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**On the role of interleukin-6 and soluble interleukin-6 receptor
as potential biomarkers for ulcerative colitis**

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Table of Contents

List of abbreviations	III
List of figures	VI
List of tables.....	VII
Introduction.....	1
Objective	1
Epidemiology of Ulcerative Colitis	1
Aetiology of Ulcerative Colitis	2
Environmental factors	2
Genetic predisposition	3
Loss of barrier function.....	4
Microbiome.....	5
Immunology	5
Clinical aspects of Ulcerative Colitis.....	6
General Information.....	6
Arriving at a diagnosis	6
Disease activity	7
Clinical examination	8
Laboratory Data	9
Endoscopy.....	9
Histology.....	10
Treatment of ulcerative colitis	10
Biomarkers in ulcerative colitis	11
Role of IL-6, IL-6R and gp130 in ulcerative colitis	12
Methods.....	15
Background	15
Ethical considerations	16
Ethical approval	16
Informed consent	16
Sample specification	16
Study Overview	16
ELISA Testing	16
Data analysis and statistics.....	20
Results.....	23
IL-6, sIL-6R and sgp130 levels in the complete patient cohorts	24

Subgroup analysis based on disease activity scores	26
Subgroup analysis based on CRP	27
IL-6, sIL-6R and sgp130 levels in sub-cohorts with matched measurements	28
Association of IL-6, sIL-6R and sgp130 levels with general, clinical and laboratory parameters	31
Discussion	37
Mucosal levels of IL-6	37
Disease activity	38
IL-6	38
sIL-6R	38
sgp130	39
Correlations with general, clinical and laboratory parameters	39
Liver enzymes	39
Medication	40
IL-6, sIL-6R and sgp130 as biomarkers	40
Outlook	40
Summary	41
Bibliography	43
Acknowledgements	50

List of abbreviations

°C	Degrees Celsius
4-PL	Four-parameter logistic
5-ASA	5-aminosalicylic acid
ALT	Alanine transaminase
ANCA	Anti-neutrophil cytoplasmic antibodies
ASCA	Anti-saccharomyces cerevisiae antibodies
AST	Aspartate transaminase
BMI	Body mass index
CAI	Colitis Activity Index
CD	Crohn's disease
CD4	Cluster of differentiation 4
CDAI	Crohn's Disease Activity Index
CDH1	Cadherin-1
CI	Confidence interval
CONARIS	CONARIS Research Institute AG
CRP	C-reactive protein
ECM1	Extracellular matrix protein 1
ELISA	Enzyme-linked immunosorbent assay
Est.	Estimate
g/dL	Grams per decilitre
GNA12	Guanine nucleotide-binding protein subunit alpha-12
GOT	Aspartate aminotransferase
gp130	Glycoprotein 130
GPT	Glutamic pyruvic transaminase
GWA	Genome wide association
HATH1	Atonal homolog 1
HBI	Harvey-Bradshaw Index
HNF4A	Hepatocyte nuclear factor 4 alpha
IBD	Inflammatory bowel disease(s)
IgG	Immunoglobulin G
IgG1	Immunoglobulin G1
IgG4	Immunoglobulin G4

IL	Interleukin
IL-13	Interleukin-13
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-9	Interleukin-9
IQR	Interquartile range
JAK	Janus kinase
kg	Kilogram
KLF4	Kruppel-like factor 4
L	Litre
LAMB1	Laminin subunit beta-1
m²	Square metre
MAPK	Mitogen-activated protein kinase
MDD	Minimum detectable dose
mg/dL	Milligrams per decilitre
mg/L	Milligrams per litre
mL	Millilitre
MUC2	Mucin 2
N/A	Not applicable / not available
ND	Not determined
ng	Nanogram
ng/mL	Nanograms per millilitre
nL	Nanolitre
no.	Number of
o.	Ordinal
OD	Optical density
OR	Odds ratio
p	p-value
pANCA	Perinuclear anti-neutrophil cytoplasmic antibodies
pg	Picogram
pg/mL	Picograms per millilitre
PMS	Partial Mayo Score

sgp130	Soluble glycoprotein 130
sgp130Fc	Olamkicept
sIL-6R	Soluble interleukin-6 receptor
sR	Soluble receptor
STAT	Signal transducer and activator of transcription
STAT3	Signal transducer and activator of transcription 3
Th-2	T helper cell type 2
TLR	Toll-like receptor
TNF-α	Tumour necrosis factor alpha
TNF-α	Anti-tumour necrosis factor-alpha
UC	Ulcerative colitis
UKSH	Universitätsklinikum Schleswig-Holstein
Var	Variance
vol%	Volume percent
y	Years (age at sampling)
μL	Microlitre

List of figures

Figure 1 Incidence of ulcerative colitis (UC) from 1990 to 2016 in different territories	1
Figure 2 Inflammatory bowel disease susceptibility loci.....	3
Figure 3 Types of IL-6 signalling	13
Figure 4 Overview of the study.....	16
Figure 5 Procedure for the ELISA testing.	18
Figure 6 Overview of IL-6, sIL-6R and sgp130 serum levels in healthy controls and patients with Crohn's disease (CD) or ulcerative colitis (UC).....	25
Figure 7 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in all patients with Crohn's disease (CD) or ulcerative colitis (UC) differentiated according to disease activity scores.....	26
Figure 8 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in all patients with Crohn's disease (CD) or ulcerative colitis (UC) differentiated according to C-reactive protein (CRP) levels.	28
Figure 9 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in selected patients with ulcerative colitis (UC) differentiated according to disease activity scores.....	29
Figure 10 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in selected patients with ulcerative colitis (UC) differentiated according to C-reactive protein (CRP) levels.....	30
Figure 11 Association heat maps for ulcerative colitis.	32

List of tables

Table 1 Mayo Score	7
Table 2 Colitis Activity Index	8
Table 3 Endoscopic Mayo Score.....	10
Table 4 Treatment according to the course or behaviour of disease	11
Table 5 Circulating levels of IL-6, sIL-6R and sgp130 in patients with ulcerative colitis (UC).	14
Table 6 Preparations for ELISA for IL-6 (D6050, Quantikine® Human IL-6 Immunoassay	17
Table 7 Preparation for ELISA testing.....	18
Table 8 General, clinical and laboratory parameters.....	20
Table 9 Number of patients and measurements per cohort and sub-cohort.	21
Table 10 Characteristics of UC patients and healthy controls. All values in this format represent median (range).....	23
Table 11 Circulating levels of IL-6, sIL-6R and sgp130.	24
Table 12 Ordinal groups of IL-6 and corresponding levels across all groups.	24
Table 13 Logarithmic levels of IL-6, sIL-6R and sgp130.....	25
Table 14 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in all patients with ulcerative colitis (UC) differentiated according to disease activity scores.....	27
Table 15 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in all patients with ulcerative colitis (UC) differentiated according to CRP levels.	27
Table 16 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in selected patients with UC differentiated according to disease activity scores.....	29
Table 17 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in selected patients with UC differentiated according to CRP levels.....	30
Table 18 Association of ordinal interleukin-6 levels with general, clinical and laboratory parameters.....	33
Table 19 Association of soluble interleukin-6 receptor levels with general, clinical and laboratory parameters.....	34
Table 20 Association of soluble glycoprotein 130 levels with general, clinical and laboratory parameters.	35
Table 21 Associations of interleukin-6, soluble interleukin-6 receptor and soluble glycoprotein 130 levels with disease location.	36

Introduction

Objective

This study aims to assess the value of serum levels of interleukin-6 (IL-6) and its soluble receptor (sIL-6R) as well as soluble glycoprotein 130 (sgp130) as biomarkers for ulcerative colitis (UC).

Epidemiology of Ulcerative Colitis

UC is a global disease with prevalence and incidence steadily increasing. As shown in Figure 1, the highest levels of incidence of UC have been described in Northern Europe, North America and Australia (1). Germany has reached an estimated 3,0-3,9 per 100,000 and epidemiological research suggests that these numbers will increase further (2).

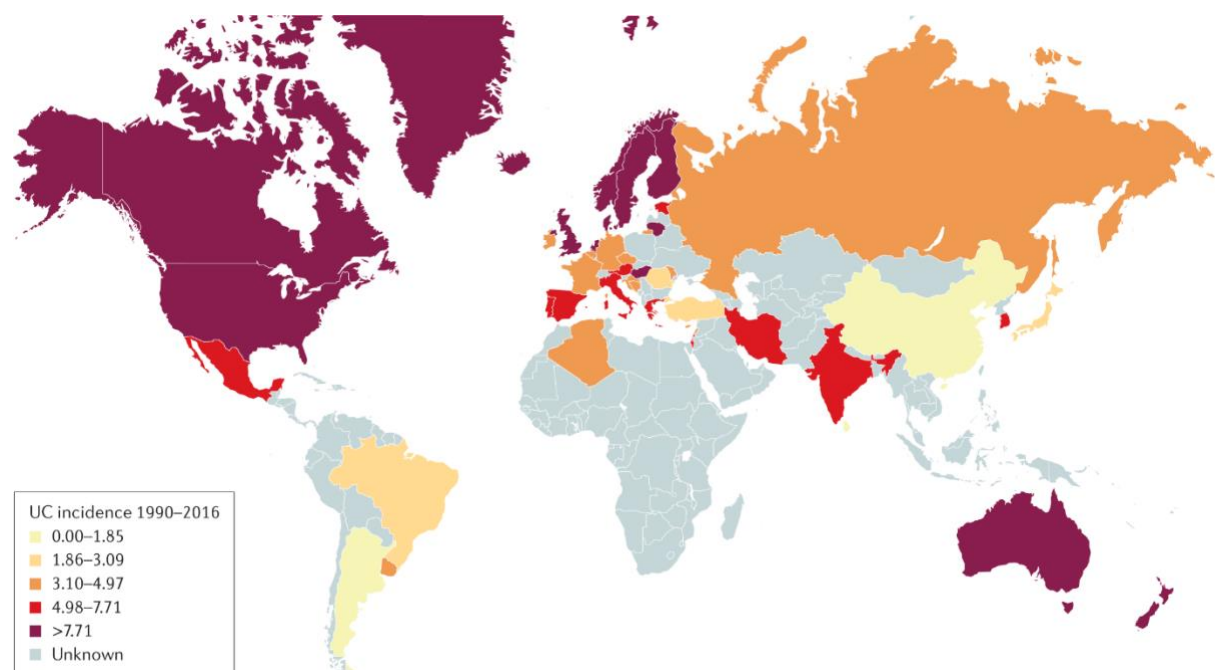


Figure 1 Incidence of ulcerative colitis (UC) from 1990 to 2016 in different territories (3).

On a global scale, the incidence and prevalence values have started to level off though in areas like Northern Europe and North America; nevertheless, levels are still rising in most parts of the developing world, Southern Europe and South America and Asia (1). A westernised lifestyle, cigarette smoking and appendectomies seem to play a major part in the distribution of the disease across the globe (4).

Aetiology of Ulcerative Colitis

The exact aetiology of UC remains elusive despite a vast amount of research being conducted in this area. The overall consensus is that there is not a single factor that can be attributed to sparking the disease but rather a poorly understood interplay of environmental, genomic, microbial and immunological factors together with an altered intestinal barrier function.

Environmental factors

Studies have shown that second generation migrants are at the same risk of developing UC compared to the native population (5–7) and according to Niv et al. (2000), the rates of prevalence of UC align after migrating into another country or region (8). These data and the fact that there is a low concordance rate in monozygotic twins underscore the influence of environmental factors in the development of UC. Moreover, a large number of environmental factors have been identified over the last few decades, some of them reducing the risk of developing UC whilst others have shown to be associated with an increased risk of UC development, more frequent flares and increased disease severity.

Smoking is by far the most researched environmental risk factor. Samuelsson (1976) was the first to describe that UC mostly affected non-smokers compared to healthy controls (9). It was initially believed that UC made patients give up smoking rather than it being a protective factor in its own right. Similar findings several years later by Jick and Walker (1983) sparked the idea of an aetiological connection between smoking status and the development of UC (10) and nowadays it is fully accepted that non-smokers and ex-smokers are at a higher risk of developing UC compared to smokers. Furthermore, a recent study indicates a reduced risk of colectomy for smokers (11).

Gastrointestinal infections have been associated with an increased risk of developing UC, whereas appendectomy seems to confer a protective effect against disease development, particularly when performed at a young age. And whilst prospective studies have not indicated a link between stress and the onset of UC, stress can affect the severity of the disease and the frequency of flares as well as hospitalisations (12). Patients suffering from severe disease are also more likely to have experienced major life events in the past 12 months compared to patients in remission (13). Furthermore, according to Barclay et al. (2009) breastfeeding can reduce the risk of UC (14) whereas the use of oral contraceptives, hormone replacement therapy

and non-steroidal anti-inflammatory drugs have all been associated with an increased risk of UC (15–17).

Genetic predisposition

Depending on the study, 9-54% of patients have been reported to have first-degree relatives suffering from UC, indicating that genetics play a role in UC development (18,19). On a genetic level, more than 47 susceptibility loci/genes have been discovered using genome wide association (GWA) studies and the identification of these loci has led to a better understanding of the pathogenesis of the disease. Epithelial barrier genes (ECM1, HNF4A, LAMB1, CDH1 and GNA12), for example, have been shown to be associated with UC (20,21). Another significant discovery was the genetic link between E-cadherin and the development of colorectal cancer in UC patients. The most pronounced susceptibility locus for IBD according to genetic association studies is the highly variable human leukocyte antigen (HLA) region and DRB1*01:03 explicitly for UC (22). Despite these advances in recent years, currently only a small percentage of disease variance can be explained by genetics.

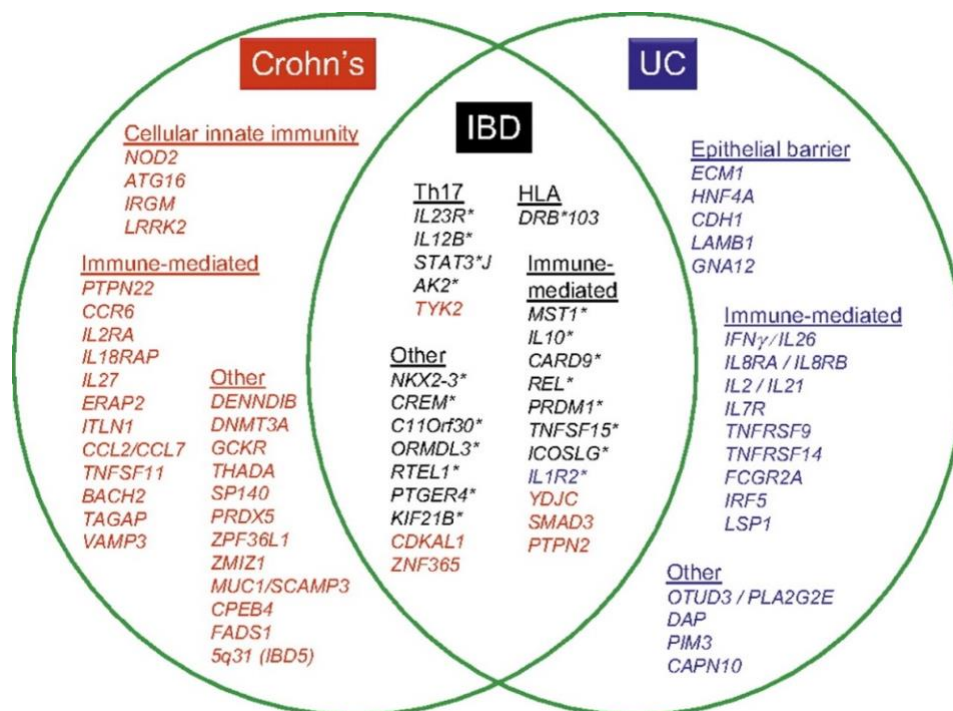


Figure 2 Inflammatory bowel disease susceptibility loci. The loci (depicted by lead gene name) attaining genome-wide significance ($P < 5 \times 10^{-8}$) are shown for Crohn's disease (red), ulcerative colitis (blue) and IBD (black where $p < 5 \times 10^{-8}$ in Crohn's disease (CD) and ulcerative colitis (UC); red where $p < 5 \times 10^{-8}$ in Crohn's disease and $< 5 \times 10^{-4}$ in ulcerative colitis; blue where $p < 5 \times 10^{-8}$ in ulcerative colitis and $< 5 \times 10^{-4}$ in Crohn's disease) (23).

‘Cross disease risk’ is a term used to describe the increased risk of developing Crohn’s disease (CD) as a relative of a UC patient (2-fold increase) and vice versa (4-fold increase). These findings have been confirmed by identifying certain susceptibility loci/genes that predispose to inflammatory bowel diseases (IBD) alone, whereas others are specific to either CD or UC (Figure 2) (23). The clinical uses of these findings are sparse at the moment but are thought to develop as research into the synergistic effect of genetic-environmental interactions onto the phenotype continues and scientists reach a better understanding of disease pathology as a whole.

