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Fecal Zonulin as an Early Predictor of Necrotizing Enterocolitis in Premature Infants: A Pilot Prospective Single-Center Cohort Study

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Abstract

Background

Zonulin is an endogenous regulator of intestinal epithelial tight junctions and a biomarker of intestinal permeability. This study evaluated the predictive value of fecal zonulin levels for the early identification of necrotizing enterocolitis (NEC) risk in premature infants.

Methods

This single-center prospective cohort study included 91 premature infants (gestational age 22–32 weeks) and 25 term controls followed for 2 months. Fecal zonulin concentrations were measured by enzyme-linked immunosorbent assay (ELISA) using stool samples collected on postnatal days 3, 7, and 14. The primary receiver operating characteristic (ROC) analysis was performed at the patient level, with a sample-level ROC analysis conducted as a sensitivity analysis. Multivariate logistic regression was performed as an exploratory analysis because of the limited number of NEC events.

Results

NEC developed in 10 of 91 (11.0%) premature infants at a mean postnatal age of 12.5 ± 0.35 days. Mean fecal zonulin concentrations on postnatal day 7 were significantly higher in infants who subsequently developed NEC than in those who did not (618.4 ± 89.9 ng/mL vs 343.5 ± 76.7 ng/mL; $p < 0.05$). Patient-level ROC analysis for day 7 fecal zonulin demonstrated an area under the curve (AUC) of 0.81, with 85% sensitivity and 72% specificity at a cutoff value of 490 ng/mL. Sample-level ROC analysis (sensitivity analysis) yielded an AUC of 0.995, with 100% sensitivity and 94.5% specificity at a cutoff value of 548.2 ng/mL. Exploratory multivariate logistic regression identified fecal zonulin as an independent predictor of NEC; however, these findings should be interpreted cautiously because of the limited number of NEC events.

Conclusions

Elevated fecal zonulin concentrations on postnatal day 7 are associated with subsequent development of NEC in premature infants and may represent a promising noninvasive biomarker for early risk stratification. Larger multicenter studies are warranted to validate these findings.

Background

Premature infants, particularly those born before 32 weeks of gestation, are highly vulnerable because of the functional immaturity of multiple organ systems, including the gastrointestinal tract. One of the most devastating complications in this population is necrotizing enterocolitis (NEC), a multifactorial disease

characterized by intestinal barrier dysfunction, abnormal microbial colonization, and exaggerated inflammatory responses that result in intestinal injury and high morbidity and mortality. Experimental and clinical studies indicate that disruption of the intestinal epithelial barrier is an early event in NEC pathogenesis, preceding bacterial translocation, uncontrolled intestinal inflammation, and tissue necrosis. Despite advances in neonatal care, NEC remains difficult to predict before the onset of clinical manifestations [1–3, 13–15].

The intestinal epithelial barrier plays a fundamental role in preventing bacterial translocation and limiting intestinal inflammation. The integrity of this barrier depends on the coordinated function of the apical junctional complex, including tight junctions and adherens junctions, which together regulate paracellular permeability and maintain epithelial cohesion. In this study, we focused on zonulin, an endogenous regulator of intestinal tight junction permeability that modulates paracellular permeability and maintains intestinal barrier homeostasis. Zonulin is released primarily by intestinal enterocytes and acts locally at the apical tight junctions between adjacent epithelial cells, where it reversibly regulates the assembly of tight junction proteins. Increased zonulin release disrupts epithelial barrier integrity, facilitating the translocation of bacteria, endotoxins, and other luminal antigens that initiate intestinal inflammation. As fecal zonulin (FZ) reflects local barrier dysfunction rather than systemic inflammation, it has emerged as a promising noninvasive biomarker of intestinal permeability. Beyond serving as an indicator of intestinal inflammation, fecal zonulin in premature infants may also represent a biomarker of delayed maturation of the intestinal epithelial barrier, reflecting developmental vulnerability before the onset of overt disease. Because intestinal barrier disruption is believed to precede the clinical manifestations of NEC, FZ may enable identification of premature infants at increased risk before the onset of irreversible intestinal injury [4–12].

Current research on the early prediction of NEC has focused primarily on inflammatory biomarkers, particularly fecal calprotectin. Although fecal calprotectin reflects intestinal inflammation, its predictive value in premature infants has been inconsistent, highlighting the need for biomarkers capable of detecting intestinal barrier dysfunction before the development of overt inflammation and clinical disease [16–22].

Because disruption of the intestinal barrier is considered a pivotal early event in the pathogenesis of NEC, fecal zonulin represents a biologically plausible candidate biomarker for early risk stratification. However, data regarding its clinical utility in premature infants remain limited. Therefore, the objective of this study was to evaluate whether fecal zonulin measured during the first two weeks of life can predict the subsequent development of necrotizing enterocolitis in premature infants.

