



## Gasdermin-D driven pyroptosis fuels oxidative stress in ulcerative colitis: Biochemical and physiological correlations

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Ulcerative colitis (UC) is a chronic inflammatory bowel disease, resulting from intricate interplay among immune dysregulation, epithelial barrier malfunction and oxidative stress. Recent evidence has identified pyroptosis, a lytic and inflammatory type of programmed cell death, which is mediated by gasdermin D (GSDMD), as an important driver of intestinal inflammation. Pyroptosis also results in the release of proinflammatory cytokines, such as IL-18, that can further activate neutrophils and increase oxidative damage. Nevertheless, there are few integrated clinical research studies on the simultaneous detection of pyroptosis and cytokine release and oxidative stress markers in UC. Thus the aim of our research is to determine the serum concentrations of GSDMD, IL-18, myeloperoxidase (MPO), malondialdehyde (MDA) and C-reactive protein (CRP) in UC patients compared with healthy controls and to investigate their relationships as well as explore the diagnostic value of these indices for UC. **Methods:** A case-control study was performed with 45 patients who were diagnosed with UC and 45 age-matched healthy controls. Serum levels of GSDMD, IL-18, MPO, and MDA were assayed by enzyme linked immunosorbent assay (ELISA), and C-reactive protein (CRP) was assessed with immunodetection methods. Statistical analysis comprised independent t-tests, correlation, receiver operating characteristic (ROC) curve and logistic regression. **Results:** The concentration of serum GSDMD, IL-18, MPO, MDA and CRP in UC patients were all obviously higher than that in controls (all  $P < 0.05$ ). IL-18 was positively correlated with CRP ( $r = 0.481$ ,  $p = 0.001$ ) and MDA ( $r = 0.389$ ,  $p = 0.008$ ). The ROC analysis showed excellent diagnostic power for GSDMD (AUC = 0.993), IL-18 (AUC = 0.995), and MDA (AUC = 0.992) in distinguishing UC patients from healthy controls. In regression analysis, GSDMD and IL-18 were independent predictors for UC status. **Conclusions:** Together, these results favor a pathogenic model of UC where GSDMD-mediated pyroptosis leads to IL-18 release and subsequent neutrophil activation and oxidative lipid damage. GSDMD, IL-18 and MDA show promise as alternative non-invasive diagnostic markers. These findings emphasize the importance of both pyroptosis and oxidative stress next to other biological mechanisms in their translation into clinical aspects of UC.

**Keywords:** ulcerative colitis; gasdermin D; pyroptosis; interleukin-18; oxidative stress; myeloperoxidase; malondialdehyde.

### Introduction

Ulcerative colitis (UC) is a type of relapsing and remitting chronic inflammatory bowel disease (IBD) in which there is diffuse mucosal inflammation of the colon and rectum. It presents clinically with bloody diarrhea, colicky abdominal pain, urgency in bowel movements, and systemic symptoms of fatigue and weight loss (Torres et al., 2017). Despite tremendous progress in diagnostic and therapeutic techniques, the exact etiology of UC continues to be multi-factorial and not well defined. There is increasing evidence that the pathogenesis of IBD involves a complex interaction between genetic predisposition, environmental factors as triggers, abnormal immune responses, changes in gut microbiota, and defective epithelial barrier function (Ungaro et al., 2017). This complex pathogenesis is associated with an important burden in the clinical management of UC, mainly because it is difficult to predict disease flares, evaluate mucosal healing and apply personalized treatment strategies (Colombel et al., 2017).

An ongoing challenge in UC management has been the absence of dependable biomarkers that properly reflect the pathophysiologic cascade behind inflammation and tissue damage. Common clinical scores and laboratory markers, including CRP and the ESR, have utility in the evaluation of systemic inflammation, however they frequently have poor correlation with endoscopic disease activity and no ability to elucidate individual pathophysiologic pathways (Magro et al., 2020). Stool calprotectin, although better suited for identifying intestinal inflammation, is a general measure of neutrophil invasion that does not delineate the cellular or molecular events responsible (D'Haens et al., 2012). Therefore, the discovery and validation of new biomarkers with diagnostic/prognostic significance and mechanistic

relevance are urgently needed to connect bench studies to clinical research.

In recent years, programmed cell death signaling cascades, in particular pyroptosis, have been proposed as key modulators of the IBD inflammatory environment. Pyroptosis is an apoptosis similar, proinflammatory form of regulated cell death with distinct characteristics from apoptosis and necrosis, such as cell swelling, membrane pore formation and the release of pro-inflammatory intracellular contents (Bergsbaken et al., 2009). The executor protein for pyroptosis is gasdermin D (GSDMD). When it is cleaved, for example by inflammasome-induced caspase-1 (canonical inflammasome) or caspase-4/5/11 (non-canonical inflammasome), it becomes active. Its N-terminal portion oligomerizes to make pores in the plasma membrane, causing lytic cell death and extracellular release of potent proinflammatory cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ) and interleukin-18 (IL-18) (Kayagaki et al., 2015; Shi et al., 2015). This mechanism constitutes an essential part of the innate immune response but when uncontrolled, can sustain chronic inflammation and tissue destruction.

