Soluble CD14 and lipopolysaccharide binding-protein are associated with clinical disease activity in inflammatory bowel disease patients; a biomarker study

Author

Sterre Wouda[1]

Supervision

Sem Geertsema[1]; M.D.

Arno R. Bourgonje[1,2]; M.D., Ph. D.

Prof. Klaas Nico Faber[1]; Ph. D.

Examiners

Prof. Harry van Goor

Prof. Klaas Nico Faber

[1] Department of Gastroenterology and Hepatology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

[2] The Henry D. Janowitz Division of Gastroenterology, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, United States

Correspondence: Sterre Wouda **Address:** Hanzeplein 1, 9700 RB Groningen

E-mail: h.t.wouda@student.rug.nl **Date:** 25-01-2025

Abstract

Introduction: Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a chronic inflammatory disease of the gastrointestinal tract. Although the exact cause is not fully understood, multiple factors are involved in IBD pathogenesis. This includes environmental factors, such as dietary patterns, and gut barrier integrity. Currently, gut barrier integrity is mostly assessed through histological analysis, an invasive procedure with high patient burden. As a non-invasive alternative, circulating biomarkers are increasingly used to assess gut barrier function, offering potential insights in disease activity and development. That is why this study aimed to characterize the prevalence of three circulating biomarkers that reflect gut barrier dysfunction and its correlation with dietary patterns and disease activity in a cohort of adult IBD patients.

Methods: Plasma of 450 patients with IBD (263 CD and 187 UC) was collected for analysis. Fatty acid-binding protein (I-FABP or FABP2), lipopolysaccharide-binding protein (LPS-BP or LBP) and soluble CD14 (sCD14) were measured using an ELISA assay. Demographic and clinical data were collected at time of plasma sampling. Data on dietary intake were collected from all patients using semi-quantitative food frequency questionnaires (FFQs). Disease activity was based on clinical parameters (clinical scores) and biochemical parameters (C-reactive protein). A new defined disease activity score and a gut barrier variable were created for further analysis. To identify associations, multiple linear regression models were used correcting for confounders (age, sex, BMI, smoking status, diagnosis, albumin levels and surgical history).

Results: No prominent associations were found between biomarkers and dietary patterns. Significant associations between biomarkers sCD14 and LBP and clinical disease activity (sCD14: $p = 0.02$ and LBP: $p < 0.001$) and biochemical disease activity (CRP <5 vs. >5 both $p < 0.001$) were identified. After adjusting for confounders, the association with sCD14 and LBP and the defined disease activity score was amplified (sCD14: $\beta = 0.11$ [0.04–1.77], $p = 0.009$ and LBP: $\beta = 3.89$ [2.84–4.94], $p < 0.001$). The gut barrier variable significantly associated with the defined disease score (intact gut barrier: 72.1% remission vs. 27.9% active disease and non-intact gut barrier: 24.4% remission vs. 75.6% active disease, both $p < 0.001$) and CRP levels (both $p < 0.001$).

Conclusion: sCD14 and LBP associate with clinical- and biochemical disease activity, but do not exhibit associations with dietary patterns of patients with IBD. Future research is necessary to validate these biomarkers and assess interventions aimed at restoring gut barrier integrity.

Keywords: IBD; gut barrier integrity; sCD14; FABP2; LBP; clinical disease activity, dietary patterns

1. Introduction

Inflammatory bowel disease (IBD) is a disorder with recurrent, chronic inflammation of the gastrointestinal tract [1]. IBD clinically consists of Crohn's disease (CD), ulcerative colitis (UC) and other conditions [2]. CD and UC can be distinguished by clinical, histopathological and endoscopic features [3]. Although it can affect the whole gastrointestinal tract, CD is characterized by patchy, transmural inflammation that mainly affects the terminal ileum and colon. UC induces widespread, superficial ulcerative inflammation limited to the mucosa of the rectum and colon [4]. Common symptoms of IBD include changes in bowel habits, diarrhoea, abdominal pain, malnutrition, and weight loss, but alco complications outside the gastrointestinal tract may occur, like anaemia, rash, and fatigue [3,5,6]. The exact cause of IBD is still unknown, however it is widely thought that a combination of genetic, environmental, microbiome-related, and immunological factors contributes to the disruption of gut homeostasis (Figure 1) [7-10].

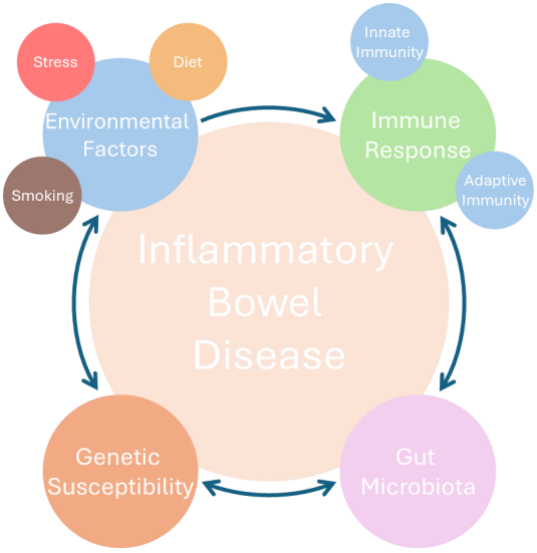

Figure 1. The interaction of different factors influencing the causes of inflammatory bowel disease (IBD). Gut microbiota dysbiosis, environmental factors and genetic susceptibility can cause abnormalities in the immune response, activating the innate and adaptive immune system. Figure created with inspiration from [11,12].

1.1. Pathogenesis of IBD

IBD is a polygenic disorder with a familial tendency in 5-10% of individuals, impacted by over 240 risk loci identified via genome-wide association studies (GWAS) [7, 13-15]. These loci, which are mostly shared by CD and UC, show common pathways in immunity, host-pathogen interactions and intestinal barrier function. Important genetic markers include *IL23R*, associated with inflammatory regulation in both diseases, *NOD2*, which is essential to microbial detection in CD, and the *HLA* complex unique to UC [1, 16-18]. Despite these genetic connections, only a small percentage of individuals with IBD risk loci develop the disease [1,15]. IBD likely arises from interactions between genetic susceptibility and environmental factors that disrupt the balance of the gut microbiota, leading to an abnormal immune response. Environmental factors include cultural, demographic, economic, lifestyle and technological influences that shape both health and disease outcomes [19]. Over the last decades, the frequency of IBD has significantly increased in the developed countries, and the occurrence and recognition of the disease has also increased in less developed countries with progressive industrialization [1]. A wide range of environmental risk factors have been explored for their interaction with IBD, this includes but is not limited to diet, smoking, breastfeeding, infections and vaccinations, oral contraceptives, antibiotic use, psychological stress and urban lifestyle [20-23]. These factors collectively reveal common vulnerabilities that may contribute to the risk of developing IBD [19]. In IBD, dysbiosis of the microbiome disrupts immune function and compromises the intestinal barrier, leading to chronic inflammation [19,24]. Overall, both bacterial diversity as well as quantity in the gut decreases in IBD

patients [24,25]. Germ-free animal studies suggest that gut bacteria contribute to intestinal inflammation, highlighting their role in IBD pathogenesis, though it remains unclear if dysbiosis is a cause or consequence of the disease [3,16]. The immune system plays a crucial role in maintaining intestinal health, with a balance between microbial tolerance and pathogen defence. In IBD, the immune defence against intestinal microbes is compromised in two ways: by changes in both innate and adaptive host immune responses and through the impairment of the epithelial mucosal barrier [19]. The innate immune system comprises a physical barrier, including mucus and epithelial cells [26]. Beneath the epithelium, innate immune cells in the lamina propria, bloodstream or other tissues, comprise the non-specific innate response [3]. The adaptive immune system depends on the specific recognition of antigens by B cell or T cell receptors and further regulates the immune response. IBD seems to result from chronic inappropriate disruptions in the immune homeostasis. This may be due to delayed immune system development, immaturity of the mucosal barrier and its innate defences, failure of the epithelial barrier, or a chronically dysregulated immune network—likely influenced by a polygenic predisposition [19,27]. This immunological dysregulation manifests as epithelial damage (including abnormal mucus production and impaired repair mechanisms), inflammation expansion driven by gut flora, and extensive infiltration of various immune cells like T cells, B cells, macrophages, dendritic cells, and neutrophils into the lamina propria. Additionally, immune regulation failure results in the inability to control the inflammatory response [1]. The chronic nature of IBD indicates that compromised epithelial barrier integrity, over time, leads to significantly disrupted mucosal homeostasis [27].

1.2 Diet and its implications for IBD

Food intake or diet is an important environmental factor affecting the pathogenesis of IBD and playing a role in intestinal permeability and immunity [28]. The first dietary exposure for humans is normally either breast milk or formula. It has been found that breastfeeding is linked to a reduced risk of IBD, however after weaning the gut microbiota undergoes significant changes [20]. Several food groups and diets are associated with IBD risk. High meat-, fat- and sugar-consumption has been associated with an increased risk in several forms of IBD. However, high fibre, fruits and vegetables intake has been linked to lower risk of IBD development [29-30]. Pro-inflammatory diets like the Western diet and the high-salt diet are associated with immune imbalances and inflammation, exacerbating the progression of IBD [31]. The Mediterranean diet might be able to modulate immune function, alleviating IBD [31]. Many dietary components have been studied in their role of regulating mucosal immune function and intestinal barrier function, including vitamins, amino acids and short-chain fatty acids. Dietary nutrients also impact the composition and function of the gut microbiota [20,32]. Next to the role in IBD pathogenesis, dietary strategies have gained attention in the management of IBD. Several factors contribute to the increased nutritional risk of these patient, including insufficient dietary intake and malabsorption due to intestinal losses. This vulnerability to nutritional deficiencies highlights the role of nutrition in both IBD pathogenesis and treatment [33].

1.3. Gut barrier integrity and its implications for IBD

The intestinal barrier serves as a physical barrier, formed by epithelial cells and secretions, and as an immunological defence against pathogens [34]. The gut barrier primarily consists of a single layer of epithelial cells with densely packed microvilli, or brush border, forming the transcellular barrier. Tight junctions (TJs) regulate the paracellular barrier (Figure 2) [35]. When mucosal damage occurs, the epithelial barrier becomes more permeable, allowing the translocation of microbiota and antigens to deeper layers of the mucosa. Increased exposure can activate the recruitment of immune cells that produce various cytokines, activating localized immune responses and inflammation, potentially disrupting homeostasis [3,36,37]. A compromised intestinal epithelial barrier is recognized as a key factor in the pathogenesis of IBD. However, it is still debated whether epithelial barrier impairment is a result of the inflammatory response or a primary defect that triggers mucosal inflammation [38,39].

The link between reduced barrier function and intestinal disease was first identified in the early 1980s. Research using both ex vivo and in vivo methods showed that permeability is increased in IBD,

independent of ulcerations [34]. Increased intestinal permeability has been shown to correlate more strongly with symptoms of IBD than with endoscopic activity and is a better predictor of relapse than other clinical or blood markers [40]. A recent study showed that within one year, an abnormal epithelial barrier is associated with an 80% risk of relapse and a 45% chance of serious complications, such as hospitalization or surgery [41]. Some structural abnormalities, like crypt changes, improve with treatment, but fluorescein leakage indicating increased permeability may persist [42]. This suggests the idea that intestinal barrier dysfunction might be a primary pathological characteristic of IBD, and current therapies may not completely restore intestinal barrier integrity [40].

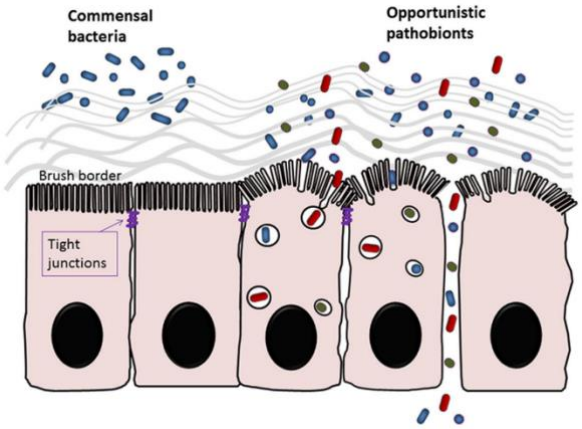

Figure 2. The epithelial barrier. The gut consists of epithelial cells with a brush border (BB) forming the transcellular barrier, and tight junctions (TJs) forming the paracellular barrier. Together the BB and TJs serve as a physical barrier to block the entry of bacteria in healthy conditions. Damage to the epithelial barrier can allow commensal and opportunistic pathological bacteria to access the lamina propria [35].

