Interleukin-1ß levels in patients with inflammatory bowel disease

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INTERLEUKIN-1β LEVELS IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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List of Abbreviations

IBD – Inflammatory bowel disease

UC – Ulcerative Colitis

CD – Crohn's disease

EIMs – extra-intestinal manifestations

CRC – Colorectal cancer

WD – western diets

MWD – maternal western diets

GWAS – genome wide association studies

IRR – incidence rate ratio

EEN – exclusive enteral nutrition

NF-kb - Nuclear factor kappa B

NOD2- Nucleotide Binding Oligomerization Domain Containing 2)

HLA – human leukocyte antigen

MHC- major histocompatibility complex

IL23R – interleukin 23 receptor

CBC – complete blood count

CRP – c-reactive protein

ESR – erythrocyte sedimentation rate

CMP – comprehensive metabolic panel

ECG – Esophagogastroduodenoscopy

LFT – liver function tests

DILI – drug induced liver injury

AIH – autoimmune hepatitis

PSC – primary sclerosing cholangitis

ALT - Alanine transaminase

AST - Aspartate transaminase

IL-1β – Interleukin-1 beta

NLRP3 – NOD-, LRR- and pyrin domain-containing protein 3

DAMPs – Damage-associated molecular pattern molecules

TLR – toll-like receptor

ASC – apoptosis-associated spec-like protein containing a caspase recruitment domain

TAK1 – Transforming growth factor-β (TGF-β)-activated kinase 1

IL-R1 – Interleukin receptor type 1

JNK – c-Jun N-terminal kinases

FPG – Fasting plasma glucose

LDL – low-density lipoprotein

HDL – high density lipoprotein

hsCRP – high sensitivity c-reactive protein

BMI – body mass index

1. INTRODUCTION

1.1 Inflammatory Bowel Diseases

Characterised by chronic inflammation of the gastrointestinal tract, inflammatory bowel disease (IBD) encompasses two distinct clinical entities; Crohn's Disease (CD) and Ulcerative Colitis (UC) which typically follow a life-long relapsing and remitting course (1,2). Although the precise aetiology underpinning IBD remains enigmatic, it is generally accepted that a complex confluence of genetic and environmental factors, coupled with aberrant immune responses to microbes in the gut precipitate IBD development (3).

Despite mechanistic overlap in pathogenesis, UC and CD display considerable clinical and pathological heterogeneity. For instance, UC lesions are confined to the colon, with mucosal and submucosal inflammation typically extending proximally from the rectum to adjacent mucosa in a continuous manner. UC can result in ulcerations, severe bleeding, toxic megacolon and fulminant colitis (4).

In contrast, while CD generally affects the terminal ileum and colon, it can impact any part of the gastrointestinal tract, often in a patchy or interrupted fashion. Moreover, CD is characterised by transmural inflammation prompting complications such as fibrotic strictures, fistulas and abscesses (4,5).

Extraintestinal manifestations (EIMs) are prevalent in IBD, with 50% of patients experiencing at least one, commonly affecting the joints, eye, skin, kidney or liver (6). Furthermore, owing to the chronic inflammatory nature of IBD and widespread use of immunosuppressive therapy in IBD, patients are at increased risk of colorectal cancer (CRC) in addition to extra-intestinal malignancies (7,8).

1.2 Epidemiology

1.2.1 Global Burden of IBD

IBD is a global disease estimated to afflict over 6.8 million people worldwide (9). In recent decades changing trends of IBD incidence and prevalence have altered the epidemiological landscape of what was traditionally considered a disease of the West (10,11). In 2020, a seminal paper by Kaplan et. al described the global evolution of IBD across four distinct epidemiological stages; (1) Emergence (2) Acceleration in Incidence (3) Compounding Prevalence and (4) Prevalence Equilibrium (11).

Countries in the Western world (Western Europe, US, Canada, Australia, New Zealand) currently exist in Stage 3 – Compounding Prevalence; characterised by stabilising incidence alongside sharply increasing prevalence secondary to improved survival (11,12).

As a chronic disease with low mortality new diagnoses of IBD expand the existing IBD population at a rate significantly greater than the rate of loss of patients (13).

Simultaneously, newly industrialised countries in Asia, South America and the Middle East are in stage 2 – Acceleration in Incidence; in which the number of incident cases rise significantly, but overall prevalence remains low. Two fundamental phenomena justify the increasing incidence of IBD observed in newly industrialised regions. First, economic growth and development fuels improved healthcare access, infrastructure and quality, fostering greater disease awareness, recognition and surveillance. As a result, rather than a true rise of IBD incidence, an unveiling of previously undetected cases occurs. Demonstrating this, in post-civil war Bosnia and Herzegovina a commensurate rise in the incidence of CD was documented which paralleled increased use of colonoscopy (14).

Second, as nations advance economically they typically adopt lifestyle and dietary customs characteristic of Western societies. Consequently, it is thought that the introduction of, and exposure to environmental risk factors associated with Westernisation negatively influences the gastrointestinal microbiota and subsequently IBD risk (11). The emerging geographical variation represents a unique opportunity to explore and elucidate key environmental contributors to IBD pathogenesis, particularly those accompanied by "Westernisation" (15).

Though the overall the prevalence of IBD in newly industrialised countries remains considerably lower than that of the West (0.3-0.5%) the gap is narrowing subsequent to the steep incline in incidence in populous countries like China and India (10). As a result, it is projected that the number of cases in these regions could approximate that of the Western world in coming decades (8,11). The rising global burden of IBD poses formidable challenges for healthcare systems and economies alike. Concerted effort and planning is essential to contend with the escalation of IBD cases, ageing IBD populations with complex comorbidities and ensuring equitable access to modern therapeutics worldwide.

1.2.2 Sex and Age Distribution of IBD

The gender distribution of UC or CD varies on the specific disease subtype and geographical region (16,17). CD generally shows a female predilection in Europe and the United states, albeit inconsistently, while in Asia, prevalence appears to be higher in males (17). In contrast, UC occurrence appears to be equally distributed between both sexes in Europe and Asia (16). IBD typically exhibits a bimodal age distribution at diagnosis, characterised by an initial peak during the second or third decades of life, followed by a second smaller peak in the fifth and six decades (18,19).

1.3 Pathogenesis

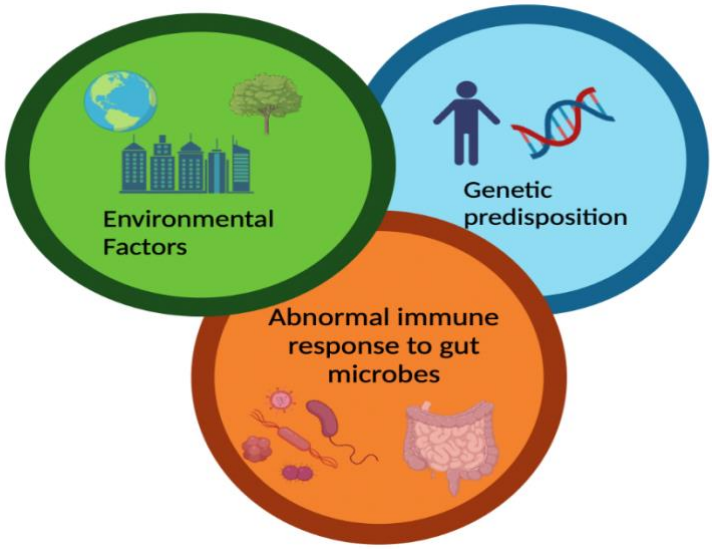

Figure 1. Pathogenesis of IBD. Created with BioRender.com

1.3.1 Genetic Risk Factors

Thought to account for 20-25% of all cases of IBD, genetic studies to date have identified over >240 susceptibility loci associated with CD, UC or both (3,5). Different genetic variants impart susceptibility to IBD through diverse inflammatory pathways. However, while many individuals harbour IBD genetic predisposition, clinical manifestation occurs only within a subset, thus it is unlikely that the presence of genetic risk loci alone is insufficient to trigger IBD (20). As such, it is considered that alongside genetic susceptibility, the synergistic effect of environmental factors and alterations to the gut microbiota and mucosal immune system are necessitated to establish disease **(Figure 1)** (3,21).

Currently, the most significant genetic associations identified include the NOD2 gene in CD, HLA complex and IL23R for both IBD forms (22).

NOD2

First characterised in 2001, nucleotide-binding oligomerization domain-containing protein 2 (NOD2) is the most widely implicated gene in CD aetiology, estimated to underpin approx. 8% of cases (23). It is expressed in granulocytes, dendritic cells, T-cells and in highest concentration in the terminal ileal Paneth cells (23). NOD2 encodes an intracellular bacterial sensor which governs immune responses, eliciting either a defensive or tolerant response toward detected pathogens. Defective NOD2 function hinders bacterial recognition and handling, compromises NF-kb pathway activation and diminishes cytokine production in response to microbial invaders, which prompts the ongoing intestinal inflammation observed in CD (24–26).

In addition to the pertinent role of NOD2 in CD initiation, it is thought that the gene exerts influence on disease phenotype, including location, extent, age-of-onset, severity, treatment and requirement for surgery (24).

HLA Complex

The Human leucocyte antigen (HLA) complex, synonymous with the major histocompatibility complex (MHC) is located on chromosome 6 (27). The HLA region encodes "classical HLA genes" (Class I- A, B, C; Class II -DR, DQ and DP) in addition to over 200 proteins involved in the induction and regulation of immune responses (28). HLA-DRB1 and HLA-DQB1 alleles are consistently linked with IBD pathogenesis, though the exact mechanism of their involvement is unclear (22,28).

