Title: Evaluation of cytokines levels as putative biomarkers to predict the pharmacological response to biologic therapy in inflammatory bowel diseases

Authors: Lorenzo BERTANI [1]*, Luca ANTONIOLI [2], Matteo FORNAI [2], Gherardo TAPETE [1], Giovanni BAIANO SVIZZERO [1], Santino MARCHI [1], Corrado BLANDIZZI [2], Francesco COSTA[3]

Affiliations: [1]Gastroenterology Unit, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; [2]Unit of Pharmacology and Pharmacovigilance, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy; [3]IBD Unit, Department of General Surgery and Gastroenterology, Pisa University Hospital, Pisa, Italy

*Corresponding Author: Lorenzo Bertani, Gastroenterology Unit, Pisa University Hospital, via Paradisa, 2, 56124 Pisa, Italy. E-mail: lorenzobertani@gmail.com

Abstract

Cytokines play a central role in the pathogenesis of inflammatory bowel diseases. For this reason, the vast majority of biological therapies are aimed to block pro-inflammatory cytokines or their receptors. Although these drugs have modified the course of the disease due to their efficacy, a high rate of non-response or loss of response over time is still an important issue for clinicians. In this perspective, many studies have been conducted in recent years to individuate a reliable biomarker of therapeutic response. In this review, we discuss the role of cytokines involved in the pathogenesis and in the therapy of inflammatory bowel diseases, and their putative use as pharmacological biomarkers of therapy responsiveness.

Keywords: IBD; Cytokines; Anti-TNF; Biomarkers

MAIN TEXT

Introduction

Inflammatory Bowel Diseases (IBD) are chronic relapsing conditions characterized by an abnormal activation of immune system, leading to a significant damage of gastrointestinal tract.[1] The two major subtypes of IBD, Crohn's Disease (CD) and Ulcerative Colitis (UC), are distinguished by some clinical and histopathological characteristics, even if they share many pathophysiological features.[2]

Nowadays, the mechanisms underlying development and pathophysiology of IBD are not fully understood. However, an overactive mucosal immune response against gut microorganisms in genetically susceptible individuals is thought to play a predominant role.[3-5] At present, current therapeutic approaches, far to be resolutive for the inflammatory process, allowed to counteract the activation of the inflammatory cascade.[6]

The characterization of the immunophenotype of mucosal cells in IBD allowed the identification of several key players in the onset and development of the pathological process, highlighting a critical involvement of cytokines in orchestrating the major immunologic events underlying IBD.[2] A better comprehension and identification of key cytokines, critically involved in the pathophysiology of IBD, paved the way to the development of several biological therapies[7]. In this regard, the introduction of the innovative single-cell analysis of samples obtained from IBD patients, in parallel with the available pre-clinical models of IBD, allowed to identify several cytokine and cellular pathways associated with intestinal inflammation, as novel and potential alternative therapeutic targets.[7] However, it remains very difficult to target therapies to those patients most likely to respond. In this regard, a number of recent studies are beginning to investigate if the assessment of tissue and circulating levels of some cytokines can be used as biomarkers, useful to predict the response to biologic therapy in IBD. The aim of this review is to illustrate the available data about how and to what extent cytokine patterns can affect the response to a biological therapy, and critically discuss their putative use as pharmacological biomarkers of therapy responsiveness.

Cytokines in the pathogenesis of IBD

The cytokines represent the key pathophysiologic elements, driving and shaping the onset and the progression of the phlogistic process occurring in IBD. At present, based on the binding to different receptors, the cytokines are divided in six families[8]: the hematopoietin (Class I) family, the interferon (Class II) family, the tumor necrosis factor (TNF) family, the interleukin (IL)-1 family, the IL-17 family and the chemokines. Such families exhibit different functions, although they share sequence similarity and some promiscuity in their reciprocal receptor systems. In particular, the receptors for the class I and II cytokines are dimers or trimers that typically consist of unique ligand-binding chains and one or more signal-transducing chains, which are often shared by receptors for different cytokines[8]. Both engage the JAK-STAT (Janus activated kinase–signal transducer and activator of transcription) signaling, and the only difference between these two families is represented by a membrane proximal peptide stretch containing a tryptophan-serine-X-tryptophan-serine motif, where X is any amino acid only in Class I receptors[8]. The TNF family receptors are trimers that share intracellular signaling mechanisms aimed to stimulate gene expression, but in some cases induce apoptosis trough the activation of caspase-8. The receptors of IL-1 family display a conserved cytosolic sequence, and engage similar signal transduction pathways that induce gene transcription. IL-17 family receptors are pre-formed oligomers that activate intra-nuclear mediators that are usually associated with innate immune signaling. Chemokine receptors are G-protein-coupled that can bind many different chemokines, as well as a chemokine can bind different receptors.

In this paragraph, we discuss the role of the main pro-inflammatory and anti-inflammatory cytokines in the pathogenesis of IBD.