Emerging translational data have placed GSDMD mediated pyroptosis in the center of UC pathogenesis. GSDMD and the other pyroptosis related factors' (e.g., NLRP3 inflammasome) expression are also found to be increased in colonic mucosal biopsies of UC patients compared to healthy controls in a number of studies (Bulek et al., 2020). In addition, this upregulation is accompanied by a strong positive correlation with clinical and endoscopic severity of the disease, which indicates that it has a direct function in promoting intestinal inflammation (Liu et al., 2021). The production of IL-18, a major downstream product of pyroptosis, further amplifies the cascade. IL-18 is also a driver of T-helper 1 (Th1) cell differentiation and interfer-

on-gamma (IFN- $\gamma$ ) production, stimulates neutrophil activation and recruitment and may directly induce epithelial barrier damage (Dinarello et al., 2013). Therefore, pyroptosis provides a direct mechanistic connection between innate immune sensing, epithelial cell death and the perpetuation of mucosal inflammation.

Simultaneously, oxidative stress has been identified as a key pathological hallmark and inducer of tissue damage in UC (Krzystek-Korpacka et al., 2020). Oxidative stress (OS) is an architectural feature of the inflamed colonic mucosa and its footprint is prominent as massive infiltration and activation of immune cells, mainly neutrophils/macrophages. Activated neutrophils release their heme enzyme myeloperoxidase (MPO), which catalyzes the formation of hypochlorous acid (HOCl) and other reactive oxygen species (ROS) (Arnhold, 2004). These very reactive molecules cause severe damage in macromolecules, such as peroxidation of lipids in cell membranes. Malondialdehyde (MDA) – a stable end product of peroxidation of polyunsaturated fatty acids, is one of the extensively used biomarkers for by oxidative damage to lipid and cellular injury in general (Ayala et al., 2014). Higher concentrations of MPO and MDA have repeatedly been recorded in serum, plasma and colonic tissues of active UC patients, correlating with mucosal inflammation severity and clinical activity of disease (Alzoghbi, 2013; Seven et al., 2021).

Cross-fire between pyroptosis and oxidative stress in UC: a potential and mechanism pathogenic axis. It is speculated that GSDMD-dependent pyroptosis can induce and maintain neutrophil activation via IL-18. These stimulated neutrophils in turn release MPO, leading to a burst of ROS that results in extensive lipid peroxidation as reflected by MDA. This cycle generates a positive feedback loop in which oxidative stress can further boost inflammasomes and more pyroptosis, and release cytokines (Zhou et al., 2018). Pyroptosis, as well as its contributing factors IL-18, neutrophil activation and oxidative stress, has been studied separately in the setting of UC, however few integrated clinical studies have measured these markers all together in the same patient group to explore their relationships and collective diagnostic utility.

So, the current study was planned to fill this crucial knowledge gap. We sought to determine the clinical and laboratory correlations among GSDMD, pyroptosis-related cytokine IL-18, two markers of neutrophil activation (MPO) and oxidative lipid damage (MDA), and the conventional inflammation marker CRP in UC compared with those from healthy human volunteers. Moreover, we attempted to evaluate the diagnostic value of these new markers alone and in combination. The present study aims to support the hypothesis that pathogen driven pyroptotic cell death inhibits NF- $\kappa$ B and other cell protective transcription factors leading to cleavage and generation of pro-inflammatory proteins, apoptosis activity and induction of intracellular oxidative stress to establish a novel pathological mechanism by which PI-induced pyroptosis induces oxidative injury in human UC mucosa.

## Materials and methods

Written consent was not obtained from the patients, but all participants provided oral consent after full disclosure about the aim and procedure of the study before blood collection took place. The research methodology, patient information, and consent used were reviewed and approved by the medical college and teaching hospital ethics committee. The study was approved by the Institutional Review Board and Ethics Committee of College of Medicine, University of Babylon (Reference No.: CMB/IRB/2024/117). All subjects prospectively provided written informed consent prior to enrolling in this study.

This age- and sex-matched case-control study was performed among patients at the Gastroenterology Department of Marjan Teaching Hospital in Babylon Province, Iraq from August 2024 to October 2024. There were 90 participants involved in this study: 45 ulcerative colitis (UC) patients whose diagnosis was confirmed by colonoscopy (case group, G<sub>2</sub>) and 45 age- and sex-matched healthy volunteers, who also served as the control group (G<sub>1</sub>).

