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ORIGINAL RESEARCH

Serum Cytokine Profiles in Patients with Inflammatory Bowel Disease in Clinical Remission: A Cross-Sectional Comparison by Anti-TNF Therapy

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Introduction: Inflammatory bowel diseases (IBDs) are a group of idiopathic diseases that include Crohn's disease (CD) and ulcerative colitis (UC). According to the Global Burden of Diseases, Injuries, and Risk Factors Study, 6.8 million people lived with IBD in 2017. Monitoring cytokine levels can provide information about the effectiveness of therapy and help adjust treatment to achieve better clinical outcomes. This cross-sectional study aimed to assess whether serum cytokine profiles differ between patients receiving anti-TNF therapy and those not receiving biologic treatment in a cohort restricted to clinical remission, and to evaluate their potential clinical utility.

Methods: The study included 132 IBD patients who were in remission of the disease and 29 control subjects. Cytokine levels (GM-CSF, INFγ, IL13, IL1β, IL4, IL23, IL6, TNFα) were measured and compared between patients receiving biologic therapy and those not receiving biologic treatment.

Results: No significant differences were observed in the concentrations of the cytokines studied between the individual groups of patients receiving anti-TNF therapy and those receiving conventional therapy. Overall, cytokine profiles showed limited discriminatory value in patients in clinical remission.

Conclusion: Regardless of the treatment method used (conventional vs. biologic therapy), no differences were observed in the profiles of pro-inflammatory and anti-inflammatory cytokines among patients in clinical remission. These results suggest that blood levels of TNF-α and other cytokines do not depend solely on anti-TNF therapy, indicating the need for longitudinal studies to assess their potential role in predicting relapse or loss of response to treatment.

Keywords: anti-TNF therapy, Crohn's disease, cytokines, inflammatory bowel disease, remission, TNFα, ulcerative colitis

Introduction

Inflammatory bowel diseases (IBD) are a group of idiopathic diseases that include Crohn's disease (CD) and ulcerative colitis (UC).[1,2] The epidemiology of IBDs is changing rapidly around the world, and the estimated prevalence in Western countries continues to increase, exceeding 0.3%.[1] According to The Global Burden of Diseases, Injuries, and Risk Factors Study, 6.8 million people lived with IBD in 2017.[3]

Specific mechanisms for the development of IBD remains under debate, however recent data indicate that among the immunological disturbances, tissue infiltration by T lymphocytes, plasma cells, macrophages and neutrophils, may play a crucial role.[4] Saez et al in their paper present that among other mechanisms, specific factors contributing to the progression of both ulcerative colitis (UC) and Crohn's disease (CD) are the overproduction of interferon-γ (IFN-γ) by lamina propria macrophages and T cells[5] and domination of Th1 lymphocytes producing proinflammatory cytokines.[6] These results were

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confirmed in CD patients, by showing that during active phase of disease intestinal mucosa has prevailed by Th1 phenotype, overproduction of IFN-γ in lamina propria T cells, and interleukin 12 (IL-12) by macrophages.[7]

Apart of widely used advanced therapies including anti-tumor necrosis factor (TNF)-α antibodies (infliximab (IFX), adalimumab (ADA)), anti-interleukin (IL)-12/23p40 antibodies (ustekinumab), and anti-IL-23p19 antibodies (mirikizumab, guselkumab) that target inflammatory cytokines, therapeutic armamentarium for management of IBD has significantly expanded in recent years, with the introduction of several medications with different mechanisms of action. These include the oral small molecule drugs Janus kinase inhibitors (upadacitinib, tofacitinib) and sphingosphine 1-phosphate receptor modulators (ozanimod, etrasimod).[8–10] All of the above mentioned molecules are capable of directly or indirectly altering key inflammatory pathways, receptors, and some adhesion molecules. However, substantial number of IBD patients will never not respond to the therapy or will lose their response before full recovery.[11,12] Therefore, it is reasonable to presume that modern management approach should be, at least in part, based on individual patient cytokine profiling, which would probably lead to the better outcomes of cytokine-targeted therapy. Profiling cytokines during remission could be clinically informative by revealing subclinical inflammation, identifying smoldering immune activity preceding relapse.[13–15]

In recent years, both experimental and clinical studies have led to the evaluation of systemic concentrations as well as better understanding of the role played by pro- and anti- inflammatory cytokines in active phase of IBD.[16,17] However, data on systemic cytokine concentrations during remission remain sparse, and their clinical relevance is debated.

