obscure [8–10].

Matrix metalloproteinase-9 (MMP-9), belonging to a class of metal-dependent enzymes, has been studied in the pathophysiology of UC primarily due to its involvement in tissue remodeling and the breakdown of the extracellular matrix [11]. Some studies have demonstrated that MMP-9 is overexpressed in the colonic mucosa of UC subjects, especially during active disease flares, and in the epithelium and inflammatory infiltrates, such as neutrophils, which are abundant in UC lesions [12].

Both LCN-2 and MMP-9 have been found stored in the granules of neutrophils, specialized leukocytes whose migration to the intestinal active site is a peculiarity of the UC disease.

The interaction of MMP-9 with LCN-2 (the MMP-9/LCN-2 complex) protects MMP-9 against auto-degradation, preserving its full enzyme activity. Serum levels of MMP-9/LCN-2 complex has been proposed as new surrogate marker of mucosal healing in UC patients after therapy with infliximab [13].

To date only few studies have investigated the three molecules (LCN-2, MMP-9 and MMP-9/LCN-2 complex) in children, in the contest of IBD [14–16]. Primary aim of the study was to determine the serum levels of LCN-2, MMP-9 and MMP-9/LCN-2 complex at the diagnosis in children with UC, and to compare them with age- and sex-matched healthy controls. Secondary aim was to investigate the correlations between LCN-2, MMP-9 and MMP-9/LCN-2 complex and the most common endoscopic, laboratory, fecal, and clinical markers of disease activity in pediatric UC, to investigate the potential clinical value in this pathological condition.

2. Materials and methods

2.1. Study design and participants

The study was a prospective cross-sectional observational study conducted at the Pediatric Gastroenterology, Hepatology and Nutrition Unit of the Umberto I Hospital in Rome.

All male and female children aged 6-18 years of age (n. 32), newly diagnosed with UC were consecutively enrolled between 2023 and 2024. The diagnosis of UC had to meet the specific clinical, endoscopic, and histologic Porto criteria [17]. Exclusion criteria included (i) use of drugs that could affect the results, such as anti-inflammatory, and immunosuppressants, (ii) presence of ongoing inflammatory conditions or (iii) having a suspected inflammatory process other than UC at the time of the enrollment. Children with less than 6 years at the diagnosis (very early onset IBD) were also excluded to avoid any possible confounding effects of genetics.

Baseline demographics, clinical, and endoscopic data were recorded prospectively using standardized and anonymized case report forms. Demographic data included age, gender, ethnicity, family history of IBD. Clinical data included: (1) endoscopic disease location according to the Paris classification [18]: E1 defined the proctitis, E2 left-sided colitis, E3 extensive colitis and E4 pancolitis; (2) disease severity at the diagnosis according to the Pediatric Ulcerative Colitis Activity Index (PUCAI), according to the validated cut-offs for disease activity (<10 clinical remission, between 10 and 34 mild disease activity, between 35 and 64 moderate disease activity, and >65 severe disease activity [19]; (3) endoscopic disease activity scores through the Ulcerative Colitis Endoscopic Index of Severity (UCEIS), using the validated cut offs for remission (UCEIS 0–1), mild (UCEIS 2–4), moderate (UCEIS 5–6), and severe disease (UCEIS 7–8) [20].

Laboratory markers of disease activity measured in each patient at the diagnosis per local practice included (normal reference ranges in parentheses): Hemoglobin (12.2 - 16.6 g/dL), erythrocyte sedimentation rate (ESR, 0-25 mm/h), C-reactive protein (CRP, 0.00-0.50 mg/dL), albumin (38-54 g/L). Moreover, children and their parents were instructed to collect a stool sample before beginning bowel preparation for ileocolonoscopy and store it at 3–5°C until the following day. On the day of the endoscopy, the stool samples were transported to the local laboratory, where FC (< 100 µg/g) analysis was conducted using a commercially available ELISA kit (Calprest; Eurospital SpA, Trieste, Italy).

The control group consisted of age- and sex-matched healthy children attending the clinic for routine blood testing (n. 38). These children had no history or clinical suspicion of acute or chronic inflammatory conditions and were free from any known diseases.

The sample size for the pilot study was estimated using a power analysis calculator. The analysis indicated that a minimum of 20 participants per group (40 in total) would be sufficient to achieve a statistical power of 0.95. A higher power was achieved with the inclusion of a few additional samples, which occurred during the recruitment of both patients and healthy individuals, resulting in a total sample size of 70. For further details, see the Supplementary Materials.