TNF

TNF is a pro-inflammatory cytokine produced mainly by activated macrophages, monocytes and T lymphocytes, and is chronically elevated both in intestinal cells and in serum of IBD patients[9, 10]. Activation of TNF receptor (TNFR)-1 induces an intracellular signaling cascade with pleiotropic effects involving apoptosis, cell proliferation or cytokine secretion. Activation of the nuclear factor kappa B (NF-κB) following stimulation of TNFR-1 results in translocation to the nucleus and transcriptional upregulation of several genes such as IL-8, IL-1, IL-6, COX-2 and TNF[11]. Alternatively, TNFR-1 engagement can activate a caspase-8 dependent signaling pathway triggering a pro-apoptotic process[11].

The TNFR-2 pathway does not contain a death domain and its stimulation can result in proliferation, migration and cytokines' production (such as IL-1 and IL-6)[12]. Therefore, TNF plays a leading role on sustaining inflammation in IBD among all the pro-inflammatory cytokines, promoting upregulation of other inflammatory cytokines, as well as up-regulating the expression of adhesion molecules in the endothelium allowing lymphocytes homing and the activation of macrophages[4]. In addition, a number of evidences highlighted its pivotal role in altering the intestinal barrier integrity eliciting the apoptosis of epithelial gut cells[13].

IL-12 group

This family includes a series of heterodimeric cytokines, such as IL-12, IL-23, IL-27 and IL-35[14]. Despite many structural similarities in the cytokines (chain pairing promiscuity is a common feature of them), their receptors and downstream signaling components, they possess vastly contrasting biological activities[14]. The two dimers of this group of cytokines consist of an α chain (p19, p28 or p35) and a β chain (p40 or Ebi3). The p40 chain can pair with p35 or p19 to form IL-12 or IL-23, respectively, while Ebi3 can pair with p28 or p35 to form IL-27 or IL-35, respectively[14].

IL-12 is a pro-inflammatory cytokine produced by dendritic cells, macrophages and B cells in response to microbial pathogens[15]. This cytokine is a Class I cytokine, so its functions are mediated by a JAK-STAT activation. IL-12 induces Interferon (IFN)-γ production by NK cells and CD8+ T cells, which activate additional antigen presenting cells for IL-12 production, and facilitates Th_1 differentiation of naïve CD4+ T cells[16]. Moreover, IL-12 antagonize Th_2 response and differentiation by inhibit IL-4 production[17]. Therefore, IL-12 secretion associated with innate recognition of pathogens was linked with the development of adaptive immune responses dominated by the production of IFN-γ, which is central in the pathogenesis of autoimmune inflammation[18].

IL-23, like IL-12, is a pro-inflammatory cytokine produced by dendritic cells and macrophages that creates a positive feedback loop that enhances IL-23 expression. However, if differs from IL-12 by playing a key role in Th_{17} development[19]. Th_{17} lymphocytes have a central role in autoimmunity and regulate inflammatory response at mucosal level[20]. They produce a milieu of cytokines, such as IL-17, IL-21, IL-22, IL-26[21]. Functionally, IL-17 and IL-21 promote inflammation through the upregulation of TNF, IL-1β, IL-6, and IL-8, recruitment of neutrophils and secretion of tissue-degrading proteases by intestinal fibroblasts[22, 23].

IL-13

IL-13 is a cytokine secreted by eosinophils, basophils, $CD8^+$ T-lymphocytes, and activated Th_2 cells, and exerts its functions trough the activation of JAK-STAT signaling in the same producing cells, as well as in fibroblasts, dendritic cells and epithelial cells[24]. The main inflammatory role of this cytokine is against helminthic infections as a part of the gut mucosal immune response system. IL-13 acts in mucosal immunity as a factor that stimulates goblet cells to produce mucus, and attracts eosinophils to release and increase the IgE production[25]. In IBD, IL-13 limits Th_{17} inflammation by blocking IL-17 production, with a subsequent result of increasing IL-10[26]. This effect has been proved by investigating related responses on both human and mouse cells, which suggests a homeostatic role of IL-13 in the gut[27]. On the other hand, IL-13 is also produced by natural killer T-cells, and exerts a negative effect on gut barrier function, promoting an increase of permeability[28]. In addition, IL-13 participate also to increase gut motility and epithelial secretion[25]. This is particularly important in UC, characterized by an increase of Th_2 population, promoted by IL-4. Indeed, IL-13 levels in patients with active UC are higher in comparison with the active CD patients and healthy controls[29, 30].