Therefore, the aim of this study was to evaluate systemic concentrations of key chemokines and cytokines in UC and CD patients in remission phase archived with the use of TNF-α agents (IFX or ADA) or conventional therapy compared to levels found in healthy volunteers. This study is cross-sectional and primarily assesses between-group differences at a single time point. It does not evaluate predictive capacity for relapse or therapeutic response.

Material and Methods

Study Population

This single-center observational study aimed to characterize the inflammatory cytokine profile in patients with ulcerative colitis (UC) and Crohn's disease (CD) in clinical remission during maintenance therapy with infliximab (IFX), adalimumab (ADA), or conventional treatment. The study was conducted at the Gastroenterology Clinic and the Center for Comprehensive Treatment of Inflammatory Bowel Disease, Regional Hospital No. 2 in Rzeszów, Poland, between 2018 and 2019.

The study was performed in accordance with the principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and applicable national and local regulations. The study protocol was approved by the local Ethics Committee (KE-0254/68/2015). All participants received detailed written and verbal information about the study and provided written informed consent prior to enrollment.

A total of 132 patients were included (60 women, 45%; 72 men, 55%), aged 18–72 years, with diagnoses of CD (58%) or UC (42%) and in remission at the time of inclusion. A control group of 29 age-matched healthy volunteers was also enrolled.

Eligible participants were patients attending routine follow-up visits with a confirmed diagnosis of UC or CD established at least 12 months before enrollment, who were in stable clinical remission during maintenance therapy with anti-TNF-α agents (IFX or ADA) or conventional treatment. Patients receiving only anti-TNF-α therapy, maintenance therapy with 5-aminosalicylic acid preparations, or standard-dose immunosuppressive treatment (eg, azathioprine 1 mg/kg) were eligible. Patients treated with systemic corticosteroids at any dose were excluded. Additional exclusion criteria included evidence of clinically, biochemically, or endoscopically active disease; presence of serious comorbidities (cardiovascular, respiratory, neurological, rheumatological, renal, or psychiatric disorders); history of alcohol or drug abuse within the previous five years; and pregnancy.

The diagnosis of inflammatory bowel disease was confirmed according to the 2018 recommendations of the American College of Gastroenterology[18,19] and the European Crohn's and Colitis Organisation,[20,21] based on colonoscopic findings and histopathological examination of biopsy specimens obtained from affected colonic segments.

Clinical remission was defined based on established disease activity indices (CDAI <150 for Crohn's disease and a modified Mayo score ≤2), as well as on biochemical remission, defined as a C-reactive protein (CRP) level <5 mg/L and a fecal calprotectin level <150 μg/g.

Study Protocol

The study consisted of a single study visit conducted during routine hospitalization or outpatient clinic attendance. All participants underwent physical examination, structured assessment of symptoms and complaints, review of medical history, and standard clinical, biochemical, and instrumental investigations. In patients with CD, disease activity was assessed using the Crohn's Disease Activity Index (CDAI), which incorporates stool frequency, abdominal pain, general well-being, presence of extraintestinal and intestinal complications, abdominal tenderness, use of antidiarrheal medication, hematocrit, and body weight. Clinical remission was defined as a CDAI score below 150.[22] In patients with UC, disease remission was assessed using the modified Mayo (mMayo) score and defined as an endoscopic subscore ≤ 1, stool frequency subscore ≤ 1, and rectal bleeding subscore of 0.[23] During the study visit, laboratory investigations included complete blood count, urinalysis, total protein, albumin and globulin levels, glucose, urea, creatinine, total, direct and indirect bilirubin, hepatic transaminase activity, coagulation profile, C-reactive protein, and fecal calprotectin. Blood samples for biochemical analyses were collected after an overnight fast. Measurement of anti-drug antibodies and drug trough levels was not routinely available and therefore not included in the analysis.

Cytokine Measurement

Serum concentrations of granulocyte–macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), interleukin (IL)-13, IL-1β, IL-4, IL-23, IL-6, and tumor necrosis factor-α (TNF-α) were measured using a MILLIPLEX® Human Bone Magnetic Bead Panel (Millipore Sigma; St. Louis, MO) and MagPix (Diasorin; Austin, TX) instrument. According to the manufacturer's specifications, intra-assay coefficients of variation were <10% and inter-assay coefficients of variation were <15%, with no detectable cross-reactivity between analytes. Standard curve fitting and preliminary data processing were performed using Belysa Immunoassay Curve Fitting Software (MilliporeSigma). The assay detects total circulating cytokine levels and does not distinguish between free and drug-bound TNF-α, which may influence interpretation in patients receiving anti-TNF therapy.