1.4. Current clinical diagnosis of IBD

Effective management of IBD requires accurate diagnosis and differentiation from other acute and chronic bowel diseases. Key treatment goals are rapid symptom control, normalization of biomarkers, and endoscopic healing, which together can slow disease progression, reduce steroid dependency, and prevent complications [43]. Challenges in diagnosing IBD arise because no single test reliably covers diagnosis, prognosis, or disease severity. Physicians rely on a mix of clinical assessments, lab tests, imaging, and endoscopy with histology, as no single method suffices [44]. Laboratory tests of blood and stool are useful for screening and tracking inflammation, especially in mild or asymptomatic phases, but they cannot confirm IBD alone. Blood tests may reveal anaemia, elevated leukocytes, and markers like C-reactive protein (CRP), while stool tests can detect calprotectin levels, which often allow for inflammation assessment without invasive procedures like colonoscopy [45,46]. For a definitive diagnosis and to evaluate disease severity, endoscopy with biopsy remains essential. However, endoscopy is costly and invasive, making it less suitable for frequent monitoring [44]. For these reasons, efforts at identifying accurate, non-invasive biomarkers have been undertaken [19].

1.5. Currently used biomarkers for IBD

Biomarkers are essential for diagnosing, monitoring, and predicting treatment responses in IBD. Because IBD often alternates between remission and unpredictable flare-ups, biomarkers in serum and faeces are especially useful for tracking disease activity and predicting relapses [47]. While an ideal biomarker would be non-invasive, specific, sensitive, and cost-effective, no single biomarker currently meets all these criteria for accurately diagnosing IBD, distinguishing between its subtypes, or monitoring disease activity [44,45]. Currently, several known biomarkers for IBD are faecal and blood-based biomarkers, which include serum biomarkers and serological antibodies [44,48]. The most common faecal biomarker is faecal calprotectin, a protein from leukocytes that indicates mucosal inflammation. Calprotectin levels correlate with mucosal healing and therapy response, making it valuable for monitoring remission and predicting relapse [49,50]. However, while faecal markers like calprotectin are specific for intestinal inflammation, they cannot confirm that the inflammation is due to IBD, as elevated levels may also result from other factors, such as medication use [51]. Blood-based markers like CRP and erythrocyte sedimentation rate (ESR) are widely used for their simplicity, low

cost, and non-invasive nature [44,48]. CRP is helpful in assessing CD activity and response to treatment but is less useful for UC. However, CRP is non-specific and can be elevated in various inflammatory conditions [44]. ESR, similarly, is an inflammation marker affected in both CD and UC, although it also lacks specificity [46]**.** Identifying reliable biomarkers can provide insights into disease activity and guide the development of more targeted therapeutic strategies [52]**.**

1.6. Gut barrier biomarkers

Because of the idea that intestinal barrier dysfunction might be a primary pathological characteristic of IBD, there is a need for studying biomarkers that are associated with gut barrier integrity [40]. Our study focuses on three biomarkers: intestinal fatty acid binding protein (I-FABP or FABP2), lipopolysaccharide-binding protein (LPS-BP or LBP) and soluble cluster of differentiation 14 (sCD14) (Figure 3).

FABP2 is produced by enterocytes and is a serum biomarker that indicates intestinal barrier damage due to its high expression in the intestine and role in fatty acid transport. Elevated FABP2 in blood or urine signals epithelial injury and has shown its utility in diagnosing acute conditions like mesenteric ischemia and necrotizing enterocolitis without invasive procedures [53]. FABP2 also shows promise as a diagnostic tool in chronic intestinal disorders, particularly in celiac disease. The rationale behind using FABP2 as a biomarker lies in its high expression in the intestine and its rapid response to epithelial damage, reflecting the extent of injury and inflammation [54]**.**

Lipopolysaccharide (LPS), primarily derived from Gram-negative gut bacteria, is a well-studied pathogen-associated molecular pattern (PAMP) linked to endotoxemia and chronic inflammation in diseases like IBD [55,56]. When gut permeability increases, elevated plasma LPS leads to a rise in LBP, an acute-phase protein produced by liver and intestinal cells in response to LPS. LBP binds LPS, activating immune pathways that produce cytokines and other inflammation markers [57]. Since the half-life of LPS is very short, measurement of the body's response to LPS via LBP is better, due to its longer half-life. This makes LBP a great serum biomarker of LPS translocation and therefore of intestinal permeability. Elevated LBP and LPS levels in active IBD support LBP as a valuable biomarker for monitoring and assessing disease severity and prognosis.

Soluble CD14 (sCD14) is produced in the liver and interacts with LPS in the bloodstream to increase immune responses, particularly by enhancing Toll-like receptor 4 (TLR4) activation, which initiates downstream signalling that releases pro-inflammatory cytokines [58,59]. This process, mediated by LBP, is crucial in the innate immune defence against Gram-negative bacteria. Elevated levels of sCD14, LPS, and LBP have been observed in IBD patients during active disease and decrease with effective treatment, correlating with disease activity. This suggests sCD14's potential as a biomarker for monitoring disease progression and treatment response [47,60].

The primary aim of this study is to investigate the prevalence of three biomarkers that reflect gut barrier dysfunction and its interaction with dietary patterns in patients with IBD. Secondary, this study aims to explore how these biomarkers relate to other key variables, including demographic, anthropometric, clinical and biochemical parameters. It also examines the associations between these biomarkers and clinical and biochemical disease activity scores. All these analyses might provide new insights into the effect of dietary patterns on the multifactorial nature of gut barrier dysfunction and its potential implications for disease management and patient outcomes in IBD.

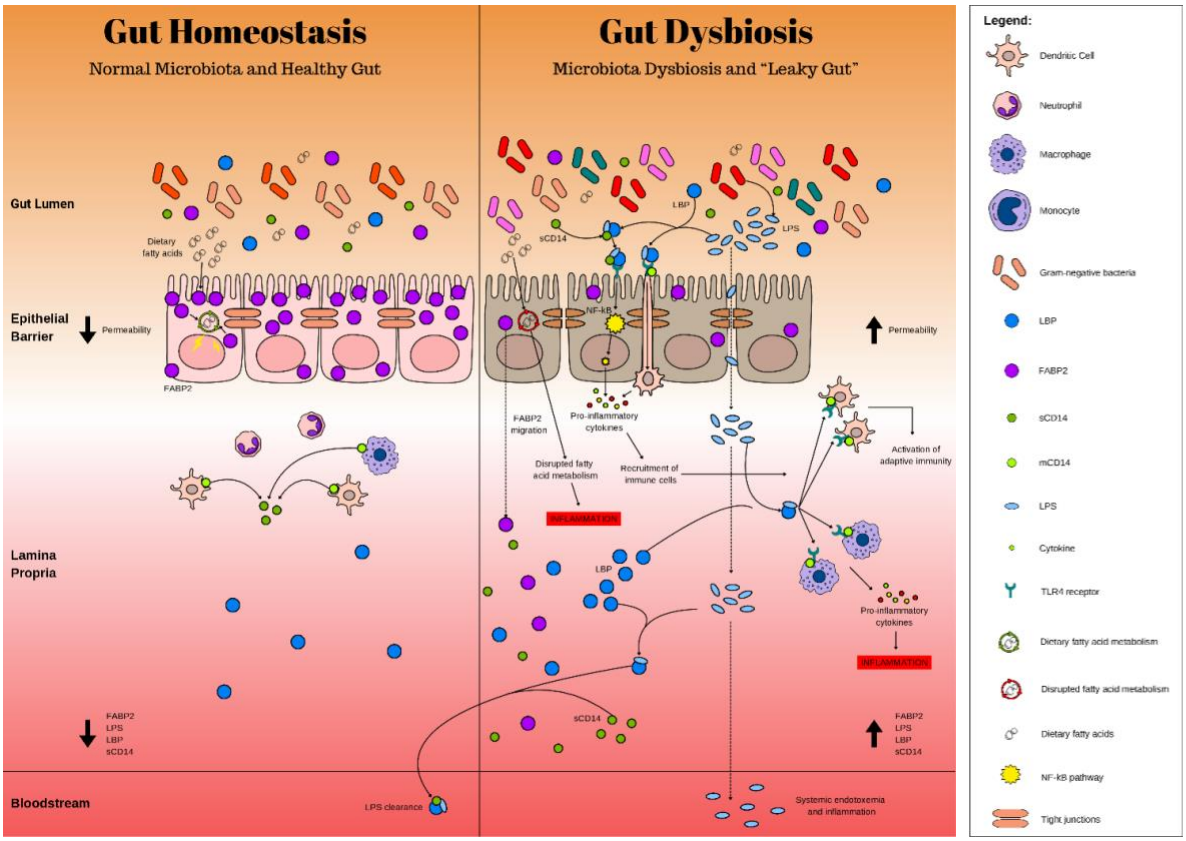

Figure 3. The role of FABP2, LBP and sCD14 in gut homeostasis and gut dysbiosis. In the healthy gut, FABP2 is primarily expressed in enterocytes, where it facilitates the transport, absorption, and metabolism of dietary fatty acids. In contrast, sCD14 circulates freely in the gut lumen, mucus layer, and bloodstream, with its main source being immune cells, which release it into a soluble form. LBP is found in the gut lumen, lamina propria, and bloodstream. When the gut barrier is compromised, LPS from Gram-negative bacteria can cross the epithelial barrier and reach the lamina propria. LBP binds to LPS and delivers it to sCD14 on epithelial cells or to mCD14 on immune cells, triggering pro-inflammatory cytokine release, increasing intestinal permeability. This leaky gut allows more LPS to enter the bloodstream, potentially leading to systemic inflammation or septic shock if not cleared by immune cells. FABP2 migrates into the lamina propria during gut damage, which disrupts fatty acid metabolism, promoting inflammation.
Abbreviations: FABP2, fatty acid-binding protein 2; LBP, lipopolysaccharide binding protein; LPS, lipopolysaccharide; mCD14, membrane cluster of differentiation 14; NF-kB, nuclear factor kappa-light chain-enhancer of activated B cells; sCD14, soluble cluster of differentiation 14; TLR4, Toll-like receptor 4.

2. Methods

2.1. Ethical considerations

This research was conducted in accordance with the ethical principles of the Declaration of Helsinki. The research protocol obtained approval from the Institutional Review Board (IRB) of the University Medical Centre Groningen (UMCG, registered as no. 2008/338). All participants involved in the study provided informed written consent for the use of their data and biological samples, ensuring that their rights and privacy were respected throughout the research.

2.2. Study participants

Patients with a confirmed diagnosis of IBD for at least one year were included at the outpatient clinic of the University Medical Centre Groningen (UMCG) in the Netherlands ($n = 450$). These patients are part of the 1000IBD / *Parelsnoer* project, a large, well-characterized prospective cohort consisting of over 1000 IBD patients living in the northern regions of the Netherlands [61]. Patients included in this study were enrolled between 2010 to 2019 and selected based on the availability of plasma samples.

2.3. Data collection and definitions

Demographic and clinical data of the study participants were collected at time of plasma sampling and included age, sex, body-mass index (BMI), smoking status, medication use, surgical history and disease activity. Disease activity was assessed using the Harvey-Bradshaw Index (HBI) for CD patients and the Simple Clinical Colitis Activity Index (SCCAI) for UC patients. Blood CRP levels were routinely measured by nephelometry at the time of plasma collection. CRP was recorded as a binary variable due to many values falling below the detection limit. Remission was defined as having both low clinical disease activity scores (HBI < 5 for CD and SCCAI ≤ 2 for UC, together with CRP < 5 mg/L), while patients with higher values were classified as having 'active disease'.