2.2. Blood withdrawal

Peripheral blood samples of 5 mL were taken and collected in BD VacutainerTM Serum Separation Tubes and centrifuged at 3000 rpm for 15 min to separate serum from blood cells. The serum was then stored at −80 °C up until the day of the analysis.

2.3. Serum LCN-2, MMP-9 and the MMP-9/LCN-2 complex analysis

Human serum LCN-2 (Cat. No. DY8556), MMP-9 (Cat. No. DY911), MMP-9/LCN-2 complex (Cat. No. DY1757) were measured using a sandwich enzyme-linked-immunosorbent assay (ELISA) kits (DuoSet ELISA, R&D Systems, Minneapolis, MN, USA), according to the protocols provided by the manufacturer. Briefly, 100 uL of standard curve and unknown samples were added in duplicate to each well and incubated for 2 h at room temperature. Optical density of each well was determined using a microplate reader (NeoBiotech, Seoul, Republic of Korea) set to 450 nm. A standard curve for each ELISA test has been created, by using a software able to generate a four-parameter logistic (4-PL) curve-fit. Final concentration of unknown samples has been extrapolated by using a data analysis tool (MyAssays; https://www.myassays.com/), considering the dilution factor (for LCN-2, 1:200 – for MMP-9, 1:500 – for MMP-9/LCN-2 complex, 1:10). Data have been represented as ng/mL.

2.4. Ethical statement

The study was approved by the Ethics Committee of the "Policlinico Umberto I" Hospital (Prot. 0520/2023). All participants and/or their guardians gave written informed consent to participate in the study. The trial was conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983, for human experimentation.

Table 1
Baseline characteristics of recruited children with newly diagnosed UC.

	Tot = 32	Normal ranges
Gender F, n (%)	11 (34)	
Age at the diagnosis (years), mean ± S.E.M.	11,86 ± 0,76	
Symptoms at the diagnosis, n (%)		
Diarrhoea	28 (87,5)	
Rectal bleeding	31 (97)	
Abdominal pain	22 (69)	
Bowel urgency	18 (56)	
Tenesmus	13 (40)	
UCEIS, mean ± S.E.M.	4,70 ± 0,41	
Mayo endoscopic subscore, n (%)		
1	2 (6)	
2	20 (62,5)	
3	10 (31,5)	
FC (μg/g), mean ± S.E.M.	722,74 ± 139,55	< 100
ESR (mm/h), mean ± S.E.M.	34,35 ± 4,66	0 - 25
CRP (mg/dL), mean ± S.E.M.	1,31 ± 0,61	0.00 - 0.50
PUCAI, mean ± S.E.M.	38,48 ± 3,07	
Albumin (g/L), mean ± S.E.M.	45,14 ± 1,01	38 - 54
Hemoglobin (g/dL), mean ± S.E.M.	11,74 ± 0,40	12.2 - 16.6
Disease location at the diagnosis, n (%)		
E1	5 (16)	
E2	3 (9)	
E3	7 (22)	
E4	17 (53)	

UCEIS (Ulcerative Colitis Endoscopic Index of Severity); FC (fecal calprotectin); ESR (erythrocyte sedimentation rate); CRP (C-reactive protein); PUCAI (Pediatric Ulcerative Colitis Activity Index)

2.5. Statistical analysis

Variables were reported as means ± standard error mean (S.E.M.) or as absolute numbers and percentages. Categorical variables were compared using χ2-test or exact Fisher's, while continuous variables were compared using Student's t-test or non-parametric Wilcoxon test in case of non-normal distribution of the variable. The results of the statistical analysis were reported for individual comparison, where appropriate, in terms of the "t" statistic (quantifying the observed difference relative to data variability) and degrees of freedom "df", reflecting the number of independent observations contributing to the estimate. Spearman's rank correlation test [rho = ρ] was performed between LCN-2, MMP-9, MMP-9/LCN-2 complex and the clinical and biological markers of disease activity in UC. A receiver operating characteristic (ROC) curve analysis was performed to determine the area under the curve (AUROC) for each comparison, sensitivity, specificity, likelihood ratio (LR), cut-off, and DeLong test (see above). Statistical significance was set at p<0.05. Analyses were conducted using GraphPad Prism (GraphPad Prism 9.5.1.733) for Windows, GraphPad software (San Diego, CA, USA).

3. Results

3.1. Characteristics of the population

Thirty-two children with a new diagnosis of UC (mean age 11,86 ± 0,76) and 38 age- and sex-matched healthy control (mean age 11.47 ± 0.68) were consecutively enrolled during the study period. Patients' baseline characteristics are listed in Table 1.