Methods

Study design and participants

This single-center pilot prospective cohort study was conducted between 2021 and 2023 at the obstetric hospitals and neonatal units of the Republican Perinatal Center, Tashkent, Uzbekistan. The study was approved by the Ethics Committees of EMU University (Approval No. IP-2021-1223) and the Republican Perinatal Center of Uzbekistan (Approval No. IP-2021-578). Written informed consent was obtained from the parents or legal guardians of all participants before enrollment.

The study evaluated intestinal barrier biomarkers in premature infants during the first two weeks of life. Inclusion criteria were birth at 22–32 weeks' gestation and admission to the neonatal intensive care unit (NICU). The study protocol, including stool collection, was completed during the first 14 days of life, and infants were subsequently followed until hospital discharge.

Exclusion criteria included major congenital heart disease, chromosomal or hereditary disorders, and death during the first week of life. NEC was diagnosed according to the modified Bell staging criteria described by Walsh and Kliegman [REF].

Clinical assessment and laboratory investigations

Maternal and neonatal demographic and clinical characteristics were prospectively recorded. All infants underwent standardized physical examinations and routine laboratory investigations according to institutional protocols, including complete blood count, serum bilirubin, blood glucose, C-reactive protein (CRP), coagulation profile, and stool analysis. Instrumental investigations included chest and abdominal radiography, cranial ultrasonography, and echocardiography when clinically indicated. Growth was assessed using the Fenton growth charts.

Fecal samples were collected on postnatal days 3, 7, and 14. Fecal zonulin (FZ) concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Immunodiagnostik AG, Germany). Fecal α1-antitrypsin was determined by immunoturbidimetry, and fecal calprotectin was measured on postnatal day 7 using ELISA (PhiCal®, Immunodiagnostik AG, Germany).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± standard error of the mean (SEM), and categorical variables as frequencies and percentages. Comparisons between groups were performed using Student's *t*-test for independent samples. A two-sided p value < 0.05 was considered statistically significant.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the predictive performance of fecal zonulin, and optimal cutoff values were determined using the Youden index [23]. Sensitivity, specificity, positive predictive value, negative predictive value, and area under the ROC curve (AUC) were calculated. Comparisons between ROC curves were performed using the DeLong test.

Because only 10 infants developed NEC, multivariable logistic regression was considered exploratory [24] and was performed to evaluate the independent association between fecal zonulin, fecal calprotectin, C-reactive protein, and NEC. Odds ratios (ORs) and corresponding P values are reported. Model discrimination was assessed using the area under the receiver operating characteristic curve (AUC).

Results

Study population

A total of 91 premature infants born between 22 and 32 weeks' gestation were enrolled in the study. A contemporaneous group of 25 healthy term newborns served as controls for developmental comparisons. Baseline maternal and neonatal characteristics are summarized in Table 1.

Among the premature infants, 10 (10.9%) subsequently developed NEC at a mean postnatal age of 12.5 ± 0.4 days, whereas 81 (89.1%) completed their hospital course without NEC. According to the modified Bell staging criteria, 6 infants developed stage IIA disease and 4 progressed to stage IIIA NEC without intestinal perforation.

Developmental profile of fecal zonulin

Fecal zonulin (FZ) concentrations increased progressively in healthy term newborns during the first two weeks of life (Table 2). Mean FZ concentrations increased from 28.5 ± 3.6 ng/mL on postnatal day (D) 3 to 64.1 ± 5.1 ng/mL on D7 and 89.8 ± 5.8 ng/mL on D14 (P for trend < 0.001).

In contrast, premature infants demonstrated substantially higher FZ concentrations throughout the study period. Mean FZ concentrations were **490.9 ± 73.5 ng/mL** on D3, **507.7 ± 77.2 ng/mL** on D7, and **430.0 ± 60.4 ng/mL** on D14, corresponding to approximately **17-fold**, **20-fold**, and **5-fold** higher values than those observed in term infants, respectively (all **P < 0.05**) (Table 2).

Fecal zonulin and subsequent development of NEC

Serial FZ concentrations were analyzed according to subsequent clinical outcome (Table 2, Fig. 1).

On D3, mean FZ concentrations were similar between infants who subsequently developed NEC (339.5 ± 78.9 ng/mL) and those who did not (373.7 ± 65.8 ng/mL, P = 0.742).

By D7, FZ concentrations were significantly higher among infants who subsequently developed NEC (618.4 ± 89.9 ng/mL) compared with infants without NEC (343.5 ± 76.7 ng/mL, P < 0.05).

By D14, FZ concentrations had decreased in infants with NEC (213.3 ± 45.9 ng/mL) whereas concentrations remained relatively stable among infants without NEC (376.9 ± 66.5 ng/mL, $P < 0.05$).

These findings demonstrate that elevated FZ concentrations preceded the clinical diagnosis of NEC by several days.

Inflammatory biomarkers

Inflammatory biomarkers are summarized in Table 3.

Infants who subsequently developed NEC demonstrated significantly higher concentrations of C-reactive protein (CRP) (36.0 ± 12.0 mg/L vs 12.0 ± 2.27 mg/L, $P < 0.05$) and fecal calprotectin (214.8 ± 38.3 µg/g vs 85.5 ± 35.7 µg/g, $P < 0.05$) than infants who did not develop NEC.