Class I and II complexes are essential in the immune system's ability to recognize and respond to pathogenic bacteria and infected host cells, as well as in maintaining tolerance to commensal bacteria and the body's own cells (28). Therefore, proposed mechanisms for the IBD-HLA relationship include: (1) an excessive immune response to commensal bacteria (hyper-immune response), (2) insufficient elimination of pathogenic bacteria leading to ongoing inflammation (hypo-immune response), (3) compromised epithelial barrier function, allowing microbes to invade the mucosa despite a normal immune response, and (4) an inability to distinguish self-cells, causing an autoimmune inflammatory response (22,28).

Interleukin 23-receptor (IL23R)

The interleukin 23-receptor (IL23R) gene encodes a subunit of the receptor for the proinflammatory cytokine IL-23, which is elevated in IBD patients (29). In recent years, IL-23, derived predominantly from macrophages and dendritic cells upon antigenic stimulation emerged as a key driver of chronic intestinal inflammation in IBD (29,30). While plausible biologic pathways via which IL-23 drive IBD exist, the exact effector mechanism is a yet to be fully elucidated. Nonetheless, the success of biologic therapies directed against IL-23 provide compelling evidence of the deleterious role of the cytokine in IBD (30). In UC and CD patients, research has demonstrated that binding of the IL-23 ligand to its cognate receptor (IL-23R) expressed on various target cells (including Th17 subset) activates a pro-inflammatory cascade, inducing IL-17 and IFN-gamma production (31), Furthermore, overexpression of IL-23 suppresses IL-10, in turn dampening IgA production and invoking epithelial barrier dysfunction (31). Genome wide association studies (GWAS) have revealed various IL23R polymorphisms capable of modifying the propensity to IBD. More specifically, the G149R, V362I, and R381Q IL23Rα chain variants are protective against IBD development mediated by reduced cell surface receptor expression (30–32). Conversely, several other IL-23R variants are purported to predispose to IBD development (30).

1.3.2 Environmental Determinants

To date, cigarette smoking is one of the most extensively investigated environmental risk factors for IBD. The divergent effects of smoking in the aetiopathogenesis of UC (protective) and CD (harmful) have been documented for over 40 years (33). Nevertheless, as the geographical landscape of IBD has evolved overtime and these findings originate from studies conducted almost exclusively in white- western populations, researchers have contested whether the association pertains to other ethnic groups (33).

In 2021, a meta-analysis of 37 studies including 9332 CD patients, confirmed that while smoking is a risk factor for CD onset in non-Jewish White population (RR: 1.95 95% CI: 1.69–12.24), the associations were not replicated for other ethnic groups (34). The precise explanation for the disparate results remains obscure, but may be attributed to an increased genetic susceptibility to the harmful effects of smoking mediated by specific NOD2 genetic variants present in non-Jewish white populations (34).

In contrast, regarding UC, current smoking conferred protection against IBD development irrespective of ethnicity (RR: 0.55, 95% CI: 0.48–0.64) consistent with previous findings (34,35).

Hygiene Hypothesis and Gastric Pathogens

According to the hygiene hypothesis first described by Strachan, stringent hygiene and sanitary conditions which limit exposure to microbes during early childhood years increase susceptibility to various maladies in later life, including IBD (36). Corroborating this hypothesis, several epidemiological studies have documented inverse associations with the risk of IBD and (1) pet owning (2) exposure to farm animals (3) larger households (4) consumption of unpasteurised milk and (5) bedsharing (10,13,33).

Moreover, evidence has emerged indicating that gastric colonization with *Helicobacter pylori (H. pylori*) exerts a unique protective effect on IBD development (37). Several studies identified decreased *H. pylori* infection rates among IBD patients compared with controls, lending further credence to concept that microbial exposure influences immune tolerance and future IBD onset. Despite these observations, other researchers have questioned the veracity of this proposition, instead owing the eradication of *H. pylori* in IBD patients to the intake of complex IBD therapies, though this is not supported by the current literature (37). In contrast, conflicting findings emanate from studies in Asian populations where access to hot water and flushing toilets instead ameliorated the risk of UC development (10). As such, it appears likely that hygiene hypothesis holds true for certain though not all populations, and exposure to specific microbes and/or pathogenic bacteria.

Antibiotic exposure

Antibiotic therapy perturbs gastrointestinal microbiota both quantitatively and quantitively, promoting a low-diversity microbiome (38). Mounting evidence implicates antibiotic use with the development of several immune-mediated diseases, including IBD (38,39). However, preliminary studies addressing the IBD association were encumbered by methodological limitations, including small sample sizes, a lack of histopathological case ascertainment and were restricted mostly to paediatric IBD cohorts (39,40). More recent investigations have mostly overcome these limitations and added credibility to prior findings (40,41).

Specifically, in 2020 a Swedish case-control study of almost 24,000 IBD patients conducted over a 10-year period reported an almost two-fold increased risk of IBD development associated with previous antibiotic use (40). Analogous findings were later observed within a large Danish cohort, with antibiotic use increasing IBD risk.

Moreover, a positive dose-response was described, with subsequent antibiotics courses incrementally increasing IBD risk (IRRs per antibiotic course were 1.11 (95% CI 1.10 to 1.12), 1.15 (95% CI 1.14 to 1.16), and 1.14 (95% CI 1.13 to 1.15) for individuals aged 10–40 years, 40–60 years, and ≥60 years, respectively. Furthermore, antibiotic classes commonly prescribed to target GI pathogens, including nitroimidazoles and fluroquinolones posed the greatest risk of developing UC and CD later (41).

Intriguingly, these findings derived from Western populations are at odds with the protective effect of antibiotic exposure reported in Asian populations and Middle Eastern migrants in Australia. Tentative explanations for these discordant findings include potential differences in the underlying IBD pathogenesis or greater resistance to antibiotic-induced gut dysbiosis in distinct populations (13,42–44). While antibiotic therapy is often warranted, these findings underscore the importance of judicious use and the need for further studies to delineate how antimicrobial therapy exposure modulates gut microbial diversity and potentially culminates in IBD onset.

Appendectomy

Previous observational research indicates an inverse association between appendectomy and UC (45). In a comprehensive case-control study spanning from 1964-1995 in Sweden, appendectomy performed for the treatment of appendicitis or mesenteric lymphadenitis was associated with decreased risk UC development (incidence rate ratio IRR 0.73, 95% confidence interval CI 0.62–0.87 for appendicitis, and IRR 0.48, 95% CI 0.27–0.83 for mesenteric lymphadenitis). On the contrary, appendectomies performed for non-specific abdominal pain failed to provide benefit (IRR 1.34, 95% CI 0.77–2.38) (45,46).

Pooling data from both Swedish and Danish cohorts later reproduced similar findings, identifying that in the absence of preceding inflammation, appendectomy was not associated with reduced risk of UC (47). Further corroborating this supposition, another study revealed that childhood appendicitis managed conservatively or surgically conferred subsequent UC protection (48).

A precise biological explanation linking the pathologies remains elusive, however it has been proposed that appendicitis promotes an immune profile which decreases UC risk. While this remains speculative, it may be mediated by altered host immune responses to the intestinal microbiome, and changes to the composition and metabolic activity of gut microbiota induced by an inflamed appendix (49).

Conversely, with regard to CD, prior appendectomy has historically been associated with elevated risk of CD. Though the literature pertaining to the relationship between CD and appendectomy has been less consistent, a 2023 meta-analyses reiterated the presence of a positive association between CD development and appendectomy, particularly early post-operatively (25). The authors noted that while the risk gradually attenuated with time elapsed from surgery, it persisted at 5-years and remained regardless of the initial indication for appendectomy, unlike in UC (25). These findings challenge earlier research which primarily attributed the connection to CD detection bias early post-operatively and/ or reverse causality rather than true causal association (50).

Nutrition and Dietary Chemicals

Diet and specific dietary components are key determinants of intestinal microbiota composition and function, the integrity of the gut barrier and subsequent immune responses (51). Consequently, intake can instil/ evoke benefit or susceptibility to IBD. Interestingly, it appears that protection from IBD can be afforded even from early life exposures, with several lines of evidence supporting a link between decreased risk of paediatric and adult onset IBD and a history of breastfeeding (52). In recent decades, the widespread consumption of western diets (WD) typically enriched with refined sugars, animal protein, saturated fat and largely devoid of fibre and adequate fruit and vegetable intake, coincided with the rising global incidence of IBD (13). These dietary constituents are thought to perturb microbial composition and function, promoting gut dysbiosis and inflammation - cardinal features of IBD pathologies (53)**.** Additionally, food additives (including colourants, sweeteners and emulsifiers), present in abundance in WD within ultra-processed foods, soft drinks, confectionary and condiments have evoked colitis in animal models (53). Moreover, Huang *et al.,* demonstrated that altered microbiota mediated by pre-natal exposure to a Maternal Western diet (MWD) later increased off-spring susceptibility to CD-colitis in mice (54).

Recent dietary trials with exclusive enteral nutrition (EEN) alone, or in conjunction with specific exclusion diets aimed at correcting Western dietary habits have illustrated remission of CD in patients with mild-to-moderate disease, reinforcing the concept that diet is a central trigger of gut inflammation in IBD (53,55–58). Thus, nutrition represents a modifiable factor in IBD onset and course. With potential implications for prevention and treatment, further research is warranted to delineate the dietary patterns of greatest benefit in IBD and the specific disease phenotypes most amenable to nutritional therapy (53).