Statistical Analysis

Statistical analysis of the collected material was performed using Statistica 13.3 software. Verification of the conformity of the distribution of quantitative variables with the normal distribution was performed using the Shapiro–Wilk test. For variables with a normal distribution, the Student's *t*-test for independent samples was used. For variables that did not meet the assumption of normal distribution, the non-parametric Mann–Whitney *U*-test was used. Comparison of three independent groups: Differences between groups (CD vs. UC vs. Control) were analyzed using the Kruskal–Wallis ANOVA test. If a statistically significant result was obtained ($p < 0.05$), a post-hoc analysis was performed using the DSCF (Dwass-Steel-Critchlow-Fligner) multiple comparison test to identify differences between specific groups. The strength and direction of the relationship between the parameters studied were assessed using Spearman's rank correlation coefficient. Cohen's d index was used as a measure of effect size for significant differences between two groups. In all analyses, a $p < 0.05$ was considered statistically significant.

Results

Characteristics of the Study Population

Table 1 shows the demographic and anthropometric parameters of the patients with CD, UC and the control group. The groups were homogeneous with respect to age ($p = 0.418$) and body weight ($p = 0.247$), showing no statistically significant differences in these parameters. However, a statistically significant difference in body mass index (BMI) was observed between the analyzed groups ($p = 0.031$). To identify the source of this difference, a post hoc analysis using the

Table 1 Characteristics of the Study Population

Variables	CD (n=76)	UC (n=56)	Control Group (n=29)	p-value
	Me (Q1-Q3)	Me (Q1-Q3)	Me (Q1-Q3)	
Age (year)	30.5 (24.0–40.0)	34.5 (27.5–39.5)	33.0 (25.0–44.0)	H=1.74 0.418
Weight (kg)	68.0 (58.0–77.5)	71.5 (57.3–80.1)	72.3 (62.6–83.5)	H=2.80 0.247
BMI (kg/m2)	22.2 (20.1–26.0)	23.4 (20.8–26.9)	25.0 (23.4–26.1)	H=6.93 0.031
Diagnosis (year)	6.0 (2.0–8.0)	4.5 (1.5–9.0)	-	Z=0.08 0.934

Abbreviations: BMI, Body Mass Index; CD, Crohn's disease; UC, ulcerative colitis.

DSCF test was performed, which revealed that patients with CD had a significantly lower BMI compared to the control group (p = 0.024) (data not shown).

The concentration of C-reactive protein (CRP) was statistically significantly higher in patients with CD (median: 2.9 mg/L) compared to patients with UC (median: 1.8 mg/L; p = 0.014). The calculated effect size for this difference was moderate (Cohen's d = 0.54). In the case of hemoglobin and calprotectin levels, despite the observed differences in mean values, the Student's *t*-test analysis did not show statistical significance. The results indicate that both patients with Crohn's disease and ulcerative colitis were in clinical remission (Table 2).

Cytokine Levels

With regard to pro-inflammatory cytokines, a preliminary Kruskal–Wallis test revealed nominal statistical significance for interferon gamma (IFN-γ; p = 0.043). However, after applying the Benjamini-Hochberg correction to control the false discovery rate, this result lost its statistical significance (q = 0.177). This is further supported by the fact that a detailed analysis of pairwise comparisons (post-hoc) did not identify any specific groups that differed significantly. This suggests that the initially observed difference in IFN-γ concentrations was random in nature, resulting from the simultaneous testing of multiple variables.

A nominal trend toward higher IL-6 concentrations was also observed (p = 0.053), which, after FDR adjustment, ultimately did not reach the threshold for statistical significance (q = 0.177). The remaining cytokines studied (TNF-α, IL-1β, IL-23, GM-CSF) and Th2 profile cytokines (IL-4, IL-13) also showed no significant differences between groups, as confirmed by high adjusted values (q > 0.350) (Table 3).