Predictive performance of fecal zonulin

Receiver operating characteristic (ROC) analysis was performed to evaluate the predictive performance of FZ measured on postnatal days 3, 7, and 14 (Table 4, Fig. 2).

The primary patient-level ROC analysis demonstrated that FZ measured on D7 provided the highest predictive accuracy for subsequent NEC, with an AUC of 0.81. The optimal cutoff value was 490 ng/mL, corresponding to a sensitivity of 85% and specificity of 72%.

Sensitivity analysis performed using all stool samples demonstrated similar findings, with a D7 AUC of 0.995, sensitivity of 100%, specificity of 94.5%, and an optimal cutoff value of 548.2 ng/mL (Additional file 1: Table S1).

Comparison with other biomarkers

The predictive performance of FZ was compared with other biomarkers of intestinal injury and inflammation (Table 5).

FZ measured on D7 demonstrated superior discriminatory performance compared with fecal calprotectin and CRP. The AUC for FZ was 0.995, compared with 0.84 for fecal calprotectin and 0.69 for CRP, indicating higher predictive performance of FZ.

Sensitivity, specificity, positive predictive value, and negative predictive value for each biomarker are summarized in Table 5.

Exploratory multivariable analysis

Because only 10 infants developed NEC, multivariable logistic regression was considered exploratory. The model incorporated fecal zonulin measured on D7, fecal calprotectin, and C-reactive protein (CRP). Fecal zonulin remained the strongest independent predictor of NEC (OR 1.015; $P < 0.001$). Fecal calprotectin (OR 1.009; $P = 0.022$) and CRP (OR 1.005; $P = 0.049$) also remained independently associated with NEC (Table 6). The overall model demonstrated excellent discrimination, with an AUC of 0.997.

Secondary analyses

No significant association was observed between feeding type (exclusive breast milk, formula feeding, or mixed feeding) and FZ concentrations on postnatal days 3, 7, or 14 (all $P > 0.05$).

Discussion

To our knowledge, this study is the first to characterize the dynamic profile of fecal zonulin (FZ) concentrations in premature and term newborn infants during the early neonatal period. In healthy term newborns, mean FZ concentrations were 28.5 ± 3.6 ng/mL on postnatal day (D) 3, 64.1 ± 5.1 ng/mL on D7, and 89.8 ± 5.8 ng/mL on D14. Sochaczewska et al. [25] previously measured FZ levels at birth and reported concentrations of 74.14 ng/g. We detected substantially lower FZ concentrations on D3, suggesting dynamic postnatal modulation of intestinal permeability during the early neonatal period. Łoniewska et al. [12] subsequently demonstrated a progressive increase in FZ concentrations throughout the first two years of life. Differences among studies may be explained by variations in age at sampling, biological specimen, laboratory methodology, units of measurement, and study populations.

We observed a gradual rise in FZ concentrations in healthy term newborns from D3 to D14, suggesting physiological regulation of intestinal permeability during early postnatal adaptation. This period is characterized by increasing enteral feeding, establishment of the intestinal microbiome, maturation of epithelial junctional complexes, and development of mucosal immune tolerance, all of which may contribute to normal remodeling of the intestinal barrier. In contrast, FZ concentrations in premature infants were markedly higher than in term infants, reaching approximately 17-fold higher levels on D3, 20-fold higher levels on D7, and fivefold higher levels on D14. These elevated concentrations likely reflect immaturity of the gastrointestinal barrier and the associated predisposition to increased intestinal permeability, thereby contributing to feeding intolerance and increased susceptibility to NEC and other gastrointestinal complications [27]. Premature birth also interrupts exposure to amniotic fluid-derived trophic factors, including hepatocyte growth factor, whereas human milk provides transforming growth factor-β2, epidermal growth factor, lactoferrin, and human milk oligosaccharides that promote epithelial maturation and barrier integrity. Consequently, persistently elevated FZ concentrations in premature infants may reflect not only intestinal injury but also delayed maturation of the intestinal epithelial barrier.

Our data demonstrate that FZ concentrations were already elevated on D7, several days before the clinical diagnosis of NEC. These findings provide biological support for the hypothesis that loss of intestinal barrier integrity is an early pathogenic event in NEC. Increased epithelial permeability may facilitate bacterial translocation, exposure of the immature intestinal immune system to luminal microbial products, and activation of inflammatory pathways that ultimately culminate in intestinal necrosis. Because the increase in fecal zonulin preceded the onset of overt disease, it is unlikely to represent merely a marker of established intestinal injury. Rather, it may identify a clinically silent phase of barrier dysfunction during which disease progression has begun but remains potentially modifiable. Early recognition of this vulnerable window could permit closer surveillance and timely implementation of preventive or therapeutic interventions in infants at greatest risk. Importantly, our findings demonstrate not only an association between elevated FZ and NEC but also a temporal sequence in which increased intestinal permeability preceded clinical disease, a prerequisite for considering FZ as a predictive rather than merely a diagnostic biomarker.