3.2. LCN-2, MMP-9, and the MMP-9/LCN-2 complex serum levels in UC patients and healthy controls

As shown in Fig. 1 (upper panels), LCN-2, MMP-9 and MMP-9/LCN-2 complex serum levels were significantly higher in UC patients compared to healthy controls [LCN-2; t=8.07, df=62. p<0.0001. MMP-9: t=5.95, df=65, p<0.0001. MMP-9/NGAL t=5.002, df=66, p<0.0001].

ROC analysis (Fig. 1, lower panels) revealed that LCN-2 had a better performance compared to that of MMP-9 and MMP-9/LCN-2 complex thus demonstrating a superior ability to discriminate healthy individuals from pathological ones. Supplementary Table 2 reports the results of the ROC analysis, underscoring that LCN-2 exhibits superior values across all assessed parameters (including AUROC, sensitivity, specificity, and likelihood ratio), when compared to the other molecules.

Finally, the pairwise DeLong test for AUROC comparison revealed statistically significant differences for LCN-2 vs MMP-9 and LCN-2 vs the MMP-9/LCN-2 complex (Supplementary Table 3).

3.3. LCN-2, MMP-9, and the MMP-9/LCN-2 complex serum levels in relation to endoscopic findings in UC

Ulcerative colitis children with a moderate-to-severe disease activity (UCEIS >4) presented higher levels of LCN-2 (t=2.30, df=20, p=0.03), MMP-9 (t=2.36, df=20, p=0.03) but not of MMP-9/LCN-2 complex (t=0.81, df=20, p=0.43), compared to those with endoscopic mild disease activity (UCEIS $\leq$ 4) (Fig. 2, upper panels).

ROC curve analysis, reported in the lower panels of Fig. 2, showed the best performance of MMP-9 compared to LCN-2, in discriminating moderate-severe from mild endoscopic disease activity.

Supplementary Table 4 presents the results of the ROC analysis, highlighting that MMP-9 consistently outperforms LCN-2 across all evaluated parameters, including AUROC, sensitivity, specificity, and likelihood ratio. However, the DeLong test indicated no statistically significant difference between their AUROCs (z = -0.2741, p = 0.784).

The ability of the three biomarkers in differentiating children with pancolitis (E4 according to the Paris classification) to those with extensive (E3), left-sided colitis (E2), or proctosigmoiditis (E1), is illustrated in Fig. 3. Both LCN-2 and MMP-9, but not MMP-9/LCN-2 complex were significantly over-expressed in the serum of E_4 UC patients, compared to $E_{1,2,3}$ UC children [LCN-2; t=4.06,