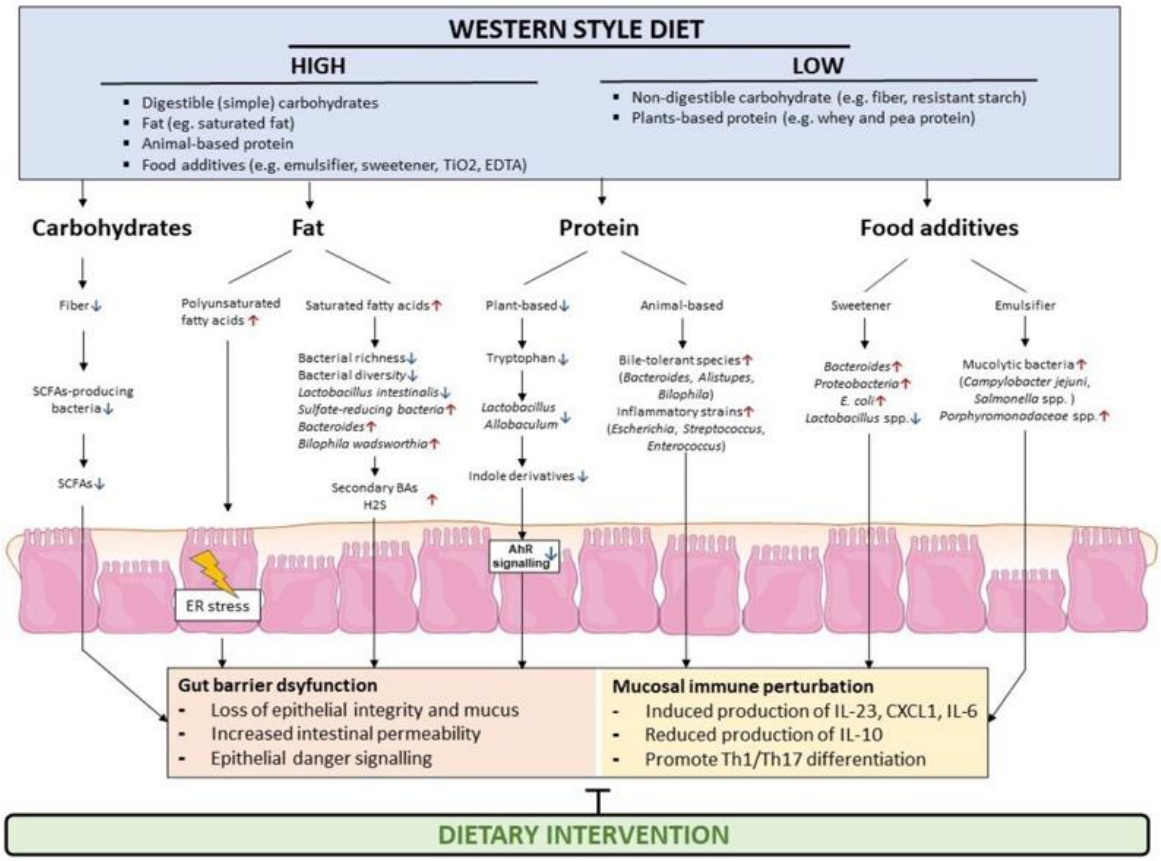

Figure 2 . Western Style Diets perturb the gut microbiome and immune response in IBD Source: Adolph TE and Zhang J. Diet fuelling inflammatory bowel diseases: preclinical and clinical concepts. Gut. 2022. 71:2574–86.

IBD: inflammatory bowel disease.

1.3.5 Gut Microbiome and the Immune System

Dysregulated immune responses (innate and adaptive arms) toward intestinal micro-organisms, leading to ongoing- inflammation and sustained mucosal damage is characteristic of IBD. The initial stimuli that incites this damage remains largely elusive, but may be attributable to infectious agents, chemical compounds or gut dysbiosis (59).

The gut microbiome is thought to play an integral role in IBD pathogenesis (13). Broadly, the composition and function of the intestinal microbiota, which is closely linked to environmental exposures and host genetics, is perturbed in IBD (13). Patients with IBD exhibit various changes in their microbial profile, including reduced butyrate- producing Firmicutes (possesses barrier protective and anti-inflammatory properties) and elevated proteobacteria levels, which can include adherent-invasive E.coli known to infiltrate the epithelium and persist within macrophages, prompting a pro-inflammatory response (13,60).

Additionally, the synthesis of mucin-2 a key component of the mucosal layer, which is normally impenetrable to bacteria, is reduced, thereby decreasing the thickness of this protective layer. Together, these alterations compromise the integrity of the mucosal barrier and permit approach of microbes (commensal and/or pathogenic) to the epithelial layer (61). Aberrant expression of tight junction proteins and the presence of apoptotic foci disrupts the intestinal epithelium and consequently permeability, which in turn enables translocation of microbes across the barrier (61)

In response, antigen presenting cells (APCs) and macrophages are activated, leading to the expression of chemokines responsible for neutrophil recruitment. Neutrophils in turn perpetuate intestinal inflammation by further impairing epithelial barrier function and via release of various inflammatory mediators. Naïve CD4+T cells are activated in response to antigen-stimulation from APC's and subsequently differentiate into effector T-helper cells (Th1), T-helper 2 (Th2), T-helper 9 (Th9), T-helper 17 (Th-17) or regulatory T-cells (Tregs) (59,62). A Th1 dominated immune response, characterised by increased IL-12 and IFN-y has historically been associated with CD, while UC was designated as an "atypical" Th2 condition, defined by increased IL-13, IL-5 and IL-9 from Th9 subset. However, since the discovery and implication of Th17 cells in IBD, this distinction is now largely considered an oversimplification (62).

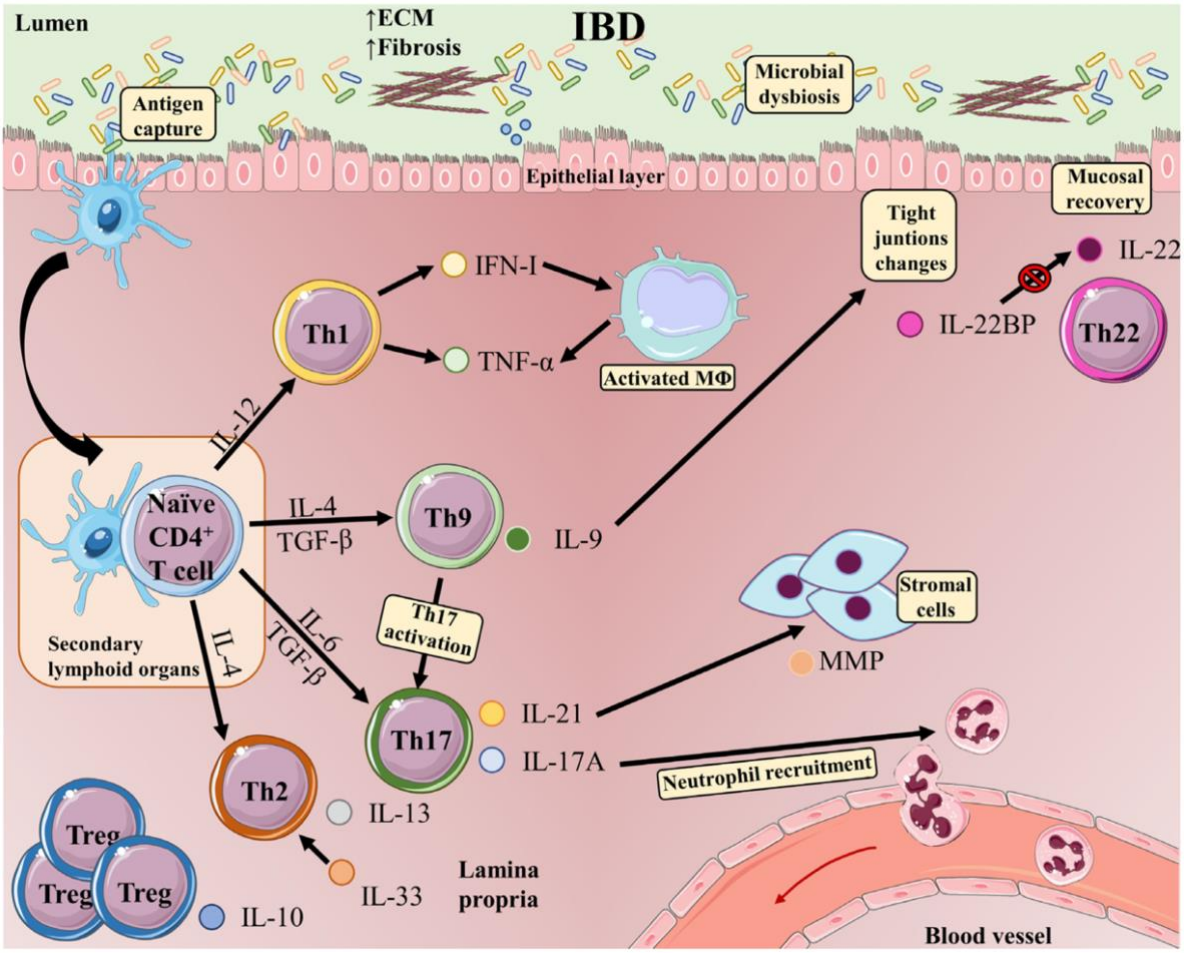

Figure 3. T-cell subsets and functions in IBD. Source: Gomez-Bris R, Saez A, Herrero-Fernandez B, Rius C, Sanchez-Martinez H, Gonzalez-Granado JM. CD4 T-Cell subsets and the pathophysiology of inflammatory bowel disease. Int J Mol Sci. 2023;24:2696.

1.4 Clinical Manifestations

1.4.1 CD – Signs and Symptoms

Patients can present with an array of non-specific symptoms which may manifest insidiously and vary according to disease location, extent and severity. The hallmark symptoms most frequently reported in CD patients include (1) prolonged diarrhoea (watery, +/- mucus or blood) (2) abdominal pain (right lower quadrant or peri-umbilical if terminal ileitis present, diffuse abdominal pain in Crohn's colitis) and (3) weight loss (63,64). Other prominent features observed include, fatigue, fever, abdominal tenderness, and peri-anal lesions (skin tags, fistulae, abscesses, scaring or sinuses) (64). Once a diagnosis of CD has been established, patients should be stratified and phenotyped according to the Montreal Classification system for CD **(Table 1)** and screened for the presence of EIM's (65).