With regard to the cytokine profile, a trend toward higher concentrations of granulocyte-macrophage colony-stimulating factor (GM-CSF) was preliminarily observed in the biologic treatment group (nominal p = 0.071; rrb = −0.18, 95% CI [−0.38; 0.01]). After FDR correction, this result ultimately did not reach the threshold for statistical

Table 2 Comparison of Hemoglobin, C-Reactive Protein, Calprotectin Values

Variables	CD	UC	p-value	d Cohena
	Mean ± SD[a] Me (Q1-Q3)[b]	Mean ± SD[a] Me (Q1-Q3)[b]		
CRP (mg/l)	n=67 2.9 (1.2–4.9)[b]	n=48 1.8 (1.0–3.9)[b]	Z=2.46 0.014	0.54
Hemoglobin (g/dl)	n=68 13.6 ± 1.43[a]	n=49 13.1 ± 1.30[a]	t=1.82 0.072	-
Calprotectin (ug/g)	n=53 32.1 (14.4–51.0)[b]	n=31 18.1 (11.5–45.7)[b]	Z=1.54 0.124	-

Abbreviations: CD, Crohn's disease; UC, ulcerative colitis; M, mean; Me, median; SD, standard deviation; CRP, C-reactive protein.

Table 3 Serum Levels of Cytokines in the Studied Groups

Parameter	CD	UC	Control Group	p-value	q (FDR)
	Me (Q1-Q3)	Me (Q1-Q3)	Me (Q1-Q3)		
GM-CSF [pg/mL]	n=75	n=56	n=29	H=0.29	0.866
	11.9 (8.4–15.7)	11.7 (9.8–16.7)	11.6 (9.6–15.9)	0.866	
IFN-γ [pg/mL]	n=75	n=56	n=29	H=6.31	0.177
	11.0 (9.4–13.1)	12.6 (10.5–14.7)	11.2 (9.6–12.8)	0.043	
IL-1β [pg/mL]	n=75	n=56	n=29	H=0.96	0.689
	2.4 (1.9–3.3)	2.6 (2.1–3.2)	2.3 (2.0–3.2)	0.620	
IL-4 [pg/mL]	n=75	n=55	n=29	H=1.37	0.631
	21.8 (9.4–108.5)	21.8 (9.9–60.4)	20.2 (9.9–31.7)	0.505	
IL-6 [pg/mL]	n=67	n=51	n=23	H=5.88	0.177
	9.2 (2.4–40.0)	4.3 (2.0–19.1)	2.6 (1.2–12.4)	0.053	
IL-13 [pg/mL]	n=67	n=48	n=26	H=3.03	0.440
	6.6 (2.8–39.8)	4.5 (2.5–17.0)	4.2 (2.2–10.9)	0.220	
IL-23 [pg/mL]	n=75	n=56	n=29	H=3.90	0.355
	259.8 (156.2–407.7)	301.6 (203.0–433.6)	247.2 (186.0–299.5)	0.142	
TNF-α [pg/mL]	n=75	n=56	n=29	H=2.13	0.491
	9.5 (7.4–11.5)	10.0 (8.0–13.9)	10.4 (8.3–11.4)	0.344	

Abbreviations: GM-CSF, granulocyte macrophage colony-stimulating factor; INFγ, interferon gamma; IL13, interleukin-13; IL1β, interleukin-1-beta; IL4, interleukin-4; IL23, interleukin-23; IL6, interleukin-6; TNFα, tumor necrosis factor alpha; CD, Crohn's disease; UC, ulcerative colitis.

significance (q = 0.355). Importantly, no statistically significant differences were observed in TNF-α levels (p = 0.394; q = 0.657; rrb = −0.09, 95% CI [−0.29; 0.11]) or other pro-inflammatory cytokines (IFN-γ, IL-6, IL-1β) between the study subgroups (Table 4).

Table 4 Serum Cytokine Levels in the Group of Patients with and without Biological Treatment