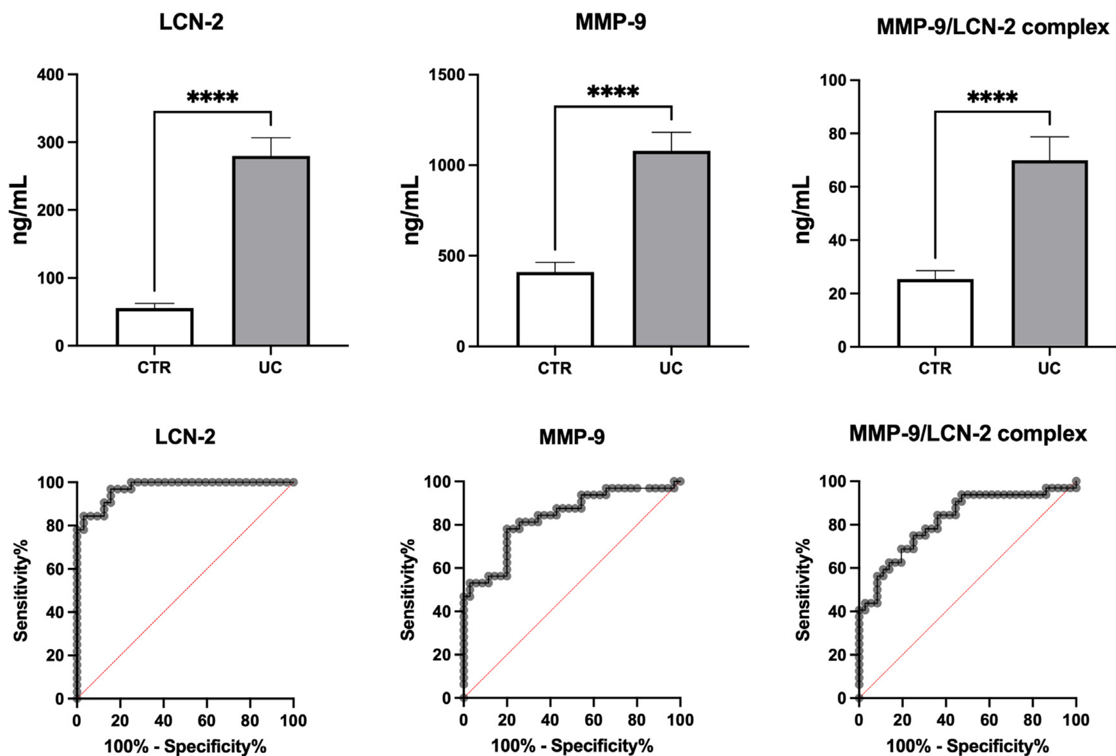

Fig. 1. LCN-2, MMP-9 and MMP-9/LCN-2 complex circulating levels in healthy controls *vs* UC pediatric patients. Histograms represent the mean ± SEM of serum levels from controls and patients (upper panels). Student's t test, **** $p < 0.0001$. Lower panels represent ROC curves for circulating levels of LCN-2, MMP-9 and MMP-9/LCN-2 complex in healthy controls vs UC pediatric patients.

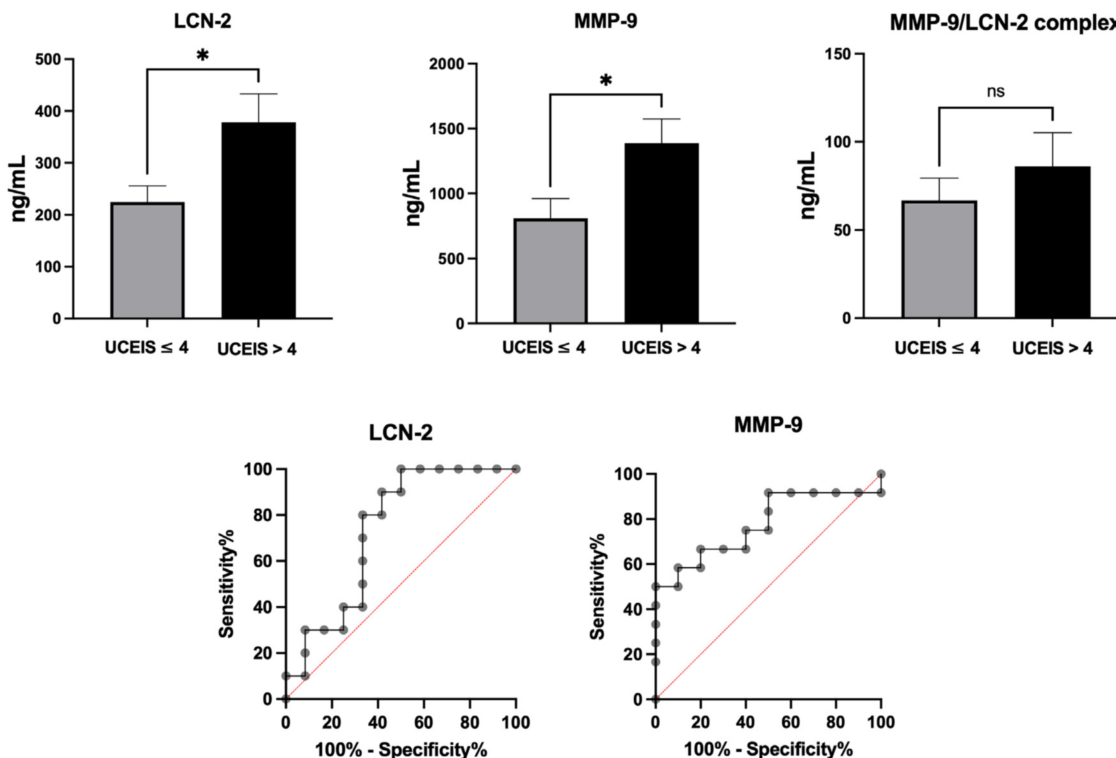

Fig. 2. LCN-2, MMP-9 and MMP-9/LCN-2 complex circulating levels in UC patients with UCEIS ≤4 *vs* those with UCEIS >4. Histograms represent the mean ± SEM of serum levels in each group (upper panels). Student's t test, *$p < 0.05$. Lower panels represent ROC curves for circulating levels of LCN-2 and MMP-9 in UCEIS≤4 *vs* UCEIS >4 UC pediatric patients.