Loss of barrier function

Contrary to CD, inflammation in UC is confined to the mucosal surface, conceivably supporting the hypothesis of an impaired mucosal barrier in the pathology of the disease (24). Recent advances in GWA studies have underscored the importance of loss of intestinal barrier function in UC development. Whilst the intestinal epithelium provides a mechanical barrier between the intestinal lumen and the rest of the body, antimicrobial peptides, as part of the innate immune system, build a host-defence system together with the intestinal mucus layer in which they are suspended. If this barrier is disrupted, the equilibrium of the mucosal barrier is shifted, leading to a disproportionate immune response towards commensal gut bacteria or a defective immune response towards pathogens. A loss of barrier function is the cause for increased bacterial adherence and invasion resulting in a heightened immune response which can ultimately lead to chronic inflammation (25).

The intestinal mucosa of UC patients has been shown to be more permeable compared to controls during active disease as well as remission (26). In patients with mild UC and macroscopically intact mucosa, extensive breakdown of the mucosal barrier, on a microscopical level, can already be observed and the leakage of the epithelial mucosa correlates with the degree of inflammation (27). Research by Schmitz et al. (1999) revealed an 80% reduction in epithelial resistance in UC patients suffering from inflamed sigmoid colons (28). This increased permeability can be in part attributed to the upregulation of the channel forming tight junction protein claudin 2. However, it remains unclear whether this upregulation is a cause or rather a consequence of intestinal inflammation (26). Intestinal permeability is further increased by epithelial apoptosis induced by IL-13 leading to the formation of micro-erosions (29).

UC patients have been shown to have thinner and less continuous mucus layers, a reduced mucin synthesis as well as reduced levels of phosphatidylcholine (30–32). The lack of mucin is due to a reduced number of Mucin 2 (MUC2) producing goblet cells (33). This can largely be attributed to a diminished induction of the goblet cell differentiation factors Atonal homolog 1 (HATH1) and Kruppel-like factor 4 (KLF4) and hence a defective maturation and migration of goblet cells to the upper crypts and surface which is the area mainly affected in UC (34).

Microbiome

The intestinal microbiome plays a central part in the pathogenesis of UC interlinking with genetic as well as environmental risk factors. Host genetics, for example, have been shown to affect the gut microbiota composition, which in turn has an effect on the host metabolism. The composition of the intestinal microbiome is further determined by factors such as diet, drugs, xenobiotics, age and disease. Consequently, each individual has a distinctive microbial fingerprint and patients suffering from IBD have been shown to have a dysbiotic intestinal flora. A study by Frank et al. (2007) shows that certain species of the commensal gut bacteria, for example members of the phyla Firmicutes and Bacteroidetes, were absent, whereas Gamma-proteobacteria and Enterobacteriaceae were increased (35). Up to this point, the question that remains unanswered is whether microbiome dysbiosis is a cause or consequence of UC.

As mentioned previously, a disrupted intestinal barrier permits the invasion and adherence of microbes to the epithelial lining. One such example are gram-negative anaerobic bacteria of the *Fusobacterium* genus. In UC patients, the levels of *Fusobacterium* spp. in the intestinal mucosa were elevated compared to controls (36,37). A study conducted by Strauss et al. (2011) demonstrated that the degree of invasiveness of the *Fusobacterium nucleatum* strain is correlated with the disease status (38).

Immunology

Physiologically, the gut's immune system maintains a tolerance towards the gut microbiota. This is accomplished by an intact barrier function, as previously described, as well as an intact immune system. In health, the innate and adaptive immune system work in unison to maintain homeostasis, whereas in chronic inflammation of the bowel, the immune system reacts inadequately to a stimulus in the form of luminal antigens or host microbiota.

Contemporary research attributes the pathogenesis of UC not only to modified T-helper-2 cells but rather an interplay of innate and adaptive cellular immunity (39). Nevertheless, Th-2 related cytokines such as IL-4, IL-5 and IL-13 play a pivotal role in disease pathogenesis. IL-4 and IL-13 were shown to correlate strongly with disease activity both clinically, endoscopically and histologically and their expression is enhanced during active UC (40,41). IL-13 produced by NK cells has cytotoxic potential for intestinal mucosa epithelial cells. This is evidenced by an increase in epithelial apoptosis and dysfunction of the epithelial barrier: increased IL-13 production leads to upregulated expression of claudin-2, a pore-forming tight junction protein (29). As a result, UC presents with epithelial leaks, which occur early in the course of the disease and are to be understood as a consequence of micro-erosions. More recent work by Gerlach et al. (2014) has also implicated a new population of IL-9 producing CD4-positive Th cells in the pathogenesis of UC (42). IL-9 interferes with the barrier function by impeding cellular proliferation and repair as well as also raising levels of TNF- α within the lamina propria of the intestinal mucosa.

Clinical aspects of Ulcerative Colitis

General Information

UC is an idiopathic inflammatory bowel disorder. In contrast to CD, UC is limited to the mucosa and mainly affects the large intestine. The incidence peak for the disease lies between the ages of 15 to 25, often with an additional peak at the age of around 60. According to the Montreal criteria, UC can be classified into proctitis, left-sided colitis or pancolitis (43) and a characteristic feature of UC is the uninterrupted inflammation of the colon that extends proximally. In up to 17% of cases, patients can develop ileal inflammation which is called back-wash ileitis. This can make an initial differentiation between UC patients and patients suffering from CD challenging.

Arriving at a diagnosis

Reaching a diagnosis is frequently a team effort and requires interdisciplinary collaboration of specialists in the fields of internal medicine, clinical chemistry, radiology and clinical pathology. The main goal of the clinical, radiological, endoscopic and histological examination is to narrow down the spectrum of possible diagnoses and establish a treatment plan that is tailored to the exact needs of the patient.

Disease activity

UC patients can be subcategorised into two main groups: patients with active colitis and patients in remission. Interestingly, the distribution of these two groups remains relatively constant with 50% of patients in each group.

According to Langholz et al. (1994), the only predictor of disease activity is activity in the previous year (44). In line with this, patients with active colitis within the first two years after diagnosis are likely to have active disease in the following five years. Clinical activity scores such as the Mayo score (Table 1) and Colitis Activity Index (CAI) (Table 2) are tools used by physicians to determine disease activity. Additionally, the partial Mayo score (PMS) can be calculated using stool frequency, rectal bleeding and physician rating of disease activity, at every patient visit without the need for endoscopy. UC can also be classified according to disease severity according to the Montreal classification. S0 corresponds to UC in remission, S1 a mild course of UC, S2 a moderate inflammation and S3 severe inflammation. The Montreal classification incorporated the following parameters: number of stools, fever, pulse-rate, haemoglobin and blood sedimentation rate (45).

Criteria	Point Value
Stool Frequency	
Normal	0
1–2 stools/day more than normal	+1
3–4 stools/day more than normal	+2
>4 stools/day more than normal	+3
Rectal bleeding	
None	0
Visible blood with stool less than half the time	+1
Visible blood with stool half of the time or more	+2
Passing blood alone	+3
Physician rating of disease activity	
Normal	0
Mild	+1
Moderate	+2
Severe	+3
Mucosal appearance at endoscopy	
Normal or inactive disease	0
Mild disease (erythema, decreased vascular pattern, mild friability)	+1
Moderate disease (marked erythema, absent vascular pattern, friability, erosions)	+2
Severe disease (spontaneous bleeding, ulceration)	+3

Table 1 Mayo Score (45).

Symptom	Score
Bowel frequency (per day)	
1–3	0
4–6	1
7–9	2
>9	3
Bowel frequency (per night)	
0	0
1–3	1
4–6	2
Urgency of defecation	
None	0
Hurry	1
Immediate	2
Incontinence	3
Blood in stool	
None	0
Trace	1
Occasionally frank	2
Usually frank	3
General well-being	
Very well	0
Slightly below par	1
Poor	2
Very poor	3
Terrible	4
Extracolonic manifestation	
Uveitis, pyoderma gangrenosum, erythema nodosum, arthropathy	1/manifestation

Table 2 *Colitis Activity Index* (47).

Clinical examination

UC patients should be fully clinically examined on a regular basis. Weight loss, for example, may indicate disease progression, whereas a stable weight or weight gain can be indicators of effective treatment. Special attention should be paid to symptoms such as tachycardia and the appearance of mucous membranes, as pathological findings can be first signs of acute anaemia. Typical pathologies during a clinical examination are abnormal bowel sounds, detection of an abdominal mass and pain on palpation in particular in the left lower quadrant as a sign of inflammation of the sigmoid colon. In order to identify perianal fistulae or fissures, which would indicate CD, an inspection of the anal region should be carried out (48). A digital rectal examination to assess sphincter function, rectal mucosa and haematochezia should also be carried out at regular intervals. The majority of patients with UC present with extra-intestinal

manifestations such as pyoderma gangraenosum or erythema nodosum. This underscores the importance of a full clinical evaluation of each patient's skin (48).

Laboratory Data

Blood tests, in particular levels of c-reactive protein (CRP), thrombocyte count and leucocyte count, are some of the diagnostic criteria for UC. As there is no single universal test for UC, laboratory tests can guide physicians in their clinical diagnosis and can also help to monitor disease activity. Further laboratory tests such as levels of haemoglobin and haematocrit are determined to rule out anaemia, which is common among UC patients. Levels of iron, transferrin as well as transferrin saturation are useful measurements for the adequately substitution of iron if this becomes necessary. Since extra-intestinal manifestations occur in 50–60% of IBD patients, liver, pancreas and kidney function also need to be evaluated on a regular basis (48). Serologic markers such as perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) have been shown to have a 55% sensitivity and 89% specificity. Rather than a screening test for UC, pANCA in combination with anti-aaccharomyces cerevisiae antibodies (ASCA) can be helpful in differentiating between UC (pANCA positive and ASCA negative) and CD (pANCA negative and ASCA positive) (49). Moreover, faecal calprotectin has been shown to be a valid inflammation parameter, particularly in the case of a mild course or pure proctitis and is particularly suitable for monitoring disease activity in adult paediatric patients due to its non-invasive nature (50).

Endoscopy

Endoscopy remains the gold standard for diagnosing UC. Patients with UC typically present with continuous inflammation starting from the rectum that is most often confined to the colon. The degree of inflammation can be estimated using the following macroscopic criteria: erythema, vascular pattern, friability, erosions, ulcers bleeding. This description of the intestinal mucosa can then be transferred to the endoscopic Mayo Score. The endoscopy part of the Mayo Score, developed by Schroeder et al. (1987), is based on a scale from 0 to 3 increasing in disease activity and is the most commonly used score to assess disease activity in UC patients during clinical trials (46).

Mucosal appearance at endoscopy	
Normal or inactive disease	0
Mild disease (erythema, decreased vascular pattern, mild friability)	+1
Moderate disease (marked erythema, absent vascular pattern, friability, erosions)	+2
Severe disease (spontaneous bleeding, ulceration)	+3

Table 3 Endoscopic Mayo Score (46).

In addition, mucosal oedema, pseudo-polyps, shortening of the colon, loss of haustral markings and muscle function can also be found. These changes can be described as the ‘lead pipe sign’. During a diagnostic endoscopy procedure, the physician will take biopsies from each segment of the colon for histological assessment.

Histology

Histological examination of biopsies plays an important part of the diagnostic process. The guidelines of the German Society for Digestive and Metabolic Diseases call for biopsy specimens from the terminal ileum, the rectum and each colon segment from macroscopically affected and macroscopically free sections of the colon (51).

Even though a definite histological diagnosis is rare due to the lack of pathognomonic features such as granulomas, the presence of typical histological features such as infiltration of the crypts, especially by neutrophilic granulocytes, a continuous inflammation of all biopsy specimens, crypt abscesses and generalized destruction of crypt architecture can support a clinical UC diagnosis (52). Other histopathological features include cryptal atrophy, Paneth cell metaplasia beyond the right flexure, and mucin loss in the margins of ulcerations.

Treatment of ulcerative colitis

So far, the disease can be controlled by treating the symptoms but there is no cure for UC. An acute episode of ulcerative colitis is characterized by bloody diarrhoea, tenesmus and imperative stool urgency. In addition to the intensity of the symptoms, the endoscopic pattern of infection (proctitis, left-sided colitis, pancolitis), the course of the disease, the response to the previous therapy, the duration of the disease, extra-intestinal manifestations as well as concomitant diseases and patients’ wishes play an important role. Treatment needs to be adapted to the patients’ needs. This is particularly important when deciding between inpatient and outpatient treatment.

The primary goal of UC therapy is to achieve rapid clinical remission followed by long-term, steroid-free clinical and endoscopic remission. Before treatment commences, drug therapy

options and risks should be weighed up against an operative therapy. Medical management options for active UC have been summarised in Table 4 below.

Disease severity	Treatment
Mild active UC	Aminosalicylate +/- oral steroids + topical therapy (steroids or 5-aminosalicylic acid (5-ASA))
Severe or chronic UC	Oral or intravenous steroids + immunosuppressants or biologics (infliximab/vedolizumab/ Ustekinumab) or small molecules (5-tetradecyl-oxy-2-furoic acid)

Table 4 Treatment according to the course or behaviour of disease (50).

Once remission has been achieved, it is vital for this state to be maintained, whereby continued treatment with 5-ASA, thiopurines, or biologic therapy has been shown to increase long-term remission rates (50).

Biomarkers in ulcerative colitis

Biomarkers have several desirable features when it comes to diagnostics, prediction of disease development, response to treatment and disease activity. A perfect biomarker would enable clinicians to find answers to their most pressing issues, all from a simple blood or faecal sample. This would not only be a more cost-effective solution, but also spare the patients invasive diagnostic procedures, such as colonoscopies, on a regular basis. Furthermore, if a biomarker were able to indicate changes in disease activity, treatments could be adjusted in a proactive rather than in a reactive manner, as is the case at the moment, meaning that treatment could take effect before a worsening of symptoms sets in.

A vast amount of research has already been conducted in search of biomarkers for IBD, but none of them can be used to accurately diagnose IBD, or to differentiate between CD and UC. To date, biomarkers can only be used as an adjunct to procedure evaluation or in disease monitoring. CRP, for example, is cheap and reliable but unspecific which means that it cannot be used on its own as a diagnostic or treatment biomarker for UC, because it lacks sufficient functional characteristics to replace clinical endoscopic or radiographic findings.

It is understood that an imbalance between cytokines, regulatory and inflammatory, contributes to the perpetuation of the mucosal inflammation in UC; one such cytokine being IL-6. Numerous studies have demonstrated an overall increase of this pleiotropic cytokine in animal models and IBD patients (53). The production and signalling of IL-6 is increased in the inflamed mucosa in UC as well as other rheumatological diseases. Furthermore, studies have

shown that in the active disease phase, levels of IL-6 production in stimulated monocytes are significantly increased in patients with IBD compared to inactive disease phases or controls (54,55). Moreover, the use of the neutralising anti-IL-6R antibody tocilizumab, a robust inhibitor of IL-6/signal transducer and activator of transcription 3 (STAT3) activity, has shown normalisation of the acute phase response (including CRP) in rheumatoid arthritis patients, as well as demonstrating promising results in a pilot phase II study for active CD (56).

Role of IL-6, IL-6R and gp130 in ulcerative colitis

During disease remission, levels of IL-6 usually range from 1–5 pg/ml, but it has been shown that these levels can increase significantly to several µg/ml in patients with active inflammation (57). Whilst such dramatic increases have been demonstrated for IL-6, one of the major inflammatory mediators, other gp130-related cytokines do not seem to follow the same pattern (57). IL-6 uses gp130 as a signal transducer within a complex signalling cascade, which can be either pro- or anti-inflammatory (58,59).

IL-6 signalling can be divided into two mechanisms: (i) classic signalling, which is thought to be mainly anti-inflammatory and protective by stimulating intestinal regeneration, inhibiting epithelial cell apoptosis, defending against bacterial infections and inducing hepatic acute phase response, and (ii) trans-signalling, which is responsible for the recruitment of mononuclear cells, inhibition of T-cell apoptosis and inhibition of the differentiation of regulatory T-cells particularly in chronic inflammation (60) (Figure 3). The type of signalling depends on the presence of the IL-6R, which is only present on the surface of a limited number of cells such as hepatocytes and leukocytes, whereas the gp130 signal transducer is expressed ubiquitously throughout the body. In classic signalling, IL-6 binds to its membrane bound cell surface receptor and forms an IL-6/IL-6R complex, which then in turn forms a complex with preformed dimers of gp130. However, this type of signalling is limited to cells that express IL-6R on their cell surface. IL-6 is also able to bind to the soluble form of the IL-6R (sIL-6R), which has been shed from the cell membrane (in most cases) or produced by alternative splicing. The IL-6/sIL-6R complex can bind to gp130 dimers and initiate intracellular signalling even on cells that do not express the membrane-bound IL-6R, which vastly enlarges the scale of IL-6 signalling. Intracellular signalling, activated by the IL-6/IL-6R/gp130 complex, takes place mainly via the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway and the mitogen-activated protein kinase (MAPK)

pathway (58). Previous studies investigating the IL-6/p-STAT3 expression in epithelial cells of biopsy specimens from UC patients with active disease showed considerably raised levels compared to controls (61).