1.4.2 CD - Location and Phenotype

CD can occur along any segment of the GI tract (66). The most commonly involved sites at clinical presentation are the terminal ileum, ileocecal valve and cecum (66,67). Disease involves both the terminal ileum and colon in 50% of CD cases, whereas another 30% of cases affect the small intestine alone. In 20% of patients CD is limited to the colon. Less often, patients present with upper GI disease, isolated perianal lesions or EIMS (66,67).

CD can be categorized into distinct phenotypic subgroups; (1) inflammatory (2) stricturing and (3) penetrating/fistulizing. Inflammatory CD is marked by GI inflammation without evidence of structuring or fistulizing disease. Overtime, inflammatory CD may progress to fibrosis and luminal narrowing, which categorizes patients under the stricturing subtype. Persistent transmural inflammation can give rise to sinus tracts or fistula development between bowel segments and adjacent organs, including the vagina and bladder (63,65).

Table 1. Montreal Classification for CD

Age at diagnosis, years (A)	• A1: ≤16 years • A2: 17-40 years • A3: > 40 years
Location (L)	• L1: terminal ileum • L2 : colon • L3: ileocolon • L4: upper gastrointestinal ○ L4 is a modifier that can be added to L1,2 and L3 when concomitant upper gastrointestinal disease is present
Behaviour (B)	• B1: non-stricturing, non-penetrating • B2: stricturing • B3: penetrating • P: perianal ○ P is a modifier that can be added to B1, 2 and 3 when concomitant perianal disease is present

Source: Satsangi J. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut. 2006;55:749–53.

1.4.3 UC – Signs and Symptoms

Akin to the presentation of CD, UC symptoms often appear gradually. Rectal bleeding with frequent stools and mucous discharge are characteristic symptoms of UC, the severity and frequency of which relate to disease severity and extent. Individuals with mild disease limited to the rectum (proctitis) or rectosigmoid area (distal colitis) frequently experience intermittent rectal bleeding associated with mucus passage and mild diarrhoea (<4 loose stool per day) (68,69)Additional UC associated symptoms include (1) urgency or tenesmus (2) abdominal pain (typically LLQ) and tenderness (3) lethargy (4) weight loss and (5) fever (68,69). When a diagnosis of UC is confirmed, disease extent and severity should be assessed and categorized using the Montreal Classification for UC (**Table 2)** alongside screening for EIM's as in CD.

1.4.4. UC – Severity and Extent of Disease

UC can be categorised by both disease severity and extent (68). The extent of UC is dynamic and may advance to involve increasing colonic segments as time elapses from diagnosis. Approximately 1/3 of patients initially diagnosed with limited disease experience proximal extension of UC within 10 years of UC diagnosis (70,71).

The rectum is involved in >95% of cases, and UC is termed proctitis if disease is limited to this region alone (30-60% cases). If mucosal inflammation extends from the rectum proximally to the splenic flexure (<50 cm from the anus) or beyond the splenic flexure, UC is described as either left-sided colitis (16-45% cases) or extensive/pancolitis (15-35% cases), respectively (**Figure 4**) (72,73).

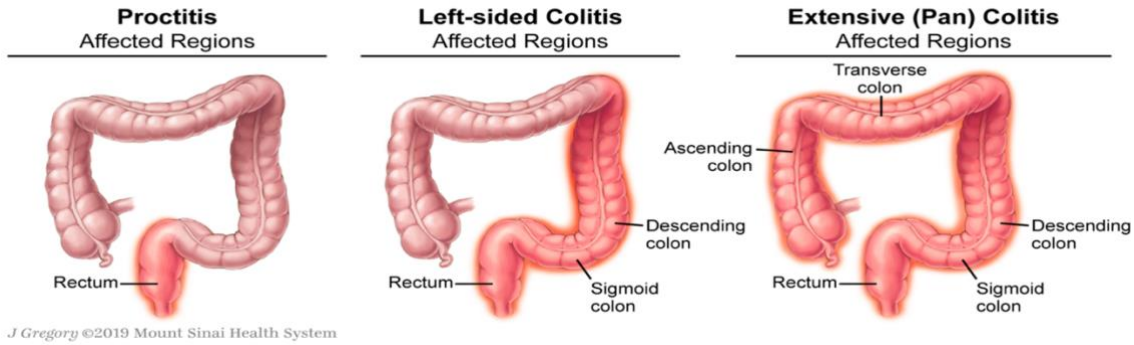

Figure 4 – Classification of UC Extent. Source: Kayal M and Shah S. Ulcerative Colitis and Emerging Treatment Strategies. J.Clin. Med. 2020;99:4.

Table 2. Montreal Classification for UC

Extent (E)	• E1: ulcerative proctitis; involvement limited to rectum (rectosigmoid junction) • E2: left sided ulcerative colitis: involvement limited to portion of colorectum distal to splenic flexure • E3: extensive ulcerative colitis: involvement extends proximal to splenic flexure
Severity (S)	• S0: ulcerative colitis in clinical remission; no symptoms of UC • S1: mild UC ≤ 4 bloody stools daily, lack of fever, pulse <90 bpm, haemoglobin >105g/L, ESR < 30mm/hr • S2: moderate ulcerative colitis: > 4-5 stools daily but with minimal signs of systemic toxicity • S3: severe ulcerative colitis: ≥ 6 bloody stools daily, pulse > 90 bpm, temperatures > 37.5°C, haemoglobin < 105 g/L, ESR > 30 mm/hr

Source: Satsangi J. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut. 2006;55:749–53. Click or tap here to enter text.
UC: ulcerative colitis, bpm: beats per minute, ESR: erythrocyte sedimentation rate.

1.5 IBD Course and Disease Monitoring

The clinical course of UC and CD is unpredictable, however both forms of IBD are typically characterised by phases of remission interrupted by acute exacerbations or flares (74). UC and CD are progressive in nature requiring timely and efficacious intervention to prevent irreversible long-term complications and the need for surgery (75). Currently, universal recommendations regarding the optimal timepoints and modalities for monitoring IBD activity and treatment efficacy are lacking. However, a composite strategy of clinical symptoms, serum and stool biomarkers, imaging and endoscopy have been proposed (76).

1.6 Extra-intestinal Manifestations

EIMs are common in IBD, affecting approximately 50-60% of patients. EIMS can affect several distant organ systems and represent a source of substantial morbidity and, in some instances mortality in IBD (6,8). The most frequently encountered EIMs of IBD are outlined in **Table 3.**

Table 3. Extra-intestinal manifestations of IBD

Cutaneous Lesions	• Erythema Nodosum • Pyoderma Gangrenosum
Oral Lesions	• Aphthous Ulcers
Ophthalmological	• Anterior uveitis* • Scleritis • Episcleritis
Bone and Joint Disease	• Arthritis* • Sacroiliitis • Ankylosing Spondylitis • Osteoporosis
Hepatobiliary	• PSC (UC – 2-8% patients) • PBC • NAFLD
Haematological	• Thrombotic events >2x as frequent in IBD • Anaemia – microcytic, normocytic or macrocytic

Source: Satsangi J. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut. 2006;55:749–53. Click or tap here to enter text.
*most frequent. PSC: primary sclerosing cholangitis; PBC: primary biliary cirrhosis; NAFLD: non-alcoholic fatty liver disease

1.7 Diagnostics

Establishing a diagnosis of IBD is complex, and a single reference standard protocol is currently lacking (77,78). According to international guidelines, the recommended diagnostic approach for IBD includes comprehensive evaluation of patients anamnestic data, physical examination and a combination of endoscopic, radiological, histological and/or biochemical analyses to support diagnosis (77,78). Suspicion of IBD should be raised in patients presenting with bloody diarrhoea and/or diarrhoea with signs of systemic inflammation >3 weeks (68).

1.7.1 Stool Assays

Stool samples for microscopy and culture should be sought at initial presentation for the presence of infective pathogens. Comprehensive microbiological analysis including Clostridium difficile toxin A and B immunoassay is indicated to exclude alternative causes of colitis (68).

Faecal calprotectin – non-invasive biomarkers

Faecal calprotectin (FCP) testing is routinely recommended to support diagnosis of IBD. Calprotectin, a neutrophil derived protein is released into faeces when neutrophils accumulate at the site of intestinal inflammation, and FCP correlates with endoscopic indices of disease severity and extent (79–81). Though elevated FCP levels (>150 µg/g stool) are useful in supporting clinicians diagnosis of IBD, a lack of specificity for IBD (61.5 - 79%) limits the diagnostic utility of FCP as a non-invasive biomarker (68,80,81). Despite this inability to discriminate between specific aetiologies of colitis, FCP appears to be particularly valuable in monitoring IBD activity, predicting disease relapse and monitoring treatment efficacy (77).

1.7.2 Biochemical Assessments

Routine biochemical assessments during IBD workup and at the time of initial diagnosis include; complete blood count (CBC), inflammatory markers – C- reactive protein (CRP) and erythrocyte sedimentation rate (ESR), liver function tests (LFTSs) and a comprehensive metabolic panel (CMP). CBC may reveal anaemia, leucocytosis and thrombocytosis (subsequent to acute inflammation) (63,68). Anaemia (HB <12-13g/dl) is a frequently encountered complication of IBD (21-27% patients), typically secondary to either chronic inflammation, chronic blood loss, or iron, B12 and folate malabsorption (82,83).

Elevated serum CRP and ESR are associated with IBD, however both inflammatory markers are not IBD-specific and increased levels occur in other causes of colitis and inflammatory disorders unrelated to the GI tract (84). Moreover, normal values do not definitively exclude disease, IBD- associated complications or flares (85). Raised CRP appears to closely correlate with CD activity and severity in some patients, which may be attributed to genetic variation, but it is less related to UC activity and severity (77,84,86).