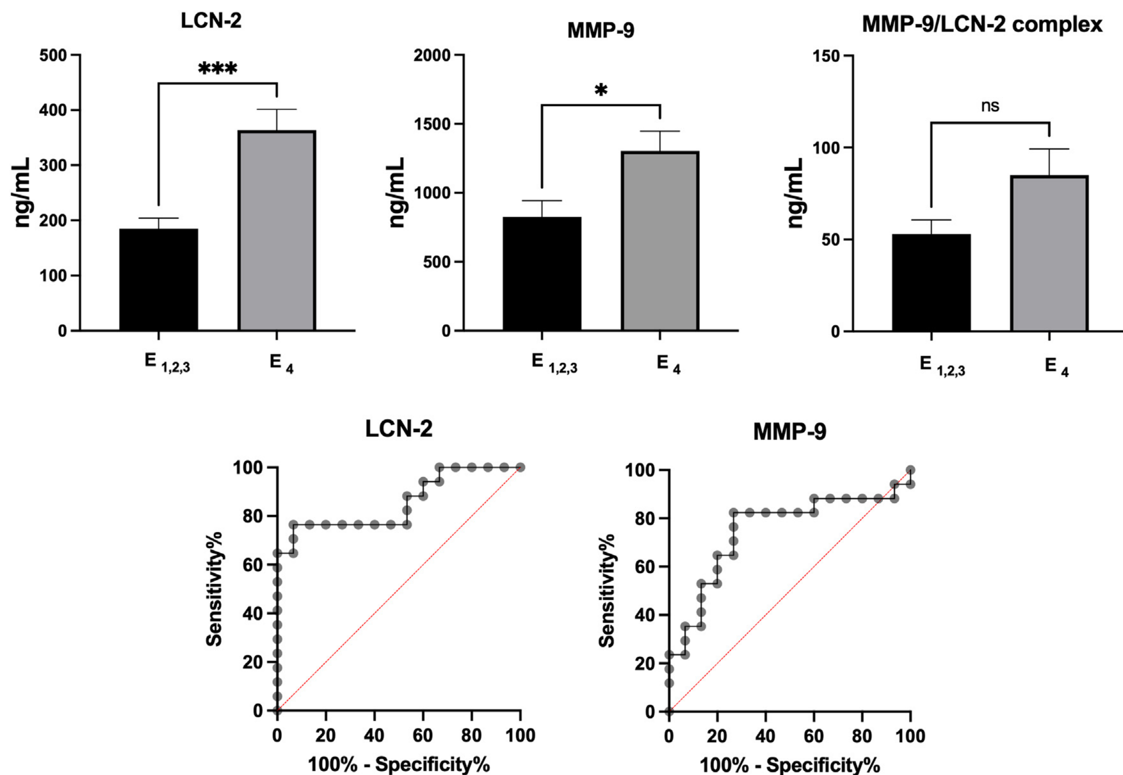

Fig. 3. LCN-2, MMP-9 and MMP-9/LCN-2 complex circulating levels in UC patients with pancolitis (E4) *vs* those with less extensive colitis (E1,2,3). Histograms represent the mean ± SEM of serum levels in each group (upper panels). Student's t test, * $p < 0.05$, *** $p < 0.001$. Lower panels represent ROC curves for circulating levels of LCN-2 and MMP-9 in E4 *vs* E1,2,3 UC pediatric patients.

df=30, p=0.0003. MMP-9: t=2.52, df=30, p=0.02. MMP-9/LCN-2 complex t=1.89, df=30, p=0.07] (Fig. 3, upper panels). Concerning the ROC curve analysis, the best performance has been obtained with LCN-2, followed by MMP-9 (Fig. 3, lower panels).

Supplementary Table 5 reports the results of the ROC analysis, underscoring that LCN-2 exhibits superior values across all assessed parameters (including AUROC, sensitivity, specificity, and likelihood ratio), when compared to MMP-9. The DeLong test indicated no statistically significant difference between their AUROCs (z = 1.1465, p = 0.252).

Supplementary Fig. 1S reports serum LCN-2 and MMP-9 levels stratified according to E1, E2, E3, and E4 scores. In both cases, no significant gradual increase was observed in our pilot study. Nevertheless, according to the data illustrated in Fig. 3, serum LCN-2 levels were markedly elevated in E4 UC patients compared with those in the other extent categories, and this increase was more pronounced than that observed for MMP-9.

Fig. 4 reports the trade-off between specificity and sensitivity for LCN-2, FC, and for PUCAI, clinically relevant parameters in the evaluation of colitis extension. The comparison of the ROC curves (Supplementary Table 6) highlights how LCN-2 and PUCAI can provide comparable information in distinguishing between pancolitis, and other disease extensions, whilst FC was unable in differentiating patients with different UC extensions.

The pairwise DeLong test for AUROC comparison showed that the difference between LCN-2 and FC was statistically significant and more pronounced than the difference between FC and PUCAI (Supplementary Table 7), supporting the hypothesis that LCN-2 may serve as a potential biomarker for distinguishing pancolitis from other disease extensions.