2.4. Gut barrier biomarkers

We assessed all biomarkers using enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's protocols. The assay kits were for human FABP2 (Thermofisher Scientific), human LBP (Thermofisher Scientific) and human sCD14 (Quantikine, R&D Systems, Inc.). Averages of duplicates were determined, and absolute values are expressed in µg/mL for sCD14 and LBP and in ng/mL for FABP2. After determination of biomarker levels, two sCD14 samples were excluded from the study due to incorrect measurements, resulting in 448 samples.

2.5. Dietary data

Habitual dietary intake was assessed using existing data from a previous study, which used semi-quantitative food frequency questionnaires (FFQs) developed and validated by Wageningen University [62] (Supplementary table 1). During that study, participants reported their dietary intake for one month, with portion sizes estimated through common household measures. Intake in grams per day was calculated by converting consumption frequencies into daily equivalents. The 110 food items were grouped into 25 standardized food groups for analysis. Five dietary patterns were identified and validated using principal component analysis (PCA) on these food groups, cumulatively explaining 40% of the total dietary variation in this study. These five principal components (PCs) from the same previous study were used in the current study:

- PC1 (Refined Carbohydrate Pattern): Characterized by a high intake of bread, spreads, pastry, sugar, sweets, savoury snacks, and juice.
- PC2 (Mediterranean-Style Pattern): Characterized by a high intake of fruit, fish, nuts, eggs, tea, and cereals, and low intake of meat and non-alcoholic beverages.
- PC3 (Processed/Ready-Made Foods Pattern): Characterized by a high intake of sauces, pasta, ready-made meals, rice, alcohol, nuts, and savoury snacks.
- PC4 (Coffee and Alcohol Pattern): Characterized by an exceptionally high intake of coffee and alcohol, along with spreads and dairy, and low intake of tea.
- PC5 (Traditional Dutch Pattern): Characterized by a high intake of vegetables, potatoes, meat, and legumes.

Biomarker levels were stratified into four quartile groups to analyse with the PCs: Q1 (lowest 25%), Q2 (25th to 50th percentile), Q3 (50th to 75th percentile), and Q4 (highest 25%).

2.6. Endoscopy data

From the patient cohort, we used the patients' medical records to examine if patients had an endoscopy within three months prior or after the sample collection (n = 111). Documentation of endoscopy procedures were used to extract further details, like the sign of inflammation and the Mayo-scores (n = 33).

2.7. Defined disease activity score and gut barrier score

A new variable for clinical activity was created for further analysis. This defined disease activity score was assessed using a stepwise algorithm and the outcomes were 'active disease' and 'remission'. Patients

were classified as having active disease if the clinical score (SCCAI/HBI) indicated activity, they were receiving induction therapy, or if faecal calprotectin levels exceeded 250 µg/g. If these criteria were not met, CRP levels were evaluated, with levels >5 mg/L indicating active disease. For patients with lower CRP levels, recent endoscopic findings were reviewed; active inflammation classified the disease as active. Patients meeting none of these criteria were classified as being in remission (Supplementary figure 1).

Next to the defined disease activity score, a new variable for gut barrier score was created for further analysis. Participants were stratified based on gut barrier function and the outcomes were 'GB+' (intact gut barrier) and 'GB-' (non-intact gut barrier). Classification was performed using quartiles (Q1–Q4) of the stratified sCD14 and LBP levels. For GB+, participants were categorized as sCD14 in Q1 & LBP in Q1, or sCD14 in Q2 & LBP in Q1, or sCD14 in Q1 & LBP in Q2. For GB-, participants were categorized as sCD14 in Q4 & LBP in Q4, or sCD14 in Q3 & LBP in Q4, or sCD14 in Q4 & LBP in Q3 (Supplementary figure 2).

2.8. Statistical analysis

For descriptive statistics, continuous variables were presented mean ± standard deviation or medians with 1st and 3rd quartile cut-offs representing the interquartile range (IQR), depending on normality of data distribution. Categorical variables were presented as proportions with percentages. The normality of continuous variables was assessed using the Shapiro–Wilk test. Comparisons between groups were performed using the Mann-Whitney U-test for continuous variables and the chi-squared test for categorical variables. Spearman's rank correlation coefficients (expressed as ρ) were used to assess associations between two variables within the complete group. When $\rho < 0.2$, correlations were considered negligible. Multiple linear regression models were used to assess associations between our biomarkers and disease activity, where age, sex, BMI, smoking status, diagnosis, albumin levels and surgical history were included as confounders. *p*-values ≤ 0.05 were considered statistically significant. Statistical analyses and data visualizations were conducted using R (v. 4.1.2).

3. Results

3.1. Descriptive data

In total, 448 patients were included, of whom 261 (58.3%) were diagnosed with CD and 187 (41.7%) with UC. Patient demographic and clinical characteristics are presented in Table 1. Patients with CD were younger (39.7 ± 14.2 vs. 44.8 ± 14.3 years, $p < 0.01$) and more often female (67.4% vs. 55.6%, $p = 0.01$), had a lower BMI (23.9 [21.4–27.6] vs. 25.4 (22.8–29.0), $p < 0.01$) and smoked more frequently (28.0% vs. 10.2%, $p < 0.01$) compared to patients with UC. The proportion of patients who underwent ileocecal resection was higher in the CD compared to the UC group ($p < 0.01$), whereas the history of (partial) colon resections was comparable between groups ($p = 0.88$). Finally, elevated CRP levels (> 5 mg/L, tested binary) were more frequent in CD compared to UC patients (26.1% vs. 16.6%, $p = 0.02$).

Table 1. Demographic and clinical characteristics of study participants and for patients with CD and UC separately.

Characteristics	Total	CD	UC	*p*-value
	n = 448	*n* = 261	*n* = 187	
Age (years)	41.8 ± 14.4	39.7 ± 14.2	44.8 ± 14.3	**<0.01**[a]
Sex, *n* (%)				**0.01**[b]
Male	168 (37.5)	85 (32.6)	83 (44.4)	
Female	280 (62.5)	176 (67.4)	104 (55.6)	
BMI (kg/m^2)	24.7 [22.0-28.0]	23.9 [21.4-27.6]	25.4 [22.8-29.0]	**<0.01**[a]
Current smoking, *n* (%)	438 (97.3)	256 (97.3)	182 (97.3)	**<0.01**[b]
Yes	92 (20.5)	73 (28.0)	19 (10.2)	
No	344 (76.8)	181 (69.3)	163 (87.2)	
Medication use, *n* (%)				

TNF-α-antagonists	113 (25.2)	98 (37.5)	15 (8.0)	**<0.01**[b]
Aminosalicylates	156 (34.8)	27 (10.3)	129 (69.0)	**<0.01**[b]
Thiopurines	169 (37.7)	111 (42.5)	58 (31.0)	**0.02**[b]
Steroids	105 (23.4)	65 (24.9)	40 (21.4)	0.45[b]
Calcineurin inhibitors	8 (1.8)	2 (0.8)	6 (3.2)	0.12[b]
Methotrexate	32 (7.1)	28 (10.7)	4 (2.1)	**<0.01**[b]
Disease activity, *n* (%)				
HBI (CD)	-	249 (95.4)	-	
<5	-	159 (60.9)	-	
≥5	-	90 (34.5)	-	
SCCAI (UC)	-	-	179 (95.7)	
≤2	-	-	129 (69.0)	
>2	-	-	50 (26.7)	
CRP	367 (81.9)	216 (82.8)	151 (80.7)	**0.02**[b]
≤5 mg/L	268 (59.8)	148 (56.7)	120 (64.2)	
>5 mg/L	99 (22.1)	68 (26.1)	31 (16.6)	
Surgical history, *n* (%)				
Ileocecal resection	87 (19.4)	86 (33.0)	1 (0.5)	**<0.01**[b]
Colon resection (or partial)	74 (16.5)	42 (16.1)	32 (17.1)	0.88[b]

Data are presented as proportions n with corresponding percentages (%), means ± standard deviation or as medians [1st-3rd quartile]. Statistical significance was tested by [a] Mann-Whitney U tests or [b] chi-squared test. Significant differences are highlighted in **bold**.
Abbreviations: BMI, body-mass index; CD, Crohn's disease; CRP, C-reactive protein; HBI, Harvey–Bradshaw index; IBD, inflammatory bowel disease; SCCAI, simple clinical colitis activity index; TNF-α, tumour necrosis factor alpha; UC, ulcerative colitis. '-': Not applicable.

3.2 No association between dietary patterns and gut barrier biomarker levels

A correlation matrix was computed to examine the relationships between the five different PCs and the gut barrier biomarkers. The correlation matrix revealed that all PCs showed no correlations ($\rho < 0.2$) with the gut barrier biomarkers (Figure 4).

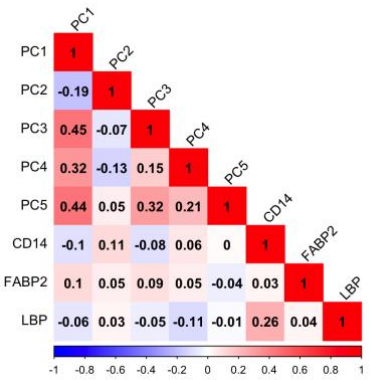

Figure 4. Correlation matrix for the relationship between the five PCs and the three biomarkers. The correlation coefficients range from -1 to +1, where values close to +1 indicate a strong positive correlation, values close to -1 indicate a strong negative correlation, and values near 0 indicate no significant correlation. No correlations between PCs or biomarkers were found. Abbreviations: PC, principal component.

After stratification of all three biomarkers into four quartile groups, analysis between the PCs and biomarkers was performed. Between sCD14 and PC4 were two significant differences; between Q1 and Q4 ($p = 0.004$) and between Q3 and Q4 ($p = 0.018$), where Q4 was significantly higher than both (Figure 5A). Between FABP2 and PC3 was one significant difference; between Q2 and Q4 ($p = 0.022$), where Q4 was significantly higher (Figure 5B). Between all other quartile groups, no significance differences were seen between biomarker levels and PCs (Supplementary figures 3A, 3B and 3C).

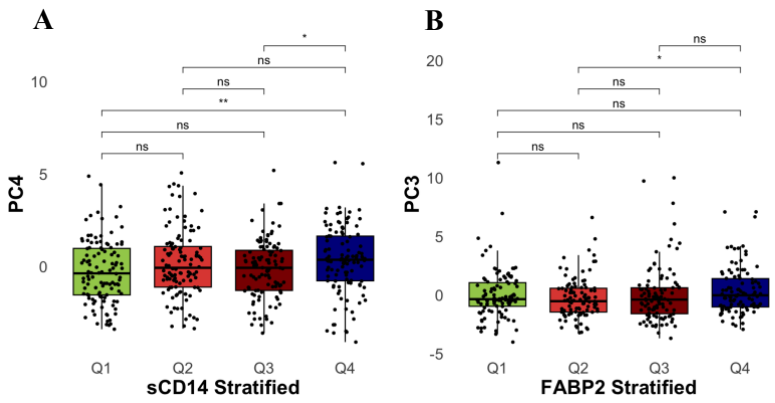

Figure 5. Stratified biomarker scores and PCs. (**A**) A significant association between PC4 and sCD14 Q1 – Q4 and sCD14 Q3 – Q4 was found. (**B**) Between PC3 and FABP2 Q2 – Q4 was another positive association. *: p < 0.05; **: p < 0.01; ns, not significant.
Abbreviations: PC, principal component.

3.3 LBP levels are significantly higher in CD patients

Three gut barrier biomarkers were analysed, and their levels are presented in Table 2 and Figure 6. Analysis of gut barrier biomarker levels between CD and UC patients showed that for sCD14 the median levels were similar between CD (1.28 [1.12-1.60] µg/mL) and UC (1.36 [1.16-1.57] µg/mL), with no significant difference ($p = 0.239$). For FABP2, UC patients exhibited slightly higher median levels (3.02 [1.76-7.86] ng/mL) compared to CD patients (2.73 [1.48-5.45] ng/mL), though this trend did not reach statistical significance ($p = 0.055$). For LBP, CD patients had significantly higher levels (13.72 [10.04-18.93] µg/mL) than UC patients (11.72 [8.40-15.42] µg/mL), with a p-value of <0.001.

Table 2. Gut barrier biomarker levels of study participants and for patients with CD and UC separately.