Comprehensive Metabolic Panel (CMP)

Deranged LFTs are frequently reported in IBD with approximately 50% of patients presenting with abnormal values during their disease course (87). Abnormalities are often transient, reflecting IBD-flares or drug-induced liver injury (DILI) secondary to immunosuppressive or immunomodulatory IBD- therapy (88). Alternatively, elevated liver enzymes (ALP, bilirubin, AST, ALT) in IBD may be suggestive of more sinister associated conditions including autoimmune hepatitis (AIH) or primary sclerosing cholangitis (PSC). Consequently, LFT's are useful in surveillance of hepatobiliary manifestations (87,89)

Diarrhoea in IBD can disturb acid-base balance, most commonly resulting in hypokalaemic metabolic acidosis secondary to intestinal bicarbonate loss (63,68,90). Likewise, excessive fluid losses and dehydration can cause elevated sodium (Na+) and urea, however, hyponatremia due to reduced intestinal absorption is also habitually observed (91)

As a negative acute phase reactant, decreased levels of albumin are often documented in cases of active IBD. Moreover, although diminished albumin levels have previously been touted as biochemical evidence of malnutrition in IBD, use of albumin as a direct marker of malabsorption is tenuous and not supported in the current European Crohn's and Colitis Organisation (ECCO) guidelines (77,92).

1.7.3 Imaging and Endoscopy

Plain abdominal radiograph

Plain abdominal X-rays are a widely accessible and inexpensive imaging modality used in IBD to assess the extent of disease and to screen for complications (63,68). In UC, non-specific findings may be present, however evidence of mural thickening or "thumbprinting" denoting large bowel thickening may occur. During complications with toxic megacolon, the transverse colon becomes dilated to at least 6 cm and evidence of pneumoperitoneum may occur if dilation advances to the point of perforation (63,68)Likewise, in CD, small or large bowel loop distension, calcifications, intra-abdominal abscesses or pneumoperitoneum may be visible on X-ray (63).

Crohn's Disease – Macroscopic Features

Ileocolonoscopy is the gold standard endoscopic procedure for investigation of CD. Hyperaemia and oedema are often noted however the characteristic endoscopic findings in CD are skip lesions (areas of inflammation interposed between normal-appearing mucosa), aphthous ulcers and a cobble-stone appearance (63,93). Esophagogastroduodenoscopy (EGD) is not routinely required in the absence of upper GI symptoms (63).

CD Histological Features

To assess for microscopic evidence of CD, a minimum of two biopsies from inflamed regions should be obtained, with additional samples from uninflamed regions and each colonic segment (63,77). Microscopic features which aid in distinguishing CD from UC include; (1) transmural inflammation (2) non-caseating granulomas (3) lack of depletion of goblet cells (4) focal crypt architectural abnormalities and (5) the specific distribution of disease (63,77,93).

Ulcerative Colitis - Macroscopic Features

Flexible sigmoidoscopy permits visualisation of the distal colon and is often the first endoscopic procedure performed in UC workup as it can performed without sedation or full bowel preparation.

During flexible sigmoidoscopy and colonoscopy features suggestive of UC include involvement of the rectum, a diffuse, continuous pattern of inflammation and (1) erythematous and oedematous mucosa with or without ulcerations (2) decreased vascular markings (4) mucosal friability and (5) luminal narrowing with pseudopolyps in long-standing disease. In severe cases, contact bleeding may be seen (68,93).

UC Histological Features

A minimum of two biopsies should be obtained from at least 5 sites along the colon, including the rectum and terminal ileum (68). Microscopic features of UC include (1) mucosal and submucosal inflammation (2) continuous distal disease (3) goblet cell mucin depletion (4) focal or diffuse basal plasmacytosis (5) crypt abscesses (7) muscular atrophy and (7) crypt architectural distortion (93,94).

1.8 Treatment Approaches in IBD

Historically the primary objectives of IBD treatment were limited to symptom alleviation and steroid-sparing, with the aim of preventing hospitalisation and need for surgical intervention (95–97). Today, with advances in IBD therapy rather than mere symptom control, modern treatment paradigms incorporate patient centred outcomes (quality of life, disability prevention and work productivity) in addition to objective endpoints of disease control such as mucosal, histological or transmural healing (75,95,98).

In 2015, the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) recommendations were published, providing a framework for IBD treatment goals in a treat-to-target (T2T) concept, later updated in 2021 (STRIDE-II) (99). The STRIDE-II recommendations and T2T concept advocate directing IBD therapy toward various pre-defined, patient-specific goals and offer time-dependant treatment targets (75,100). Close and regular monitoring of these targets facilitates timely therapeutic adjustments, aimed at achieving disease remission if treatment goals are unmet.

Although the approach is promising, T2T has garnered considerable criticism, with clinicians and researchers citing that suggested endpoints, specifically mucosal healing (MH), for which no standardised definition exists is out-of-reach for the majority of patients. Moreover, the T2T approach is cumbersome for both physicians and patients.

In addition, patients who achieve symptom control are less likely to remain compliant with the intense surveillance required for T2T (8,98).

1.8.1. Stepwise Therapy

Conventional Step Up Approach – UC

The therapeutic approach in UC is dictated primarily by disease extent and the presence of EIM's. However, existing evidence generally does not support the early initiation of biologic agents (top-down approach) in mild-moderate UC patients, which contrasts recent findings in the context of CD (101,102). For UC patients with mild to moderate disease limited to the rectum, topical amino salicylates (5-ASA) compounds administered rectally constitute the cornerstone of treatment, whereas patients with left-sided or extensive colitis are typically prescribed oral forms. If treatment response is inadequate at this level, patients can be stepped up incrementally to more complex and expensive regimens, including steroids, immunomodulators (azathioprine, 6-mercaptopurine, methotrexate) and various biologic agents (e.g. anti-TNF) (**Figure 5)** (101,102).

To induce remission in patients hospitalised with acute flares intravenous steroids are administered, subsequently followed by oral steroids. In cases of uncontrolled disease refractory to the aforementioned treatments, total colectomy may be indicated or proctocolectomy with ileal pouch-anal anastomosis (IPAA) (18).

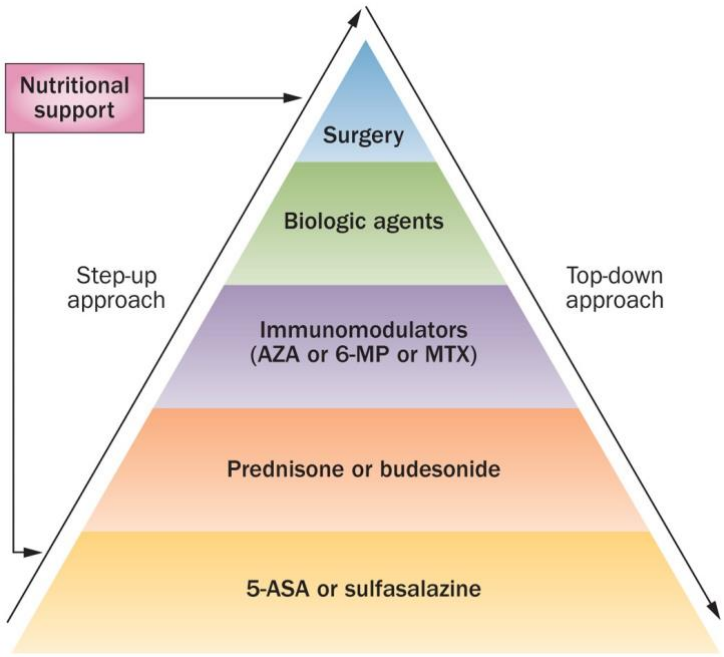

Figure 5. Step-up and Top-down approaches in IBD treatment. Source: Aloi M, Nuti F, Stronati L, Cucchiara S. Advances in the medical management of paediatric IBD. Nat Rev Gastroenterol Hepatol. 2014;11:99-108.

Step Up and Top-Down in CD

Although the advent of infliximab and other modern biologic therapies revolutionised IBD management and greatly improved patient outcomes, surgical resection is still required within 5-years of diagnosis in approximately 17-25% of CD patients (103). Several studies have illustrated significant benefits of early advanced treatment in CD (104–106). Yet despite this, biologics are typically not prescribed at the time of diagnosis, and are introduced only when conventional management (step-up) fails - an approach endorsed by various international guidelines (103).

However, recently published findings from the PROFILE (PRedicting Outcomes For Crohn's disease using moLecular biomarker) study which stratified newly diagnosed CD patients to either top-down (infliximab plus thiopurine or methotrexate) or accelerated step-up therapy call into question the merit of this current standard of care (103).

Within this trial, the rate of sustained corticosteroid-free and surgery-free remission at 1 year was notably higher in the top-down group compared to the accelerated step-up cohort (149 [79%] of 189 patients vs. 29 [15%] of 190 patients). Moreover, the top-down group experienced fewer adverse events including disease flares, as well as serious adverse events (hospitalisation, surgery for disease complications, medication related events or serious infection). These findings lend further credence to earlier findings and provide convincing evidence for earlier initiation of biologic agents in CD, rather than the conventional step-up approach. In the future, biomarkers to identify patients who would derive the most benefit from advanced therapy at earlier stages of CD could enable more targeted administration of these treatments while minimizing unnecessary use in less suitable candidates (103).

1.9 Interleukin-1β

IL-1β, encoded by the IL-1β gene on Chromosome 2 is one of eleven members of the IL-1 cytokine family. Sentinel cells of the innate immune system including macrophages and monocytes are the dominant source of IL-1β, however fibroblasts, neutrophils, epithelial and endothelial cells additionally contribute (107). IL-1β, akin to other members of the IL-1 group is initially generated as an inactive 31 kDA precursor molecule, pro- IL-1β, which uniquely requires proteasomal cleavage by the multiprotein complex known as the Inflammasome (108).