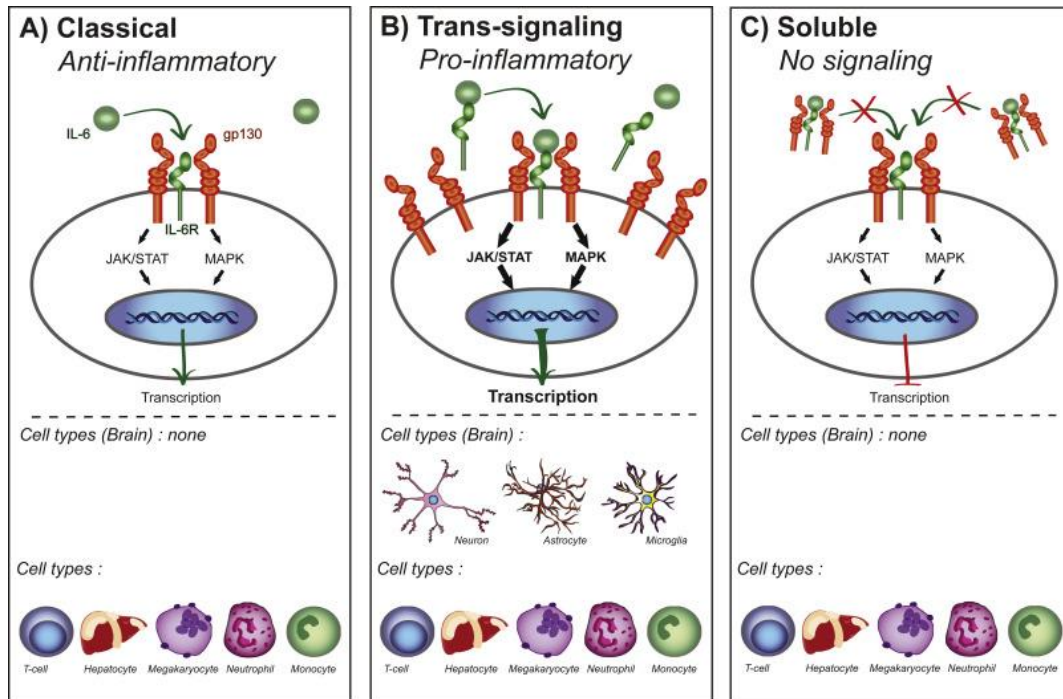


Figure 3 Types of IL-6 signalling (60).

To maintain homeostasis, soluble forms of gp130 (sgp130) in human serum at concentrations of up to 400 ng/ml (62) buffer the IL-6/sIL-6R complex and hence inhibit the trans-signalling pathway (59,63,64). Differential mRNA splicing and alternative polyadenylation (65) leads to a natural variety of sgp130 variants in human serum, and hence a spectrum of IL-6 trans-signalling inhibition effectiveness. Considering this, it has been suggested that the different variants of sgp130 exist to modulate the degree of IL-6 trans-signalling inhibition (58,66) whilst avoiding a dampening effect on classical signalling by sequestration of IL-6 in IL-6/sIL-6R/sgp130 complexes at high levels of sgp130 (67). In UC patients who are in remission, it has been shown that levels of IL-6 remain in the low picogram range, whilst levels of sIL-6R and sgp130 are significantly higher (low to mid nanogram range (68).

Due to the different pattern of distribution of IL-6R, sIL-6R and gp130, pro-inflammatory trans-signalling increases the types of cells through which IL-6 can signal. Specific inhibition of trans-signalling, not affecting the anti-inflammatory activities of IL-6 via classic signalling using an Fc-dimerized version of sgp130 (sgp130Fc) has already been shown to be effective in several inflammatory diseases and cancer (69) and has shown superior survival rates in a

murine sepsis model compared to global blockade of IL-6 signalling using neutralising antibodies (64). As such, selective blockade of IL-6 trans-signalling could prove an invaluable therapeutic instrument in the treatment of UC.

Numerous studies have described significantly elevated levels in circulating IL-6 and sIL-6R in UC patients, although with a wide range of concentrations (Table 5). Moreover, IL-6 and sIL-6R serum levels have been found to be positively associated both with activity indices and biomarkers of UC. This being said, associations of biomarkers in UC of IL-6, sIL-6R and sgp130 are inconsistent (Table 5) and warrant a systematic analysis on a larger scale.

Publication	Groups	IL-6 (pg/mL) [median (range)] [mean \pm SEM]	sIL-6R (ng/mL)	sgp130 (ng/mL) [median (range)]	Selected correlations ($p < 0.05$) \ddagger
Mahida <i>et al.</i> 1991 (70)	Healthy controls (n = 16) Active UC (n = 20)	<20 (<20–30) <20 (<20–80)	ND ND	ND ND	ND
Hyams <i>et al.</i> 1993 (71)	Healthy controls (n = 29) Paediatric UC (n = 26)	0.4 \pm 0.2 1 \pm 0.3 \dagger	ND ND	ND ND	Significant association between IL-6 levels and disease activity.
Mitsuyama <i>et al.</i> 1995 (72)	Healthy controls (n = 69) Inactive UC (n = 16) Active UC (n = 16)	ND 4 \pm 1 10 \pm 9*	107 \pm 2 116 \pm 7 148 \pm 5* \dagger	ND ND ND	(+) IL-6 & CRP; (+) IL-6 & leukocyte counts; (+) sIL-6R & CRP.
Gustot <i>et al.</i> 2005 (68)	Healthy controls (n = 15) Inactive UC (n = 11) Active UC (n = 13)	2 (0–4) 6 (0–12) 21 (0–293)* \dagger	33 (21–50) 35 (21–59) 37 (22–72)	273 (213–375) 265 (182–437) 176 (96–374)* \dagger	(–) sgp130 & CRP.
Mitsuyama <i>et al.</i> 2006 (73)	Healthy controls (n = 17) Inactive UC (n = 13) Active UC (n = 14)	ND ND ND	ND ND ND	43 (27–59) 81 (25–137) \dagger 94 (26–125) \dagger	ND

Table 5 Circulating levels of IL-6, sIL-6R and sgp130 in patients with ulcerative colitis (UC). All numbers above 1 were rounded to the nearest integer. * $p < 0.05$ or less vs. inactive disease. $\dagger p < 0.05$ or less vs. healthy controls. \ddagger Positive (+) or negative (–) correlations between the indicated parameter combinations.

Methods

Background

This study was carried out in a collaboration with CONARIS Research Institute AG (CONARIS), a spin-off company from the University Hospital Schleswig-Holstein (UKSH) that is mainly involved in preclinical and early-stage drug development and has developed olamkicept (sgp130Fc), the first specific trans-signalling inhibitor based on a prototype from Prof. Stefan Rose-John (Biochemical Institute, Kiel University). Existing serum samples from a previous study conducted by the UKSH on changing levels of tryptophan (74) as well as freshly collected serum samples from both healthy volunteers and patients with confirmed IBD were utilised. The control group was made up of 100 age-matched healthy individuals who had donated blood at the Department of Transfusion Medicine of the UKSH.

IBD patients were regularly clinically examined during their visits at the IBD outpatient clinic of the Department of Internal Medicine I at the UKSH. Their disease activity was evaluated by determining the Colitis Activity Index (CAI) and (Partial) Mayo Score and carrying out routine endoscopies at set intervals. Blood was drawn at each visit to monitor molecular markers of disease activity. Blood count measurements, liver parameters and CRP were determined by the in-house laboratory of the UKSH. Measurements of IL-6, sIL-6R and sgp130 were conducted by CONARIS.

CONARIS utilised enzyme-linked immunosorbent assays (ELISA) to determine the quantity of IL-6, sIL-6R and sgp130 contained within the serum samples. The measurements were carried out using the Quantikine kits from R&D Systems and took place on the same day for any given sample in order to obtain reliable results. To ensure accuracy, the Quantikine handbooks were followed precisely. To further ensure consistency of the ELISA results over the period of analysis, any changes in the kit instructions were recognised and employed in CONARIS' standard operating procedures.

Ethical considerations

Ethical approval

The ethics committee of the medical faculty of Kiel University granted ethical approval for this study (AZ D489/14). Any procedures conducted as part of this study that involved human participation were carried out in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

All participants who took part in this study gave informed consent and Kiel has recently implemented the ethical concept of healthcare-embedded biobanking with broad informed consents (75).

Sample specification

Study Overview

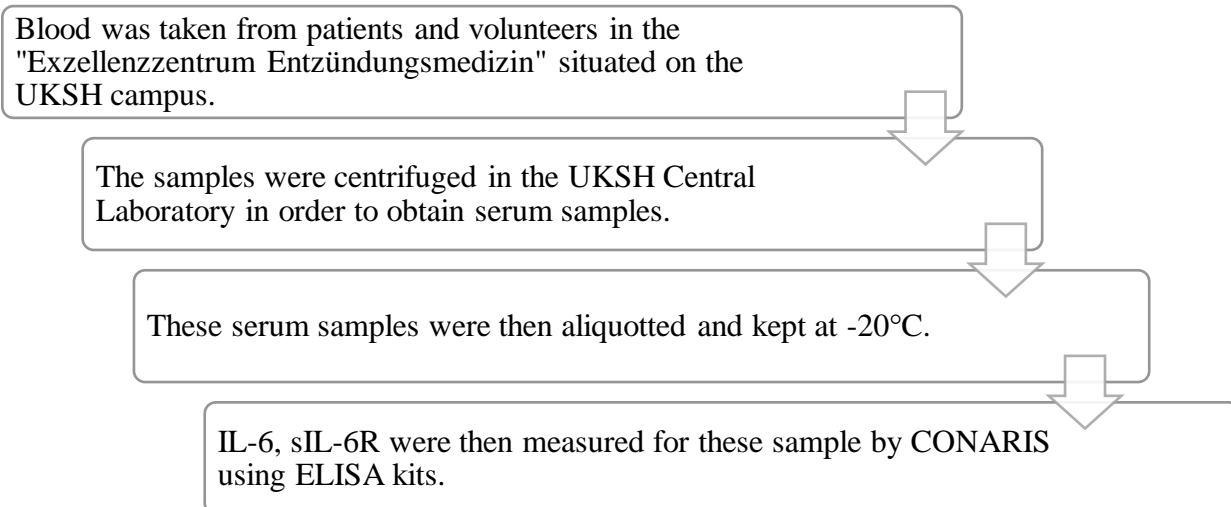


Figure 4 Overview of the study.

ELISA Testing

Principle

To determine the concentration of IL-6, sIL-6R and soluble gp130 in the serum samples of volunteers and UC patients in both Part 1 and Part 2, ELISA Quantikine sets from R&D Systems were used. These immunoassays use a method referred to as the quantitative sandwich enzyme technique. The technique uses a microplate to which IL-6, sIL-6R or soluble gp130

specific antibodies have been attached. As soon as the samples are added, any IL-6, sIL-6R or soluble gp130 contained within it will bind to the antibodies. The plate is then washed repeatedly to remove any excess material. In the next step, an enzyme-linked polyclonal antibody is added. After further washing steps to remove any excess enzyme-linked polyclonal antibody, a substrate solution is added. The substrate solution will undergo a colour change, depending on the amount of IL-6, sIL-6R or soluble gp130 that was initially bound to the plate. The enzymatic colour change is stopped, and the colour intensity is recorded.

Equipment

ELISA	Equipment
IL-6	Human IL-6 microplate, human IL-6 standard, human IL-6 conjugate, assay diluent RD1W, calibrator diluent RD6F, human IL-6 controls
sIL-6R	Human IL-6 sR microplate, human IL-6 sR standard, human IL-6 sR conjugate, assay diluent RD1-1, calibrator diluent RD5C concentrate, human IL-6 sR controls
sgp130	Human sgp130 microplate, human sgp130 standard, human sgp130 conjugate, assay diluent RD1W, calibrator diluent RD5P concentrate, human sgp130 controls
All ELISA testing	Wash buffer concentrate, colour reagent A, colour reagent B, stop solution, plate sealers, microplate reader, pipettes & pipette tips, deionized / distilled water, 500 mL graduated cylinder, squirt bottles / automated microplate washer, test tubes for dilution of standards and samples

Table 6 Preparations for ELISA for IL-6 (D6050, Quantikine® Human IL-6 Immunoassay)

Reagent Preparation

ELISA for IL-6 (D6050, Quantikine® Human IL-6 Immunoassay) (76)

Wash buffer	Wait until wash buffer concentrate has reached room temperature, then add 20 mL of concentrate to deionized / distilled water to make up 500 mL of wash buffer.
Substrate solution	Mix equal amounts of colour reagent A and colour reagent B up to 15 minutes prior to use (200 µL per well).
Human IL-6 Standard	Mix 5 mL of calibrator diluent RD6F together with the human IL-6 standard. This should produce a solution of 300 pg/mL. Allow to rest for 15 minutes and stir gently before use.

ELISA for sIL-6R (DR600, Quantikine® Human IL-6 sR Immunoassay) (77)

Wash buffer	Wait until wash buffer concentrate has reached room temperature, then add 20mL of concentrate to deionized / distilled water to make up 500 mL of wash buffer.
Substrate solution	Mix equal amounts of colour reagent A and colour reagent B up to 15 minutes prior to use and protect from light (200µL per well).

Calibrator Diluent RD5C (diluted 1:5)	For the preparation of 100 mL Calibrator Diluent RD5C (diluted 1:5), add 20 mL of Calibrator Diluent RD5C concentrate to 80 mL of deionised / distilled water.
Human IL-6sR Standard	Mix 5 mL of calibrator diluent RD5C (diluted 1:5) together with the human IL-6 R α standard. This should produce a solution of 2000 pg/mL. Allow to rest for 15 minutes and stir gently before use.
ELISA for sgp130(DGP00, Quantikine® Human soluble gp130 Immunoassay) (78)	
Wash buffer	Wait until wash buffer concentrate has reached room temperature, then add 20 mL of concentrate to deionized / distilled water to make up 500 mL of wash buffer.
Substrate solution	Mix equal amounts of colour reagent A and colour reagent B up to 15 minutes prior to use (200 μ L per well).
Calibrator Diluent RD5P (diluted 1:5)	For the preparation of 100 mL Calibrator Diluent RD5P (diluted 1:5), add 20 mL of Calibrator Diluent RD5P concentrate to 80 mL of deionised / distilled water.
Human sgp130 Standard	Mix 5 mL of calibrator diluent RD5C (diluted 1:5) together with the human sgp130 standard. This should produce a solution of 16 ng/mL. Allow to rest for 15 minutes and stir gently before use.

Table 7 Preparation for ELISA testing.