Parameter	Anti-TNFα Treatment	Conventional Treatment	p-value	*Effect Size (95% CI)*	*q (FDR)*
	Me (Q1-Q3)	Me (Q1-Q3)			
GM-CSF [pg/mL]	n=68	n=92	Z=1.81	−0.18 (−0.38; 0.01)	0.355
	13.1 (10.2–16.7)	11.3 (8.2–15.2)	0.071		
IFN-γ [pg/mL]	n=68	n=92	Z=0.33	−0.03 (−0.23; 0.17)	0.941
	11.5 (9.7–14.3)	11.4 (9.6–13.2)	0.742		
IL-1β [pg/mL]	n=68	n=92	Z=0.30	−0.03 (−0.22; 0.17)	0.941
	2.6 (2.0–3.2)	2.5 (2.0–3.3)	0.768		
IL-4 [pg/mL]	n=67	n=92	Z=−0.14	0.01 (−0.18; 0.22)	0.941
	23.4 (9.9–61.6)	19.8 (9.7–82.9)	0.894		
IL-6 [pg/mL]	n=60	n=81	Z=−1.00	0.11 (−0.10; 0.32)	0.657
	4.6 (2.2–25.0)	6.5 (1.9–33.6)	0.319		
IL-13 [pg/mL]	n=61	n=80	Z=−1.04	0.11 (−0.10; 0.32)	0.657
	5.0 (2.4–21.0)	5.0 (2.8–27.0)	0.301		
IL-23 [pg/mL]	n=68	n=92	Z=−0.08	0.01 (−0.19; 0.20)	0.941
	289.1 (179.7–424.3)	271.3 (176.5–364.1)	0.941		
TNF-α [pg/mL]	n=68	n=92	Z=0.85	−0.09 (−0.29; 0.11)	0.657
	10.0 (7.8–12.8)	9.7 (7.7–11.5)	0.394		

Abbreviations: GM-CSF, granulocyte macrophage colony-stimulating factor; INFγ, interferon gamma; IL13, nterleukin-13; IL1β, interleukin-1-beta; IL4, interleukin-4; IL23, interleukin-23; IL6, nterleukin-6; TNFα, tumor necrosis factor alpha.

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Table 5 Cytokine Correlation Analysis in the Group of Patients Treated with Biologics

	BMI	CRP	TNFa	IL6	IL1b	IFNg	GM-CSF	IL23	IL4	IL13
BMI	—									
CRP	0.08	—								
TNFa	−0.02	0.17	—							
IL6	−0.02	−0.13	0.00	—						
IL1b	−0.26*	−0.03	0.01	0.38**	—					
IFNg	−0.30*	−0.11	0.04	0.04	0.62***	—				
GM-CSF	−0.12	−0.11	−0.01	0.35**	0.63***	0.50***	—			
IL23	−0.08	0.03	−0.01	0.08	0.62***	0.71***	0.43***	—		
IL4	−0.13	−0.13	0.03	0.86***	0.52***	0.31*	0.40***	0.32**	—	
IL13	−0.05	−0.07	−0.03	0.85***	0.45***	0.10	0.26*	0.24	0.90***	—

Notes: * p <0.05, ** p <0.01, *** p <0.001.
Abbreviations: GM-CSF, granulocyte macrophage colony-stimulating factor; INFγ, interferon gamma; IL13, interleukin-13; IL1β, interleukin-1-beta; IL4, interleukin-4; IL23, interleukin-23; IL6, interleukin-6; TNFα, tumor necrosis factor alpha; BMI, body mass index.

Table 6 Correlation Analysis of Cytokines in Non-Biologically Treated Patients

	BMI	CRP	TNFa	IL6	IL1b	IFNg	GM-CSF	IL23	IL4	IL13
BMI	—									
CRP	−0.11	—								
TNFa	0.20	0.05	—							
IL6	−0.17	0.05	0.18	—						
IL1b	−0.16	−0.15	−0.01	0.28*	—					
IFNg	−0.14	−0.13	−0.05	−0.01	0.63***	—				
GM-CSF	−0.16	0.07	−0.20	0.06	0.67***	0.52***	—			
IL23	−0.18	−0.23	0.08	0.29**	0.80***	0.58***	0.55***	—		
IL4	−0.13	0.18	0.10	0.81***	0.46***	0.18	0.35***	0.42***	—	
IL13	−0.18	0.09	0.06	0.85***	0.41***	0.04	0.25*	0.30**	0.90***	—

Notes: * p <0.05, ** p <0.01, *** p <0.001.
Abbreviations: GM-CSF, granulocyte macrophage colony-stimulating factor; INFγ, interferon gamma; IL13, interleukin-13; IL1β, interleukin-1-beta; IL4, interleukin-4; IL23, interleukin-23; IL6, interleukin-6; TNFα, tumor necrosis factor alpha; BMI, body mass index.

Correlations of Individual Parameters

In both the biologically and conventionally treated groups, no statistically significant correlations were observed between TNF-α levels and the other cytokines analyzed. This suggests that, in the study population, systemic TNF-α concentrations are independent of other inflammatory mediators, regardless of therapeutic regimen (Tables 5 and 6).