Assembly and activation of the NLRP3 inflammasome, which is "sensor of cell injury" relies upon two distinct signals. The initial signal, referred to as "priming" is triggered by tissue damage leading to the release of Damage-associated molecules patterns (DAMPs), including the IL-1a precursor and extracellular ATP (eATP). DAMPs activate various membrane receptors including toll-like receptors (TLR) or IL-1 receptor type 1, which result in translocation of NF-kB to the cell's nucleus. Binding of Nf-kb induces the expression of pro-inflammatory genes, particularly the precursors of IL-1B (pro- IL-1β) and IL-18 (pro-IL-18), in addition to NLRP3 inflammasome components. The newly translated precursor molecules accumulate in the primed cells cytosol, however the inflammasome is not directly activated from signal 1 alone. Instead, the second signal, which is promoted by eATP or intracellular DAMPS (reactive oxygen species and lysosomal contents) serve to activate the complex (109,110)

Activation prompts NLRP3 to oligomerise and form a complex with ASC (apoptosis-associated spec-like protein containing a caspase recruitment domain) and pro-caspase-1. This subsequently leads to autolytic cleavage of procaspase-1 into the active form, caspase-1.

Caspase-1, the "effector" component of the inflammasome then cleaves the 269- amino acid pro- IL-1β molecule, generating the bioactive and mature form, a 17 kDA IL-1β molecule. Furthermore, Inflammasome-independent processing of proIL-1β in acute inflammatory states is well-documented and typically occurs when neutrophils are the predominant cell-type within the lamina propria (111). For instance, during arthritic flares, neutrophil-derived proteases play a key role in the generation of bioactive IL-1B. Likewise, in murine models of osteomyelitis, neutrophil derived serine proteases drive the inflammatory response and production of mature IL-1β (111,112). Once cleaved, IL-1β gains an overall positive charge, enabling localisation with the negatively charged molecule, phosphatidylinositol 4,5-bisphosphate (PIP2), present within the plasma membrane. Subsequently, microvesicles shed from the cellular membrane, facilitating the secretion of IL-1β into the extracellular space (110).

IL-1β binds to the Interleukin-1 receptor (IL-1R) which consists of an extra-cellular immunoglobulin domain and intra-cellular Toll/IL-1 receptor (TIR) domain. The receptor is expressed by several cell types including epithelial and endothelial cells, hepatocytes, and various innate and adaptive leukocytes including B and T-cells, dendritic cells, monocytes, macrophages, granulocytes and mast cells (109). Binding of IL-1β induces conformational change of the receptor and recruitment of Myd88 which associates with IRAK 1, IRAK 4 and/ or IRAK2 (109,113).

IRAK4 phosphorylates IRAK1 and IRAK2, which facilitates their interaction with TRAF6, which functions as a scaffold to recruit and activate TAK1 (transforming growth factor β-activated kinase 1). TAK1 can then activate p38 and JNK (c-Jun N-terminal kinase), which results in the activation of the transcription factor AP-1. Alternatively TAK1 can activate the IKK (inhibitor of NF-κB kinase) complex, which consists of two kinases (IKKα and IKKβ) and a regulatory subunit IKKy (113). Subsequently, the IKb component is phosphorylated and degraded, which renders NF-KB free to translocate to the cell nucleus. Activation of p38/JNK and NF-κB leads to the transcription of genes involved in diverse biological processes, varying by the cell type stimulated by IL-1β (109,110).

1.9.1 The role of IL-1B

IL-1β is a potent pro-inflammatory cytokine involved in both local and systemic responses to injury, infection and inflammation (109). Historically, IL-1β has been considered

central in the effective initiation of innate and shaping of adaptive immune response responsible for resolving acute inflammation (109).

However, the hypothesis that IL-1β is essential and solely a beneficial immune regulator has been challenged by evidence demonstrating that gain-of-function mutations in components of the inflammasome complex leads to excessive cytokine production and contribute to the development of autoimmune and chronic inflammatory conditions (111,114).

Systemically, IL-1β has the capacity to evoke fever and hypotension, and is involved in the regulation of various central nervous system functions including sleep, pain and appetite. At a local level, IL-1β stimulates the activation and effector functions of several immune cell types, including macrophages, dendritic cells and neutrophils (115). Moreover, IL-1β facilitates neutrophil recruitment and migration to sites of injury or infection in addition to playing a crucial role in T cell activation and survival.

Lastly, IL-1β works synergistically alongside other pro-inflammatory cytokines to facilitate the differentiation of CD4+ Th17 cells (115).

In the context of infection, these functions act to enhance the clearance of pathogens, however when IL-1β is present in excess, or chronically, it provokes excessive inflammation and tissue damage, as seen in IBD (114) .

1.9.2 IL-1β in IBD

Numerous clinical and animal studies have demonstrated markedly increased expression of IL-1β in IBD and other inflammatory disorders of the gut (114,116).

Mononuclear cells, primarily macrophages residing within the lamina propria constitute the chief source of elevated IL-1β levels in tissue biopsies from patients with active IBD. Furthermore, a positive correlation between the severity of mucosal inflammation, IBD disease activity and the extent of IL-1β elevation has been reported (115,116). Though several studies have linked IBD intestinal inflammation with heightened IL-1β levels, fewer studies have successfully characterised the precise mechanisms which underlie the pro-inflammatory cytokines contribution to the IBD inflammatory milieu.

However, using two complementary models of intestinal inflammation Coccia *et al.,* 2019 elucidated three potential cellular mechanisms involved. The researchers identified that IL-1β was a critical driver of severe innate immune pathology induced by the intestinal pathogen *Helicobacter hepaticus*, through increased granulocyte and innate lymphoid cell recruitment and activation (115).

Additionally, employing a T-cell transfer colitis model, a pivotal contribution of IL-1R signalling in facilitating the accumulation and survival of pathogenic CD4+ T cells within the intestine was noted. Lastly, the research highlighted that IL-1β induces Th17 responses from CD4+ T cells and innate lymphoid cells. Consistent with these findings, targeting and blockade of IL-1β has been successful in attenuating colitis in animal models though the limited findings available in human studies have produced less promising results (114,115).

Furthermore, in addition to the direct immunomodulatory effects of IL-1β, other studies have demonstrated that the cytokine disrupts the integrity of the intestinal tight junction barrier, resulting in increased paracellular permeation of luminal antigens (117). Research conducted by Al-Sadi *et al*., 2008 revelated that this mechanism was mediated via IL-1β activation of NF-κB, and stimulation of MLCK gene transcription and protein expression, a pathway which culminates in the opening of Caco-2 tight junction barriers. Subsequently, diffusion of luminal contents into the lamina propria provokes local inflammatory response, secretion of cytokines, matrix metalloproteases, epithelial degradation and inflammation (117,118).

2. OBJECTIVES

The primary aim of this study was to investigate the IL-1β levels in ulcerative colitis and Crohn's patients, and to compare them to healthy controls. An additional goal was to explore correlations of IL-1β levels with various laboratory parameters.

2.1. Hypotheses

1. IL-1β levels will be significantly higher in IBD group of patients when compared to healthy control group
2. IL-1β levels will positively correlate with fecal calprotectin levels
3. IL-1β levels will positively correlate with CRP levels
4. There will not be statistical difference in IL-1β levels between ulcerative colitis and Crohn's disease patients

3. MATERIALS AND METHODS

3.1 Study Design and Ethical Considerations

This single-centre, cross-sectional study was conducted at the Laboratory for Cardiometabolic Research, University of Split School of Medicine and the Department of Gastroenterology, University Hospital of Split. Participants were recruited from May 2022 to January 2023 and informed of the study's objective, procedure and course. All participants provided written informed consent prior to inclusion. The study adhered to the ethical principles outlined in the Declaration of Helsinki and its amendments, as well as the Good Clinical Practice guidelines from the International Conference on Harmonisation. Ethical approval was granted by the Ethics Committee of the University Hospital of Split (Class: 500-03/21-01/186; No: 2181-147/01/06/M.S.-21-02; Date: 22 December 2021).

3.2 Study Population

We recruited 50 patients with IBD with from the Department of Gastroenterology at the University Hospital of Split, along with 50 age- and sex-matched healthy control subjects aged between 18-65 years. A diagnosis of IBD was established based on the most recent European Crohn's and Colitis Organization and the European Society of Gastrointestinal and Abdominal Radiology guidelines. Exclusion criteria for the study included the following; history of cardiovascular or metabolic disorders, corticosteroid use within the preceding three months, use of psychoactive medications, or alcohol and substance misuse.

3.3 Clinical assessment and laboratory analysis

Body weight (kg) and height (m) were measured using a calibrated stadiometer with an integrated weight scale (Seca, Birmingham, UK). Body mass index (BMI) was then derived by dividing body weight (kg) by the square of body height (m^2). Further data was obtained from patient medical records. Following an overnight fast, venepuncture samples from the cubital vein were obtained for analysis. Blood samples designated for IL-1β biomarker analysis were aliquoted and stored at −80°C. Samples obtained for baseline analysis (albumins, hsCRP, lipid profile) were analysed immediately after collection by the laboratory. IL-1β biomarker analysis was performed using ProcartaPlex multiplex immunoassays (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). These assays utilise Luminex xMAP technology, which enables concurrent detection and quantification of up to 80 protein targets within a single 25–50 µL sample of body fluids.

Luminex technology employs differentially dyed capture beads for each target in a multiplex ELISA-like assay, and the beads are individually read using an xMAP instrument. An experienced biochemist, who was blinded to participant allocation, conducted the blood sample analyses following standard protocols in the institutional biochemical laboratory. Lastly, fecal calprotectin levels were quantified from stool samples after appropriate collection according to standard laboratory protocols.

3.4 Statistical analysis

The statistical program MedCalc (MedCalc Software, Ostend, Belgium) was used for statistical evaluation of the results. Normality of data distribution was tested with the D'Agostino-Pearson test, and, consequently, continuous data were presented as mean and standard deviation due to a normal data distribution pattern. Categorical data were presented as whole numbers and percentages, with the chi-squared test used for testing statistical differences. Finally, the correlation between IL-1β levels and other study parameters was tested with the Pearson's correlation coefficient. Statistical significance was set at $P<0.05$.