Procedure

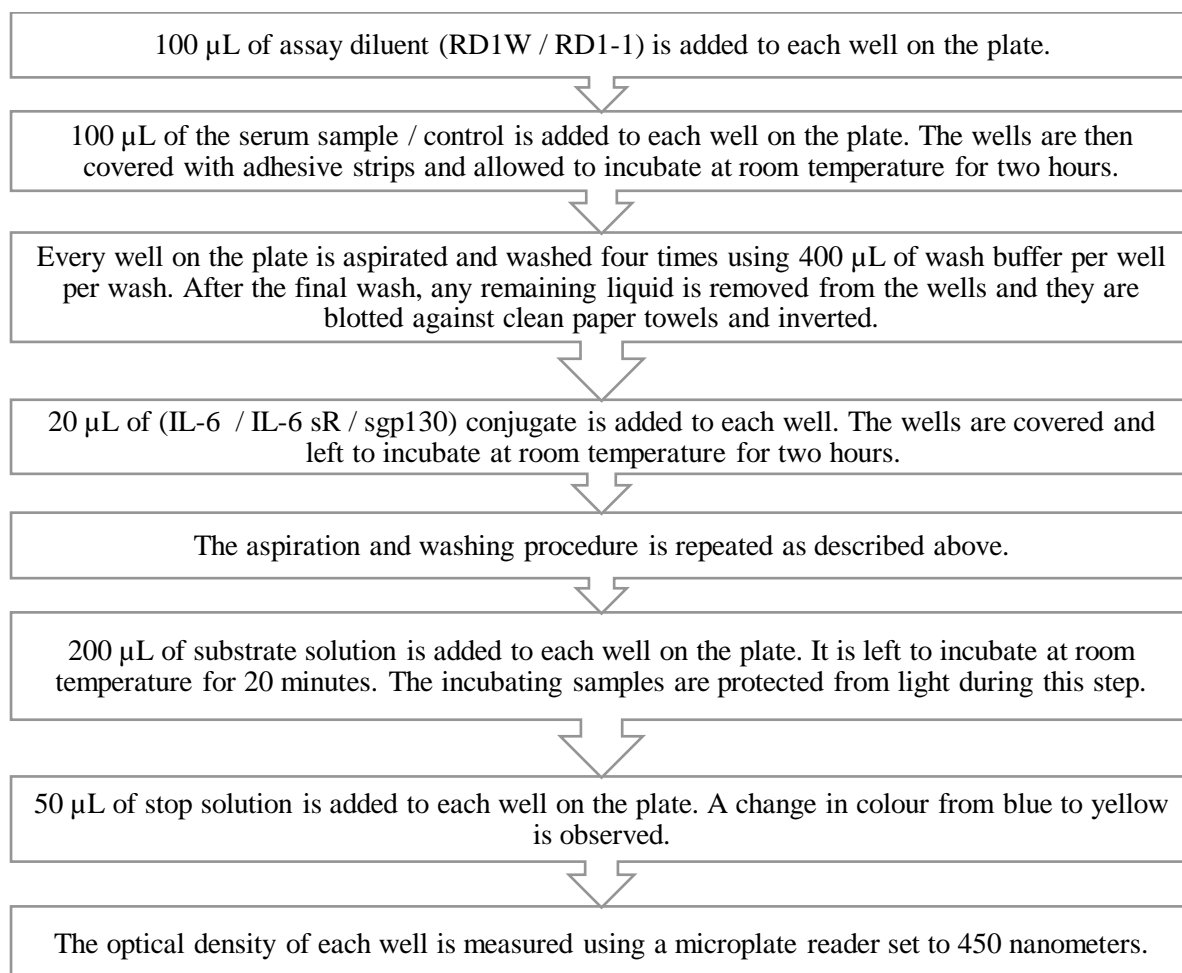


Figure 5 Procedure for the ELISA testing.

Calculation of results

Any duplicate readings were averaged, and the average zero standard optical density was subtracted (OD). Utilising a computer software which was able to produce a four-parameter logistic (4-PL) curve fit, a standard curve was created. For the eight diluted samples, the reading off the curve was corrected using the dilution factor.

Sensitivity

For this method, the minimum detectable dose (MDD) for human IL-6 is approximately 0.70 pg/mL, for human IL-6 sR is 6.5 pg/mL and for sgp130 is approximately 0.08 ng/mL according to the manufacturer (76–78).

Data analysis and statistics

Table 8 encompasses the parameters studied.

Data group	Parameter
General data	Age at sampling [y]
	Allergy/asthma
	Appendectomy
	Arthritis as a comorbidity
	Body mass index (BMI), sorted into overlapping groups: group 1: high ($\text{BMI} \geq 25$) or normal ($18.5 \leq \text{BMI} < 25$) group 2: low (< 18.5) or normal ($18.5 \leq \text{BMI} < 25$)
	Food allergy
	Gender
	Positive family history of inflammatory bowel diseases
	Smoker (current)
	Smoker (former or current)
	Surgery on gastrointestinal tract (ever)
	Time after diagnosis [y]
Activity (UC)	Colitis Activity Index (CAI)
	Mayo Score
	Partial Mayo Score (PMS)
Laboratory data	Alanine transaminase (ALT; formerly glutamic-pyruvic transaminase, GPT) [U/L]
	Aspartate transaminase (AST; formerly glutamic-oxaloacetic transaminase, GOT) [U/L]
	C-reactive protein (CRP) [mg/L]
	Creatinine [mg/dL]
	Haematocrit [vol%]
	Haemoglobin [g/dL]
	Interleukin-6 [pg/mL]
	Leukocytes [/nL]
	Soluble glycoprotein 130 [ng/ml]
	Soluble interleukin-6 receptor [ng/ml]
	Thrombocytes [/nL]
Medication	Amino salicylate therapy
	Immunosuppressive therapy
	Monoclonal antibody therapy, except for anti-tumour necrosis factor-alpha (TNF- α) antibodies
	Steroid therapy
	TNF- α inhibitor therapy

Table 8 General, clinical and laboratory parameters.

More than half of the IL-6 measurements were found to lie below the detection limit of the Quantikine® Human IL-6 Immunoassay. Subsequently, each IL-6 measurement was allocated one of three categories: (1) below the detection limit of 3.13 pg/mL, (2) between the detection limit and the third quartile and (3) above the third quartile. Any statistical analysis carried out compared group 1 to group 3 and utilised all the data obtained previously.

For the analysis of associations with clinical and serological parameters, all available data was used. Univariate mixed models with the independent variable (*i.e.* clinical parameter) as the fixed effect and the patient identity code as random effect were employed to account for any multiple observations per patient. For the continuous variables sIL-6R and sgp130, linear mixed models were fitted while the ordinal variable IL-6 required logistic regression models to be estimated. Dichotomisation of all independent continuous variables was based on the median for all mixed model analyses.

Moreover, a comparison of IL-6, sIL-6R and sgp130 was carried out within patients, between patient groups and between patients and healthy controls. To determine the active and/or inactive time points for each patient, disease activity scores (active: CAI >4 points; extremely active: CAI >6 points; inactive: CAI ≤4 points) and CRP levels (active: CRP >5 mg/L; inactive: CRP ≤5 mg/L) were used. Subsequently, the dataset was divided into two cohorts; a full dataset/cohort (all active and inactive time points from all patients) and a reduced data set/cohort (most active and most inactive time point from patients if both of these were available). Table 9 displays the number of patients and measurements for the full dataset and the reduced data set.

Group	UC: no. of patients	UC: no. of measurements
All	166	514
All: activity by score	163	495
All: activity by CRP	166	514
Subset: activity by score	32	64
Subset: activity by CRP	41	82

Table 9 Number of patients and measurements per cohort and sub-cohort.

A nonparametric bootstrap approach was employed to compare active, inactive and healthy control samples. Sampling with replacement was performed on the proband level, and a suitable test statistic was calculated on each bootstrap sample. Confidence intervals were

estimated using the 2.5% and 97.5% quantiles of the distribution of the bootstrap test statistics. P-values were determined by 2* the proportion of bootstrap test statistics larger or smaller than zero, depending on the sign of the test statistic based on the original sample. As test statistics, the difference in medians or the median of the differences for the continuous variables sIL-6R and sgp130 in the unpaired or paired comparisons were utilised. The difference in proportions or the proportion of disagreement were applied for IL-6.

Statistical analysis was conducted by Dr. Silke Szymczak at the Institute of Medical Informatics and Statistics of Kiel University. All statistical tests were carried out two-sided and a nominal significance level of 5% was used. The statistical software R (version 3.3.1) was used to perform analyses. Linear mixed models were estimated using R packages name (version 3.1-128) and MuMIn (version 1.15.6) and the variance explained by the fixed effect (= marginal R²). Data in boxplots was presented using the standard settings in R: the box represents the interquartile range, the bold line signifies the median, the whiskers at either side of the box denote the highest and lowest values within the 1.5-fold of the interquartile range, and any values outside this range are shown as single dots.

Results

Overall, 166 UC patients and 100 controls took part in this study. Characteristics of study participants are summarised in Table 10 below.

	Ulcerative colitis	Controls
Number of individuals	166	100
Number of visits	2 (1–20)	1
Males	45.1%	68.00%
Age at sampling [y]	42.5 (17–78)	44 (19–68)
Body mass index [kg/m ²]	25.3 (16.8–48.3)	ND
Weight [kg]	78 (41–148)	ND
Smokers	37.9%	ND
Time after diagnosis [years]	4.5 (0–41)	N/A
Crohn's Disease Activity Index (CDAI)	N/A	N/A
Harvey-Bradshaw Index (HBI)	N/A	N/A
Colitis Activity Index (CAI)	5 (0–17)	N/A
Mayo Score	3 (0–11)	N/A
Partial Mayo Score (PMS)	2 (0–9)	N/A
Aminosalicylate therapy	91.0%	N/A
Immunosuppressive therapy	19.0%	N/A
Monoclonal antibody therapy, except for anti-tumour necrosis factor-alpha (TNF- α) antibodies	3.2%	N/A
Steroid therapy	63.6%	N/A
Tumour necrosis factor-alpha (TNF- α) inhibitor therapy	54.1%	N/A

Table 10 Characteristics of UC patients and healthy controls. All values in this format represent median (range).

IL-6, sIL-6R and sgp130 levels in the complete patient cohorts

The levels of IL-6, sIL-6R and sgp130 measured in this study are displayed in Table 11. The table acts as an overview and levels of IL-6, sIL-6R and sgp130 from this study can be compared with levels from the literature provided in Table 5.

Groups	IL-6 (pg/mL)	sIL-6R (ng/mL)	sgp130 (ng/mL)
Controls	$\leq 3^{\ddagger}$ ($\leq 3-6$) (n = 100)	35 (20–53) (n = 100)	217 (136–432) (n = 100)
Inactive UC	≤ 3 ($\leq 3-27$) [†] (n = 222)	39 (19–87) [†] (n = 222)	197 (112–306) (n = 32)
Active UC	≤ 3 ($\leq 3-195$) ^{*†} (n = 273)	39 (15–83) [†] (n = 273)	249 (115–341) (n = 41)

Table 11 Circulating levels of IL-6, sIL-6R and sgp130. All values represent median (range) and were rounded to the nearest integer. * $p < 0.05$ or less for active (CAI > 4) vs. inactive disease (CAI ≤ 4). [†] $p < 0.05$ or less vs. healthy controls. [‡]Detection limit of the IL-6 ELISA: 3.13 pg/mL. IL-6 and sIL-6R were measured in a total of 166 patients with UC; sgp130 was measured in 22 patients with UC.

Due to the large proportion of IL-6 measurements below the detection limit of 3.13 pg/mL, ordinal IL-6 data was used for further analysis (Table 12). Statistical evaluation compared the group with values below the detection limit with the top quartile group. The control group has the largest proportion of measurements below the detection limit and whilst there are still more than 50% of measurements below the IL-6 detection limit in the UC group, the proportion of values above the detection limit is greater; this is illustrated in Figure 6a.

Ordinal groups for IL-6 Control	Control	Ulcerative colitis
Values < detection limit (3.13 pg/mL)	0.97	0.66
Detection limit \leq values \leq 3rd quartile	0.01	0.09
3 rd quartile < values	0.02	0.25

Table 12 Ordinal groups of IL-6 and corresponding levels across all groups.

Median levels of IL-6 were found to be below 3.13 pg/mL across all groups, but the range for active and inactive UC was greater compared to controls (Table 13). Hence, quantitative IL-6 data was log transformed in Figure 6b. The median and interquartile range for both sIL-6R (Figure 6c) and sgp130 (Figure 6d) were greater for UC compared to the control group. Despite this, median levels for sIL-6R and sgp130 were not increased by more than 15% compared to controls and the increase in median was not consistent with disease activity (Figure 6c, d, Table 11).

Variables	Control	Ulcerative colitis
Interleukin-6 (logarithmic; pg/mL)	0.5 (0.5–0.8)	0.5 (0.5–2.29)
Soluble interleukin-6 receptor (ng/mL)	35 (20–53)	39 (15–87)
Soluble glycoprotein 130 (ng/mL)	217 (136–432)	229 (112–341)

Table 13 Logarithmic levels of IL-6, sIL-6R and sgp130. All values represent median (range) and were rounded to the nearest integer.

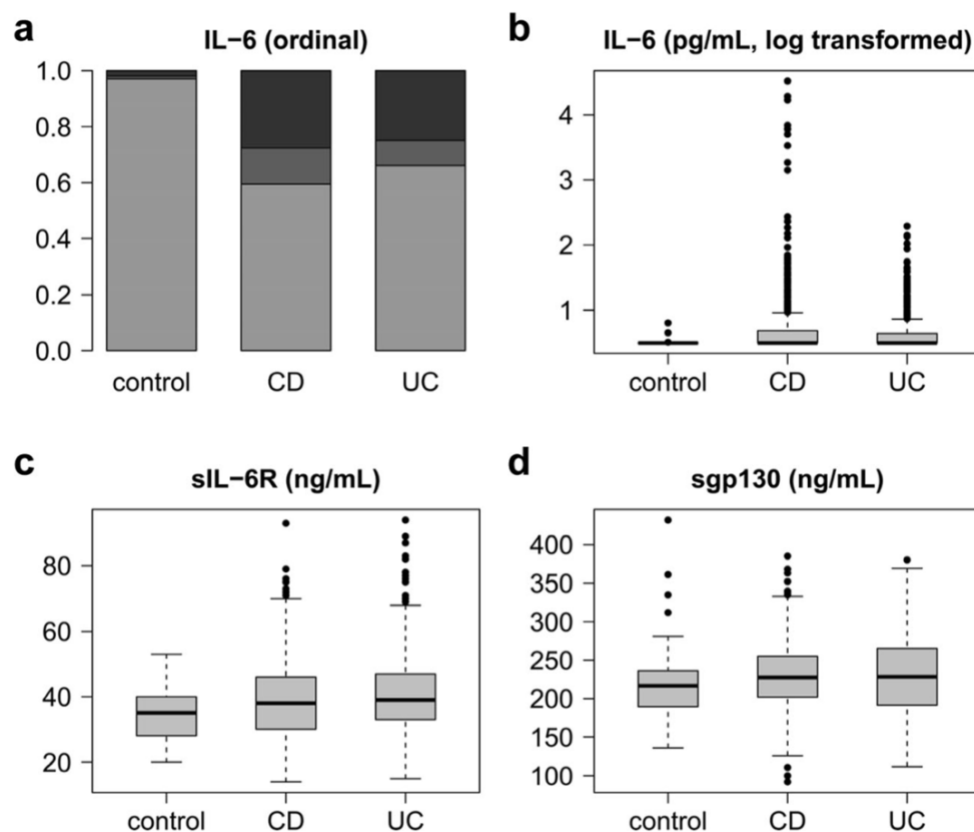


Figure 6 Overview of IL-6, sIL-6R and sgp130 serum levels in healthy controls and patients with Crohn's disease (CD) or ulcerative colitis (UC). a Ordinal representation of IL-6 levels across all groups: below the detection limit of 3.13 pg/mL (light grey), between the detection limit and the third quartile (grey) and above the third quartile (black). b Logarithmic representation of IL-6 levels. c sIL-6R levels. d sgp130 levels (79).

Subgroup analysis based on disease activity scores

The analysis of patient subgroups based on their disease activity scores revealed a significant association with regards to IL-6 (Table 14, Figure 7). The data shows that significantly more UC patients had IL-6 levels in the upper quartile when compared to controls; this is true for both active and inactive disease. Likewise, UC patients with active disease (CAI > 4) had a significantly greater proportion of IL-6 measurements in the upper quartile versus patients with inactive disease (CAI < 4). In a small group of patients with moderate to severe disease activity scores (CAI > 6), levels of IL-6 were higher than the sum of IL-6 levels from all active patients, which also included patients with mild activity. (Figure 7a). Despite this, more than 50% of active patients did not show elevated levels of IL-6. With regards to sIL-6R, levels were significantly higher in UC patients versus controls, but this change was independent of disease activity according to disease activity scores (Figure 7c). Levels of sgp130 did not differ significantly between UC patients and controls nor between active and inactive disease status (Figure 7d).