A limited number of studies have evaluated circulating zonulin concentrations in premature infants. Serum zonulin levels were not consistently elevated in infants with NEC, even in cases complicated by intestinal perforation. As reported by Tarko et al. [29], increased serum zonulin concentrations may reflect the extent of intestinal involvement rather than the severity of intestinal injury. Similar findings have been reported by Li et al. [30], who suggested that plasma zonulin may serve as an early indicator of inflammatory responses and intestinal dysfunction in severe NEC. These observations support the concept that fecal zonulin may represent a more sensitive and localized marker of intestinal barrier dysfunction during the earliest stages of disease. Because circulating zonulin may originate from multiple organs, fecal zonulin is likely to provide greater specificity for assessing intestinal permeability and epithelial barrier dysfunction [31]. Furthermore, serum zonulin has important biological and analytical limitations. Zonulin (pre-haptoglobin 2) is synthesized by both enterocytes and hepatocytes, and circulating concentrations may be influenced by systemic inflammatory and metabolic conditions. In addition, several commercially available ELISA assays marketed for zonulin have been shown to detect structurally related proteins rather than pre-haptoglobin 2 itself. Consequently, serum zonulin concentrations should be interpreted cautiously. In contrast, FZ reflects proteins released directly into the intestinal lumen and therefore provides a more localized assessment of epithelial barrier integrity. Although similar analytical considerations apply to fecal assays, fecal measurements are less likely to be influenced by extraintestinal sources and may offer greater biological specificity for disorders characterized by primary intestinal injury, such as NEC.

The pathophysiology of NEC is multifactorial, involving intestinal barrier immaturity, dysbiosis, altered immune responses, and microvascular dysfunction [28]. Unlike fecal calprotectin, which reflects neutrophil-mediated intestinal inflammation, zonulin regulates epithelial tight junctions and intestinal permeability. Elevated FZ therefore reflects early epithelial barrier dysfunction rather than established inflammation. By disrupting barrier integrity, zonulin facilitates bacterial translocation and innate immune activation. In the 10 infants who subsequently developed NEC, FZ concentrations were already elevated on D7, before the onset of clinical manifestations, supporting the concept that barrier

dysfunction precedes the inflammatory cascade leading to NEC. As microbial colonization rapidly evolves during the first weeks of life, increased FZ may identify premature infants with delayed intestinal barrier maturation before overt disease develops. These findings suggest that fecal zonulin is both a biomarker of epithelial barrier dysfunction and a marker of delayed intestinal barrier maturation, providing an opportunity for earlier risk stratification and preventive intervention.

In our study, fecal calprotectin also demonstrated prognostic value for the early detection of NEC. Calprotectin is a well-established marker of neutrophil-mediated intestinal inflammation, and our findings are consistent with recent studies by ZongLi et al. (2025) and Aydemir et al. (2025), which demonstrated that fecal calprotectin levels are significantly elevated in premature infants with NEC and may be useful for the early diagnosis and assessment of disease severity [34, 35]. However, these findings should be interpreted cautiously, particularly in small cohorts, because intestinal dysbiosis alone, even in the absence of NEC, may increase fecal calprotectin concentrations [36]. This limitation suggests that inflammatory biomarkers may be most informative when interpreted alongside biomarkers that reflect intestinal barrier function. To explore this concept, we developed a multivariable logistic regression model incorporating fecal zonulin, fecal calprotectin, and C-reactive protein. Although this analysis should be considered exploratory because of the limited number of NEC events, the combined model improved predictive performance and identified FZ as the strongest independent predictor of subsequent NEC. These findings support the complementary roles of biomarkers reflecting epithelial barrier dysfunction and intestinal inflammation and provide a rationale for incorporating fecal zonulin into multimarker strategies for early risk stratification and individualized management of premature infants at increased risk for NEC.

Comparable results regarding the prognostic relevance of fecal calprotectin have been reported by Godovanets et al. [21], who demonstrated that, based on ROC analysis, several fecal markers—including calprotectin, albumin, polymorphonuclear elastase, and α1-antitrypsin—exhibited the highest sensitivity and specificity in premature infants with digestive disorders associated with various perinatal pathologies. Taken together, these data support the complementary role of inflammatory and permeability-related fecal biomarkers in improving the early identification and risk assessment of NEC. Future studies should evaluate whether combining FZ with additional biomarkers of intestinal permeability, inflammation, epithelial injury, and microbial dysbiosis further improves the early prediction and risk stratification of NEC.

Limitations

This study has several limitations. First, it was conducted at a single tertiary referral center with a relatively small sample size, including only 10 infants who developed NEC, which may limit the generalizability of the findings. Consequently, the multivariable logistic regression analysis should be considered exploratory, and validation in larger multicenter cohorts is required to confirm the predictive performance of fecal zonulin and the proposed multimarker model.

Second, the degree of prematurity was not incorporated as an independent stratification variable. Inclusion of gestational age as a covariate could have provided a more precise assessment of its influence on intestinal permeability, inflammatory biomarkers, and the subsequent risk of NEC.