Biomarker	Total	CD	UC	*p*-value
	n = 448	*n* = 261	*n* = 187	
sCD14 (µg/mL)	1.33 [1.13-1.58]	1.28 [1.12-1.60]	1.36 [1.16-1.57]	0.239
FABP2 (ng/mL)	2.81 [1.60-6.54]	2.73 [1.48-5.45]	3.02 [1.76-7.86]	0.055
LBP (µg/mL)	12.79 [9.49-17.34]	13.72 [10.04-18.93]	11.72 [8.40-15.42]	**<0.001**

Data are presented as medians [1st-3rd quartile]. Statistical significance was tested by Mann-Whitney U test. Significant differences are highlighted in **bold**.
Abbreviations: FABP2, fatty acid-binding protein; LBP, lipopolysaccharide binding protein; sCD14, soluble CD14.

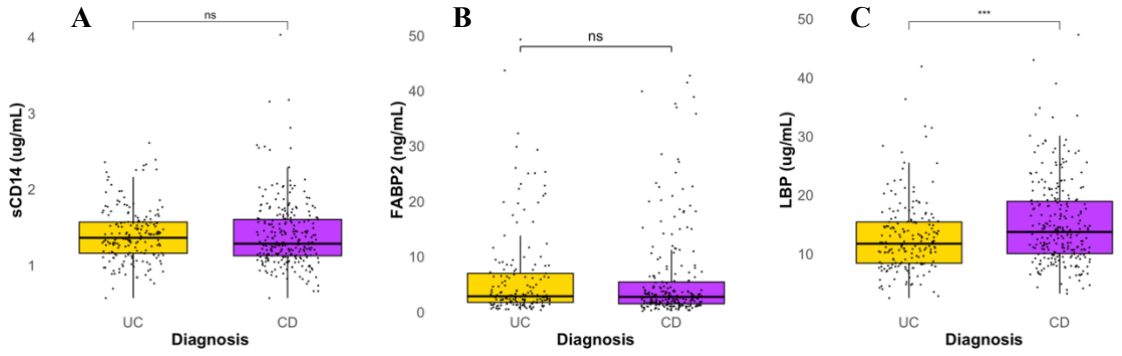

Figure 6. Comparison of gut barrier biomarker concentrations between CD (n = 261) and UC (n = 187). (**A**) sCD14 levels show no difference between UC and CD. (**B**) FABP2 levels in UC patients are slightly higher but show no significant difference compared to CD. (**C**) LBP levels are significantly higher in CD patients compared to UC patients. ***: p < 0.001; ns, not significant.
Abbreviations: CD = Crohn's disease; FABP2, fatty acid-binding protein 2; LBP = lipopolysaccharide binding protein; sCD14, soluble CD14; UC = ulcerative colitis.

3.4 Gut barrier biomarkers levels are affected by age and BMI

A Spearman correlation analysis was conducted to examine the relationship between demographic and anthropometric variables and gut barrier biomarker levels in the total cohort and for UC and CD separately. A weak, but significant positive correlation was observed between age and sCD14 levels in the total cohort ($\rho = 0.220$, $p < 0.001$), and in both UC ($\rho = 0.237$, $p = 0.001$) and CD ($\rho = 0.186$, $p = 0.003$) (Figure 7A). No significant correlation was found between age and the other two biomarkers in the total cohort (FABP2: $\rho = -0.076$, $p = 0.127$ and LBP: $\rho = 0.054$, $p = 0.253$) (Supplementary figures 4A and 4B). A significant positive correlation was observed between BMI and LBP levels in the total cohort ($\rho = 0.183$, $p < 0.001$). In UC this correlation was stronger ($\rho = 0.246$, $p < 0.001$) compared to CD ($\rho = 0.185$, $p = 0.003$) (Figure 7B). No significant correlation was found between BMI and the other two biomarkers in the total cohort (sCD14: $\rho = 0.027$, $p = 0.567$ and FABP2: $\rho = 0.024$, $p = 0.632$) (Supplementary figures 5A and 5B).

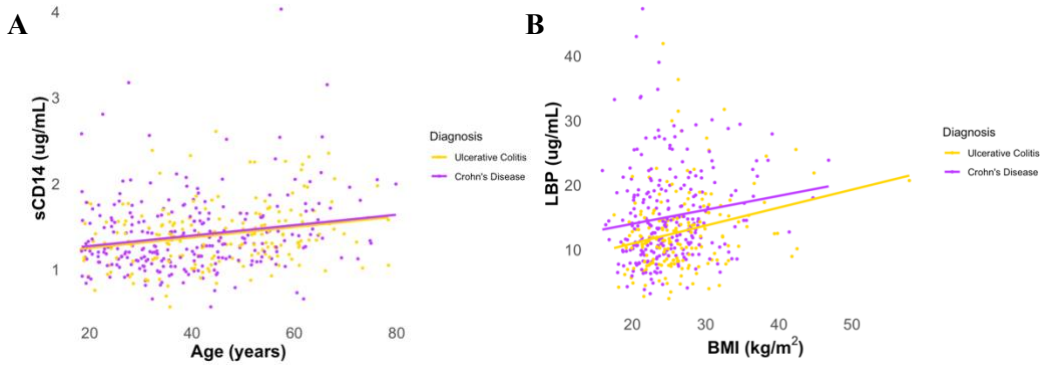

Figure 7. Biomarker levels compared to continuous variables. (**A**) When age increases, sCD14 increases slightly in both UC and CD (**B**) When BMI increases, LBP levels increase strongly in UC and moderately in CD.
Abbreviations: BMI, body-mass index; LBP = lipopolysaccharide binding protein; sCD14, soluble CD14.

3.5 Gut barrier biomarker levels are affected by gender, smoking status and surgical history

Analysis of gut barrier biomarker levels and sex showed that for sCD14 the levels were significantly higher in women compared to men ($p = 0.039$) (Figure 8A). For FABP2, men showed higher levels compared to women ($p = 0.042$) (Figure 8B). For LBP, women had significantly higher levels than men ($p = 0.024$) (Figure 8C).

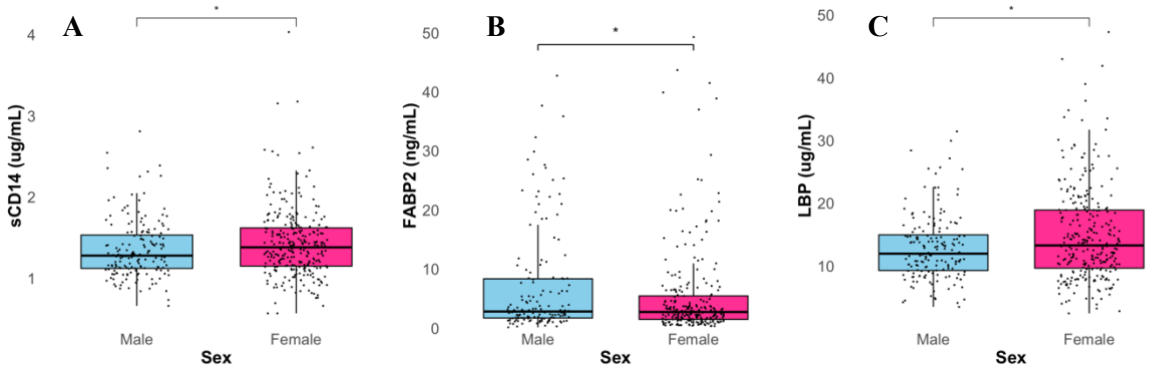

Figure 8. Comparison of gut barrier biomarker concentrations between men ($n = 168$) and women ($n = 280$). (**A**) sCD14 levels are significantly higher in women compared to men. (**B**) FABP2 levels are significantly higher in men compared to women. (**C**) LBP levels are significantly higher in women compared to men. *: $p < 0.05$.

Current smokers have increased sCD14 levels ($p = 0.034$) (Figure 9A). No effect of smoking is seen for FABP2 and LBP levels ($p = 0.321$ and 0.823 respectively) (Supplementary figures 6A and 6B). Patients who previously had surgery (ileocecal resection and/or colectomy) have decreased sCD14 levels ($p =$

0.001) (Figure 9B). Surgical history had no effect on FABP2 and LBP levels (p = 0.907 and 0.120 respectively) (Supplementary figures 7A and 7B).

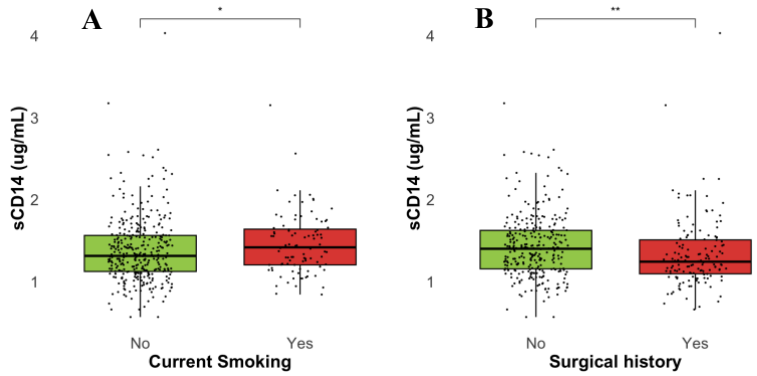

Figure 9. Comparison of sCD14 concentrations against smoking and surgical history. (**A**) sCD14 levels are significantly higher in patients who currently smoke (n = 92) compared to non-smokers (n = 344). (**B**) sCD14 levels are significantly lower in patients with a surgical history (n = 143) compared to patients without previous surgeries (n = 305). *: $p < 0.05$, **: $p < 0.01$.

3.6 sCD14 and LBP levels decrease when albumin levels increase

A Pearson correlation analysis was conducted to examine the relationship between albumin and gut barrier biomarker levels in the total cohort and for UC and CD separately. A negative correlation was observed between albumin and sCD14 levels in the total cohort (ρ = -0.150, p = 0.002). This effect was stronger in CD patients (ρ = -0.218, $p < 0.001$) compared to UC patients (ρ = -0.019, p = 0.796) (Figure 10A). A strong negative correlation was observed between albumin and LBP levels in the total group (ρ = -0.420, $p < 0.001$). This strong correlation was seen in both CD patients (ρ = -0.454, $p < 0.001$) and in UC patients (ρ = -0.326, $p < 0.001$) (Figure 10B). No significant correlation was found between albumin and FABP2 levels in the total cohort (ρ = -0.002, p = 0.9736) (Supplementary figure 8).

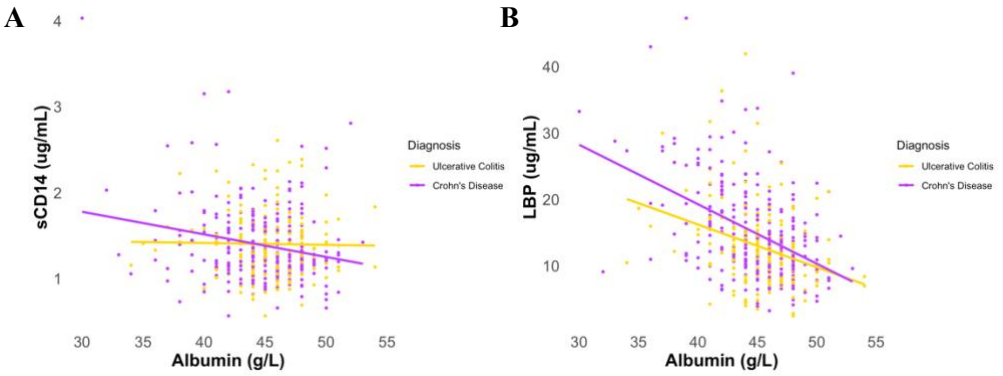

Figure 10. Biomarker levels compared to albumin levels. (**A**) When albumin levels increase, sCD14 levels decrease in CD patients (**B**) When albumin levels increase, LBP levels decrease in both UC and CD patients.

3.7 sCD14 and LBP are associated with clinical activity

Patients were classified in having active disease or remission by either HBI for CD patients or SCCAI for UC patients. The results of these clinical assessments were grouped together and this showed that both sCD14 and LBP concentrations were increased in patients with active disease compared to patients in remission (p = 0.019 and $p < 0.001$ respectively). FABP2 concentrations were similar between patients with active disease and remission (p = 0.938) (Figure 11A and Supplementary table 2).