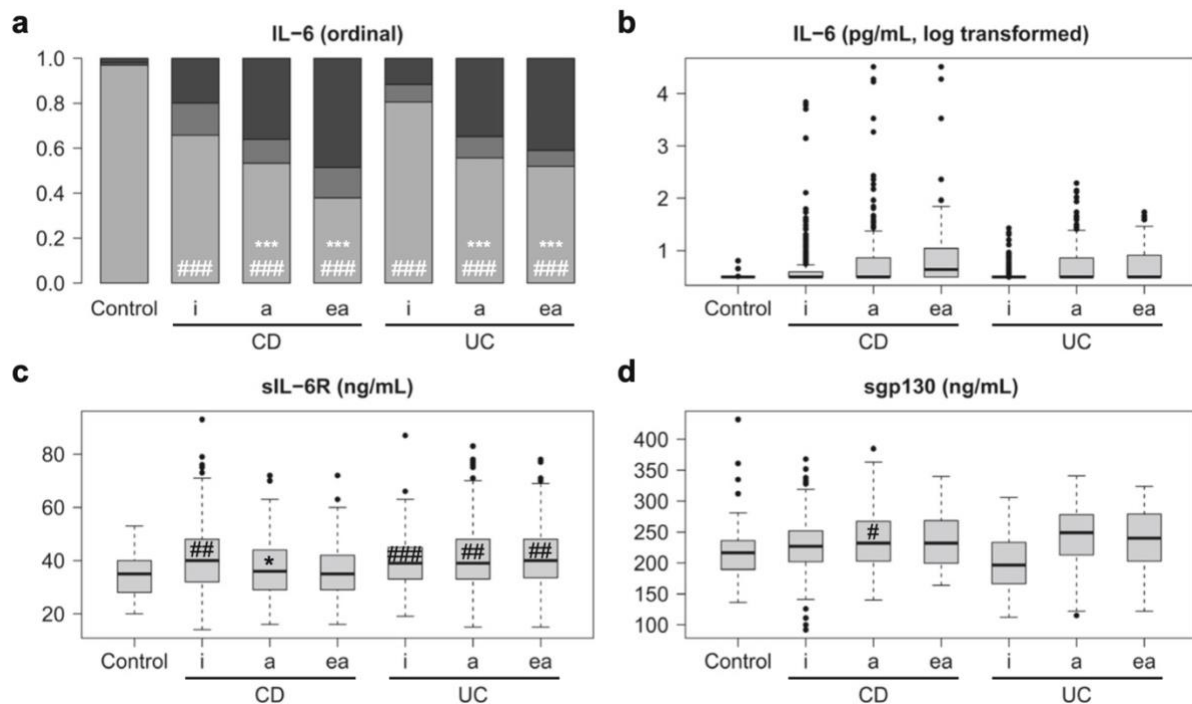


Figure 7 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in all patients with Crohn's disease (CD) or ulcerative colitis (UC) differentiated according to disease activity scores. (A) Ordinal representation of IL-6 levels across all groups: below the detection limit of 3.13 pg/mL (light grey), between the detection limit and the third quartile (grey) and above the third quartile (black). For significance analyses, the two extreme groups were compared. (B) Logarithmic representation of IL-6 levels. (C) sIL-6R levels. (D) sgp130 levels. a, active disease [Crohn's Disease Activity Index (CDAI) > 150 or Colitis Activity Index (CAI) > 4]; ea, extremely active disease (CDAI > 250 or CAI > 6); i, inactive disease (CDAI ≤ 150 or CAI ≤ 4). Significant differences between inactive and active disease: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Significant differences between inactive or active disease and controls: #, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$ (79).

	Active vs controls			Inactive vs controls			Inactive vs active			Enhanced activity vs controls			Inactive vs enhanced activity		
Var.	Est.	95% CI	p	Est.	95% CI	p	Est.	95% CI	p	Est.	95% CI	p	Est.	95% CI	p
IL-6 o.	0.36	0.26–0.46	<0.001	0.11	0.05–0.16	<0.001	0.36	0.18–0.55	<0.001	0.42	0.29–0.55	<0.001	0.51	0.24–0.75	<0.001
sIL-6R	4.00	1–10	0.006	4.00	1.5–9	<0.001	1.00	–3–5	0.51	5.00	1–11	0.004	1.00	–3–5	0.47
sgp130	32.50	–18–49	0.092	–20.00	–51–26	0.17	18.00	–26–36	0.23	23.50	–22–50	0.18	27.00	–34–41	0.13

Table 14 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in all patients with ulcerative colitis (UC) differentiated according to disease activity scores. All data except for small p values were rounded to the second decimal place.

Subgroup analysis based on CRP

The aforementioned associations between IL-6 and disease activity were even more pronounced when disease activity was assessed using CRP levels. Whilst there was no significant difference in the levels of IL-6 between inactive UC patients and controls, more than 50% of active UC patients had IL-6 levels in the upper quartile (Table 15, Figure 8a). While the increase in sIL-6R levels is only minor and largely independent of disease activity, it is more noticeable when disease activity is expressed in terms of CRP levels rather than disease activity scores.

	Active vs controls			Inactive vs controls			Inactive vs active		
Var.	Est.	95% CI	p	Est.	95% CI	p	Est.	95% CI	p
IL-6 o.	0.62	0.5–0.73	<0.001	0.04	–0.01–0.09	0.072	0.36	0.18–0.55	<0.001
sIL-6R	4.00	0–9.5	0.024	5.00	2–9	<0.001	0.00	–2–3	0.86
sgp130	11.50	–41–46	0.63	12.50	–10–45	0.19	–12.00	–29–5	0.44

Table 15 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in all patients with ulcerative colitis (UC) differentiated according to CRP levels. All data except for small p values were rounded to the second decimal place.

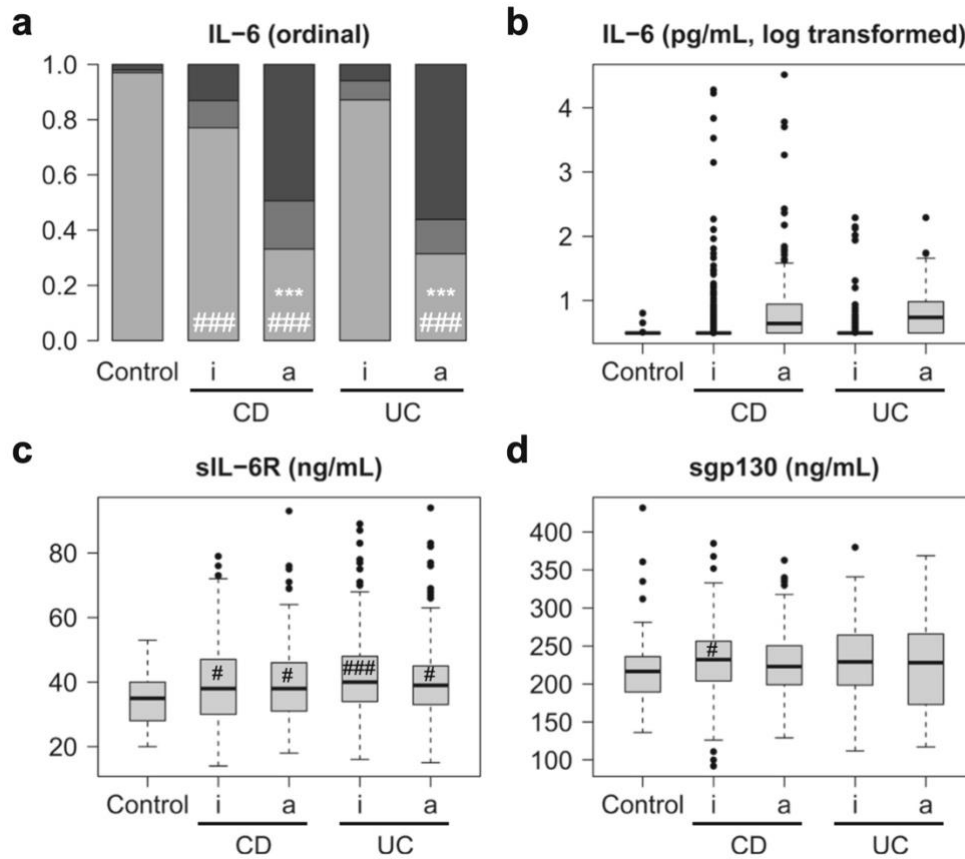


Figure 8 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in all patients with Crohn's disease (CD) or ulcerative colitis (UC) differentiated according to C-reactive protein (CRP) levels. (A) Ordinal representation of IL-6 levels across all groups: below the detection limit of 3.13 pg/mL (light grey), between the detection limit and the third quartile (grey) and above the third quartile (black). For significance analyses, the two extreme groups (below the detection limit and above the third quartile) were compared. (B) Logarithmic representation of IL-6 levels. (C) sIL-6R levels. (D) sgp130 levels. a, active disease (CRP > 5 mg/L); i, inactive disease (CRP ≤ 5 mg/L). Significant differences between inactive and active disease: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Significant differences between inactive or active disease and controls: #, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$ (79).

IL-6, sIL-6R and sgp130 levels in sub-cohorts with matched measurements

Data from the most active and the most inactive time point, based on disease activity score or CRP levels, from patients for whom both active and inactive time points were available (reduced dataset) were analysed to ensure that patients with inactive disease and multiple visits were not confounding conclusions drawn from the complete data set (Table 9). For patients with more than one active or inactive timepoint, the highest and lowest disease activity scores (Table 16, Figure 9) or CRP levels (Table 17, Figure 10) were selected for statistical analysis. This subcohort analysis revealed similar results to those of the full dataset (Figure 9 and Figure 10 cf. Figure 7 and Figure 8).

Var.	Active vs controls			Inactive vs controls			Inactive vs active		
	Est.	95% CI	p	Est.	95% CI	p	Est.	95% CI	p
IL-6 o.	0.42	0.22–0.61	<0.001	0.15	0.02–0.29	0.02	0.42	0.16–0.73	<0.001
sIL-6R	2.50	-2.5–11	0.41	5.00	1–10	0.038	1.00	-5–5	0.78
sgp130	-10.50	-68–100	0.85	-5.50	-99–200	0.61	N/A	N/A	N/A

Table 16 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in selected patients with UC differentiated according to disease activity scores. All data except for small *p* values were rounded to the second decimal place.

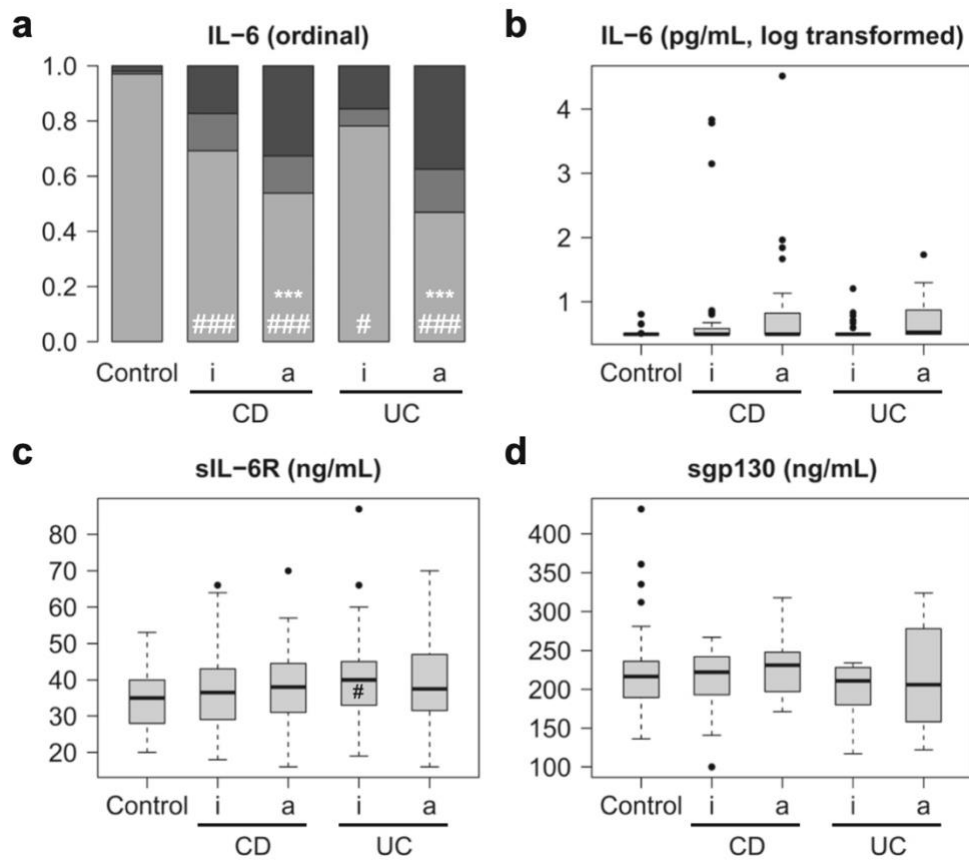


Figure 9 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in selected patients with ulcerative colitis (UC) differentiated according to disease activity scores. Only patients with at least one time point with active and one with inactive disease were included. If more than one time point per condition was available, only data from the two most extreme time points (highest and lowest disease activity score) were included. (A) Ordinal representation of IL-6 levels across all groups: below the detection limit of 3.13 pg/mL (light grey), between the detection limit and the third quartile (grey) and above the third quartile (black). For significance analyses, the two extreme groups (below the detection limit and above the third quartile) were compared. (B) Logarithmic representation of IL-6 levels. (C) sIL-6R levels. (D) sgp130 levels. a, active disease [Crohn's Disease Activity Index (CDAI) > 150 or Colitis Activity Index (CAI) > 4]; ea, extremely active disease (CDAI > 250 or CAI > 6); i, inactive disease (CDAI ≤ 150 or CAI ≤ 4). Significant differences between inactive and active disease: *, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.001. Significant differences between inactive or active disease and controls: #, *p* < 0.05; ##, *p* < 0.01; ###, *p* < 0.001 (79).

Var.	Active vs controls			Inactive vs controls			Inactive vs active		
	Est.	95% CI	p	Est.	95% CI	p	Est.	95% CI	p
IL-6 o.	0.66	0.5–0.8	<0.001	0.03	-0.04–0.12	0.43	0.63	0.36–0.85	<0.001
sIL-6R	1.00	-2–7	0.37	3.00	-1–9	0.13	-1.00	-4–2	0.48
sgp130	6.00	-47–66	0.86	11.50	-11–66	0.12	-5.50	-36–4	0.78

Table 17 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in selected patients with UC differentiated according to CRP levels. All data except for small *p* values were rounded to the second decimal place.

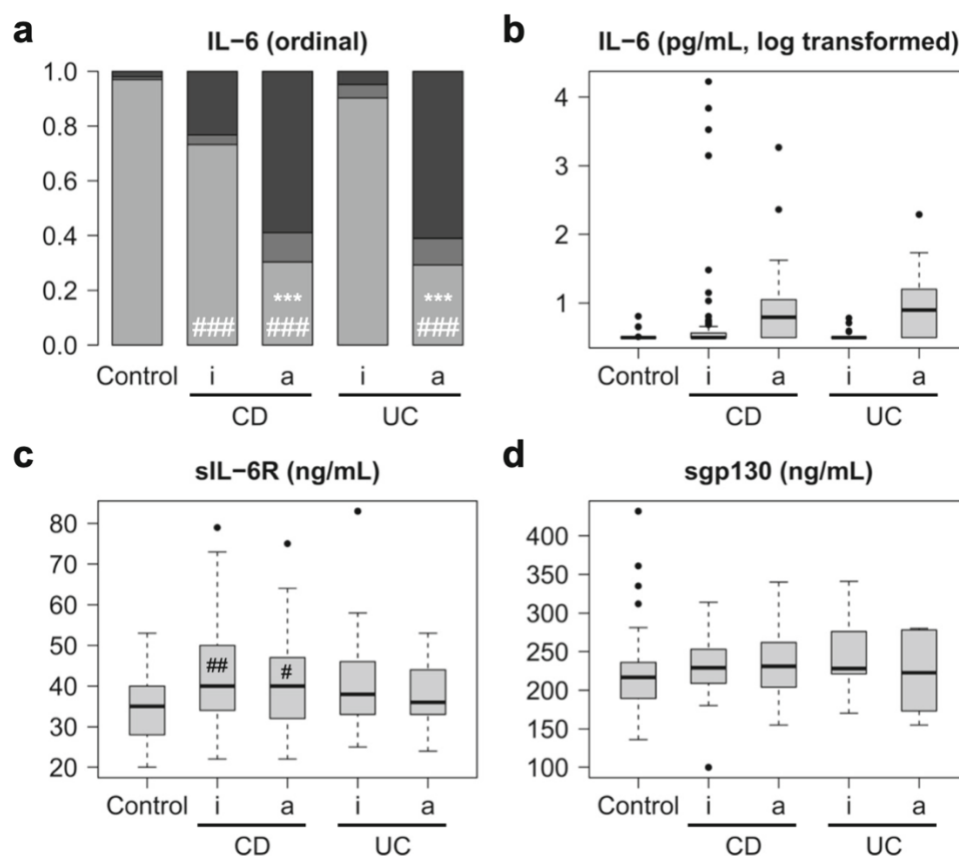


Figure 10 IL-6, sIL-6R and sgp130 serum levels in healthy controls and in selected patients with ulcerative colitis (UC) differentiated according to C-reactive protein (CRP) levels. Only patients with at least one time point with CRP > 5 mg/L and one time point with CRP ≤ 5 mg/L were included. If more than one time point per condition was available, only data from the two most extreme time points (highest and lowest CRP levels) were included. (A) Ordinal representation of IL-6 levels across all groups: below the detection limit of 3.13 pg/mL (light grey), between the detection limit and the third quartile (grey) and above the third quartile (black). For significance analyses, the two extreme groups (below the detection limit and above the third quartile) were compared. (B) Logarithmic representation of IL-6 levels. (C) sIL-6R levels. (D) sgp130 levels. a, active disease (CRP > 5 mg/L); i, inactive disease (CRP ≤ 5 mg/L). Significant differences between inactive and active disease: *, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.001. Significant differences between inactive or active disease and controls: #, *p* < 0.05; ##, *p* < 0.01; ###, *p* < 0.001 (79).