Third, the intestinal microbiome was not characterized. Because intestinal dysbiosis, particularly expansion of Enterobacteriaceae and other Gammaproteobacteria, is closely linked to epithelial barrier dysfunction and NEC, future studies integrating longitudinal microbiome profiling with fecal zonulin measurements may further clarify the temporal relationship between microbial colonization, barrier dysfunction, and disease development.

Fourth, fecal zonulin was measured using a commercially available ELISA assay. Recent studies have demonstrated that some assays marketed for zonulin may detect structurally related proteins in addition to pre-haptoglobin 2. Although this analytical limitation should be considered when interpreting absolute concentrations, all specimens were analyzed using the same assay under identical conditions, preserving the validity of the observed between-group comparisons.

Finally, several potentially important clinical variables, including the severity of respiratory disease, the presence and grade of intraventricular hemorrhage, duration of antibiotic therapy, feeding practices, and other neonatal comorbidities, were not incorporated into the multivariable analysis. These factors may influence inflammatory responses, intestinal barrier function, and the risk of NEC and should be considered in future investigations.

Despite these limitations, the prospective design, serial longitudinal fecal sampling during the first two weeks of life, inclusion of a healthy term control group, and demonstration that fecal zonulin increased before the clinical onset of NEC represent important strengths of this study. Together, these findings provide novel evidence supporting fecal zonulin as a promising early biomarker of intestinal barrier dysfunction and a potential tool for risk stratification in premature infants at risk for necrotizing enterocolitis.

Conclusions

This prospective study demonstrates that FZ is a promising noninvasive biomarker of early intestinal barrier dysfunction in premature infants. Elevated FZ concentrations preceded the clinical onset of necrotizing enterocolitis, supporting the concept that disruption of epithelial barrier integrity is an early event in disease pathogenesis. Compared with inflammatory biomarkers that primarily reflect established mucosal inflammation, FZ may identify a preclinical phase of disease during which preventive interventions remain feasible. Furthermore, combining FZ with inflammatory biomarkers such as fecal calprotectin and C-reactive protein may improve risk stratification in premature infants. Although these findings require confirmation in larger multicenter studies, they provide a strong rationale for incorporating biomarkers of intestinal permeability into future predictive models and prevention strategies for NEC.

Abbreviations

AUC: Area under the (ROC) curve

CRP: C-reactive protein

D3 / D7 / D14: Postnatal day 3 / 7 / 14

ELISA: Enzyme-linked immunosorbent assay

FZ: Fecal zonulin

NEC: Necrotizing enterocolitis

NICU: Neonatal intensive care unit

OR: Odds ratio

ROC: Receiver operating characteristic

SEM: Standard error of the mean

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committees of EMU University (Approval No. IP-2021-1223) and the Republican Perinatal Center of Uzbekistan (Approval No. IP-2021-578). Written informed consent was obtained from the parents or legal guardians of all participants before enrollment.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. [Please confirm this default statement is accurate, or update it to reflect your actual data-sharing plan / repository.]

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

[To be completed by the corresponding author, using initials for each author — e.g., “SK conceived and designed the study, collected data, and drafted the manuscript. AK contributed to study design, data interpretation, and critical revision of the manuscript. AF contributed to data collection and statistical analysis. All authors read and approved the final manuscript.” Please replace with the accurate contribution statement.]

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References

1. Khasanova SS, Kamilova AT. Dynamics of fecal zonulin levels in preterm infants during the first two weeks of life. Russian Bull Perinatol Pediatr. 2019;64(2):52–6.
2. Khasanova SS, Kamilova AT. Prognostic value of fecal calprotectin in preterm infants with a gestational age at birth of less than 32 weeks. Mod Pediatr (Ukraine). 2019;1(97):26–30.
3. Szymanska E, Bierla J, Dadalski M, Wierzbicka A, Konopka E, Cukrowska B, Kierkus J. New noninvasive biomarkers of intestinal inflammation and increased intestinal permeability in pediatric inflammatory bowel diseases and their correlation with fecal calprotectin: a pilot study. Minerva Gastroenterol. 2023;69(4):504–10.
4. Szymanska E, Wierzbicka A, Dadalski M, Kierkus J. Fecal zonulin as a noninvasive biomarker of intestinal permeability in pediatric patients with inflammatory bowel diseases: correlation with disease activity and fecal calprotectin. J Clin Med. 2021;10:3905.
5. Geller S, Umarnazarova Z, Azimova N, Usmonova K, Kamilova A. Markers of enterocyte damage in celiac disease in children: is there an association with the clinical manifestations of the disease? Front Pediatr. 2025;13:1566149.
6. Salvo Romero E, Alonso Cotoner C, Pardo Camacho C, Casado Bedmar M, Vicario M. The intestinal barrier function and its involvement in digestive disease. Rev Esp Enferm Dig. 2015;107:686–96.
7. Voniewska B, Węgrzyn D, Adamek K, Kaczmarczyk M, Skonieczna-Żydecka K, Adler G, et al. The influence of maternal foetal parameters on concentrations of zonulin and calprotectin in the blood and stool of healthy newborns during the first seven days of life: an observational prospective cohort study. J Clin Med. 2019;8(4):473.