To further substantiate this hypothesis, a two-way ANOVA was conducted to evaluate the ability of LCN-2 and/or MMP-9 to differentiate E4 colitis from E1–E3 colitis in patients with either mild (UCEIS ≤4) or moderate-to-severe (UCEIS >4) disease activity.

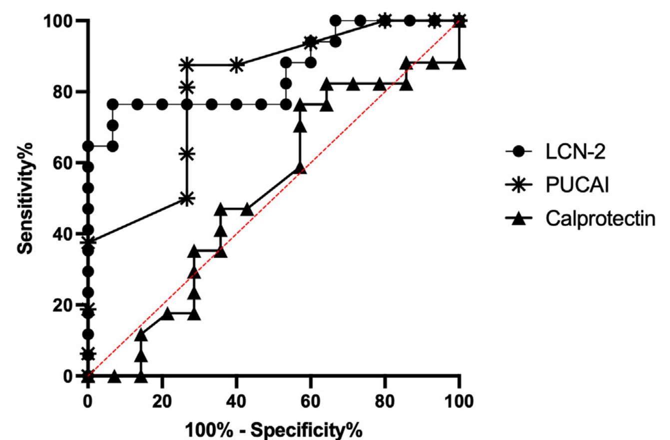

Fig. 4. ROC curves comparison for circulating levels of LCN-2, PUCAI index and fecal calprotectin, in E4 vs E1,2,3 UC pediatric patients.

Interestingly, Fig. 5 shows that in the presence of a UCEIS > 4, LCN-2 [F (1,18) = 13,24, p = 0.0104], but not MMP-9 [F (1,18) = 19.41, p = 0.1329], was able to distinguish children with pancolitis from those with less extensive forms of colitis.

3.4. Correlation analysis

Supplementary Table 8 reported Spearman's correlation analysis between LCN-2, MMP-9, MMP-9/LCN-2 complex and the main clinical (PUCAI), endoscopic (UCEIS) and laboratory indexes (serum albumin and CRP, and FC). A significant inverse correlation was

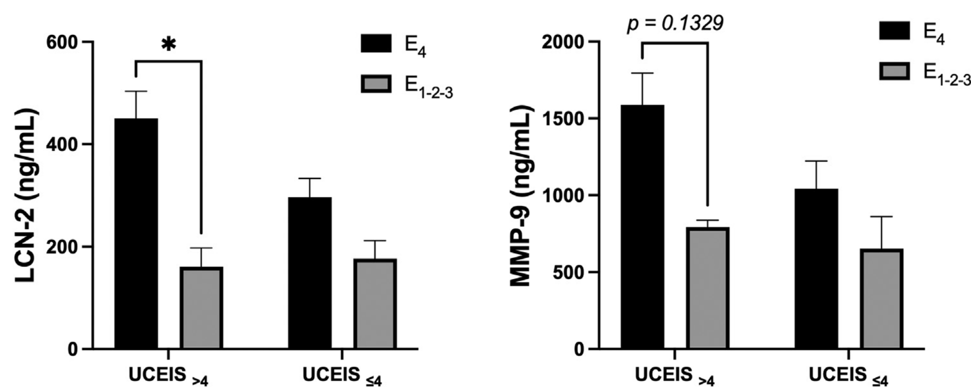

Fig. 5. LCN-2, and MMP-9 circulating levels in UC pediatric patients with pancolitis (E4) and with less extensive colitis (E1,2,3), in the presence of moderate-severe activity score (UCEIS >4) or mild activity score (UCEIS ≤4). Histograms represent the mean ± SEM of serum levels from each group of patients. Two-way ANOVA, followed by Tukey's multiple comparisons test, * $p < 0.01$.

observed between LCN-2 and albumin levels at the diagnosis ($r = -0.47$; $p = 0.01$) while a direct (although not significant) correlation was found between LCN-2 and CRP ($r = 0.34$; $p = 0.06$), PUCAI ($r = 0.33$; $p = 0.07$) and UCEIS ($r = 0.38$; $p = 0.07$). No correlation existed between LCN-2 and FC ($r = 0.12$; $p = 0.52$). Regarding MMP-9, a positive correlation has been found with CRP ($r = 0.42$; $p = 0.02$) and UCEIS ($r = 0.57$; $p = 0.004$). No other correlations were found between MMP-9/LCN-2 complex and the considered parameters.