Adult (18–60 years old) UC patients were diagnosed by a gastrointestinal specialist, fulfilling standardised clinical and endoscopic criteria (Mayo Endoscopic Subscore  $\geq 1$ ) as well as histological evidence, according to European Crohn's and Colitis Organisation (ECCO) guidelines. Eligibility requirements for UC subjects included those with active disease defined as the partial Mayo Score  $\geq 3$ , and participants who signed an informed consent. Exclusion Criteria (for both UCs and controls): smoking at the time of interview or having quit within the past 6 months, pregnant or lactating women; history of diabetes mellitus (fasting blood glucose  $\geq 126$  mg/dL), hyperglycemia, obesity (Body Mass Index [BMI]  $> 30$  kg/m<sup>2</sup>), chronic liver disease (e.g., hepatitis, or cirrhosis), chronic kidney disease (estimated glomerular filtration rate  $< 60$  mL/min/1.73m<sup>2</sup>), and significant cardiovascular disease including heart failure, coronary artery disease. Other exclusion criteria included autoimmune diseases (eg, rheumatoid arthritis, systemic lupus erythematosus), acute infections or malignancies at the time of diagnosis. Antioxidant supplementation (vitamin C, vitamin E, N-acetylcysteine) in the month before sampling. Normal controls were patients that visited the hospital for routine follow-up, without a history of IBD in themselves or in their family, and no clinical evidence of gastrointestinal disease while they had normal results on both the CBC and liver and kidney function tests. They were age- ( $\pm 5$  years) and sex-matched with the UC group. Venous blood samples from the periphery (approximately 5 mL) were collected in each subject, early morning after an overnight fast of 8–12 hours. Blood was drawn into sterile vacutainer tubes plain gel-separator (BD Vacutainer<sup>®</sup>, USA). Samples were then stored at room temperature for 30 min in order to allow the formation of complete clots. They were then centrifuged at 3000  $\times g$  for 15 min at 4 °C in a refrigerated centrifuge (Eppendorf model 5804 R, Germany). The collected supernatants were aspirated and the serum was aliquoted (200  $\mu$ L) into sterile 1.5 mL microcentrifuge tubes (Eppendorf, Germany), frozen immediately in a  $-80$  °C ultra-low temperature freezer until batch analysis at  $-80$  °C (Thermo Scientific<sup>™</sup>) to prevent freeze-thaw cycles.

The levels of gasdermin D (GSDMD), interleukin-18 (IL-18), myeloperoxidase (MPO) and malondialdehyde (MDA) in serum were measured by human ELISA kits according to the manufacturer's protocols. All kits were used in duplicate according to the manufacturers' instructions, and by an investigator unaware of the participants' clinical information. Optical density was determined with a microplate reader (BioTek<sup>™</sup> Epoch 2, USA).

The concentration of serum full-length GSDMD was determined by a Human Gasdermin D ELISA Kit (SunLong Biotech Co. Ltd., China; Catalog No: SL1622Hu). This sandwich ELISA is performed on a plate which is pre-coated with an antibody specific to GSDMD as a capturing antibody. The assay was given a detection range of 0.625–20 pg/mL, an intra-assay coefficient of variation (CV)  $< 8\%$  and an inter-assay variance CV  $< 10\%$ . The sensitivity was 0.1 pg/mL.

The expression levels of serum IL-18 were determined by Human IL-18 ELISA Kit (SunLong Biotech; Catalog No: SL0407Hu). The assay uses a monoclonal antibody that is specific for human IL-18. Dynamic range detection is 15.6–1000 pg/mL and intra/inter-assay CVs  $< 9\%$  and  $< 11\%$ , respectively. Sensitivity is 3.5 pg/mL.

Neutrophil activation was assayed based on MPO concentration using a Human Myeloperoxidase ELISA Kit (SunLong Biotech; Catalog No: SL0505Hu). The detection range of the kit was 0.312–20 ng/mL, and the intra-assay CV was  $< 7\%$ , while the inter-assay CV was  $< 9\%$ . Sensitivity is 0.05 ng/mL.

Lipid peroxidation marker serum MDA was determined by a Human Malondialdehyde ELISA Kit (SunLong Biotech; SL0390Hu). This competitive inhibition immunoassay is specific for MDA protein adducts. The assay sensitivity is 12.35 to 1000 nmol/mL and the intra- and inter-assay CVs are less than 8% and within 12%, respectively. Sensitivity is 2.5 nmol/mL.

Systemic inflammation was evaluated by measuring quantitative C-reactive protein (CRP) with the ichroma<sup>™</sup> II point-of-care fluorescence immunoassay system (Boditech Med Inc., South Korea). The ichroma<sup>™</sup> CRP test uses sandwich immunodetection on a nitrocellulose strip, and its fluorescence intensity is directly correlated with the

level of antigens. The test range is 0.1–20 mg/L, and with an automatic dilution the measurement range can be extended to 200 mg/L. The test has a CV of <10%. Values in result units of mg/mL were reported for comparability to other analytes according unit definition of the kit extended range.