8. Ohlsson B, Orho-Melander M, Nilsson PM. Higher levels of serum zonulin may rather be associated with increased risk of obesity and hyperlipidemia than with gastrointestinal symptoms or disease manifestations. Int J Mol Sci. 2017;18(3):582.
9. Scheffler L, Crane A, Heyne HO, Toenjes A, Schleinitz D, Ihling CH et al. Widely used commercial ELISA for human zonulin reacts with complement C3 rather than pre-haptoglobin 2. *bioRxiv* [Preprint]. 2017:157578.
10. Kim AS, Ko HJ. Plasma concentrations of zonulin are elevated in obese men with fatty liver disease. Diabetes Metab Syndr Obes. 2018;11:149–57.
11. Barengolts E, Green SJ, Chlipala GE, Layden BT, Eisenberg Y, Priyadarshini M, Dugas LR. Predictors of obesity among gut microbiota biomarkers in African American men with and without diabetes. Microorganisms. 2019;7(9):320.
12. Łoniewska B, Adamek K, Węgrzyn D, Kaczmarczyk M, Skonieczna-Żydecka K, Clark J, Adler G, Tousty J, Uzar I, Tousty P, et al. Analysis of faecal zonulin and calprotectin concentrations in healthy children during the first two years of life: an observational prospective cohort study. J Clin Med. 2020;9:777.
13. Xu G, Ma T, Zhou C, et al. β-Carotene attenuates apoptosis and autophagy via PI3K/AKT/mTOR signaling pathway in necrotizing enterocolitis model cells IEC-6. Evid Based Complement Alternat Med. 2022;2022:2502263.
14. Shu B, Zhang S, Gao J, Wang L, Wang X. Worldwide research tendency and collaborations on models of necrotizing enterocolitis: bibliometric exploration over the past three decades. Ann Med Surg (Lond). 2024;87:2840.
15. Dickison L, Gonzalez-Shalaby C. Reducing risk factors for necrotizing enterocolitis: what is the recent evidence and biologic plausibility supporting probiotics? Adv Neonatal Care. 2022;22(6):513–22.
16. Orivuori L, Mustonen K, de Goffau MC, Hakala S, Paasela M, Roduit C, et al. High level of fecal calprotectin at age 2 months as a marker of intestinal inflammation predicts atopic dermatitis and asthma by age 6. Clin Exp Allergy. 2015;45:928–39.
17. Kamilova AT, Azizova GK, Umarnazarova ZE, Abdullaeva DA, Geller SI. The activity of antimicrobial peptides in pediatric celiac disease. Front Pediatr. 2022;10:873793.
18. Berstad A, Arslan G, Folvik G. Relationship between intestinal permeability and calprotectin concentration in gut lavage fluid. Scand J Gastroenterol. 2000;35:64–9.
19. Willers M, Ulas T, Völlger L, Vogl T, Heinemann AS, Pirr S et al. S100A8 and S100A9 are important for postnatal development of gut microbiota and immune system in mice and infants. Gastroenterology. 2020;S0016-5085(20)35058-7.
20. Costa S, Patti ML, Perri A, Cocca C, Pinna G, Tirone C, Tana M, Lio A, Vento G. Effect of different milk diet on the level of fecal calprotectin in very preterm infants. Front Pediatr. 2020;8:552.
21. Godovanets O, Nechytaylo Y. Comparative characteristics of clinical and laboratory markers of nutrition in premature children taking into account gestational age at birth. East Ukr Med J.