4. Discussion

In this pilot cross-sectional study, we analyzed the expression of three serum biomarkers (LCN-2, MMP-9, and the MMP-9/LCN-2 complex) in a cohort of children with a new diagnosis of UC and prior to the initiation of any pharmacological treatment, investigating their potential utility as non-invasive biomarkers. We found upregulation of all the biomarkers in children with UC compared to healthy controls, along with a significant performance in terms of sensitivity and specificity of LCN-2 in discriminating between healthy and pathological conditions. Intriguingly, when analyzing the results in relation to the endoscopic findings (severity index and extension), LCN-2 emerged as a potential biomarker to distinguish children with pancolitis from those with different extents of colonic involvement. In this regard, ROC analysis showed that LCN-2 performed comparably to the PUCAI index in discriminating colonic section involvement in UC pediatric patients and had a significantly higher capacity than FC. MMP-9, but not the MMP-9/LCN-2 complex, was found to perform better than the other biomarkers in differentiating moderate/severe from mild disease activity. Preliminary findings further indicated that, based on the UCEIS score, LCN-2 levels could discriminate, within the subgroup of patients with more severe disease, between those with pancolitis and those with less extensive colonic involvement.

To date, only few serum and fecal biomarkers are used in clinical practice for the diagnosis, monitoring, and assessment of disease activity in UC, including CRP, a key indicator of inflammation, and FC. However, these biomarkers have several limitations. Notably, CRP lacks both sensitivity and specificity in detecting endoscopic inflammation in IBD, particularly in UC, where CRP responses can be modest or even absent. Furthermore, CRP levels can rise in most systemic inflammatory diseases, reducing its specificity. Additionally, up to 15% of patients may not exhibit a CRP response, further limiting its reliability [21,22]. Fecal calprotectin, while more sensitive than CRP in detecting colonic active disease, is influenced by other inflammatory conditions such as gastrointestinal infections, diverticulitis, and celiac disease. Although FC levels correlate with neutrophil migration to the gastrointestinal tract, its variability, lack of therapeutic thresholds, and poor specificity for endoscopic inflammation represent a limit [23]. Particularly in patients with low-grade inflammation, there is considerable overlap between organic and functional gastrointestinal disease, generating not only a great variability between different patients, but also making it difficult to use FC as a severity biomarker [24,25]. Moreover, FC concentration in different stool samples collected from the same patients during the day show a great variability, since FC levels increase with longer time between the bowel movements [26,27]. Furthermore, stool testing often faces low patient acceptance and low compliance, mostly for forgetfulness [28]. Both CRP and FC are unable to assess disease extent or complications, making it necessary to find new ones.

LCN-2 is a protein whose chemical stability makes it a good candidate as a new biomarker. In our study, we investigated the role of the LCN-2 system by assessing the serum levels of both its free form (LCN-2) and its conjugated complex with MMP-9 (the MMP-9/LCN-2 complex), revealing intriguing differences in the function of these two forms when considering distinctive aspects of the disease (inflammation, severity, extension).

The possible function of LCN-2 in gastrointestinal (GI) disorders has mostly been studied on adult population, showing that it has a pleiotropic role. In line with our results, several studies conducted on adult population showed increased levels of LCN-2 both in serum, colonic biopsies and fecal samples of patient with UC when compared to control population [17,29]. This upregulation was associated with a compensative anti-inflammatory and anti-microbic role of this biomarker, in addition to a microbiota-regulating one [30–32]. A positive correlation between LCN-2 expression and Enterobacteriaceae has been demonstrated, and it may suggest that these bacteria induce the expression of the siderophore binding protein as a host defense mechanism to limit the availability of iron and subsequently bacterial growth [33]. A similar mechanism occurs in patients suffering from GI infections, where LCN-2 prevents iron acquisition from the pathogen [33–35]. A study conducted by Stallhofer et al., in a population of adult patients with UC, LCN-2 was found to be a better biomarker than CRP in distinguish patients with active UC from patients in remission [10]. Janas et al., obtained similar results in a study conducted on pediatric population, showing elevated levels of LCN-2 in IBD patients [15]. Most of the studies conducted, showed that LCN-2 can be used as a biomarker of colonic inflammation, representing a more useful tool in patient with UC rather than with CD [36]. The strength of this potential new biomarker can be also demonstrated by LCN-2 protein reduction in biopsies from IBD patients treated with 5-amino salicylic acid or anti-TNF drugs, when compared to

untreated ones [29]. In our study, the inverse correlation between LCN-2 and serum albumin further highlights the significance of our findings in relation to intestinal disease severity. Moreover, the good specificity and sensitivity of LCN-2 in differentiating children based on colitis extension underscore the potential clinical value of its free form.