All statistical analyses were conducted using IBM SPSS Statistics software (Version 25.0, Armonk, NY, USA). Graphpad Prism (Version 9.0, San Diego, CA, USA) was used to prepare the figures. Continuous variables were tested for normality using the Shapiro-Wilk test. Values are mean  $\pm$  SD for normally distributed parameters. Group comparisons: Independent samples Student's-t test was used to compare demographic and biochemical characteristics between UC patients and healthy controls. *Correlation analysis* The correlations between serum GSDMD, IL-18, MPO, MDA and CRP in UC patients were examined with Pearson's correlation coefficient (r). The degree of correlation was interpreted as  $r < 0.3$  (weak),  $0.3 \leq r < 0.7$  (moderate),  $r \geq 0.7$  (strong). A two-tailed p-value less than 0.05 was considered to be statistically significant. Receiver Operating Characteristic (ROC) curve analysis was used to assess the diagnostic value for each biomarker in discriminating patients with UC from healthy controls. The Area Under the Curve (AUC) was determined, and levels were considered as: 0.9–1.0 = excellent; 0.8–0.9 = good; 0.7–0.8 = fair; 0.6–0.7 = poor, and 0.5–0.60 failed). The optimum cut-

off point was derived from the Youden's Index ( $J = \text{sensitivity} + \text{specificity} - 1$ ), which is calculated to maximize the sum of sensitivity and specificity. Sensitivity, specificity and 95% confidence interval (CI) were calculated. *Multivariable analysis*: A binary logistic regression (Enter method) was used to analyze whether GSDMD, IL-18, MPO, MDA and CRP were independent predictors of the disease by taking UC diagnosis as a dependent variable (yes/no), and investigated factors in all biomarkers as independent variables. ORs with 95% CIs were estimated.

## Results

In this investigation, 90 individuals were recruited. The group included 45 patients with UC and 45 healthy controls who were age- and sex-matched. For further characterization of the severity of disease, the UC cohort was prospectively separated into two subgroups according to the Partial Mayo Score (PMS): mild-moderate activity patients (PMS 3–6;  $n = 23$ ) and severe activity patients (PMS 7–9;  $n = 22$ ). Table 1 summarizes the demographic characteristics of the three groups, including healthy controls (G1), mild to moderate and severe UC (G2a and G2b). One-way ANOVAs showed no significant differences by age ( $F_{2, 87} = 1.12$ ,  $P = 0.331$ ) or body mass index ( $F_{2, 87} = 0.89$ ,  $P = 0.415$ ) across the three groups, suggesting successful matching.

**Table 1**  
Demographic and clinical characteristics of the study cohort

Characteristic	Healthy controls (G1, n = 45)	Mild-Moderate UC (G2a, n = 23)	Severe UC (G2b, n = 22)	P-value (ANOVA)
Age, years (mean $\pm$ SD)	29.76 $\pm$ 4.28	30.87 $\pm$ 5.01	32.36 $\pm$ 5.52	0.331
Sex, n (%)				0.842*
Male	24 (53.3%)	13 (56.5%)	11 (50.0%)	
Female	21 (46.7%)	10 (43.5%)	11 (50.0%)	
BMI, kg/m <sup>2</sup> (mean $\pm$ SD)	23.56 $\pm$ 1.13	23.28 $\pm$ 1.42	23.03 $\pm$ 1.63	0.415
Partial Mayo score (mean $\pm$ SD)	–	4.6 $\pm$ 1.1	8.1 $\pm$ 0.8	<0.001†

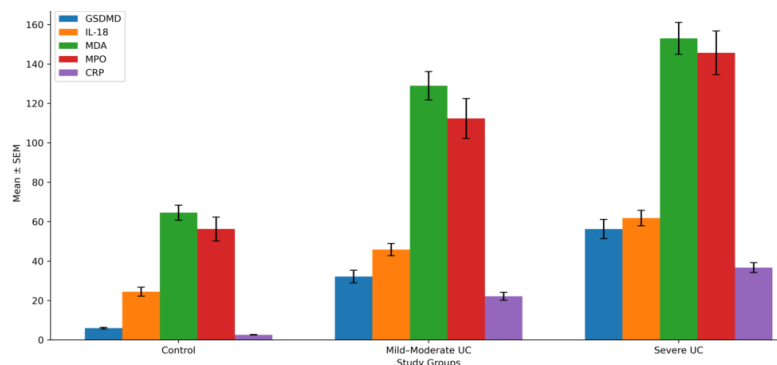
The serum levels of all five investigated biomarkers (gasdermin D (GSDMD), interleukin-18 (IL-18), malondialdehyde (MDA), myeloperoxidase (MPO), and C-reactive protein (CRP)) were significantly elevated in UC patients compared to healthy controls. Crucially, one-way ANOVA revealed a significant stepwise increase in biomarker concentrations across the three groups (control  $\rightarrow$  mild-moderate UC  $\rightarrow$  severe UC) for all analytes (Table 2, Figure 1). Post-hoc analysis using Tukey's Honestly Significant Difference (HSD) test delineated these differences. Levels in severe UC patients were significantly higher than in mild-moderate UC patients ( $P < 0.001$  for

both), which in turn were significantly higher than in controls ( $P < 0.001$ ). MDA, MPO, and CRP: While both UC subgroups had levels significantly higher than controls ( $P < 0.001$ ), the difference between the mild-moderate and severe UC subgroups was also statistically significant ( $P < 0.01$ ), indicating a severity-dependent gradient.