Next to clinical assessment scores, CRP was shown to associate with sCD14 and LBP as well. Patients with CRP levels >5 mg/L showed that both sCD14 and LBP concentrations were increased compared to patients with CRP <5 mg/L (both $p < 0.001$). FABP2 concentrations were similar between patients with CRP >5 and <5 (p = 0.376) (Figure 11B and Supplementary table 2). This effect was again seen when

CRP was stratified into three categories: <5, 5-25 and >25. Statistically significant increases are seen in sCD14 levels as CRP levels increase from the <5 group to the 5–25 and >25 groups (both $p < 0.001$). However, there is no significant difference in sCD14 levels between the 5–25 and >25 groups ($p = 0.143$). LBP levels are significantly higher in groups with higher CRP levels. There is a strong positive trend in LBP levels as CRP increases from <5 to 5–25 and then to >25 (all $p < 0.001$) (Figure 11C). For FABP2 no associations were found between any categories (<5 to 5-25, $p = 0.335$; <5 to >25, $p = 0.989$ and 5-25 to >25, $p = 0.471$) (Supplementary table 3).

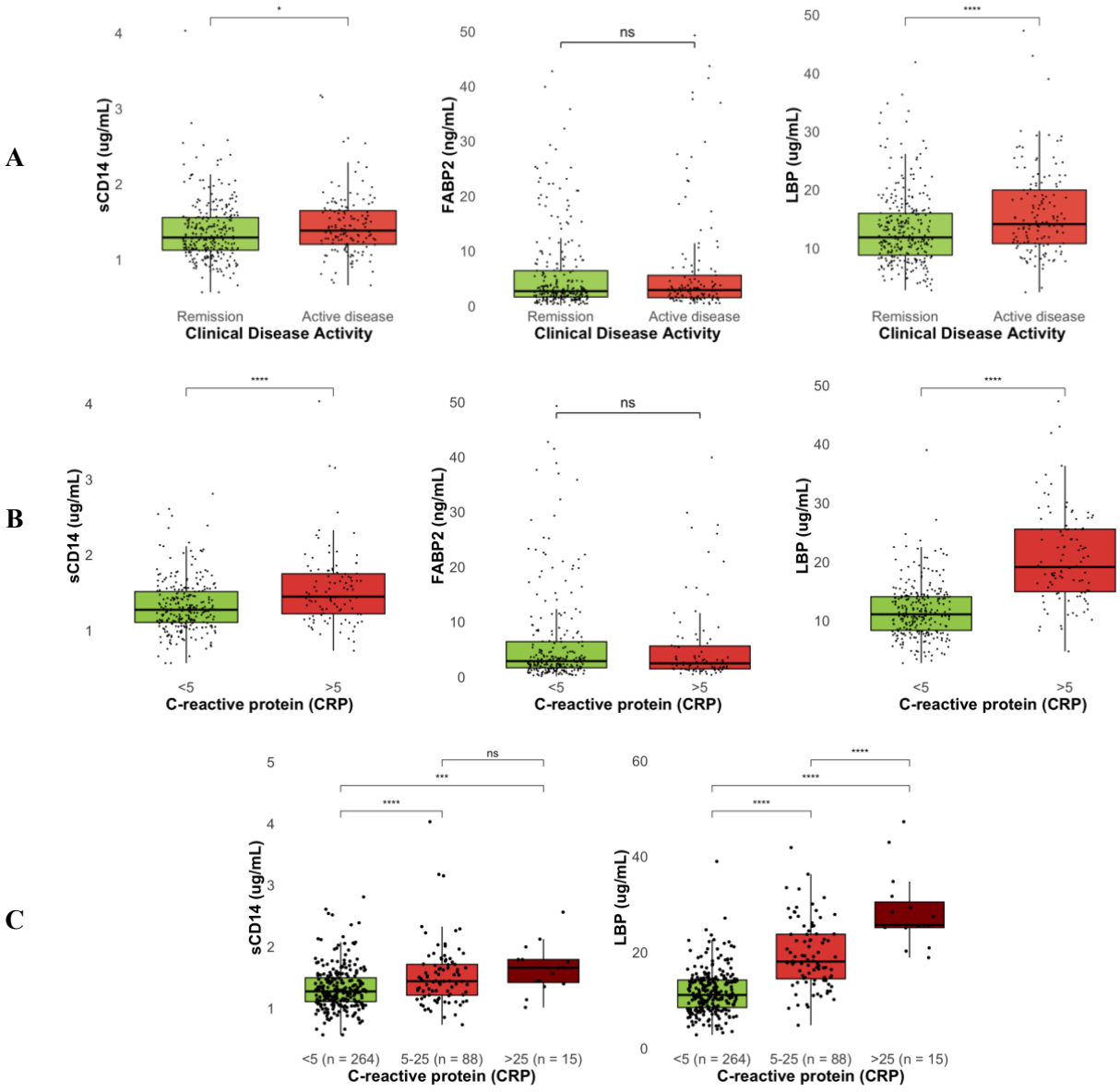

Figure 11. Biomarker levels, clinical activity scores and CRP levels. (**A**) Patients with active disease ($n = 90$) have higher sCD14 and LBP levels compared to patients in remission ($n = 159$). (**B** and **C**) Increased CRP levels are associated with higher sCD14 levels. For CRP < 5, $n = 274$; for CRP > 5, $n = 103$. CRP is expressed in mg/L. *: $p < 0.05$; ***: $p < 0.001$; ****: $p < 0.0001$; ns, not significant.
Abbreviations: HBI, Harvey–Bradshaw index; SCCAI, simple clinical colitis activity index.

3.8 No association between endoscopic inflammation and gut barrier biomarker levels

Patients with registered endoscopy data were divided between inflammation or no inflammation. This score showed that there was no significant difference between having inflammation or not for all biomarkers (sCD14, $p = 0.321$; FABP2, $p = 0.597$; LBP, $p = 0.824$) (Figure 12A). Also, there were no

differences in Mayo score for all biomarkers (Figure 12B). Corresponding *p*-values can be found in Supplementary table 4.

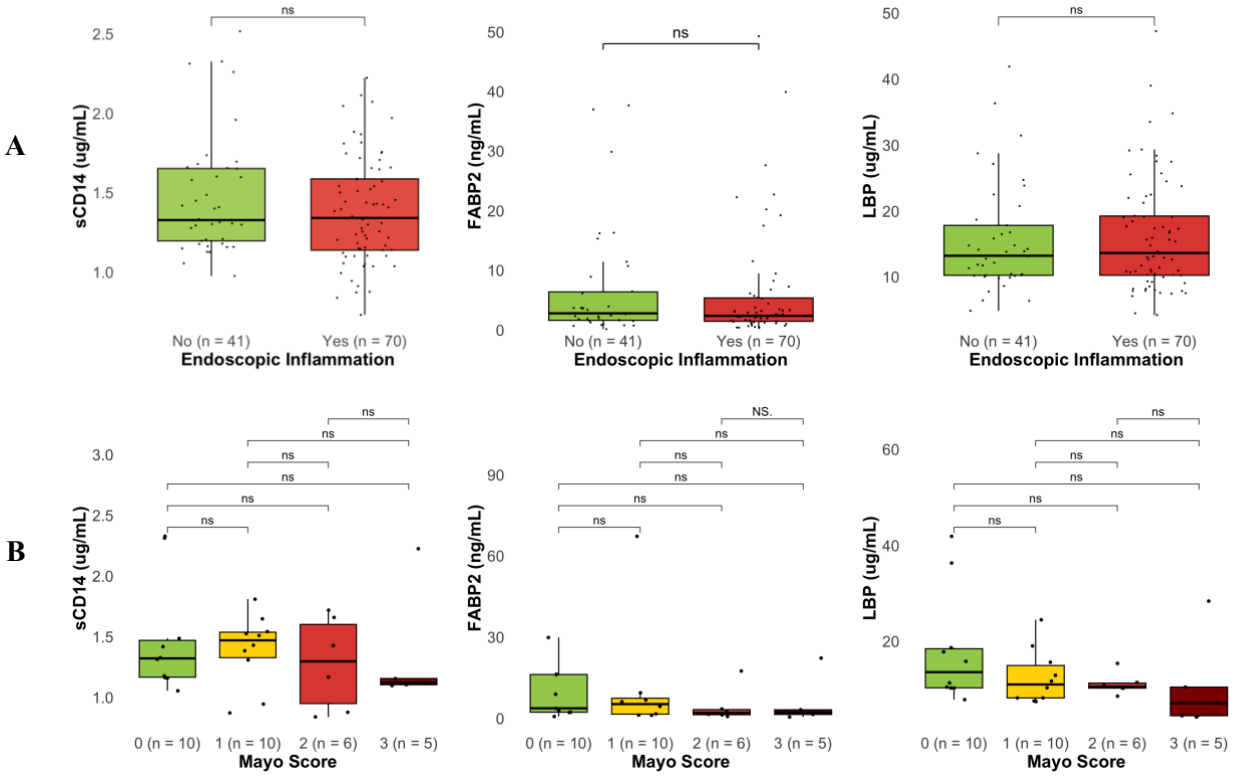

Figure 12. Endoscopy outcomes and biomarker levels. (**A**) No significant differences between endoscopic inflammation and no inflammation for all biomarkers. (**B**) No significant differences between Mayo scores and biomarker levels. ns, not significant.

3.9 Defined disease activity score is associated with sCD14 and LBP

After adjusting the disease activity score to a new defined disease activity variable, analysis between the biomarker levels and the outcomes of the new variable was conducted. It was shown that for the outcome 'active disease' the levels of sCD14 and LBP were significantly higher compared to the outcome 'remission' (both $p = 0.001$). For FABP2 the outcome was similar between active disease and remission ($p = 0.366$) (Figure 13).

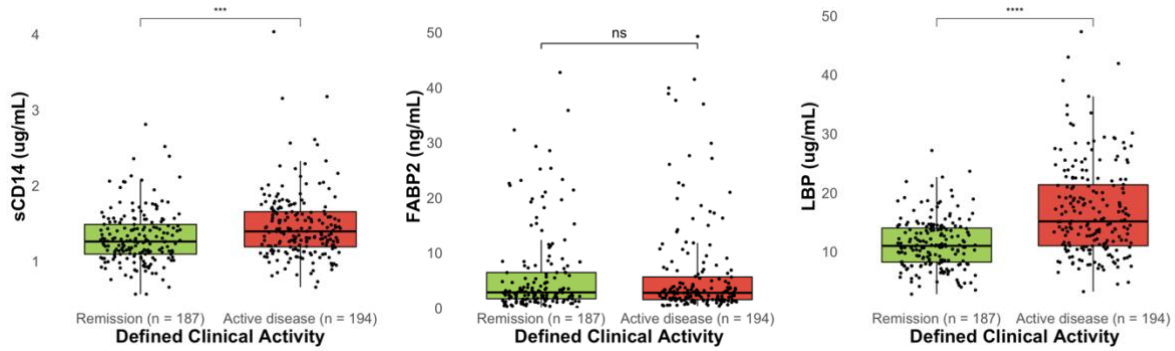

Figure 13. Defined disease activity score and biomarker levels. Patients with active disease ($n = 194$) have significantly higher sCD14 and LBP levels compared to patients in remission ($n = 187$). For FABP2 this difference was not found. ***: $p < 0.001$; ****: $p < 0.0001$; ns, not significant.

Multiple linear regression analyses again revealed significant associations between biomarkers and clinical activity (sCD14: β = 0.11 [0.04–1.77], $p = 0.009$ and LBP: β = 3.89 [2.84–4.94], $p < 0.001$),

after adjusting for age, BMI, sex, smoking, diagnosis, albumin and surgical history (Figure 14 and Supplementary table 5).

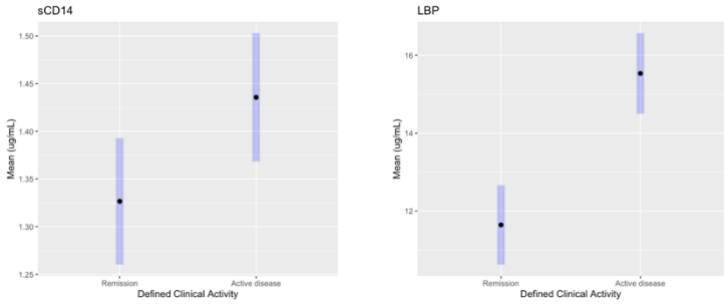

Figure 14. Mean biomarker levels and defined disease activity score. The mean biomarker levels for sCD14 and LBP are higher when patients have active disease compared to remission.