In the subcohort analysis, there was only a minor increase in levels of sIL-6R and gp130. Furthermore, serum levels of IL-6 were significantly higher in patients with active disease versus inactive disease with regards to both CRP levels and disease activity scores. Nonetheless, a large proportion of patients with active disease presented with IL-6 serum levels within the normal range.

While there was no statistically significant increase in sIL-6R or sgp130, the increase in the serum levels of IL-6 was both biologically and statistically significant on a population level. With regards to disease activity, IL-6 levels were detectable in most UC patients with active disease according to CRP levels (Figure 7a and Figure 9a), whereas less than 50% of patients with active disease, in terms of disease activity scores, showed detectable level of IL-6 (Figure 8a and Figure 10a).

Association of IL-6, sIL-6R and sgp130 levels with general, clinical and laboratory parameters

To identify associations of ordinal IL-6, sIL-6R and sgp130 serum levels with a wide variety of parameters for UC, an association heat map was created (Figure 11, Table 8, Table 18, Table 19, Table 20 and Table 21). Indicators of UC disease activity (colitis activity index, mayo score and partial mayo score) were all significantly associated with serum levels of IL-6. Furthermore, CRP, laboratory parameters linked to disease activity, leukocytes and thrombocytes were found to be positively associated with serum levels of IL-6, whereas haematocrit and haemoglobin showed a negative association (Figure 11, Table 18). Even though a decrease in the serum levels of IL-6 was associated with levels of sIL-6R and sgp130, this association was not significant (Figure 11, Table 18, Table 19 and Table 20) and the aforementioned IL-6 buffering ability of sIL-6R and sgp130 was underscored by the significant positive association between the two buffer components (Figure 11, Table 19 and Table 20). Liver function tests in the form of alanine transaminase and aspartate transaminase were observed to be significantly positively associated with sgp130 (Figure 11, Table 20), while IL-6 was significantly negatively associated with alanine transaminase (Figure 11, Table 18). Sgp130 was also significantly increased in current or former smokers (Figure 11, Table 20). Patients with a high ($BMI \geq 25$) or normal ($18.5 \leq BMI < 25$) BMI demonstrated significantly increased levels of sIL-6R (Figure 11, Table 19). Also, patients treated with monoclonal antibodies exhibited significantly lower levels of sIL-6R (Figure 11, Table 19), whilst the use

of amino salicylates was significantly associated with reduced IL-6 levels (Figure 11, Table 18). Finally, in terms of disease location, no significant associations were found for IL-6, sIL-6R or sgp130 levels (Table 21).

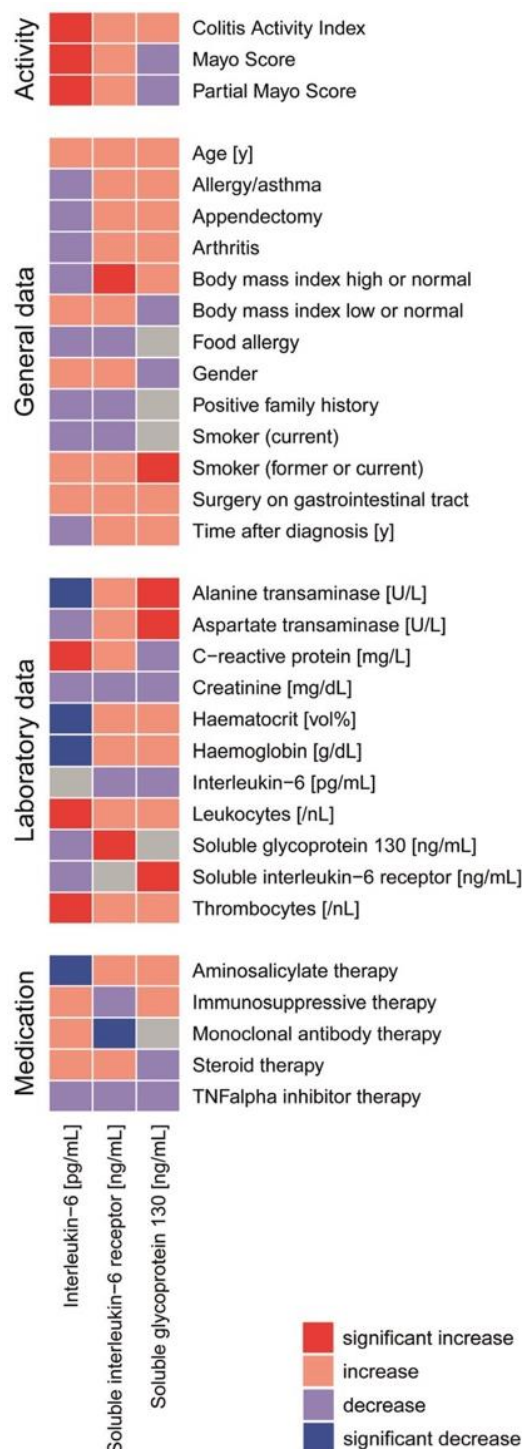


Figure 11 Association heat maps for ulcerative colitis. Tendentially or significantly positive (increase) or negative (decrease) associations of ordinal interleukin-6, soluble interleukin- 6 receptor or soluble glycoprotein 130 serum levels with other parameters are visualised by colour code. Grey fields indicate parameters with only one characteristic value or self-associations (79).

Data Group	Parameter	OR	95% CI	P-value
General data	Age at sampling [y]	1.23	0.50 – 3.03	0.66
	Allergy/asthma	0.22	0.04 – 1.35	0.079
	Appendectomy	0.55	0.06 – 5.46	0.61
	Arthritis as a comorbidity	0.58	0.23 – 1.47	0.23
	Body mass index (BMI) high (BMI ≥ 25) or normal ($18.5 \leq \text{BMI} < 25$)	0.74	0.32 – 1.72	0.49
	Body mass index low (<18.5) or normal ($18.5 \leq \text{BMI} < 25$)	18.24	0.65 – 511.66	0.052
	Food allergy	0.48	0.03 – 6.54	0.57
	Gender	1.14	0.45 – 2.91	0.78
	Positive family history of inflammatory bowel diseases	0.87	0.27 – 2.87	0.82
	Smoker (current)	0.96	0.23 – 4.06	0.96
	Smoker (former or current)	1.40	0.53 – 3.66	0.49
	Surgery on gastrointestinal tract	1.66	0.53 – 5.18	0.38
	Time after diagnosis [y]	0.58	0.23 – 1.47	0.25
Activity (UC)	Colitis activity index (CAI)	2.98	1.53 – 5.80	0.0011
	Mayo Score	3.27	1.32 – 8.06	0.0099
	Partial Mayo Score (PMS)	8.61	3.92 – 18.95	1.5e-09
Laboratory data	Alanine transaminase (ALT) [U/L]	0.44	0.22 – 0.89	0.019
	Aspartate transaminase (AST) [U/L]	0.82	0.43 – 1.56	0.54
	C-reactive protein (CRP) [mg/L]	46.27	16.16 – 132.51	9e-24
	Creatinine [mg/dL]	0.83	0.38 – 1.83	0.65
	Haematocrit [vol%]	0.30	0.15 – 0.59	0.0003
	Haemoglobin [g/dL]	0.34	0.17 - 0.67	0.0015
	Leukocytes [/nL]	4.12	2.06 - 8.24	2.1e-05
	Soluble glycoprotein 130 [ng/mL]	0.82	0.19 - 3.51	0.79
	Soluble interleukin-6 receptor [ng/mL]	0.81	0.40 - 1.62	0.55
	Thrombocytes [/nL]	3.82	1.87 - 7.81	0.00021
Medication	Amino salicylate therapy	0.12	0.03 - 0.56	0.0036
	Immunosuppressive therapy	1.89	0.50 - 7.14	0.34
	Monoclonal antibody therapy, except for tumour necrosis factor alpha (TNF- α) inhibitors	1.82	0.41 - 8.10	0.43
	Steroid therapy	1.12	0.31 - 4.09	0.86
	TNF- α inhibitor therapy	0.97	0.43 - 2.20	0.95

Table 18 Association of ordinal interleukin-6 levels with general, clinical and laboratory parameters.

Data Group	Parameter	Beta	95% CI	R ²	P-value
General data	Age at sampling [y]	2.11	-0.93 – 5.14	0.0087	0.17
	Allergy/asthma	2.59	-1.38 – 6.56	0.0036	0.2
	Appendectomy	4.46	-3.16 – 12.08	0.0076	0.25
	Arthritis as a comorbidity	1.03	-1.29 – 3.36	0.0019	0.38
	Body mass index (BMI) high (BMI ≥ 25) or normal (18.5 ≤ BMI < 25)	3.79	1.37 – 6.21	0.029	0.0023
	Body mass index low (<18.5) or normal (18.5 ≤ BMI < 25)	0.12	-6.15 – 6.38	6.9e-06	0.97
	Food allergy	-1.68	-8.15 – 4.79	0.00047	0.61
	Gender	0.95	-2.23 – 4.13	0.0018	0.56
	Positive family history of inflammatory bowel diseases	-1.16	-5.27 – 2.96	0.0014	0.58
	Smoker (current)	-3.96	-8.77 – 0.85	0.0092	0.11
	Smoker (former or current)	0.59	-2.66 – 3.84	0.00064	0.72
	Surgery on gastrointestinal tract	0.64	-2.86 – 4.14	0.00031	0.72
	Time after diagnosis [y]	0.48	-2.49 – 3.45	0.00041	0.75
Activity (UC)	Colitis activity index (CAI)	0.30	-1.41 – 2.00	0.00018	0.73
	Mayo Score	0.75	-1.61 – 3.10	0.0011	0.53
	Partial Mayo Score (PMS)	0.05	-1.68 – 1.78	5.1e-06	0.95
Laboratory data	Alanine transaminase (ALT) [U/L]	1.27	-0.44 – 2.98	0.0032	0.14
	Aspartate transaminase (AST) [U/L]	0.64	-0.99 – 2.26	0.00081	0.44
	C-reactive protein (CRP) [mg/L]	0.03	-1.62 – 1.67	1.2e-06	0.98
	Creatinine [mg/dL]	-1.38	-3.50 – 0.74	0.0038	0.2
	Haematocrit [vol%]	0.08	-1.64 – 1.80	1.3e-05	0.93
	Haemoglobin [g/dL]	0.49	-1.34 – 2.32	0.00047	0.6
	Interleukin-6 [pg/mL]	-0.31	-1.04 – 0.42	0.0009	0.41
	Leukocytes [/nL]	1.35	-0.21 – 2.91	0.0036	0.089
	Soluble glycoprotein 130 [ng/mL]	6.31	1.90 – 10.73	0.062	0.0057
	Thrombocytes [/nL]	0.56	-1.44 – 2.57	0.00063	0.58
Medication	Amino salicylate therapy	2.97	-1.59 – 7.52	0.0059	0.2
	Immunosuppressive therapy	-2.19	-6.19 – 1.81	0.0057	0.28
	Monoclonal antibody therapy, except for tumour necrosis factor-alpha (TNF-α) inhibitors	4.08	-7.83 – 0.32	0.0042	0.034
	Steroid therapy	2.20	-1.32 – 5.72	0.0078	0.22
	TNF-α inhibitor therapy	-1.45	-3.60 – 0.71	0.0043	0.19

Table 19 Association of soluble interleukin-6 receptor levels with general, clinical and laboratory parameters.

Data Group	Parameter	Beta	95% CI	R ²	P-value
General data	Age at sampling [y]	21.99	-20.94 – 64.93	0.033	0.3
	Allergy/asthma	79.53	-36.92 – 195.97	0.022	0.17
	Appendectomy	1.24	-71.44 – 73.92	5.1e-05	0.97
	Arthritis as a comorbidity	24.18	-11.14 – 59.50	0.031	0.18
	Body mass index (BMI) high (BMI ≥ 25) or normal (18.5 ≤ BMI < 25)	19.05	-12.50 – 50.59	0.032	0.23
	Body mass index low (<18.5) or normal (18.5 ≤ BMI < 25)	-31.36	-88.84 – 26.12	0.017	0.27
	Food allergy	Only one characteristic value			
	Gender	-33.86	-73.53 – 5.80	0.092	0.09
	Positive family history of inflammatory bowel diseases	Only one characteristic value			
	Smoker (current)	Only one characteristic value			
	Smoker (former or current)	55.43	20.76 – 90.10	0.24	0.0033
	Surgery on gastrointestinal tract	41.51	-9.45 – 92.48	0.09	0.1
	Time after diagnosis [y]	10.81	-32.81 – 54.43	0.0083	0.61
Activity (UC)	Colitis activity index (CAI)	3.96	-17.35 – 25.28	0.0012	0.71
	Mayo Score	-15.61	-54.09 – 22.87	0.018	0.41
	Partial Mayo Score (PMS)	-2.15	-25.60 – 21.30	0.0004	0.85
Laboratory data	Alanine transaminase (ALT) [UIL]	31.74	10.94 – 52.53	0.075	0.0033
	Aspartate transaminase (AST) [UIL]	21.69	3.80 – 39.59	0.041	0.018
	C-reactive protein (CRP) [mg/L]	-7.21	-29.00 – 14.57	0.0042	0.51
	Creatinine [mg/dL]	-2.09	-31.66 – 27.48	0.00025	0.89
	Haematocrit [vol%]	2.07	-18.75 – 22.89	0.00035	0.84
	Haemoglobin [g/dL]	4.26	-18.21 – 26.74	0.0015	0.71
	Interleukin-6 [pg/mL]	-1.89	-10.08 – 6.30	0.0015	0.65
	Leukocytes [/nL]	0.97	-17.93 – 19.88	7.1 e-05	0.92
	Soluble interleukin-6 receptor [ng/mL]	26.06	5.16 – 46.97	0.057	0.015
	Thrombocytes [/nL]	13.58	-10.38 – 37.54	0.015	0.26
Medication	Amino salicylate therapy	3.70	-36.08 – 43.48	0.0008	0.85
	Immunosuppressive therapy	7.36	-46.82 – 61.53	0.0034	0.78
	Monoclonal antibody therapy, except for tumour necrosis factor-alpha (TNF-α) inhibitors	Only one characteristic value			
	Steroid therapy	-12.28	-50.89 – 26.32	0.011	0.52
	TNF-a inhibitor therapy	-9.74	-38.69 – 19.21	0.0069	0.5

Table 20 Association of soluble glycoprotein 130 levels with general, clinical and laboratory parameters.

Location	IL-6 (ordinal data)		sIL-6R		sgp130	
	OR	P-value	Effect estimates	P-value	Effect estimates	P-value
Only rectum and sigma affected	1.30	0.82	1.90	0.43	-66.97	0.32
Left-sided colitis (up to splenic flexure)	6.27	0.09	1.56	0.51	-29.04	0.57
Subtotal colitis (up to hepatic flexure)	7.33	0.09	2.72	0.33	-47.67	0.48
Pancolitis	5.54	0.10	0.74	0.74	-39.69	0.41

Table 21 Associations of interleukin-6, soluble interleukin-6 receptor and soluble glycoprotein 130 levels with disease location.

Discussion

The primary aim of this study was to investigate the potential use of IL-6, sIL-6R and sgp130 as biomarkers for UC and identify any limitations for their use in daily practice since previous studies in this field, with smaller sample sizes, have shown elevated levels of these markers in IBD patients, but without consensus with regard to the magnitude and impact of IL-6, sIL-6R and sgp130 levels (68,70–73). This large-scale systematic analysis included 166 patients with UC and 100 controls and was able to confirm increased levels of IL-6 in patients with UC, particularly in those with active disease, in line with previous reports from Hyams et al. (1993) and Mitsuyama et al. (1995, 2006), but in contrast to the results from Mahida et al. (1991) (70–73). Overall, >50% of IL-6 measurements in this study were below the detection limit of 3.13 pg/mL and this larger patient sample demonstrated a much wider range of IL-6 levels compared to those stated in previous studies (68,70,80).