2025;13(2):526–35.
22. Akhmedova DI, Fazilova AS. The inflammatory status of the intestine in premature infants depending on gestational age. Ro J Pediatr. 2023;72(3):153–6.
23. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem. 1993;39(4):561–77.
24. Hosmer DW, Lemeshow S. Goodness-of-fit tests for the multiple logistic regression model. Commun Stat Theory Methods. 1980;9(10):1043–69.
25. Sochaczewska D, Ziętek M, Dołęgowska B, Kordek A, Szczuko M. Implications of indirect biomarkers of intestinal permeability in the stools of newborns and infants with perinatal risk factors for intestinal colonization disorders and infant feeding patterns. Nutrients. 2022;14:2224.
26. Kaczmarczyk M, Löber U, Adamek K, et al. The gut microbiota is associated with the small intestinal paracellular permeability and the development of the immune system in healthy children during the first two years of life. J Transl Med. 2021;19:177.
27. dos Lima Oliveira A, Salles Goulart N, Monteiro Leite PN, Craveiro de Paula L, de Nogueira Gomes AM. Fatima da Conceição Veríssimo Lopes J. Necrotizing enterocolitis in neonatal intensive care units: a systematic review of biochemical markers and nursing interventions. INTERFERENCE: J Audio Cult. 2025;11(2):8770–95.
28. Ma B, McComb E, Gajer P, Yang H, Humphrys M, Okogbule-Wonodi AC, et al. Microbial biomarkers of intestinal barrier maturation in preterm infants. Front Microbiol. 2018;9:2755.
29. Tarko A, Suchojad A, Michalec M, Majcherczyk M, Brzozowska A, Maruniak-Chudek I. Zonulin: a potential marker of intestine injury in newborns. Dis Markers. 2017;2017:2413437.
30. Li C, et al. Zonulin regulates intestinal permeability and facilitates enteric bacteria permeation in coronary artery disease. Sci Rep. 2016;6:29142.
31. Moser AM, Spindelboeck W, Halwachs B, Strohmaier H, Kump P, Gorkiewicz G, et al. Effects of an oral synbiotic on the gastrointestinal immune system and microbiota in patients with diarrhea-predominant irritable bowel syndrome. Eur J Nutr. 2018;58(7):2767–78.
32. Odenwald MA, Turner JR. Intestinal permeability defects: is it time to treat? Clin Gastroenterol Hepatol. 2013;11:1075–83.
33. Faubion WA, Camilleri M, Murray JA, Kelly P, Aadi B, Kosek MN, et al. Improving the detection of environmental enteric dysfunction: a lactulose, rhamnose assay of intestinal permeability in children aged under 5 years exposed to poor sanitation and hygiene. BMJ Glob Health. 2016;1(1):e000066.
34. ZongLi C, KeYong L, Liang J. Value of single-center fecal calprotectin in the early diagnosis and assessment of necrotizing enterocolitis in premature infants. Front Pediatr. 2025;13:1598448.
35. Aydemir G, Cekmez F, Tanju I, Canpolat F, Genc FA, Yildirim S et al. Increased fecal calprotectin in preterm infants with necrotizing enterocolitis. Clin Lab. 2013;59.
36. Rougé C, Butel MJ, Piloquet H, Ferraris L, Legrand A, et al. Fecal calprotectin excretion in preterm infants during the neonatal period. PLoS ONE. 2010;5(6):e11083.

Tables

Tables 1 to 6 are available in the supplementary files section.