3.10 Gut barrier scores associate with clinical activity

After combining the stratified Q-scores for every biomarker, the gut barrier score was analysed with the defined disease activity score and the CRP levels. The percentage of patients in remission and with active disease across two gut barrier states (GB+ and GB-) was analysed. In the GB+ group, a higher proportion of patients are in remission (72.1%) compared to those with active disease (27.9%). In the GB- group, a larger proportion of patients are in an active disease state (75.6%) compared to remission (24.4%) (Figure 15A). The CRP levels were analysed between the two gut barrier scores, which showed that the for GB- the CRP levels were significantly higher compared to GB+ ($p < 0.001$) (Figure 15B).

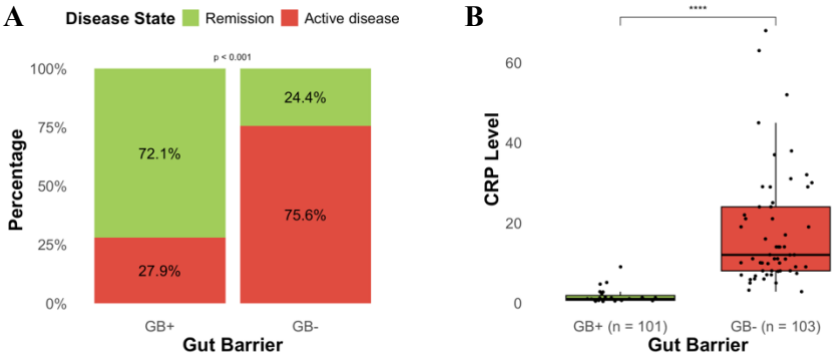

Figure 15. Gut barrier score variable, defined disease activity score and CRP levels. (**A**) For GB+, remission was higher than active disease. For GB-, active disease was higher than remission. (**B**) CRP levels were significantly higher in GB- ($n = 103$) compared to GB+ ($n = 101$). ****: $p < 0.0001$.

4. Discussion

4.1. Main findings

In this study, we evaluated the possibilities for sCD14, FABP2 and LBP as gut barrier biomarkers in IBD. There was no observed association between gut barrier biomarker levels and dietary patterns. Multiple confounders were detected that influenced biomarker levels, including age, BMI, diagnosis, sex, smoking status, and surgical history. sCD14 and LBP levels were found to decrease with increasing albumin levels. No associations between biomarkers and endoscopic inflammation were seen. We found a strong association between sCD14 and LBP and clinical- and biochemical disease activity. However, no association was observed between FABP2 and clinical- or biochemical disease activity. After creating a new defined disease activity score, a gut barrier score and adjusting for confounders, sCD14 and LBP levels were found to correlate even more with the defined disease activity score and with the new gut barrier variable.

4.2 Previous literature and possible explanations

Dietary patterns

The aim of this study was to investigate whether dietary patterns, as reflected by five PCs, were associated with three gut barrier biomarkers. Gut barrier integrity is important for maintaining overall health, and gut barrier biomarkers are indicative of intestinal permeability in the gut. Understanding whether diet influences these biomarkers is important for developing dietary interventions that may improve gut health. We found no significant correlations between the five PCs and the gut barrier biomarkers. The correlation matrix revealed weak to negligible correlations, suggesting that overall dietary patterns did not seem to have a direct impact on our biomarkers. This finding suggests that, at least within the context of this study, the general dietary patterns captured by PCs may not be strongly associated with gut barrier integrity as indicated by our biomarkers. When stratifying the biomarkers into quartile groups, a few significant differences were observed. PC4 showed significant associations between Q1 and Q4, and Q3 and Q4 for sCD14. Q4 indicates the quartile with the highest levels of sCD14 and is associated with a higher compatibility to the PC4 diet, compared to quartiles with lower sCD14 levels (Q1 and Q3). PC4 is a pattern defined by high coffee and alcohol intake, and this association may indicate the impact of these dietary components on gut barrier function. Both coffee and alcohol might affect gut permeability in different ways. Excessive alcohol intake can change the gut microbiota composition and compromise gut epithelial integrity, leading to increased intestinal permeability [63]. Moderate coffee consumption has been associated with beneficial effects on gut motility and microbiota; however, excessive intake may have adverse effects. Studies showed that coffee consumption could aggravate symptoms and was associated with the recurrence of IBD [64]. The observed association might imply that high intake of coffee and alcohol may contribute to gut barrier dysfunction, as indicated by increased sCD14 levels. PC3 showed a significant association between Q2 and Q4 for FABP2. PC3 is a pattern defined by high intake of processed foods and ready-made meals, and this association may reflect a potential damaging effect of these dietary components on gut barrier function. Diets rich in processed foods are often linked to reduced microbiome diversity and an increase in harmful gut bacteria, which can lead to inflammation and increased intestinal permeability [65]. The association with higher FABP2 levels suggests that consuming more processed foods may compromise the gut barrier. These insights suggest that specific dietary patterns, may be associated with changes in gut barrier biomarkers. However, the associations observed in this study were not particularly prominent, as most of the PCs showed no significant interaction with our biomarkers.

Demographic and clinical variables

Understanding the relationship between gut barrier biomarkers and demographic, anthropometric, lifestyle and clinical variables is essential to contextualize their potential as diagnostic or monitoring tools for IBD. We found that LBP levels were higher in patients with CD compared to UC. This is in line with previously published studies [66,67]. In CD, deeper intestinal wall involvement and frequent small intestinal disease contribute to increased gut permeability, promoting translocation of microbial LPS into circulation and inducing LBP production. This contrasts with UC, which primarily involves superficial inflammation limited to the colonic mucosa, resulting in lower LPS translocation and reduced LBP levels [68,69]. We found that gut barrier biomarkers were affected by several variables. The weak association between sCD14 and age we found might be attributed to age-related changes. One of these changes is chronic low-grade inflammation, frequently referred to as "inflammaging." This condition is characterized by chronic, systemic immune activation, driven by increased intestinal permeability and microbial translocation [70]. We also found a positive association between BMI and LBP. Elevated BMI is associated with chronic low-grade inflammation, as adipose tissue produces proinflammatory cytokines such as IL-6 and TNF-α, which enhance LBP expression [71]. We found differences in biomarker levels between sexes. This might be attributed to sex-specific physiological and metabolic factors. Women, particularly premenopausal women, tend to have higher sCD14 and LBP levels due to the modulation of oestrogen on immune responses, increasing immune cell activity and microbial translocation [72,73]. Combined with generally higher systemic inflammation in women, this might

contribute to elevated levels of sCD14 and LBP [72-74]. Conversely, men exhibit higher FABP2 levels, possibly due to their metabolic profiles, including greater lipid absorption and storage, driven by visceral fat and testosterone dominance [75,76]. These findings highlight the gender-specific interplay of immune, hormonal, and metabolic processes. We found that sCD14 levels were elevated in current smokers compared to nonsmokers. This is in line with other literature, were they found this association too [77,78]. One explanation might be that elevated sCD14 levels result from smoking-induced upregulation of CD14 expression on airway epithelial cells and macrophages, resulting in an elevation of CD14 in the bloodstream [79]. Lower systemic levels of sCD14 in patients who have undergone (partial) colectomy or ileocecal resection may be attributed to changes in intestinal physiology and immune signalling caused by the removal of important gut regions. We hypothesized that the absence of large intestinal segments might result in an imbalance or reduction of diversity of the gut microbiome, possibly contributing to a reduced microbial translocation into the bloodstream. This might diminish the stimulus for sCD14 production by monocytes and macrophages [80-82]. However, more research must be done to support this hypothesis. In our study, sCD14 and LBP levels were positively associated with albumin levels. Albumin plays a critical role in modulating the inflammatory response to bacterial infections by forming complexes with PAMPs. By binding to bacterial components such as peptidoglycan, lipoteichoic acid, and LPS, it can affect the immune response and diminish inflammation. Albumin has also been reported to be a facilitator for the binding of LPS to LBP and CD14 [83-85]. These findings emphasize the multifactorial nature of gut barrier biomarkers and their dependence on demographic, metabolic, and clinical factors.

Clinical disease activity

Our strongest finding was the association between sCD14 and LBP with both clinical- and biochemical disease activity in IBD. While this association highlights their potential as biomarkers, it raises an important consideration for their interpretation as gut barrier biomarkers. Specifically, both sCD14 and LBP are closely linked to (systemic) inflammation and disease activity, which might suggest they are not specific markers of gut barrier dysfunction. Instead, they could be involved in a wider immune response. This distinction is important, because biomarkers intended to reflect gut permeability should provide direct insights into gut barrier dysfunction, instead of serving as general indicators of systemic inflammation. This leads to the discussion about the future role of biomarkers like sCD14 and LBP. Although promising, it is important to dive deeper into understanding their general role in the body. We expect that these biomarkers can play an important role in the management of IBD, by predicting disease activity, including flare-ups, by monitoring disease progression and by assessing the effectiveness of treatments. Challenges for using these biomarkers may include improving specificity for gut barrier dysfunction, standardizing measurement techniques, and integrating biomarker data into personalized treatment plans.

We found strong associations of sCD14 and LBP with clinical indices of disease activity (HBI and SCCAI) and CRP, while no such association was observed with FABP2. Both sCD14 and LBP are markers of immune activation and microbial translocation, produced by monocytes/macrophages and hepatocytes in response to LPS derived from gut microbiota. The positive association between these markers and CRP, an acute-phase protein elevated during inflammation, suggests that sCD14 and LBP levels reflect the degree of immune activation and systemic inflammation. This is further supported by their correlation with clinical activity scores, reinforcing their role as biomarkers of disease activity [72]. In contrast, FABP2, a marker of gut epithelial dysfunction, is primarily involved in lipid absorption and reflects gut permeability. It is produced by enterocytes and released when the gut epithelium is damaged. While it indicates gut barrier dysfunction, FABP2 is less directly involved in the inflammatory cascade triggered by microbial translocation, which could explain its weaker association with CRP and clinical activity indices [72]. Contradictory outcomes for FABP2 as a gut barrier biomarker were found in previous studies. One study showed that, while plasma FABP2 correlates with disease activity in some contexts, it does not significantly differ between active disease and remission in CD and UC, making it unreliable as a non-invasive biomarker for disease activity in IBD [86]. In contrast, urinary FABP2 has

been suggested as a potential marker for disease activity in adults with CD [87]. Another study found that FABP2 levels were associated with active disease in CD, and a positive correlation with clinical activity indices like the CDAI and CRP was observed [88]. Additionally, FABP2 levels decrease with effective treatments like infliximab, indicating mucosal healing [89]. These findings highlight FABP2 in specific contexts, but its overall utility as a marker for disease activity in IBD remains limited. The analysis between biomarker levels and the new defined disease activity score revealed increased sCD14 and LBP levels in patients with active disease compared to remission. In contrast, FABP2 again did not show a difference between the two groups. These findings are in line with our previous findings and might further strengthen the hypothesis that sCD14 and LBP are more closely associated with the inflammatory processes underlying active disease in IBD, where immune activation and microbial translocation play a central role in disease progression. Next to that, even after adjusting for potential confounders, these findings were only intensified. This suggests that the association between sCD14, LBP, and clinical outcomes is robust and primarily driven by the underlying inflammatory processes rather than by other confounding variables. Patients with a possible compromised gut barrier (GB-) were more likely to be in an active disease state. Conversely, in the GB+ group, which indicates a more intact gut barrier, a higher proportion of patients were in remission. This suggests that a compromised gut barrier is closely associated with disease activity and may play a critical role in disease progression. Additionally, CRP levels were significantly higher in the GB- group. These findings highlight the importance of gut barrier integrity in maintaining disease remission, emphasizing the potential therapeutic benefits of strategies aimed at restoring or maintaining gut barrier function in IBD. Further research into interventions targeting gut permeability and microbial translocation could provide valuable insights for improving disease management.