Mucosal levels of IL-6

The present study reveals that elevated IL-6 serum levels are only present in a small percentage of patients with active UC. Previous work, however, has demonstrated that colonic mucosal IL-6 concentrations are significantly higher in patients with active disease compared to inactive disease and controls (61,81–84). And while IL-6 accurately reflects the degree of inflammation when measured within colonic biopsies, serum levels of IL-6 in blood are sampled from the cubital fossa and do not necessarily mirror IL-6 levels in the inflamed intestinal mucosa, because the blood sampled from UC patients has to pass the liver, the main site of IL-6 production and storage in the body, on its way from the intestine to the sampling site. Consequently, it can be assumed that high local levels of IL-6 in the inflamed intestine may not necessarily and not proportionally lead to increased IL-6 levels in the circulation, depending on the inflamed region. Previous attempts to measure the difference in IL-6 concentration in mesenteric and peripheral samples in a small group of UC patients failed to detect any levels of IL-6, both in peripheral and mesenteric blood samples (70). One possible explanation for this is the use of aminosalicylate therapy in all but one patient, which has been shown to be associated with a significant decrease in IL-6 concentration in the current study. Given the findings of the present study, it is plausible that localised inflammation of the colon will not raise levels of IL-6 enough to be measurable in serum obtained from peripheral blood,

whereas a more wide-spread level of colonic and also extra-intestinal inflammation could give rise to IL-6 levels above the detection limit of 3.13 pg/mL.

Disease activity

IL-6

While the levels of IL-6 in UC patients were found to be significantly higher compared to the controls, the median levels of IL-6 for active and inactive disease in this study did not differ significantly from the controls, contrary to the literature (68,70,80). This can be attributed to the fact that even in patients with active disease, the majority of patients had levels of IL-6 below the detection limit, meaning that whilst high levels of IL-6 can be observed in some patients with active disease, more than 50% of active disease stays undetected. IL-6 levels corresponded more closely to disease activity measured in terms of CRP levels in serum compared to the disease activity scores. This was to be expected, as it is well known that IL-6 is an inducer of CRP synthesis in the liver (85). Previous work in this field has shown that the blockade of IL-6R or neutralisation of IL-6 inhibits the production of acute phase proteins in the liver, including CRP (86).

sIL-6R

A similar, yet more discrete, increase in sIL-6R was observed in patients with both active and inactive UC in this study. This similar pattern in the increases of IL-6 and its soluble receptor has previously been attributed to the enhancer function of sIL-6R for pro-inflammatory IL-6 trans-signalling as well as its role as a buffer (72). sIL-6R levels were higher compared to controls but there was no significant difference between groups of patients with active and inactive disease according to disease activity scores. The same is true for disease activity measured using serum CRP levels, even though there is a more obvious increase in sIL-6R levels in patients with UC compared to the controls. These results contradict the study by Mitsuyama et al. (1995) who found significant differences in the level of sIL-6R for active vs. inactive patients and these differences were also present in paired samples where data was available both from an active and an inactive phase of disease (72). In the present study, however, sIL-6R was not significantly elevated in the active disease phase compared to phases of inactive disease in paired samples.

sgp130

For sgp130, serum levels were found to be marginally increased in UC patients. Generally, the levels of sgp130 detected match findings from the literature (68,87), but in contrast to the pilot study by Gustot et al. (2005) when comparing levels of sgp130 between patients with active and inactive disease, there was no significant difference (68). While Mitsuyama et al. (2006) measured much smaller quantities of sgp130 in controls as well as UC patients, they found significantly elevated levels of sgp130 in UC patients compared to controls (73). Concurrent with results from the current study, Mitsuyama et al. did not find significant differences in sgp130 levels between active (n=14) and inactive (n=13) UC patients (73). Nevertheless, in a selection of six patients with both active and inactive disease timepoints, Mitsuyama et al. demonstrated a significant increase in the level of sgp130 immediately after resolution of an UC exacerbation episode (73). Since the current study only evaluated the most extreme timepoints for patients with both active and inactive disease serum samples, longitudinal observations were not available for comparison.

Correlations with general, clinical and laboratory parameters

Results from the present study corroborate previous findings with regards to a positive correlation between levels of IL-6 and disease activity, CRP levels together with leukocyte and platelet counts.

Liver enzymes

ALT showed a significant negative association with levels of IL-6 in UC patients, whereas ALT and AST were significantly and positively associated with levels of sgp130. Albeit abnormal liver function tests are commonly reported in this patient group, they usually refer to increases in ALT and AST (88–90). Interestingly, Cappello et al. (2014) found that UC patients presenting with abnormal liver function tests were less likely to be on aminosalicylate therapy (90). In line with these findings, IL-6 in the present study was significantly negatively associated with both ALT and aminosalicylate therapy. Given the large number of patients in the current study, these findings warrant further investigations into the relationship between IL-6 and liver parameters.

Medication

In terms of medication, significant negative correlations between IL-6 levels and aminosalicylate therapy and between sIL-6R and monoclonal antibody therapy were found. At this time, it is unlikely that these associations will have an impact on the treatment of UC patients, but more in-depth research into this area may be warranted to ascertain whether there are causal relationships.

IL-6, sIL-6R and sgp130 as biomarkers

While sIL-6R and sgp130 are tendentially increased in UC, they do not appear to have potential as biomarkers for UC. IL-6 does show some qualities of a biomarker; however, it does not seem to be superior to existing biomarkers such as CRP. Even though existing biomarkers have significant limitations of their own, the small percentage of patients that actually have detectable levels of IL-6 renders IL-6 level measurements impracticable for the general UC population. This being said, in certain cases where IL-6 blockage may be considered as a treatment option, measurement of IL-6 and sIL-6R levels may give an indication of the level of treatment success.

Outlook

Research into the pleiotropic functions of IL-6 has led to the development and approval of several neutralizing monoclonal antibodies targeting the IL-6 signalling pathway including tocilizumab and siltuximab with many more in late-phase development. Whilst these have been shown to be more effective compared to TNF α blockers such as adalimumab in clinical trials (91), not all patients show an effective response. A further drawback of these therapies is the interference with the immune response which is vastly influenced by both IL-6 and TNF α . This, in turn, makes patients more susceptible to bacterial or fungal infections (92). Consequently, research in recent years has focused on the specific IL-6 trans-signalling blockade using the fusion protein olamkicept (sgp130Fc), for which the colon has been demonstrated to be a prominent target (86). By reducing symptoms of disease whilst preserving classical IL-6 signalling and its role in the acute immune response, the recombinant protein olamkicept has successfully undergone phase I clinical trials, and an investigator-initiated, exploratory, open-label phase 2a trial has shown promising results (93).

Summary

The purpose of this study was to evaluate the use of IL-6, its soluble receptors, sIL-6R, and sgp130 as biomarkers in patients with UC. A total of 166 adult UC patients (514 measurements) from the outpatient clinic of the Department of Internal Medicine I at the UKSH were analysed for this study using both existing serum samples from a previous study together with freshly collected samples; all of which were compared to 100 healthy age-matched controls. Patients were regularly examined at the outpatient clinic and disease activity was monitored using disease activity scores, laboratory assessments and endoscopies. Enzyme-linked immunosorbent assays (Quantikine kits from R&D Systems) were used to determine the quantity of IL-6, sIL-6R and sgp130 contained within the serum samples.

A large proportion of IL-6 measurements were found to be below the detection limit of 3.13 pg/mL. Nevertheless, when comparing UC patients to healthy controls, levels of IL-6 were found to be significantly higher. Similarly, IL-6 levels of patients with active UC were significantly increased compared to patients with inactive disease, even though more than 50% of active UC IL-6 measurements were below control levels. Furthermore, increases of up to 15% in median levels of sIL-6R and sgp130 were identified but these were not consistent with disease activity and deemed to be of negligible clinical significance. These findings were confirmed in an analysis of sub-cohorts with matched measurements.

Indicators of UC disease activity (colitis activity index, mayo score and partial mayo score) were all significantly associated with serum levels of IL-6 and, as expected, positive associations were also found with CRP, leukocytes and thrombocytes. Interestingly, IL-6 in the present study was significantly negatively associated with ALT whereas liver function tests in the form of ALT and AST were observed to be significantly positively associated with sgp130. Also, the IL-6 buffering ability of sIL-6R and sgp130 was underscored by the significant positive association between the two buffer components.

Given that only a small percentage of UC patients presented with increased levels of IL-6, this cytokine is not a suitable biomarker for the UC patient population at large, but rather a good additional indicator of disease activity in patients with levels above the detection threshold. Whilst sIL-6R and sgp130 do not show potential as valuable biomarkers for UC, further research is required to determine whether a greater percentage of patients show elevated levels

of IL-6 in inflamed mucosa. Soon, UC patients that qualify to receive treatment targeting IL-6 signalling, whether this is global or trans-signalling inhibition, could benefit from determining levels of IL-6 in serum during flare-ups to predict treatment success and thereby personalising drug treatments and reducing unnecessary side-effects.

Bibliography

1. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* [Internet]. 2012 Jan;142(1):46-54.e42; quiz e30. Available from: <http://www.sciencedirect.com/science/article/pii/S0016508511013783>
2. Ott C, Obermeier F, Thiel S, Kemptner D, Bauer A, Schölmerich J, et al. The incidence of inflammatory bowel disease in a rural region of Southern Germany: a prospective population-based study. *European journal of gastroenterology & hepatology*. 2008 Sep;20(9):917–23.
3. Kobayashi T, Siegmund B, le Berre C, Wei SC, Ferrante M, Shen B, et al. Ulcerative colitis. *Nature Reviews Disease Primers* [Internet]. 2020 Dec 1;6(1):1–20. Available from: www.nature.com/nrdp
4. Loftus E v. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* [Internet]. 2004 May;126(6):1504–17. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15168363>
5. Carr I, Mayberry JF. The effects of migration on ulcerative colitis: a three-year prospective study among Europeans and first- and second-generation South Asians in Leicester (1991-1994). *The American Journal of Gastroenterology*. 1999 Oct;94(10):2918–22.
6. Benchimol EI, Manuel DG, To T, Mack DR, Nguyen GC, Gommerman JL, et al. Asthma, Type 1 and Type 2 Diabetes Mellitus, and Inflammatory Bowel Disease amongst South Asian Immigrants to Canada and Their Children: A Population-Based Cohort Study. Zhang H, editor. *PLOS ONE*. 2015 Apr 7;10(4):e0123599.
7. Benchimol EI, Mack DR, Guttman A, Nguyen GC, To T, Mojaverian N, et al. Inflammatory Bowel Disease in Immigrants to Canada And Their Children: A Population-Based Cohort Study. *The American Journal of Gastroenterology*. 2015 Apr 10;110(4):553–63.
8. Niv Y, Abuksis G, Fraser GM. Epidemiology of ulcerative colitis in Israel: a survey of Israeli Kibbutz settlements. *The American Journal of Gastroenterology* [Internet]. 2000 Mar;95(3):693–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10710059>
9. Samuelsson S. *Ulceros colit och proktit*. University of Uppsala, Sweden; 1976.
10. Jick H, Walker AM. Cigarette Smoking and Ulcerative Colitis. *New England Journal of Medicine*. 1983 Feb 3;308(5):261–3.
11. Szamosi T, Banai J, Lakatos L, Czegledi Z, David G, Zsigmond F, et al. Early azathioprine/biological therapy is associated with decreased risk for first surgery and delays time to surgery but not reoperation in both smokers and nonsmokers with Crohn's disease, while smoking decreases the risk of colectomy in ulcerative colitis. *European Journal of Gastroenterology & Hepatology*. 2010 Jul;22(7):872–9.
12. Levenstein S. Psychosocial factors in peptic ulcer and inflammatory bowel disease. *Journal of consulting and clinical psychology* [Internet]. 2002 Jun;70(3):739–50. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12090380>
13. Tocchi A, Lepre L, Liotta G, Mazzoni G, Costa G, Taborra L, et al. Familial and psychological risk factors of ulcerative colitis. *Italian journal of gastroenterology and hepatology*. 1997 Oct;29(5):395–8.

14. Barclay AR, Russell RK, Wilson ML, Gilmour WH, Satsangi J, Wilson DC. Systematic Review: The Role of Breastfeeding in the Development of Pediatric Inflammatory Bowel Disease. *The Journal of Pediatrics*. 2009 Sep;155(3):421–6.
15. Khalili H, Higuchi LM, Ananthakrishnan AN, Manson JE, Feskanich D, Richter JM, et al. Hormone Therapy Increases Risk of Ulcerative Colitis but not Crohn's Disease. *Gastroenterology*. 2012 Nov;143(5):1199–206.
16. Ananthakrishnan AN, Higuchi LM, Huang ES, Khalili H, Richter JM, Fuchs CS, et al. Aspirin, Nonsteroidal Anti-inflammatory Drug Use, and Risk for Crohn Disease and Ulcerative Colitis. *Annals of Internal Medicine*. 2012 Mar 6;156(5):350.
17. Cornish JA, Tan E, Simillis C, Clark SK, Teare J, Tekkis PP. The Risk of Oral Contraceptives in the Etiology of Inflammatory Bowel Disease: A Meta-Analysis. *The American Journal of Gastroenterology*. 2008 Sep;103(9):2394–400.
18. Moller FT, Andersen V, Wohlfahrt J, Jess T. Familial Risk of Inflammatory Bowel Disease: A Population-Based Cohort Study 1977–2011. *The American Journal of Gastroenterology*. 2015 Apr 24;110(4):564–71.
19. Halme L, Paavola-Sakki P, Turunen U, Lappalainen M, Farkkila M, Kontula K. Family and twin studies in inflammatory bowel disease. *World journal of gastroenterology* [Internet]. 2006 Jun 21;12(23):3668–72. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16773682>
20. Barrett JC, Lee JC, Lees CW, Prescott NJ, Anderson CA, Phillips A, et al. Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. *Nature Genetics* [Internet]. 2009 Dec 15;41(12):1330–4. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19915572>
21. Anderson CA, Boucher G, Lees CW, Franke A, D'Amato M, Taylor KD, et al. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nature Genetics* [Internet]. 2011 Mar 6;43(3):246–52. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21297633>
22. Degenhardt F, Mayr G, Wendorff M, Boucher G, Ellinghaus E, Ellinghaus D, et al. Transethnic analysis of the human leukocyte antigen region for ulcerative colitis reveals not only shared but also ethnicity-specific disease associations. *Human molecular genetics* [Internet]. 2021 Mar 1;30(5):356–69. Available from: <https://pubmed.ncbi.nlm.nih.gov/33555323/>
23. Lees CW, Barrett JC, Parkes M, Satsangi J. New IBD genetics: common pathways with other diseases. *Gut* [Internet]. 2011 Dec 1;60(12):1739–53. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21300624>
24. Thompson AI, Lees CW. Genetics of ulcerative colitis. *Inflammatory Bowel Diseases*. 2011 Mar 1;17(3):831–48.
25. Antoni L, Nuding S, Wehkamp J, Stange EF. Intestinal barrier in inflammatory bowel disease. *World journal of gastroenterology* [Internet]. 2014 Feb 7;20(5):1165–79. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24574793>
26. Welcker K, Martin A, Kölle P, Siebeck M, Gross M. Increased intestinal permeability in patients with inflammatory bowel disease. *European journal of medical research*. 2004 Oct 29;9(10):456–60.
27. Gitter AH, Wullstein F, Fromm M, Schulzke JD. Epithelial barrier defects in ulcerative colitis: characterization and quantification by electrophysiological imaging. *Gastroenterology* [Internet]. 2001 Dec;121(6):1320–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11729111>
28. Schmitz H, Barmeyer C, Fromm M, Runkel N, Foss HD, Bentzel CJ, et al. Altered tight junction structure contributes to the impaired epithelial barrier function in