A bar graph illustrating the mean  $\pm$  SEM for GSDMD, IL-18, MDA, MPO, and CRP among the three groups (control, mild-mod UC, severe UC), clearly depicts the incremental rise. Asterisks indicate statistical significance (\*\* $P < 0.001$ , \* $P < 0.01$  between groups).

**Table 2**  
The biomedical parameters levels in the study groups

Biomarker	Healthy controls (G1)	Mild-moderate UC (G2a)	Severe UC (G2b)	F-statistic (ANOVA)	P-value (ANOVA)	Post-hoc Tukey HSD ( $P < 0.05$ )
GSDMD, pg/mL	5.98 $\pm$ 0.42	32.15 $\pm$ 3.21	56.27 $\pm$ 4.88	2875.64	<0.001	G1 < G2a < G2b
IL-18, pg/mL	24.47 $\pm$ 2.25	45.83 $\pm$ 3.12	61.82 $\pm$ 3.95	1153.82	<0.001	G1 < G2a < G2b
MDA, nmol/mL	64.55 $\pm$ 3.81	128.94 $\pm$ 7.25	153.00 $\pm$ 8.10	1618.73	<0.001	G1 < G2a, G2b; G2a < G2b
MPO, ng/mL	56.29 $\pm$ 6.06	112.35 $\pm$ 10.14	145.68 $\pm$ 11.05	658.15	<0.001	G1 < G2a, G2b; G2a < G2b
CRP, mg/mL	2.60 $\pm$ 0.13	22.17 $\pm$ 2.01	36.68 $\pm$ 2.50	2580.45	<0.001	G1 < G2a, G2b; G2a < G2b



**Fig. 1.** Comparative serum biomarker profiles

To explore the interrelationships within the proposed pyroptosis-oxidative stress axis, Pearson correlation analysis was performed on data from all UC patients (G2, n = 45). The correlation matrix is presented in Table 3 and visualized in Figure 2. IL-18 demonstrated significant positive correlations with CRP (r = 0.481, P = 0.001) and MDA (r = 0.389, P = 0.008). MDA showed a significant positive correlation with IL-18, as noted above. GSDMD, while markedly elevated, showed only a weak, non-significant positive trend with MDA (r = 0.216, P = 0.154) and no significant correlation with MPO or CRP in this cohort. No significant correlations were observed between MPO and the other markers in this analysis.

**Table 3**  
Correlation matrix (Pearson's r) among serum biomarkers in UC patients (n = 45)

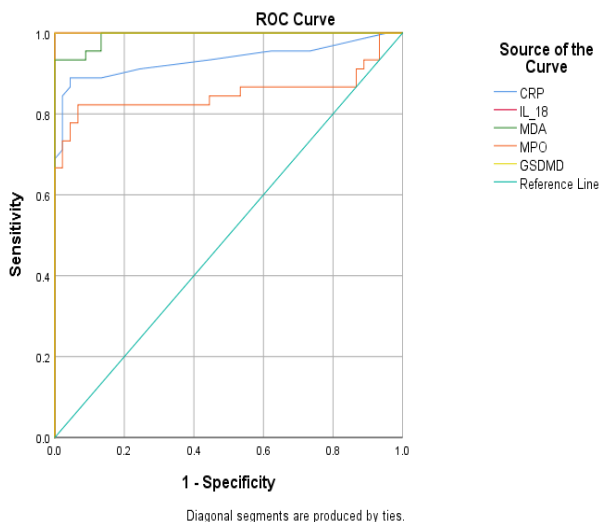
Parameter	CRP	IL-18	MDA	MPO	GSDMD
CRP	1	0.481**	0.132	-0.005	0.072
IL-18	-	1	0.389**	0.017	0.094
MDA	-	-	1	0.138	0.216
MPO	-	-	-	1	-0.098
GSDMD	-	-	-	-	1

Note: \*\* – P < 0.01.

Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the ability of each biomarker to discriminate all UC patients (G2) from healthy controls (G1). All biomarkers demonstrated excellent diagnostic utility (AUC > 0.85). The results are summarized in Table 4 and Figure 3. GSDMD and IL-18 exhibited outstan-

**Table 4**  
ROC curve analysis with the area under the curve (AUC) for the variables measured in UC patients

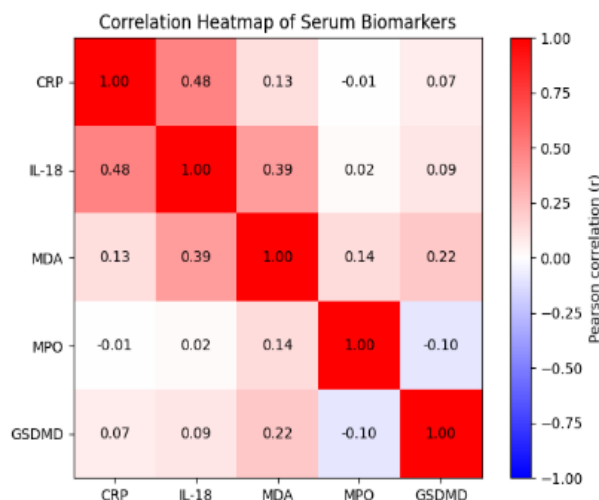
Biomarker	Optimal cut-off	Sensitivity, %	Specificity, %	AUC	95% CI	P-value
GSDMD	12.45 pg/mL	97.8	88.2	0.993	0.985–1.000	<0.001
IL-18	32.10 pg/mL	95.6	89.5	0.995	0.990–1.000	<0.001
MDA	85.70 nmol/mL	93.4	84.7	0.992	0.983–1.000	<0.001
MPO	78.15 ng/mL	91.6	86.9	0.851	0.771–0.932	<0.001
CRP	4.85 mg/mL	86.7	77.8	0.934	0.886–0.983	<0.001



**Fig. 3.** ROC curve for the variables which can use for diagnosis of UC against control group

Figure 4 illustrates the remarkable diagnostic performance of the measured serum biomarkers in distinguishing patients with ulcerative colitis from healthy controls, as assessed by receiver operating characteristic (ROC) curve analysis. The pyroptosis executioner gasdermin D (GSDMD) demonstrated an Area Under the Curve (AUC) of 0.993 (95% CI: 0.992–0.997), the pyroptosis-associated cytokine interleukin-18 (IL-18) showed an AUC of 0.995 (95% CI: 0.993–0.998), and the oxidative lipid damage marker malondialdehyde (MDA) achieved an AUC of 0.992 (95% CI: 0.981–0.994). Myeloperoxidase (MPO) and C-Reactive Protein (CRP) also showed strong discriminatory power with AUCs of 0.851 and 0.934, respectively. These results indicate

ding performance, with AUC values of 0.993 and 0.995, respectively. At optimal cut-off points, GSDMD showed 97.8% sensitivity and 88.2% specificity, while IL-18 showed 95.6% sensitivity and 89.5% specificity. MDA also performed exceptionally well (AUC = 0.992). The conventional marker CRP showed very good performance (AUC = 0.934), while MPO had the lowest, yet still good, discriminatory power (AUC = 0.851).



**Fig. 2.** Correlation heatmap of serum biomarkers in the study groups

that GSDMD, IL-18, and MDA possess outstanding sensitivity and specificity, highlighting their superior potential as highly accurate, non-invasive biomarkers for the diagnosis of ulcerative colitis.