4. RESULTS

4.1 Baseline study characteristics

Comparison of baseline study characteristics including age, sex, anthropometric markers, family history of IBD and relevant laboratory findings between patients with IBD and healthy controls are presented in **Table 3**. A total of 100 subjects were included in the study, comprising 50 patients with IBD and 50 healthy controls. The mean age of patients with IBD was 39.4± 14.7 years, and 76% of participants were male. Of the 50 subjects in the control group, 64% were male and the mean age was 36.2 ± 11.9 years. No statistically significant differences were observed between study groups in age or anthropometric biomarkers (P >0.05 for all analyses) **(Table 3).** However, in comparison with the control group, IBD patients were more likely report a positive family history of IBD (2% versus 18%; P = 0.007). Finally, laboratory analysis revealed higher hsCRP (1.9 ± 0.91. vs 0.98 ± 0.65 mg/L; P <0.001), lower serum albumin (41.6 ± 5.2 vs 45.9 ± 3.8 g/L; P <0.001), lower LDL (2.6 ± 1.2 versus 3.4 ± 1.3 mmol/L; P = 0.002) and total cholesterol (4.7 ± 1.4, vs 5.3 ± 1.6 mmol/L; P = 0.047) in patients with IBD. Other characteristics, including triglycerides and HDL did not show statistically significant differences between both groups as outlined in **Table 3.**

Table 3. Baseline characteristics of the study population.

Parameter	**Healthy controls (n = 50)**	**IBD group (n = 50)**	***P****
Age (years)	36.2 ± 11.9	39.4± 14.7	0.242
Male sex, n (%)	32 (64.0)	38 (76.0)	0.190
Body mass index (kg/m^2)	23.7 ± 2.9	24.1 ± 3.6	0.542
Waist-to-hip ratio	0.88 ± 0.05	0.87 ± 0.06	0.367
Family history of IBD, n (%)	1 (2.0)	9 (18.0)	0.007
hsCRP (mg/L)	0.98 ± 0.65	1.9 ± 0.91	<0.001
Albumins (g/L)	45.9 ± 3.8	41.6 ± 5.2	<0.001
Total cholesterol (mmol/L)	5.3 ± 1.6	4.7 ± 1.4	0.047
LDL (mmol/L)	3.4 ± 1.3	2.6 ± 1.2	0.002
HDL (mmol/L)	1.32 ± 0.5	1.41 ± 0.6	0.417
Triglycerides (mmol/L)	1.27 ± 0.7	1.37 ± 0.5	0.413

LDL: low-density lipoprotein; HDL: high density lipoprotein, hsCRP: high sensitivity c-reactive protein. Data are presented as mean±standard deviation
* Student's t-test or Chi-squared test

4.2 IL-1β concentrations in IBD patients and controls

Serum IL-1β concentrations were increased in patients with IBD (1.2±0.3 vs 0.38 ±0.12 pg/mL; *P* <0.001) compared to the age-matched healthy control group. This finding is consistent with the understanding that IL-1β is a pro-inflammatory cytokine, and that increased levels of IL-1β are reflective of the ongoing inflammatory response which is characteristic of IBD.

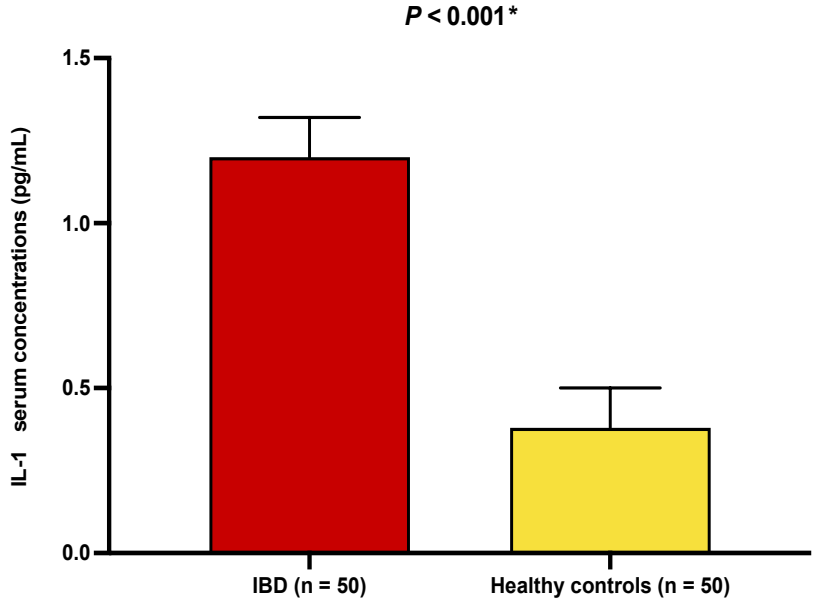

Figure 7. Comparison of IL-1β concentrations between patients with IBD and healthy controls. Data is shown as mean ± standard deviations.
*Student's t-test

4.3 IL-1β concentrations in Crohn's Disease and Ulcerative Colitis

As presented in **Figure 8** there was no statistically significant difference identified in serum IL-1β concentrations between patients with CD and patients with UC (1.18±0.14 vs 1.14 ±0.15 pg/mL; *P* = 0.864) **(Figure 8).**

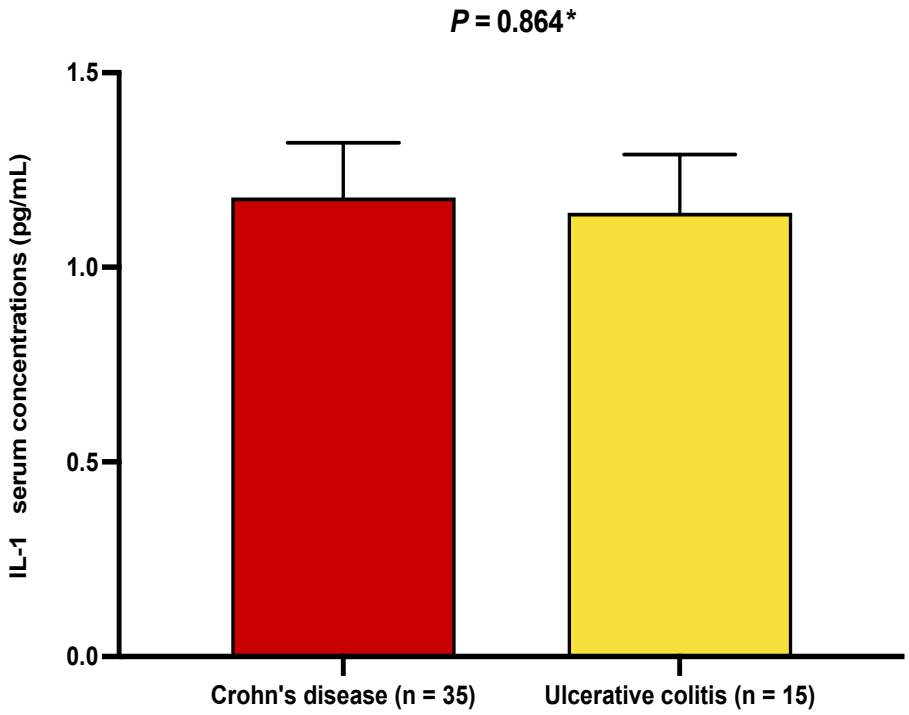

Figure 8. Comparison of IL-1β concentrations between patients with Crohn's disease and patients with ulcerative colitis. Data is shown as mean ± standard deviations.
*Student's t-test

4.4. Association of IL-1β and various laboratory variables

Lastly, we performed a correlation analysis between IL-1β serum concentrations and several anthropometric and clinical laboratory variables. Evaluation revealed that there was a significant positive correlation between serum concentrations of IL-1β with hsCRP (r=0.322; $P < 0.001$) and calprotectin (r= 0.465; $P < 0.001$) in patients with IBD. Other parameters including age, BMI, albumin, LDL, total cholesterol, and disease duration did not show a statistically significant correlation with IL-1β serum concentration.

Table 4. Correlation analysis between IL-1β serum concentrations and multiple clinical and laboratory parameters.

Parameter	**r–correlation coefficient**	***P****
Age	-0.211	0.578
BMI	0.199	0.434
hsCRP	0.322	<0.001
Calprotectin†	0.465	<0.001
Albumins	0.194	0.436
LDL	-0.082	0.946
Total cholesterol	0.214	0.419
Disease duration	-0.083	0.894

BMI: body mass indexs; hsCRP: high sensitivity C-reactive protein; LDL: low-density lipoprotein.
*Pearson's correlation coefficient † Only in patients with IBD

5. DISCUSSION

Our study included a total of 100 participants, 50 of whom were previously diagnosed with IBD alongside 50 age-matched healthy controls. The findings demonstrated that serum IL-1β levels were >3-fold higher in patients with IBD compared to healthy controls. However no appreciable difference was identified in IL-1β concentration between patients with CD and patients with UC. Additionally, in comparison to controls, patients with IBD exhibited increased hsCRP but had lower serum albumin, LDL and total cholesterol. Higher triglyceride and HDL-c levels were also observed, both of which are commonly elevated in IBD compared to the general population (119). However in our research, the differences in the aforementioned parameters did not reach statistical significance. Furthermore, patients with IBD were more likely to have a positive family history of IBD consistent with previous studies (120). Significant positive correlations were observed between IL-1β levels and both hsCRP and calprotectin in patients with IBD. More specifically, a weak positive correlation was identified between IL-1β serum concentration and hsCRP, while calprotectin level moderately correlated with IL-1β. No significant association was observed between IL-1β and other analysed parameters including age, BMI, albumins, LDL, total cholesterol and disease duration.