- ulcerative colitis. *Gastroenterology* [Internet]. 1999 Feb;116(2):301–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9922310>
29. Heller F, Florian P, Bojarski C, Richter J, Christ M, Hillenbrand B, et al. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterology*. 2005 Aug;129(2):550–64.
30. Johansson ME V, Gustafsson JK, Holmén-Larsson J, Jabbar KS, Xia L, Xu H, et al. Bacteria penetrate the normally impenetrable inner colon mucus layer in both murine colitis models and patients with ulcerative colitis. *Gut*. 2014 Feb;63(2):281–91.
31. Braun A, Treede I, Gotthardt D, Tietje A, Zahn A, Ruhwald R, et al. Alterations of phospholipid concentration and species composition of the intestinal mucus barrier in ulcerative colitis: A clue to pathogenesis. *Inflammatory Bowel Diseases*. 2009 Nov;15(11):1705–20.
32. Pullan RD, Thomas GA, Rhodes M, Newcombe RG, Williams GT, Allen A, et al. Thickness of adherent mucus gel on colonic mucosa in humans and its relevance to colitis. *Gut*. 1994 Mar;35(3):353–9.
33. Strugala V, Dettmar PW, Pearson JP. Thickness and continuity of the adherent colonic mucus barrier in active and quiescent ulcerative colitis and Crohn's disease. *International Journal of Clinical Practice*. 2008 Jan 8;62(5):762–9.
34. Gersemann M, Becker S, Kübler I, Koslowski M, Wang G, Herrlinger KR, et al. Differences in goblet cell differentiation between Crohn's disease and ulcerative colitis. *Differentiation*. 2009 Jan;77(1):84–94.
35. Frank DN, Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proceedings of the National Academy of Sciences* [Internet]. 2007 Aug 21;104(34):13780–5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17699621>
36. Ohkusa T, Yoshida T, Sato N, Watanabe S, Tajiri H, Okayasu I. Commensal bacteria can enter colonic epithelial cells and induce proinflammatory cytokine secretion: a possible pathogenic mechanism of ulcerative colitis. *Journal of Medical Microbiology*. 2009 May 1;58(5):535–45.
37. Ohkusa T, Sato N, Ogihara T, Morita K, Ogawa M, Okayasu I. *Fusobacterium varium* localized in the colonic mucosa of patients with ulcerative colitis stimulates species-specific antibody. *Journal of gastroenterology and hepatology*. 2002 Aug;17(8):849–53.
38. Strauss J, Kaplan GG, Beck PL, Rioux K, Panaccione R, DeVinney R, et al. Invasive potential of gut mucosa-derived *fusobacterium nucleatum* positively correlates with IBD status of the host. *Inflammatory Bowel Diseases*. 2011 Sep;17(9):1971–8.
39. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. *The Lancet* [Internet]. 2017 Apr 29;389(10080):1756–70. Available from: [/pmc/articles/PMC6487890/](http://www.ncbi.nlm.nih.gov/pubmed/28450500)
40. Fuss IJ, Neurath M, Boirivant M, Klein JS, de la Motte C, Strong SA, et al. Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. *The Journal of Immunology*. 1996;157(3).
41. Inoue S, Matsumoto T, Lida M, Mizuno M, Kuroki F, Hoshika K, et al. Characterization of cytokine expression in the rectal mucosa of ulcerative colitis: Correlation with disease activity. *American Journal of Gastroenterology* [Internet]. 1999 Sep;94(9):2441–6. Available from: <https://pubmed.ncbi.nlm.nih.gov/10484006/>

42. Gerlach K, Hwang Y, Nikolaev A, Atreya R, Dornhoff H, Steiner S, et al. T H 9 cells that express the transcription factor PU.1 drive T cell-mediated colitis via IL-9 receptor signaling in intestinal epithelial cells. *Nature Immunology* [Internet]. 2014;15(7):676–86. Available from: <https://pubmed.ncbi.nlm.nih.gov/24908389/>
43. Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. *The Lancet*. 2007 May 12;369(9573):1641–57.
44. Langholz E, Munkholm P, Davidsen M, Binder V. Course of ulcerative colitis: Analysis of changes in disease activity over years. *Gastroenterology* [Internet]. 1994 Jul 1;107(1):3–11. Available from: <https://www.sciencedirect.com/science/article/pii/001650859490054X>
45. Silverberg MS, Satsangi J, Ahmad T, Arnott IDR, Bernstein CN, Brant SR, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Canadian journal of gastroenterology = Journal canadien de gastroenterologie*. 2005 Sep;19 Suppl A:5A-36A.
46. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated Oral 5-Aminosalicylic Acid Therapy for Mildly to Moderately Active Ulcerative Colitis. *New England Journal of Medicine* [Internet]. 1987 Dec 24;317(26):1625–9. Available from: <http://www.nejm.org/doi/abs/10.1056/NEJM198712243172603>
47. Walmsley RS, Ayres RCS, Pounder RE, Allan RN. A simple clinical colitis activity index. *Gut*. 1998;43(1):29–32.
48. Nikolaus S, Schreiber S. Diagnostics of Inflammatory Bowel Disease. *Gastroenterology* [Internet]. 2007 Nov 1;133(5):1670–89. Available from: [http://www.gastrojournal.org/article/S0016-5085\(07\)01642-3/pdf](http://www.gastrojournal.org/article/S0016-5085(07)01642-3/pdf)
49. Reese GE, Constantinides VA, Simillis C, Darzi AW, Orchard TR, Fazio VW, et al. Diagnostic precision of anti-Saccharomyces cerevisiae antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. *The American journal of gastroenterology*. 2006 Oct;101(10):2410–22.
50. Kucharzik T, DignaB A, Siegmund B. Aktualisierung der Colitis ulcerosa Leitlinie 2020. *Zeitschrift fur Gastroenterologie*. 2020 Dec 1;58(12):1209–32.
51. Kucharzik T, Dignass A, Atreya R, Bokemeyer B, Esters P, Herrlinger K, et al. S3-Leitlinie-Colitis ulcerosa [Internet]. Available from: https://www.dgvs.de/wp-content/uploads/2018/05/CU_Leitlinie-2018.pdf
52. Nikolaus S, Schreiber S. REVIEWS IN BASIC AND CLINICAL GASTROENTEROLOGY Diagnostics of Inflammatory Bowel Disease.
53. Yamamoto M, Yoshizaki K, Kishimoto T, Ito H. IL-6 is required for the development of Th1 cell-mediated murine colitis. *Journal of immunology (Baltimore, Md : 1950)* [Internet]. 2000 May 1;164(9):4878–82. Available from: <https://pubmed.ncbi.nlm.nih.gov/10779797/>
54. Suzuki Y, Saito H, Kasanuki J, Kishimoto T, Tamura Y, Yoshida S. Significant increase of interleukin 6 production in blood mononuclear leukocytes obtained from patients with active imflammatory bowel disease. *Life Sciences*. 1990;47(24):2193–7.
55. Andus T, Gross V, Casar I, Krumm D, Hosp J, David M, et al. Activation of monocytes during inflammatory bowel disease. In: *Pathobiology*. 1991. p. 166–70.
56. Ito H, Takazoe M, Fukuda Y, Hibi T, Kusugami K, Andoh A, et al. A Pilot Randomized Trial of a Human Anti-Interleukin-6 Receptor Monoclonal Antibody in Active Crohn's Disease. *Gastroenterology* [Internet]. 2004;126(4):989–96. Available from: <https://pubmed.ncbi.nlm.nih.gov/15057738/>
57. Jones SA, Scheller J, Rose-John S. Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling [Internet]. Vol. 121, *Journal of Clinical Investigation*. American

- Society for Clinical Investigation; 2011. p. 3375–83. Available from: [/pmc/articles/PMC3163962/?report=abstract](#)
58. Garbers C, Aparicio-Siegmund S, Rose-John S. The IL-6/gp130/STAT3 signaling axis: recent advances towards specific inhibition. *Current Opinion in Immunology*. 2015 Jun 1;34:75–82.
 59. Scheller J, Garbers C, Rose-John S. Interleukin-6: From basic biology to selective blockade of pro-inflammatory activities. *Seminars in Immunology*. 2014 Feb 1;26(1):2–12.
 60. Hodes GE, Ménard C, Russo SJ. Integrating Interleukin-6 into depression diagnosis and treatment. Vol. 4, *Neurobiology of Stress*. Elsevier Inc; 2016. p. 15–22.
 61. Li Y, De Haar C, Chen M, Dearing J, Gerrits MM, Smits R, et al. Disease-related expression of the IL6/STAT3/SOCS3 signalling pathway in ulcerative colitis and ulcerative colitis-related carcinogenesis. *Gut*. 2010 Feb;59(2):227–35.
 62. Narazaki M, Yasukawa K, Saito T, Ohsugi Y, Fukui H, Koishihara Y, et al. Soluble Forms of the Interleukin-6 Signal-Transducing Receptor Component gp130 in Human Serum Possessing a Potential to Inhibit Signals Through Membrane-Anchored gp130. *Blood*. 1993 Aug 15;82(4):1120–6.
 63. Jostock T, Müllberg J, Özbek S, Atreya R, Blinn G, Voltz N, et al. Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses. *European Journal of Biochemistry*. 2001;268(1):160–7.
 64. Barkhausen T, Tschernig T, Rosenstiel P, van Griensven M, Vonberg RP, Dorsch M, et al. Selective blockade of interleukin-6 trans-signaling improves survival in a murine polymicrobial sepsis model. *Critical Care Medicine*. 2011;39(6):1407–13.
 65. Sommer J, Garbers C, Wolf J, Trad A, Moll JM, Sack M, et al. Alternative Intronic Polyadenylation Generates the Interleukin-6 Trans-signaling Inhibitor sgp130-E10. *Journal of Biological Chemistry*. 2014 Aug 8;289(32):22140–50.
 66. Wolf J, Waetzig GH, Chalaris A, Reinheimer TM, Wege H, Rose-John S, et al. Different Soluble Forms of the Interleukin-6 Family Signal Transducer gp130 Fine-tune the Blockade of Interleukin-6 Trans-signaling *. *Journal of Biological Chemistry* [Internet]. 2016 Jul 29;291(31):16186–96. Available from: <http://www.jbc.org/article/S0021925820424433/fulltext>
 67. Wolf J, Rose-John S, Garbers C. Interleukin-6 and its receptors: a highly regulated and dynamic system. Vol. 70, *Cytokine*. Academic Press; 2014. p. 11–20.
 68. Gustot T, Lemmers A, Louis E, Nicaise C, Quertinmont E, Belaiche J, et al. Profile of soluble cytokine receptors in Crohn's disease. *Gut* [Internet]. 2005 Apr;54(4):488–95. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1774443/>
 69. Chalaris A, Garbers C, Rabe B, Rose-John S, Scheller J. The soluble Interleukin 6 receptor: Generation and role in inflammation and cancer. *European Journal of Cell Biology*. 2011 Jun 1;90(6–7):484–94.
 70. Mahida YR, Kurlac L, Gallagher A, Hawkey CJ. High circulating concentrations of interleukin-6 in active Crohn's disease but not ulcerative colitis. *Gut*. 1991;32(12):1531–4.
 71. Hyams JS, Fitzgerald JE, Treem WR, Wyzga N, Kreutzer DL. Relationship of functional and antigenic interleukin 6 to disease activity in inflammatory bowel disease. *Gastroenterology*. 1993 May 1;104(5):1285–92.
 72. Mitsuyama K, Toyonaga A, Sasaki E, Ishida O, Ikeda H, Tsuruta O, et al. Soluble interleukin-6 receptors in inflammatory bowel disease: Relation to circulating interleukin-6. *Gut* [Internet]. 1995;36(1):45–9. Available from: <https://pubmed.ncbi.nlm.nih.gov/7890234/>

73. Mitsuyama K, Tomiyasu N, Suzuki A, Takaki K, Takedatsu H, Masuda J, et al. A form of circulating interleukin-6 receptor component soluble gp130 as a potential interleukin-6 inhibitor in inflammatory bowel disease. *Clinical and Experimental Immunology* [Internet]. 2006 Jan;143(1):125–31. Available from: <http://doi.wiley.com/10.1111/j.1365-2249.2005.02960.x>
74. Nikolaus S, Schulte B, Al-Massad N, Thieme F, Schulte DM, Bethge J, et al. Increased Tryptophan Metabolism Is Associated With Activity of Inflammatory Bowel Diseases. *Gastroenterology* [Internet]. 2017 Dec 1;153(6):1504-1516.e2. Available from: <http://www.gastrojournal.org/article/S0016508517360614/fulltext>
75. Richter G, Krawczak M, Lieb W, Wolff L, Schreiber S, Buyx A. Broad consent for health care-embedded biobanking: Understanding and reasons to donate in a large patient sample. *Genetics in Medicine* [Internet]. 2018 Jan 1;20(1):76–82. Available from: <https://pubmed.ncbi.nlm.nih.gov/28640237/>
76. R&D Systems Inc. Quantikine ELISA: Human IL-6 Immunoassay [Internet]. Available from: <https://resources.rndsystems.com/pdfs/datasheets/d6050.pdf>
77. R&D Systems Inc. Quantikine ELISA: Human IL-6 R α Immunoassay [Internet]. Available from: <https://resources.rndsystems.com/pdfs/datasheets/dr600.pdf>
78. R&D Systems Inc. Quantikine ELISA: Human Soluble gp130 Immunoassay [Internet]. Available from: <https://resources.rndsystems.com/pdfs/datasheets/dgp00.pdf?v=20210219>
79. Nikolaus S, Waetzig GH, Butzin S, Ziolkiewicz M, Al-Massad N, Thieme F, et al. Evaluation of interleukin-6 and its soluble receptor components sIL-6R and sgp130 as markers of inflammation in inflammatory bowel diseases. *International Journal of Colorectal Disease* [Internet]. 2018 Jul 11;33(7):927–36. Available from: <http://link.springer.com/10.1007/s00384-018-3069-8>
80. Reinisch W. Clinical relevance of serum interleukin-6 in Crohn's disease: Single point measurements, therapy monitoring, and prediction of clinical relapse. *American Journal of Gastroenterology*. 1999;94(8):2156–64.
81. Mitsuyama K, Sasaki E, Toyonaga A, Ikeda H, Tsuruta O, Irie A, et al. Colonic Mucosal Interleukin-6 in Inflammatory Bowel Disease. *Digestion*. 1991;50(2):104–11.
82. Reinecker HC, Steffen M, Witthoef T, Pflueger I, Schreiber S, MacDermott RP, et al. Enhanced secretion of tumour necrosis factor- α , IL-6, and IL-1 β by isolated lamina propria mononuclear cells from patients with ulcerative colitis and Crohn's disease. *Clinical and Experimental Immunology*. 1993;94(1):174–81.
83. Mudter J, Weigmann B, Bartsch B, Kiesslich R, Strand D, Galle PR, et al. Activation pattern of signal transducers and activators of transcription (STAT) factors in inflammatory bowel diseases. *American Journal of Gastroenterology*. 2005 Jan;100(1):64–72.
84. Bernardo D, Vallejo-Díez S, Mann ER, Al-Hassi HO, Martínez-Abad B, Montalvillo E, et al. IL-6 promotes immune responses in human ulcerative colitis and induces a skin-homing phenotype in the dendritic cells and Tcells they stimulate. *European Journal of Immunology*. 2012 May;42(5):1337–53.
85. Heinrich PC, Castell J V., Andus T. Interleukin-6 and the acute phase response. *Biochemical Journal*. 1990;265(3):621–36.
86. Garbers C, Thaiss W, Jones GW, Waetzig GH, Lorenzen I, Guilhot F, et al. Inhibition of classic signaling is a novel function of soluble glycoprotein 130 (sgp130), which is controlled by the ratio of interleukin 6 and soluble interleukin 6 receptor. *Journal of Biological Chemistry*. 2011 Dec 16;286(50):42959–70.
87. Narazaki M, Yasukawa K, Saito T, Ohsugi Y, Fukui H, Koishihara Y, et al. Soluble forms of the interleukin-6 signal-transducing receptor component gp130 in human

- serum possessing a potential to inhibit signals through membrane-anchored gp130. *Blood*. 1993;82(4):1120–6.
88. Hyams J, Markowitz J, Treem W, Davis P, Grancher K, Daum F. Characterization of Hepatic Abnormalities in Children with Inflammatory Bowel Disease. *Inflammatory Bowel Diseases*. 1995;1(1):27–33.
 89. Goyal A, Hyams JS, Lerer T, Leleiko NS, Otley AR, Griffiths AM, et al. Liver enzyme elevations within 3 months of diagnosis of inflammatory bowel disease and likelihood of liver disease. *Journal of Pediatric Gastroenterology and Nutrition*. 2014;59(3):321–3.
 90. Cappello M, Randazzo C, Bravatà I, Licata A, Peralta S, Craxì A, et al. Liver function test abnormalities in patients with inflammatory bowel diseases: A hospital-based survey. Vol. 7, *Clinical Medicine Insights: Gastroenterology*. Libertas Academica Ltd.; 2014. p. 25–31.
 91. Gabay C, Emery P, van Vollenhoven R, Dikranian A, Alten R, Pavelka K, et al. Tocilizumab monotherapy versus adalimumab monotherapy for treatment of rheumatoid arthritis (ADACTA): A randomised, double-blind, controlled phase 4 trial. *The Lancet* [Internet]. 2013 May 4;381(9877):1541–50. Available from: <http://dx.doi.org/10.1016/>
 92. Tanaka T, Narazaki M, Ogata A, Kishimoto T. A new era for the treatment of inflammatory autoimmune diseases by interleukin-6 blockade strategy. Vol. 26, *Seminars in Immunology*. Academic Press; 2014. p. 88–96.
 93. Schreiber S, Aden K, Bernardes JP, Conrad C, Tran F, Höper H, et al. Therapeutic Interleukin-6 Trans-signaling Inhibition by Olamkicept (sgp130Fc) in Patients With Active Inflammatory Bowel Disease. *Gastroenterology* [Internet]. 2021 Jun 1;160(7):2354–2366.e11. Available from: <http://www.gastrojournal.org/article/S0016508521004674/fulltext>

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