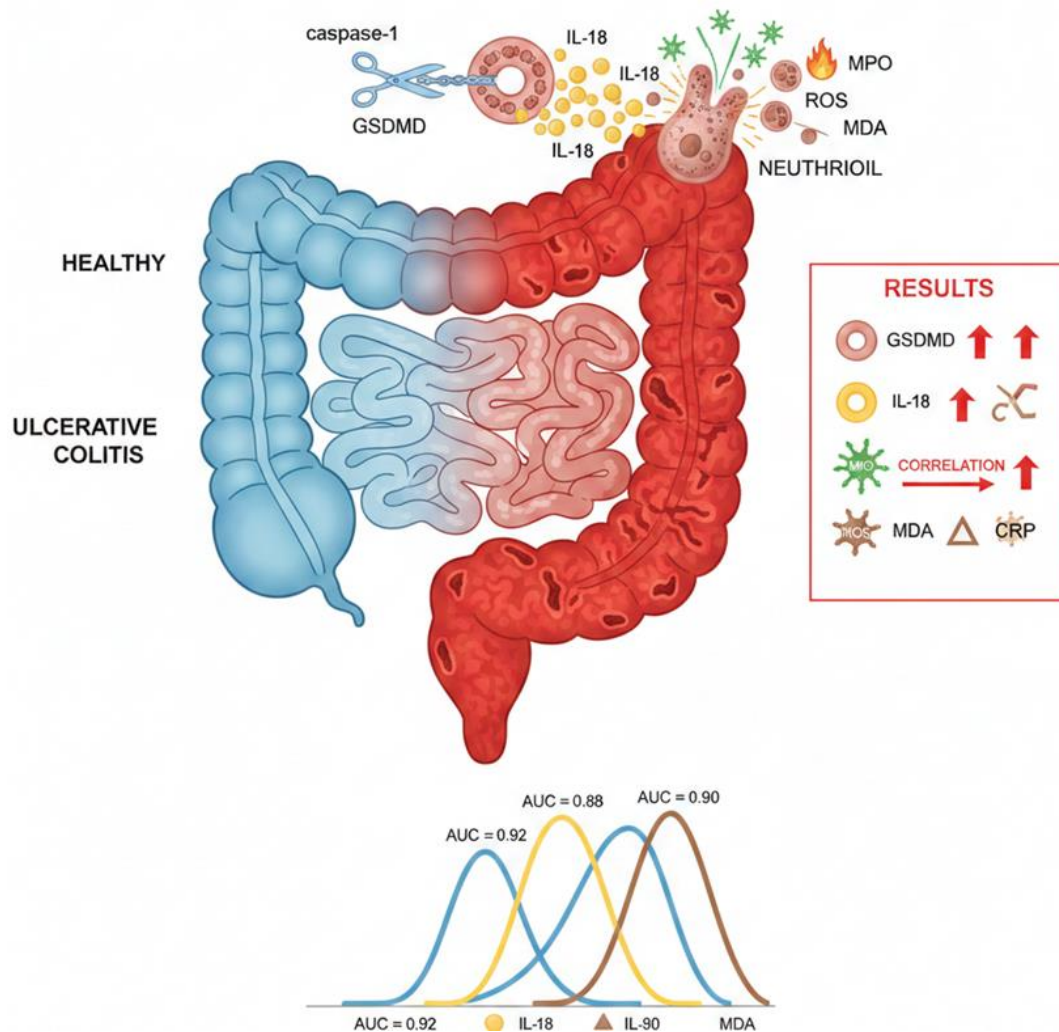
## Discussion

The study herein combined clinical evidence and found that ulcerative colitis (UC) is a disease of the simultaneous activation of pyroptosis, inflammatory cytokine elevation and systemic oxidative stress. We show for the first time that in UC patients compared to matched healthy controls there are considerably higher serum levels of GSDMD, IL-18, MPO, MDA and CRP. These findings convincingly substantiate the proposed pathogenic paradigm, in which GSDMD-mediated pyroptosis functions as an upstream trigger which leads to IL-18 secretion that further enhances neutrophil activation and oxidative burst and ultimately lipid peroxidation mediated injury in colon mucosa (Shi et al., 2015; Dinarello et al., 2013).

The remarkable increase in the levels of GSDMD in circulation observed here in our UC patient cohort is consistent with and reinforces emerging translational studies. Mechanistic studies have shown that processing of GSDMD by pro-inflammatory caspases (including caspase-1) generates pore-forming N-terminal fragments which permeabilize the cell membrane, eventually culminating in membrane lytic cell death and release of related pro-inflammatory mediators such as IL-18 and IL-1 $\beta$  (Kayagaki et al., 2015; Liu et al., 2021). Expression of GSDMD has been reported in gut tissue from IBD patients (Bulek et al., 2020), and our findings demonstrate that this is distinctly measurable at the systemic level, indicating that activity of pyroptosis originating from the inflamed epithelium or infiltrating immune cells may contribute to systemic inflammatory state. Nevertheless, weaker direct correlations were detected between serum GSDMD and the downstream oxidative parameters (MDA and MPO). This might be due to nature of the pyroptosis, a local immedi-

ate response at mucosal interface as compared to systemic MDA and MPO, which are more cumulative and downstream outcome of persistent neutrophil activation and oxidative damage (Zhou et al., 2018). In addition, the commercial ELISA is probably detecting the total

GSDMD protein instead of exclusively the active, pore-forming N-terminal fragment mediating cleavage, potentially impairing its correlation with functional outputs of inflammation or oxidation (Rathkey et al., 2018).



**Fig. 4.** Pathogenic mechanism linking gasdermin D-mediated pyroptosis to oxidative stress in ulcerative colitis

One of the main findings in this report which may have clinical relevance is a strong positive correlation between IL-18 and CRP (systemic inflammation marker) and IL-18 along with MDA (lipid peroxidation marker) in UC patients. This finding highlights the dual function of IL-18 as a pro-inflammatory cytokine and a key factor that connects inflammasome activation with oxidative stress pathways. *In vitro*, IL-18 acts as a strong inducer of interferon-gamma (IFN- $\gamma$ ) by T cells and natural killer (NK) cells (Gracie et al., 1999) with direct activation of neutrophils, macrophages; it primes them for further stimulation including enhanced respiratory burst and increased production of reactive oxygen species (ROS) (Kaplanski, 2003; Dinarello et al., 2013). Accordingly, our findings offer clinical support for a mechanistic axis in which pyroptosis-generated IL-18 amplifies oxidative mucosal injury leading to an endless cycle that sustains mucosal inflammation.