Figures

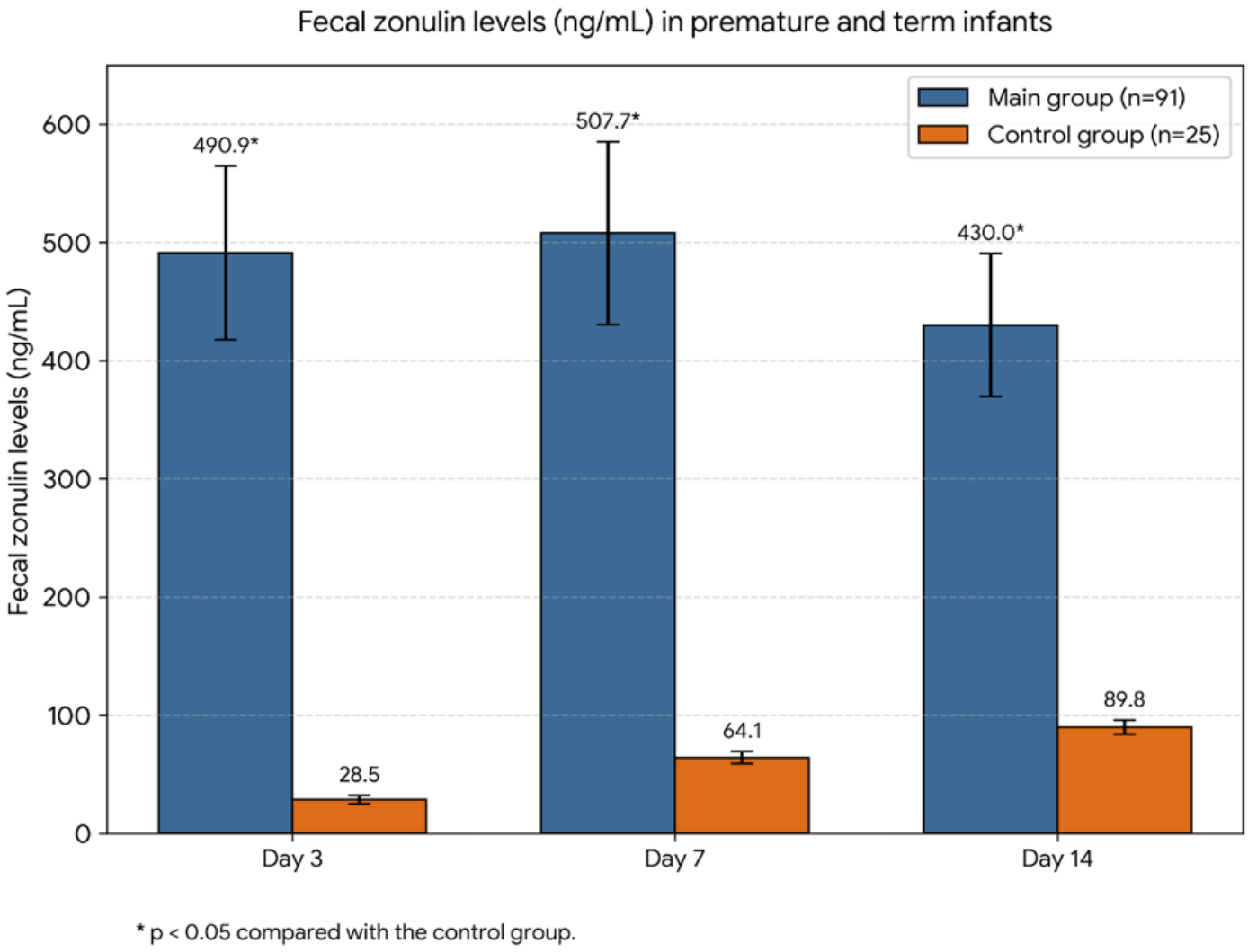

Figure 1

Longitudinal changes in fecal zonulin concentrations.

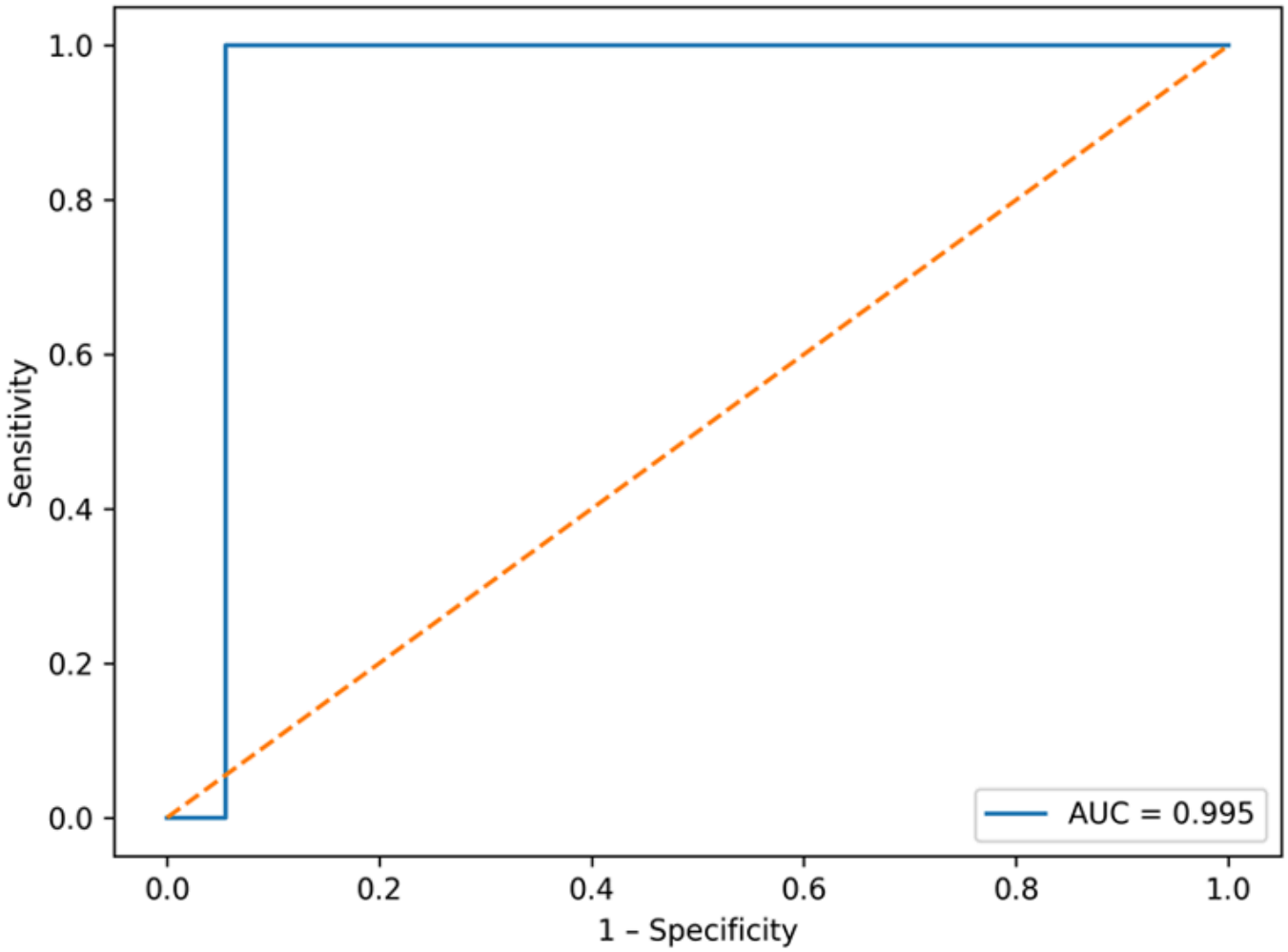

Figure 2

ROC curves for prediction of NEC.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.docx
- Table2.docx
- Table3.docx
- Table4.docx
- Table5.docx
- Table6.docx