LCN-2 exists not only as a single protein but also forms homodimers (LCN-2/LCN-2) and heterodimers, specifically binding to MMP-9 (forming the MMP-9/LCN-2 complex) [37]. Yan's study provided evidence that NGAL modulates MMP-9 activity by protecting it from degradation [38] and increasing the MMP-9-induced demolition of extracellular matrix. To obtain a more comprehensive understanding of the lipocalin system in pediatric UC, we also analyzed both MMP-9 and the MMP-9/LCN-2 complex in our clinical cohort. The overexpression of both biomarkers in the overall cohort of UC patients clearly suggested that these two biological systems are activated during the acute phase of the disease. Furthermore, the increased levels of serum MMP-9 in UC patients with high disease severity (UCEIS > 4) and pancolitis (E4), as opposed to those with milder disease and a more limited extent (E1, E2, E3), lead to intriguing insights.

Elevated MMP-9 levels have been observed in the intestinal mucosa of individuals with IBD compared to normal tissue, as well as in the serum of patients compared to healthy controls [39,40]. This increase, which is associated with extracellular matrix degradation, may contribute to the progression of mucosal ulceration and inflammation in IBD patients. Moreover, the angiogenic properties of MMP-9 could facilitate the recruitment of inflammatory cells, thereby amplifying the inflammatory response in the intestine [41]. In this context, our study demonstrated a positive correlation between MMP-9 and CRP, confirming the link between these two circulating biomarkers in response to inflammation, even in the pediatric population.

While MMP-9 expression was higher both in severe UC cases and in those with pancolitis, we found a stronger positive correlation between MMP-9 and UCEIS (severity) than between MMP-9 and pancolitis (extension). Additionally, classifying pediatric patients based on the UCEIS endoscopic activity score revealed that circulating MMP-9 outperformed LCN-2 in terms of sensitivity and specificity for distinguishing the two subgroups (as shown by ROC analysis), further supporting the biological role of MMP-9 in the context of mucosal colonic lesions.

Differently from LCN-2 and MMP-9, serum MMP-9/LCN-2 complex, upregulated in UC patients compared to healthy people, did not show significant difference when patients were stratified based on endoscopic severity or extension. To date few studies have investigated the potential clinical value of MMP-9/LCN-2 complex in GI disorders. MMP-9/LCN-2 complex has been proposed as a surrogate serum marker of mucosal healing in UC adult patients [13,42]. This circulating complex, increased in the acute phase of the disease, was reduced in patients responding to infliximab. In children, MMP-9/LCN-2 complex, as well as MMP-9 and MMP-2, were found upregulated in the urine of patients, both CD and UC [14,43]. Interestingly, their sensitivity and specificity were higher than those reported for CRP and ESR for monitoring UC [14].

As a pilot study, our research is inherently limited by a small sample size; however, the application of stringent inclusion criteria (newly diagnosed UC in children) enhances its clinical relevance. Moreover, in this preliminary phase, we did not perform tissue or fecal analyses to confirm whether the serum findings accurately reflect the inflammatory status of the colon.

The absence of longitudinal data and a validation cohort is a limitation of this study, and their inclusion in future research would broaden the interpretative scope and generalizability of the findings. Nevertheless, we believe that our results provide promising insights for future, larger-scale studies, potentially including patients in remission to further explore the clinical utility of these biomarkers in disease monitoring.

5. Conclusions

Our findings suggest that the serum free form of LCN-2, rather than its conjugated form with MMP-9 (the MMP-9/LCN-2 complex), could have clinical utility. First, it may help distinguish the extent of colonic involvement, and second, it could assist in assessing its relationship with UC severity, potentially addressing the limitations of current biomarkers. Moreover, while MMP-9 and the MMP-9/LCN-2 complex are closely related, they seem to play distinct roles in the inflammatory process. Specifically, serum MMP-9 strongly correlated with the UCEIS score, consistent with its role in extracellular matrix breakdown, whereas no such evidence was found for the MMP-9/LCN-2 complex.

Overall, our findings support further investigation into the role of the free form of LCN-2 in pediatric UC. Our research expands the current understanding of the LCN-2 system by evaluating its role as a marker of disease severity and extent, laying the foundation for its potential clinical value as a candidate biomarker for monitoring disease progression.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the "Policlinico Umberto I" Hospital (Prot. 0520/2023). All participants and/or their guardians gave written informed consent to participate in the study. The trial was conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983, for human experimentation.

Consent for publication

All authors reviewed and approved the final manuscript.

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Authors' contributions

GDA, MA and CP conceived the study. GDA, GA and CP performed experiments and analyzed data. GDA, RP, FT, LT, MA enrolled patients and managed patient sample collection. GDA, AG and CP wrote the original draft. GDA, AG, CP and MA carried out review and editing.

Declaration of competing interest

The authors declare that they have no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2026.01.224.

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