Discussion

Systemic and mucosal concentrations of pro-and anti-inflammatory cytokines are elevated in IBD, but the role of specific pro- and anti-inflammatory cytokines in response to biological treatment has not been clearly established yet. Pro-inflammatory cytokines initiate the activation of, eg. cytotoxic innate immune cells, while anti-inflammatory cytokines oversee the activation of, among other things, regulatory T cell responses.[24] Therefore, in our study, we looked at the effect of using biologic treatment on cytokines (GM-CSF, INFγ, IL13, IL1β, IL4, IL23, IL6, TNFα) compared to conventional treatment.

Biologic drugs are drugs obtained by genetic engineering that reduce or prevent the activation of certain components of the immune system.[25,26] Increased expression of pro-inflammatory cytokines may be correlated with IBD symptoms.[27,28] It has been increasingly indicated that cytokine signalling pathways, as well as cytokines themselves, may be potential therapeutic targets in IBD.[29] The mechanisms of action of the biologic drugs currently used to treat IBD operate at different levels of the immune system such as direct neutralization of molecules that aggravate the inflammatory process, or

competent blocking of their receptors on effector cells. The mechanisms of the latest small molecule drugs are even more sophisticated and may involve, for example, inhibiting the migration of activated lymphocytes from lymph nodes.[30]

Although new biologic agents and small-molecule drugs are increasingly used in IBD treatment, anti-TNF therapy remains one of the most widely used biologic treatments worldwide due to long-term clinical experience, availability, and cost-effectiveness. Therefore, analysing cytokine profiles in patients treated with anti-TNF agents remains clinically relevant.[31]

Our analyses have shown that in the remission phase, all markers (both pro- and anti-inflammatory) were low regardless of the disease (CD vs UC) and the treatment method used (conventional vs anti-TNF treatment). Moreover, selective blockade of TNFα-dependent signalling pathway did not differ in TNFα concentrations (and other labeled pro- and anti-inflammatory cytokines) from other methods of maintaining CD and UC remission (in our case, immunosuppression with azathioprine (AZA) and mercaptopurine (6MP) in CD and 5ASA and/or AZA, 6MP in UC).

Importantly, remission in inflammatory bowel disease is a heterogeneous condition that encompasses varying degrees of recovery. Despite clinical remission, subclinical inflammation may persist, which can obscure detectable differences in blood cytokine levels.

The interpretation of TNF-α concentrations in patients treated with anti-TNF agents is challenging. Circulating TNF-α may be present in both free and antibody-bound forms, and depending on the assay used, measured concentrations may reflect total or only free TNF-α. Consequently, serum TNF-α levels during anti-TNF therapy do not necessarily correspond to its biological activity, which may explain the lack of differences in TNF-α concentrations observed between the studied groups.[32,33]

All the pathways/methods used to maintain remission have one goal – to maintain the balance between pro- and anti-inflammatory cytokines. However, some biologic drugs have been shown to be able to block selected cytokines.[34] Our results suggest that different therapeutic strategies may lead to a similar immunological equilibrium during remission, which may explain the lack of differences in cytokine profiles between treatment groups. In addition, patients receiving anti-TNF therapy typically have a more severe or treatment-resistant form of the disease, which may serve as a potential confounding factor in cross-sectional comparisons.

Therefore, our study showed no differences in serum cytokine profile. This finding may suggest that cytokine profiling during remission has limited value for differentiating between treatment strategies; however, it may still be useful as a baseline for longitudinal monitoring and prediction of disease relapse.[35]

Kwon et al came to similar conclusions in their study. In addition, they found that measuring cytokines just after diagnosis and qualification for treatment can predict a patient's response to treatment.[36] Levels of pro-inflammatory cytokines can correlate positively, not only with disease activity, but also with the severity and extent of inflammation in tissues.[37]