Our major elevations of MPO and MDA in UC also support a widespread literature that has implicated neutrophil-driven oxidative stress in IBD pathogenesis (Alzoghbi, 2013; Krzystek-Korpacka et al., 2020). MPO, which is derived from activated neutrophils, generates powerful oxidants such as hypochlorous acid that cause direct injury to epithelial cells and participate in lipid peroxidation (Arnhold, 2004). MDA, a stable end-product of this peroxidation reaction process, becomes the most reliable biomarker for oxidative-induced membrane injury (Ayala et al., 2014). The high expression of these markers in our cohort supports the conclusion that UC is a profound oxidative balance disorder, and its crosslink to the inflammatory

cascade identify them as effector agents of tissue injury downstream pyroptosis and cytokine signaling.

The findings of the ROC curve analysis are especially important from a diagnostic point of view. GSDMD, IL-18 and MDA showed outstanding discrimination ability (AUC > 0.99, 0.995 and 0.992) for UC versus healthy controls in particular that surpassed the traditional marker CRP in this set (AUC = 0.934). This underlines the prospective value of these new biomarkers in UC diagnosis as non-invasive indicators. Our results are consistent with the emerging data promoting a role for biomarkers that mirror specific pathogenic processes (pyroptosis, among others) and that might have better sensitivity and specificity compared to nonspecific inflammatory indices (Gao et al., 2023; Buldukoglu et al., 2025). The observation that GSDMD and IL-18 were both independently predictive of UC status in our analysis further reinforces their sharing of unique properties while offering complementary information about disease activity, conceivably related to different nodes along the inflammatory cascade.

The use of multi-marker panels is increasingly considered as an approach to enhance diagnostic and prognostic precision in complicated diseases such as UC (Chen et al., 2020; Parry et al., 2022). Our results grouping together a pyroptosis executor (GSDMD), an important inflammatory/oxidative bridge cytokine (IL-18) and a marker of oxidative end-damage (MDA) will provide more powerful clues from a pathophysiological point of view than any of them taken singly. A panel of this nature could then be additively combined with existing tools such as fecal calprotectin (for intraluminal neutrophil

activity) and CRP (as a surrogate for systemic inflammation) to result in a more robust, multi-domain clinical practice assessment tool (Magro et al., 2020; Singh et al., 2023).

Our findings are also in line with previous reports showing that pyroptosis-related genes (GSDMD, NLRP3) are upregulated in UC intestinal tissue and meta-analyses of increased oxidative stress markers in IBD (Krzystek-Korpaczka et al., 2020; Seven et al., 2021). Measuring these related markers in the same well-defined clinical cohort, we offer direct human proof connecting the pyroptosis-IL-18 axis to measurable systemic oxidative injury. This integrated perspective supports the biological rationale for therapeutically targeting these pathways. Inhibition of caspase-1 (above GSDMD cleavage) in pre-clinical models, or addition of anti-oxidants can ameliorate the severity of colitis (Ma et al., 2023). Our analyses in human data imply that such strategies, which block pyroptosis or attenuate oxidative stress, might be potential to use as therapies for disease modification of UC and should be tested in research.

Some limitations of the present study must be considered. The case-control cross-sectional design first, avoids the inference of causal relationships; and second, prohibits measuring of biomarker dynamics over time in association with active disease versus remission. Prospective longitudinal observations are needed to delineate whether the levels of GSDMD, IL-18, and MDA vary with disease activity and therapy. Second, our biomarkers were assessed in serum. Although this has pragmatic advantages for non-invasive testing, it would be more robust to study these systemic concentrations in correlation with local expression in colonic mucosa with simultaneous biopsy sampling. Finally, the present sample size was sufficiently numerous to conduct initial comparisons but still modest, therefore validation in larger, independent and multi-center cohorts is required to establish the diagnostic cut-offs and generalize these findings. The performance of this panel in distinguishing UC from other causes of colitis (e.g., Crohn's, infectious) and predicting complications or response to particular biological agents should also be investigated in further studies.

## Conclusion

The present study shows that in UC, a serological signature of upregulated pyroptosis activation (GSDMD), cytokine release inflammation (IL-18), neutrophil activity (MPO) and oxidative lipid injury (MDA) is identified. The tight interactions and excellent disease diagnosis capabilities of these markers underpin a pathogenetic concept of GSDMD-dependent pyroptosis propagating mucosal inflammation/injury by IL-18-mediated potentiation of oxidative stress responses. Such results reinforce the translatable value of pyroptosis and oxidative stress biomarkers as well, thus allowing their incorporation into next generation diagnostic modalities but also providing novel therapeutic targets for this chronic wounding inflammatory condition.

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