4.3 Strengths and Limitations

This study has several limitations that should be considered. First, the patient cohort for endoscopy data was relatively small and lacked documentation of inflammation and Mayo-scores. This limited the analysis of endoscopy findings with biomarker levels. Next to that, we chose a cut-off of three months before and after sample collection for our endoscopy group. This might have influenced the outcome too, because patients with documented inflammation three months before sample collection might have received medication or other therapy to improve/manage this. Consequently, by the time of sample collection, their inflammatory scores could have improved, potentially underestimating the association between biomarker levels and actual disease activity at the time of sampling. This highlights the need for aligning biomarker measurements more closely with endoscopic findings in future research. Second, since we used principal components for analysis of dietary patterns and biomarker levels, another limitation is the potential for oversimplification and loss of nuanced dietary behaviours. While PCA helps identify key dietary patterns, it simplifies complex dietary data into just a few key factors. This simplification could overlook specific dietary details that might have a significant effect on biomarker levels. Next to that, the time gap between the FFQs and biomarker measurements might limit the relevance of the dietary patterns to the current state of gut barrier function or disease activity. Dietary intake can vary over time, influenced by disease flares, therapeutic interventions, or other lifestyle changes, potentially weakening the association between dietary patterns and biomarker levels. Future studies might benefit from choosing more dynamic and comprehensive dietary assessment tools, such as food diaries, to better capture the interplay between dietary intake and biomarker dynamics.

Despite these limitations, the strengths of this study lie in the evaluation of biomarkers and their relationship with several parameters for disease activity. By adjusting for confounders such as age, BMI, and smoking status, the reliability of the results is increased. The new gut barrier score showed how gut barrier integrity associates with clinical disease activity, highlighting its potential clinical relevance. Strong correlations between sCD14 and LBP with CRP and clinical disease activity further highlight their importance as markers of immune activation and inflammation in IBD.

4.4 Conclusions and Future Perspectives

This study provides new insights into the role of three biomarkers—sCD14, LBP, and FABP2—in the context of IBD. While no significant associations were observed between overall dietary patterns and the biomarkers, specific dietary components, such as high coffee, alcohol and processed food consumption, showed modest associations with sCD14 and FABP2. These findings suggest that certain dietary habits may influence gut barrier integrity, however their impact appears limited in this study. Demographic, clinical, and lifestyle factors significantly influenced biomarker levels, emphasizing their complex, multifactorial nature. Furthermore, strong associations were observed between sCD14 and LBP levels and both clinical and biochemical disease activity, highlighting their potential as reliable biomarkers for monitoring IBD. The new defined gut barrier score revealed that a compromised gut barrier is strongly associated with active disease states. These biomarkers demonstrated strong correlations even after adjusting for confounding factors, suggesting their utility in reflecting systemic inflammation and microbial translocation, rather than gut barrier dysfunction alone. In contrast, FABP2 showed weak to no associations with clinical disease activity, limiting its utility as a biomarker in this context.

These findings offer a foundation for future research, particularly in longitudinal studies, to validate these biomarkers, improve our understanding of the interplay between diet, gut barrier function and disease activity in BID, and assess interventions aimed at restoring gut barrier integrity as a strategy for improving disease management and for predicting flare-ups.

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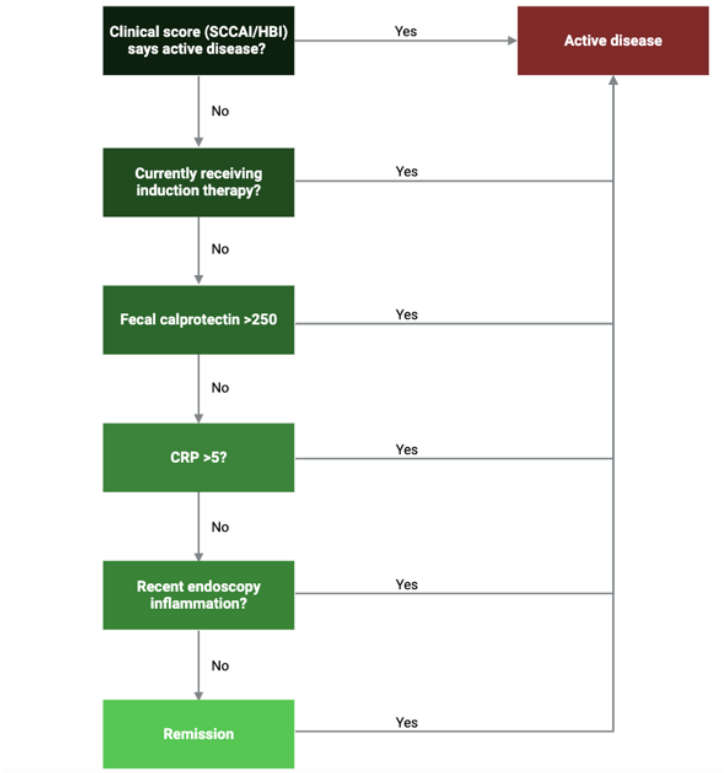
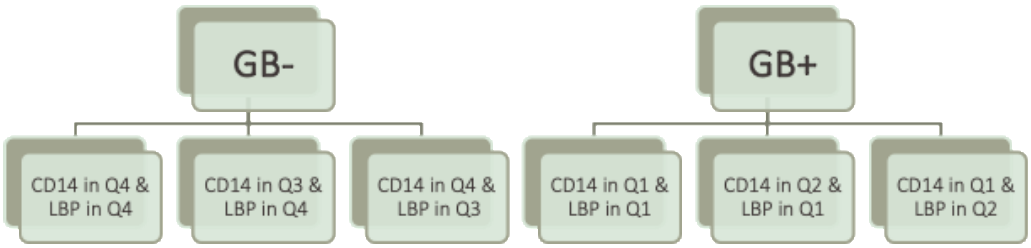
Supplementary Material

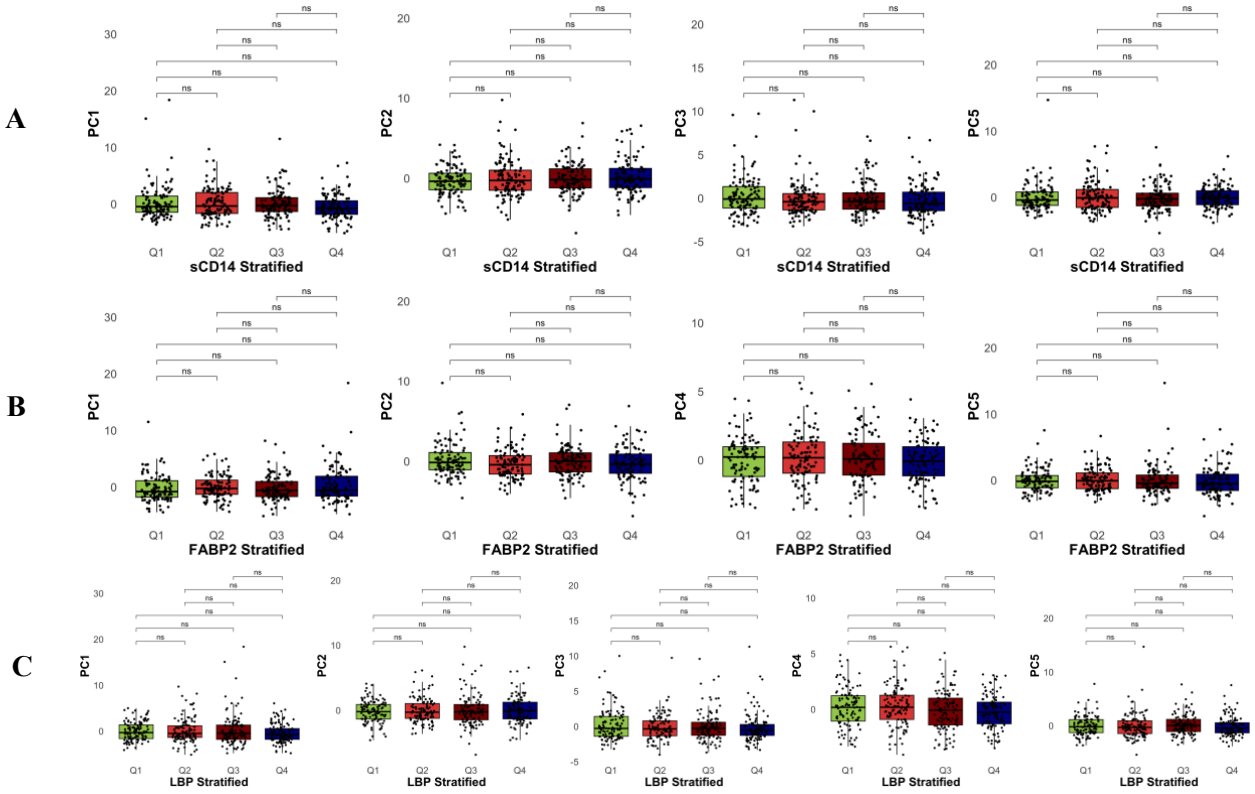
Figures



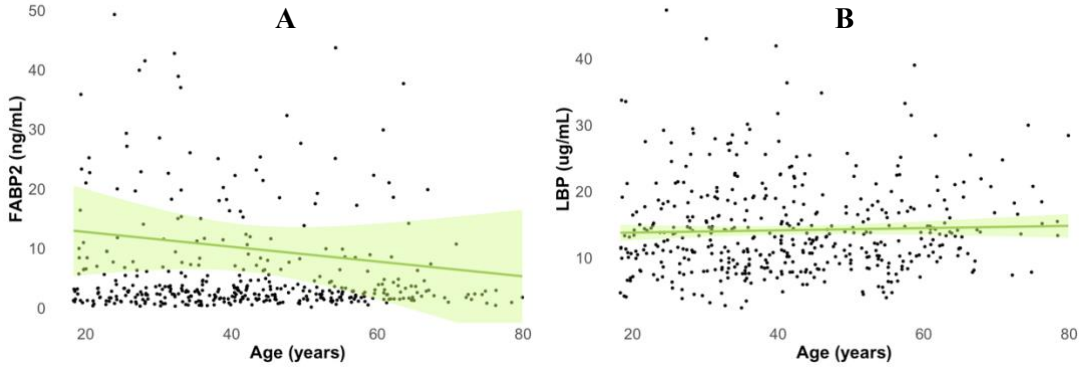
Supplementary figure 1. Flowchart for determining the new defined disease activity score, using clinical scores (SCCAI/HBI), biomarkers (faecal calprotectin and CRP), recent endoscopy inflammation, and treatment status to differentiate between active disease and remission.



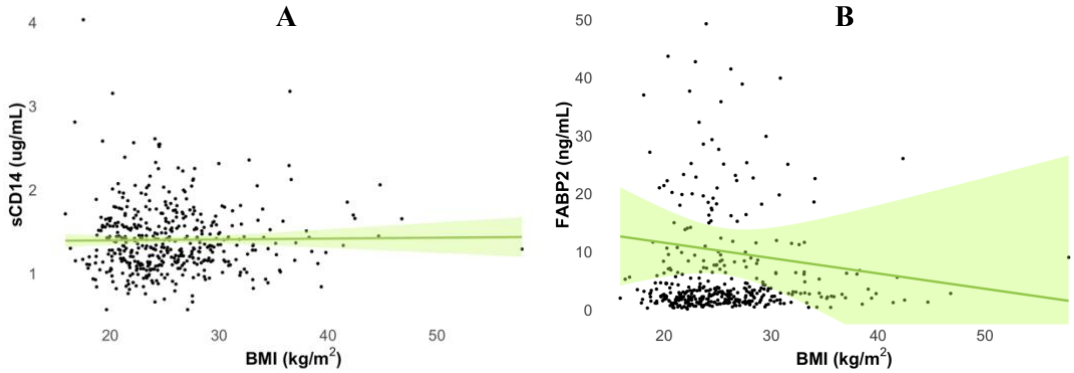
Supplementary figure 2. Flowchart describing how the outcomes, GB- and GB+, were determined for the gut barrier (GB) variable.