Produced by monocytes, macrophages, neutrophils and dendritic cells, IL-1β is an inducible cytokine acknowledged to play central role in development and prolongation of intestinal inflammation in IBD (117,121–124). Under normal physiologic conditions, IL-1β levels are low or absent, however during disease states, IL-1β gene expression markedly increases (125). In concordance with our findings, heightened concentration of IL-1β in both the intestinal mucosa and plasma of patients with IBD have been reported in previous studies (123,126,127). Furthermore, a positive association between the severity of mucosal inflammation and the extent of IL-1β elevation has been observed in animal models (117,126). Similarly, our study illustrated a positive correlation between serum IL-1β concentration and two surrogate markers of inflammation, namely, hsCRP and calprotectin level in patients with IBD. Typically, raised CRP levels more closely correlate with CD activity and severity but appear to be less related to both activity and severity in UC (77,84,86). In contrast, no difference in serum IL-1β concentrations between patients with UC and CD was noted in our study.

Though the clinical significance of the aforementioned results cannot be drawn from our current study and requires further research, these findings suggest that in the future IL-1β could be an another useful non-invasive biomarker for monitoring disease activity in UC and CD and the overall extent of inflammation in IBD, with the potential to overcome the current pitfalls associated with CRP in IBD. With that said, like CRP, IL-1β is non-specific and elevated levels occur during infection and other inflammatory conditions (84).

The precise mechanisms by which IL-1β initiates the chronic inflammation characteristic of IBD has been investigated in several animal studies. Purported mechanisms include though are not limited to (1) the recruitment of granulocytes and innate lymphoid cells and their activation (2) the promotion of pathogenic T cell responses including Th17 cell differentiation and IFN-gamma production and (3) an IL-1β intestinal tight junction barrier modulating action resulting in increased permeability (117,126,128).

Given these findings, IL-1β appeared to constitute an attractive target to attenuate the chronic inflammation observed in IBD. Indeed, while IL-1β blockade has ameliorated colitis in in-vitro and animal models, the results in human studies available to date have been less promising (129). In 2023, the effectiveness of Anakinra, a recombinant form of IL-1Ra which functions as a natural decoy for the biological activity of IL-1β was investigated in a large, multi-centre. randomised, placebo-controlled, double blinded trial. In an interim analysis of The Interleukin 1 (IL-1) blockade in Acute Severe Colitis (IASO) trial, the authors illustrated that the addition of anakinra to standard care (IV corticosteroids) failed to reduce the requirement for rescue therapy or colectomy in acute severe ulcerative colitis (130). However, in a single-centre retrospective study, a paediatric population with very-early onset IBD responded clinically to treatment with Canakinumab (anti-IL-1β monoclonal antibody) for >6 months (114,131). Interestingly, recent research highlighted that circulating IL1β levels closely associate with failure of anti-TNF therapy in UC. Consequently, IL-1β may be a clinically useful predictor for identifying infliximab non-responders, and guiding alternative therapeutic choices (132). Although IL-1β blockade in the context of IBD treatment has not yet demonstrated the desired results, findings from the CANTOS trial illustrated significant benefit of anti-IL-1β monoclonal antibody treatment on cardiovascular outcomes, which were independent of blood lipid concentration and blood pressure but were closely related to the level of reduction in inflammatory parameters in participants (125,133,134).

Thus, it is possible that patients with IBD, who constitute a vulnerable group in terms of exposure to chronic systemic inflammation may still benefit from IL-1β antagonism, though without a direct effect of decreasing gut inflammation (125,133,134).

In line with previous studies, we identified altered lipid profiles in patients with IBD. Although the data available to date has been somewhat inconsistent and describes various changes, the main lipid patterns documented in studies of IBD populations include reduced total cholesterol, LDL-c and increased triglycerides and HDL when compared with healthy controls or patients without active disease (119,135,136).

In a large cohort study conducted in the USA, Koutroumpakis *et al.,* 2015 reported that patients with IBD exhibited a significantly lower prevalence of high total cholesterol and high LDL-c (6% vs. 13% and 5% vs. 10%, respectively) and more frequent low HDL and high TG (24% vs. 17% and 33% vs. 25%, respectively) when compared with the general population (all $p < 0.001$) (137). These alterations are typically ascribed to complex interactions between the presence of pro-inflammatory cytokines in IBD**,** including IL-1β and lipid metabolism, in addition to the presence of malnutrition and malabsorption (119,135–137). However in our study, no significant correlation was noted between serum IL-1β concentration and lipid parameters in our subset of IBD patients. It is plausible that the observed dyslipidaemia was mediated instead by other inflammatory molecules which were not investigated in the present study or via alternative unrelated mechanisms. Despite the decreased prevalence of a well-established cardiovascular risk factor, namely cholesterol, research indicates that IBD patients are heightened risk of thromboembolic events, including myocardial infarction and stroke (138). A 2013 systematic review and meta-analysis found that patients with IBD have a 19% increase in risk of ischemic heart disease and an 18% higher risk of cerebrovascular events (138). The combination of increased cardiovascular disease and increased HDL, reduced total and LDL cholesterol present in IBD is referred to as the "lipid paradox". This paradoxical association has been mirrored in other immune-mediated inflammatory disorders and may obscure physicians assessment of cardiovascular disease risk in patients with IBD, thus requires additional vigilance for early risk stratification (139,140).

The present study has several limitations. Firstly, as a result of the cross-sectional study design, it was not possible to establish causality or follow longitudinal changes in IL-1β concentrations. Moreover, the relapsing and remitting nature of IBD may have confounded the level of the investigated parameter in patients. Finally, the small sample size (n=100) hinders the generalisability of the findings to the entire IBD population.

6. CONCLUSION

1. IL-1β levels were significantly higher in IBD patient group when compared to healthy control group.
2. There were no significant difference in IL-1β levels between ulcerative colitis and Crohn's disease groups.
3. IL-1β levels had significant positive correlation with calprotectin levels in the investigated population.
4. IL-1β levels had significant positive correlation with hsCRP levels in the investigated population.

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8. SUMMARY

Objectives: The primary objective of this study was to investigate the IL-1β levels in patients with inflammatory bowel disease, and to compare them to healthy controls.

Subjects and methods: This cross-sectional cohort study enrolled a total of 100 subjects between May 2022 and January 2023 - 50 with inflammatory bowel disease alongside 50 healthy age-matched controls. Following an overnight fast, venepuncture was performed to obtain samples for baseline analyses and IL-1β concentration, which was determined using ProcartaPlex multiplex immunoassays.

Results: A statically significantly higher concentration of IL-1β was identified in the IBD group compared to the control group (1.2±0.3 vs 0.38 ±0.12 pg/mL; *P* <0.001). Furthermore, higher hsCRP (1.9 ± 0.91. vs 0.98 ± 0.65 mg/L; *P*< 0.001), lower serum albumin (41.6 ± 5.2 vs 45.9 ± 3.8 g/L; *P* <0.001), and total cholesterol (5.3 ± 1.6 vs 4.7 ± 1.4 mmol/L; *P* = 0.047) among patients with IBD was noted. There was no significant difference in serum IL-1β concentration between patient with Crohn's disease and patients with ulcerative colitis (1.18 ± 0.14 vs 1.14 ± 0.15 pg/mL; *P* = 0.864). Lastly, correlation analysis revealed serum concentrations of IL-1β positively correlated with both hsCRP (r = 0.322; *P* < 0.001) and calprotectin (r = 0.465; *P* < 0.001) in patients with IBD.

Conclusions: IL-1β serum concentrations were increased in patients with IBD when compared with healthy controls. However, there was no significant difference in serum concentration of IL-1 β between patients with ulcerative colitis and Crohn's disease. Levels of hsCRP and faecal calprotectin positively correlated with serum IL-1β concentration in inflammatory bowel disease patients.

9. CROATIAN SUMMARY

Naslov: Razine interleukina-1β u bolesnika s upalnom bolesti crijeva

Cilj: Glavni cilj ovog istraživanja bio je ispitati razine IL-1 u bolesnika s upalnim bolestima crijeva (IBD) te ih usporediti sa zdravim kontrolama.

Materijali i metode: Ova presječna kohortna studija obuhvatila je ukupno 100 ispitanika između svibnja 2022. i siječnja 2023. - 50 s IBD-om i 50 zdravih kontrola usklađenih po dobi. Nakon noćnog posta, venepunkcija je izvedena kako bi se dobili uzorci za osnovne analize i koncentraciju IL-1β, koja je određena korištenjem ProcartaPlex multipleks imunoloških testova.

Rezultati: Statistički značajno viša koncentracija IL-1β pronađena je u IBD grupi u usporedbi s kontrolnom grupom (1,2 ± 0,3 vs. 0,38 ± 0,12 pg/mL; *P* <0,001). Nadalje, zabilježene su više vrijednosti hsCRP-a (1,9 ± 0,91 vs. 0,98 ± 0,65 mg/L; *P* <0,001), niže vrijednosti serumskog albumina (41,6 ± 5,2 vs. 45,9 ± 3,8 g/L; *P* <0,001) i ukupnog kolesterola (5,3 ± 1,6 vs 4,7 ± 1,4 mmol/L; *P* = 0,047) u pacijenata s IBD-om. Nije bilo značajne razlike u koncentraciji IL-1β u serumu između pacijenata s Crohnovom bolešću i pacijenata s ulceroznim kolitisom (1,18 ± 0,14 vs 1,14 ± 0,15 pg/mL; *P* = 0,864). Za kraj, analiza korelacije pokazala je da su serumske koncentracije IL-1β pozitivno korelirale s hsCRP-om (r = 0,322; *P* < 0,001) i kalprotektinom (r = 0,465; P < 0,001) u pacijenata s IBD-om.

Zaključak: Serumske koncentracije IL-1β bile su povećane kod pacijenata s IBD-om u usporedbi s zdravim kontrolama. Međutim, nije bilo značajne razlike u serumskoj koncentraciji IL-1β između pacijenata s ulceroznim kolitisom i Crohnovom bolešću. Razine hsCRP-a i fekalnog kalprotektina pozitivno su korelirale sa serumske koncentracije IL-1β u pacijenata s IBD-om.