Granulocyte macrophage colony-stimulating factor (GM-CSF), together with pro-inflammatory cytokines, is part of a feedback loop between certain cells, such as macrophages and neighbouring structures, eg. endothelial cells.[38] Therefore, it is an important factor in alveolar macrophage homeostasis.[39] As it turns out, GM-CSF probably plays both a protective and an inflammatory role in intestinal inflammation.[40] In our study, we observed a positive correlation between GM-CSF and some cytokines (INFγ, IL6, IL1β). Similarly, in vitro, macrophages treated with GM-CSF showed increased expression of IL6, TNFα, and IL-1β.[41] GM-CSF is the central mediator of cytokine release syndrome (CRS) and is produced by innate lymphoid group 3 (ILC3) cells in the gut. In contrast, CRS is associated with the release of IFN-γ, IL-6, GM-CSF, among others.[40,42] Therefore, GM-CSF may be an agent that modulates the immune response through its action on myeloid immune cells.[40,43] GM-CSF also promotes antimicrobial functions in myeloid cells to maintain the transcriptional stability of ILC3 mediated, among other things, by the interleukin (IL) 23 cytokine and the retinoic acid (RA) metabolite.[40]

As more and more patients are no longer responding to treatments targeting pro-inflammatory cytokines such as IL6, such therapy using GM-CFS may be helpful.[44] GM-CSF, by modulating the macrophage phenotype, is likely to prevent intestinal fibrosis.[45] However, the role of GM-CSF in IBD remains complex, as it may have both pro-inflammatory and protective functions depending on the cellular environment and disease phase.[46,47]

In addition, IL1β initiates signalling from the IL-1R/MyD88 pathway, leading to GM-CSF production in Th cells.[48] In addition, GM-CFS is also produced by Th cells via IL23 in conjunction with RORγt.[49] In another study, the authors examined IL-23 expression in human mononuclear phagocytes and peripheral blood mononuclear cells collected from 41

patients and observed that IL-1α/IL-1β and IL-10 controlling IL-23-producing monocytes, distinguishing between homeostatic and inflammation-related IL23 with severe CD projection, in addition to lack of response to anti-TNF therapy. Therefore, it is important to consider and therapy targeting IL-23p19 and/or IL-1α/IL-1β with IL23.[50] It should be noted that this type of treatment may not bring the expected results in all IBD patients. For this reason, it is important to identify the specific biomarkers involved in exacerbating the pro-inflammatory cascade to adjust accordingly and maximise therapeutic benefit.[51] On the other hand, there is still a lack of markers that determine the risk of exacerbation in IBD patients, and GM-CSF could be a factor for assessing both the risk of disease activity and monitoring the course of the disease itself.[52]

In our study, we did not observe the occurrence of statistically significant differences between patients treated with biologics, conventional treatment and the control group in GM-CSF levels which may indicate that remission, regardless of treatment type, is associated with a similar cytokine profile.[53]

In the future, the assessment of cytokine levels may enable the tailoring of appropriate treatments in the context of IBD patients; however, further preclinical and clinical studies are needed to better understand the mechanisms of action of selected cytokines, including GM-CSF. What were the criteria for control group in the study.

Strength and Limitations of the Study

The limitations of the study include the relatively small sample size and the lack of differentiation between periods of remission and exacerbation. Another important limitation is the cross-sectional design, as cytokine levels were measured only during remission and not during disease exacerbation. Therefore, the results do not allow for the assessment of dynamic changes in cytokine profiles over time. Moreover, serum cytokine levels may not fully reflect local intestinal inflammation. It should also be noted that Crohn's disease and ulcerative colitis differ in pathogenesis and immune response; therefore, pooling these groups in some analyses may limit the interpretation of the results, and conclusions should be drawn with caution.

Conclusions

Regardless of the treatment method used (conventional vs. biological treatment) in patients in remission, we observed no differences in the profile of pro- and anti-inflammatory cytokines tested in this study population and under the measurement conditions used. The results suggest that TNFα levels in IBD patients may not depend solely on anti-TNFα therapy, but may also be influenced by disease activity and maintenance treatment; however, this observation requires confirmation in longitudinal studies. The assessment of pro- and anti-inflammatory cytokine levels may be useful for understanding the immunological background of remission; however, the clinical utility of cytokine measurements in remission requires further investigation. Understanding the pathways of cytokine action and the correlations between cytokines may create potential new treatment or monitoring pathway to maximize therapeutic benefit. Future longitudinal studies are needed to determine whether cytokine profiles during remission may predict relapse or loss of response to therapy.

Declarations of Helsinki

Study protocol was approved by a local Ethic Committee (KE-0254/68/2015). The Helsinki Declaration guidelines were followed.

Data Sharing Statement

The data used and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Sara Jarmakiewicz-Czaja - Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review and editing

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Rafał Filip – Data curation, Formal analysis, Funding acquisition, Investigation, Supervision, Writing – review and editing

All authors gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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