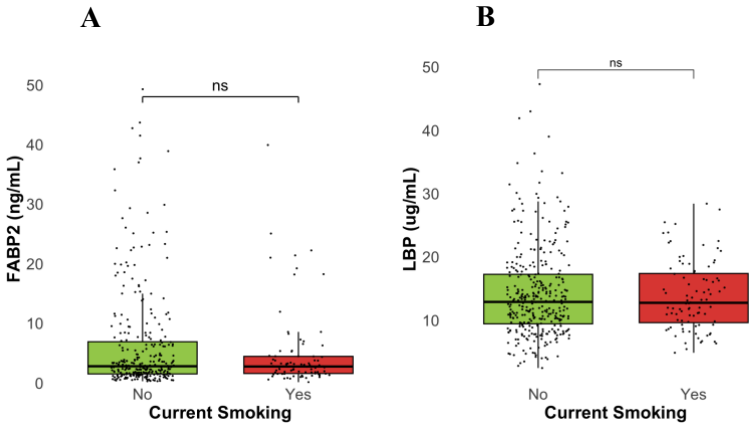
Supplementary figure 3. Stratified biomarker scores and PCs. (**A**) No significant association between PC1, PC2, PC3, PC5 and sCD14. (**B**) No significant association between PC1, PC2, PC4, PC5 and FABP2. **(C**) No significant association between PC1-5 and LBP.



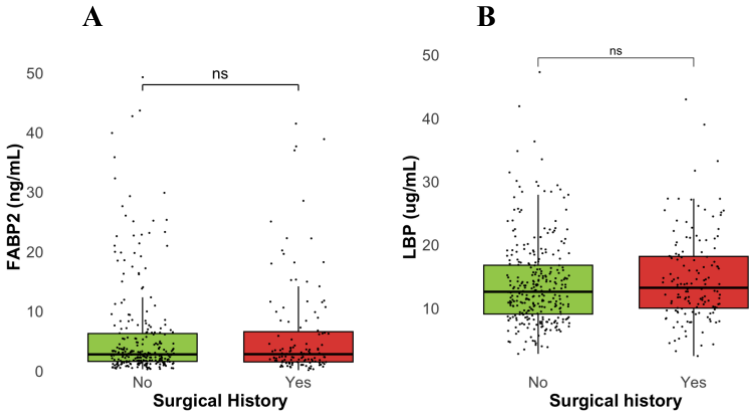
Supplementary figure 4. Biomarker levels and age. **(A)** FABP2 levels and age. **(B)** LBP levels and age.



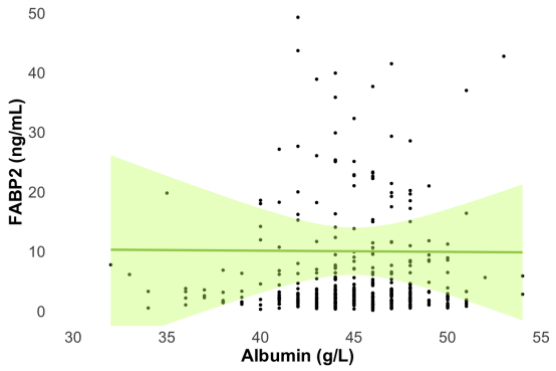
Supplementary figure 5. Biomarker levels and BMI. **(A)** sCD14 levels and BMI. **(B)** FABP2 levels and BMI.



Supplementary figure 6. Biomarker levels and smoking status. **(A)** No significant difference between FABP2 levels and smoking status. **(B)** No significant difference between LBP levels and smoking status.



Supplementary figure 7. Biomarker levels and surgical history. **(A)** No significant difference between FBAP2 levels and surgical history. **(B)** No significant difference between LBP levels and surgical history.



Supplementary figure 8. FABP2 levels and albumin levels.

Tables

Supplementary table 1. Habitual dietary intake of study participants

	Total	CD	UC	*p*-value
Food groups (g/day)	*n* = 450	*n* = 263	*n* = 187	
Alcoholic beverages	13.0 [0.0-68.2]	11.8 [0.0-62.64]	15.6 [0.0-74.5]	0.284
Breads	129.9 [80.2-164.6]	127.5 [78.1-158.3]	133.7 [91.5-174.1]	0.069
Cereals	0.0 [0.0-3.5]	0.0 [0.0-2.9]	0.0 [0.0-4.65]	0.322
Cheese	21.0 [8.5-36.9]	20.7 [8.1-32.9]	21.5 [9.2-38.3]	0.340
Coffee	232.3 [17.9-464.5]	232.3 [11.1-464.5]	232.3 [35.8-464.5]	0.792
Dairy	182.7 [96.5-329.8]	159.6 [87.7-296.2]	243.2 [135.0-347.2]	**0.001**
Eggs	8.9 [4.5-17.9]	8.9 [4.5-17.9]	8.9 [4.5-17.9]	0.875
Fish	11.1 [4.4-17.5]	10.9 [4.2-17.1]	11.3 [4.9-18.1]	0.281
Fruits	188.5 [93.4-304.7]	175.4 [89.6-325.3]	216.7 [108.3-264.4]	0.860
Legumes	2.2 [0.0-11.0]	0.0 [0.0-10.96]	4.4 [0.0-16.4]	**0.027**
Meat	86.6 [59.1-111.1]	84.3 [51.3-108.6]	92.2 [65.9-113.1]	0.097
Non-alcoholic beverages	104.3 [20.9-278.5]	135.7 [26.5-282.9]	52.8 [13.0-269.4]	**<0.001**
Nuts	5.4 [1.7-13.1]	4.3 [1.4-12.3]	6.5 [2.2-14.2]	0.069
Pasta	12.7 [7.9-25.5]	12.7 [7.9-25.5]	15.9 [7.9-31.8]	0.070
Pastry	23.8 [12.4-40.1]	21.8 [11.5-39.0]	26.5 [14.7-43.5]	**0.020**
Potatoes	72.3 [41.0-111.2]	71.3 [41.1-104.1]	85.6 [41.1-119.8]	0.198
Prepared meals	32.0 [12.9-58.6]	32.4 [12.9-59.9]	27.8 [12.9-53.5]	0.265
Rice	14.7 [6.0-24.8]	14.9 [5.0-24.8]	14.9 [6.0-24.3]	0.560
Sauces	10.0 [4.6-20.8]	10.6 [4.5-21.3]	9.2 [4.7-20.5]	0.713
Savoury snacks	13.3 [5.4-23.9]	13.4 [5.2-24.7]	12.8 [5.7-23.5]	0.689
Soup	35.8 [9.0-71.5]	35.8 [9.0-44.5]	35.8 [18.0-71.5]	0.258
Spreads	20.8 [8.3-31.5]	19.2 [7.8-30.7]	22.6 [9.9-32.7]	0.133
Sugar/Sweets	29.9 [14.9-49.8]	30.4 [13.2-48.7]	29.7 [15.8-50.1]	0.984
Tea	232.3 [44.6-464.5]	232.3 [44.6-348.4]	232.3 [62.5-464.5]	0.742
Vegetables	107.4 [62.4-147.4]	81.8 [61.7-114.4]	108.3 [63.2-150.6]	0.169

Data are presented as medians [1st-3rd quartile]. Statistical significance was tested by Mann-Whitney U test. Significant differences are highlighted in **bold**.
Abbreviations: CD, Crohn's disease; UC, ulcerative colitis.

Supplementary table 2. Gut barrier biomarker concentrations and clinical outcomes.

Clinical Scores	sCD14 (ug/mL)	*p*-value	FABP2 (ng/mL)	*p*-value	LBP (ug/mL)	*p*-value
Remission						
Active (*n* = 141)	1.38 [1.20-1.65]	**0.02**	2.90 [1.52-6.27]	0.94	14.14 [10.79-19.97]	**< 0.001**
Remission (*n* = 288)	1.29 [1.12-1.56]		2.77 [1.66-7.24]		11.86 [8.82-15.97]	
HBI (CD)						
Active (*n* = 90)	1.38 [1.19-1.65]	**0.02**	2.81 [1.48-5.78]	0.99	14.92 [11.25-21.35]	**0.001**
Remission (*n* = 159)	1.24 [1.10-1.56]		2.73 [1.60-5.54]		12.63 [9.80-16.58]	
SCCAI (UC)						
Active (*n* = 51)	1.40 [1.27-1.63]	0.28	3.18 [2.02-6.38]	0.91	12.93 [10.33-16.04]	**0.03**
Remission (*n* = 129)	1.34 [1.14-1.56]		2.81 [1.72-8.74]		11.32 [7.98-14.67]	

CRP						
<5 (*n* = 274)	1.27 [1.10-1.51]	**< 0.001**	2.94 [1.70-6.98]	0.38	11.09 [8.37-14.08]	**< 0.001**
>5 (*n* = 103)	1.45 [1.22-1.75]		2.61 [1.45-6.45]		19.13 [14.94-25.56]	

Statistical significance between gut barrier biomarker levels and clinical parameters was tested by Mann-Whitney U test. Significant differences are highlighted in **bold**.
Abbreviations: CRP, C-reactive protein; HBI, Harvey–Bradshaw index; SCCAI, simple clinical colitis activity index.

Supplementary table 3. Gut barrier biomarker concentrations and stratified CRP scores.

Biomarker	**CRP Category** <5 (*n* = 264)	5-25 (*n* = 88)	>25 (*n* = 15)	**Comparison**	*p*-value
sCD14 (ug/mL)	1.27 [1.11-1.49]	1.44 [1.21-1.71]	1.66 [1.42-1.79]	<5 vs 5-25	**<0.001**
				<5 vs >25	**<0.001**
				5-25 vs >25	0.143
FABP2 (ng/mL)	2.91 [1.70-7.06]	2.42 [1.42-6.45]	2.90 [1.71-4.54]	<5 vs 5-25	0.335
				<5 vs >25	0.989
				5-25 vs >25	0.471
LBP (ug/mL)	11.12 [8.50-14.24]	18.09 [14.49-23.79]	25.71 [25.16-30.52]	<5 vs 5-25	**<0.001**
				<5 vs >25	**<0.001**
				5-25 vs >25	**<0.001**

Statistical significance between gut barrier biomarker levels and CRP tested by Mann-Whitney U test. Significant differences are highlighted in **bold**.

Supplementary table 4. The *p*-values of the Mayo score for all biomarkers.

Biomarker	**Mayo 0 vs 1**	**Mayo 0 vs 2**	**Mayo 0 vs 3**	**Mayo 1 vs 2**	**Mayo 1 vs 3**	**Mayo 2 vs 3**
sCD14	0.631	0.713	0.129	0.635	0.440	0.931
FABP2	0.815	0.328	0.298	0.414	0.524	1.000
LBP	0.315	0.264	0.129	0.958	0.254	0.329

Statistical significance was tested by Mann-Whitney U test between all Mayo scores for each biomarker.

Supplementary table 5. Linear regression for biomarker analyses.

Predictor	**Outcome: sCD14 (β, 95% CI)**	***p*-value**	**Outcome: LBP (β, 95% CI)**	***p*-value**
Age	0.0063 (0.0039; 0.0086)	**< 0.001**	-0.0087 (-0.0454; 0.0280)	0.696
BMI	-0.0021 (-0.0088; 0.0045)	0.597	0.1781 (0.0764; 0.2798)	**0.004**
Sex	0.1065 (0.0370; 0.1760)	**0.012**	0.3588 (-0.7084; 1.4261)	0.580
Smoking	0.0910 (0.0116; 0.1705)	0.060	-1.1455 (-2.3647; 0.0738)	0.122
Diagnosis	-0.0043 (-0.0774; 0.0687)	0.922	2.0976 (0.9771; 3.2184)	**0.002**
Albumin	-0.0148 (-0.0248; -0.0047)	**0.016**	-0.7258 (-0.8804; -0.5712)	**< 0.001**
Surgical History	-0.1057 (-0.1802; -0.0311)	**0.020**	-0.0067 (-1.1504; 1.1369)	0.992
Clinical Activity	0.1091 (0.0409; 0.1773)	**0.009**	3.8913 (2.8444; 4.9383)	**< 0.001**

Linear regression model with outcome = gut barrier biomarkers sCD14 and LBP. Adjusted for: age, BMI, sex, smoking status, diagnosis, albumin and surgical history. Significant differences are highlighted in **bold**.