IL-6

IL-6 is a pleiotropic cytokine involved in the differentiation and proliferation of $CD4^+$ and $CD8^+$ T-cells via JAK-STAT signaling pathway[31, 32]. For this reason, IL-6 is classified into the Class I family. Macrophages and dendritic cells are the main source of IL-6 in response to infections and tissue injuries, and the secretion of this cytokine contributes to immune defense through the stimulation of acute phase proteins and differentiation of T-cells[33]. IL-6 is indispensable for Th_{17} differentiation from naïve $CD4^+$ T cells, and this is particularly important in the pathogenesis of CD[34]. Moreover, the synergic action with IL-1β promotes the survival of T-cells, and facilitate granulocytes recruitment and the subsequent promotion of Th_{17} activity, especially in CD.[20] Indeed, IL-6 levels are significantly higher in CD than in UC.[35] Furthermore, IL-6 also inhibits transforming growth factor (TGF)-β-induced T_{reg} differentiation[19]. T_{reg} are suppressive $CD4^+$ T-cells, which plays an important role in maintaining gut immune homeostasis.[36, 37] Despite the molecular suppressive mechanism is not completely know, these cells boost the production of two important anti-inflammatory cytokines, IL-10 and TGF-β.[38] The alteration of the Th_{17}/T_{reg} balance is considered to be responsible for the disruption of immunological tolerance, and, thus to be pathologically involved in the development of IBD[32].

TGF-β

TGF-β is produced by many cell types: epithelial cells, immune cells, and fibroblasts[39, 40]. TGF-β is an anti-inflammatory cytokine, with the activation of SMAD nuclear pathway, but is not able to exert its anti-inflammatory role in IBD patients, due to the increased levels of SMAD7[39]. Although the mechanism underlying modulation of TGF-β in the human intestine remains to be elucidated, TGF-β production is upregulated by various factors, such as bacteria, viruses, cytokines, apoptotic cells, and the autocrine/paracrine loop[41, 42]. Intestinal TGF-β exerts its anti-inflammatory function by modulating adhesion molecule expression, preventing goblet cell depletion and dysbiosis, IL-33 production, and enhancing epithelial tight junction expression[43].

IL-10

IL-10 is a major immunosuppressive cytokine, which belongs to Class II family. It is produced by nearly all leukocytes, including macrophages, dendritic cells, natural killer cells, neutrophils, eosinophils, mast cells, B cells and a large number of CD4+ T-cell subsets (including Th_1, Th_2, Th_{17}, Th_{22}, T_{reg})[44]. IL-10 binding to its receptor activates the JAK1/STAT3 cascade, where phosphorylated STAT3 homodimers translocate to the nucleus to activate the expression of target genes (such as Bcl3, etv3, hdac1) which suppress the expression of pro-inflammatory genes[45, 46]. The IL-10/STAT3-mediated anti-inflammatory response is activated in macrophages ad dendritic cells, where it is in opposition to the pro-inflammatory IL-6 pathway, which is also mediated by STAT3[47]. On the other hand, the anti-inflammatory effect of IL-10 in macrophages is mediated by the transcriptional inhibition of the LPS-induced genes, including pro-inflammatory mediators, such as cell surface receptors, chemokines and cytokines[48]. In addition, IL-10 also suppresses cytokine production in neutrophils[49] and regulates apoptosis in B cells[50]. One of the crucial function of this cytokine in IBD is related to its capability to inhibit the release of TNF, which is critical to the maintenance of immune homeostasis in gastrointestinal tract[51].

Cytokines as targets of treatment

Based on the increasing knowledge about the molecular mechanisms underlying IBD, over the years several molecules have been developed to modify the intra- and extra-cellular signaling pathways of cytokines. In particular, the modulation of cytokine signaling in IBD recognizes different types of approaches: 1) direct inhibition of single cytokines or their receptors, such as TNF, IL-12, IL-23; 2) indirect modulation through the blockade of lymphocyte trafficking towards the intestinal mucosa through the blockade of specific integrins; 3) blockade of the superfamily of JAK receptors. In this paper, only therapies based on a direct action on cytokines are discussed.

At present, the blockade of TNF is the most common way to manage IBD patients. Since introduction of Infliximab (IFX) in the clinical practice in 1998,[52] the use of anti-TNF drugs has dramatically changed the management of IBD, as these drugs have been shown to be effective in inducing and maintaining remission in both CD and UC. Nowadays, several anti-TNF drugs are approved for the treatment of CD and UC: IFX and Adalimumab for both diseases, Golimumab only for UC, and Certolizumab Pegol for CD. These drugs are IgG monoclonal antibodies, able to restore the integrity of the intestinal epithelial tight junctions, and to reduce neo-angiogenesis that contributes to the recruitment of cytokines.[12] However, the evidence that over 60% of moderate-to-severe IBD patients does not respond to anti-TNF drugs, in parallel with the high rates of loss of response,[53] spurred the scientific community toward the development of drugs targeting other pathways involved in the onset and development of intestinal inflammation. In this regard, the evidence of the involvement of IL-12 and IL-23 in promoting a Th_1 and Th_{17} driven inflammation in IBD led to the design and development of specific drugs against this molecular target.[54] Ustekinumab, a human IgG antibody that blocks the p40 protein, (the subunit shared by IL-12 and IL-23), is currently approved for the treatment of moderate-to-severe CD in anti-TNF failure patients cohort (UNITI-1), conventional therapy failure patients cohort (UNITI-2)[55] and in the maintenance phase study (IM-UNITI)[56]. The phase 3 trial evaluating the efficacy and safety of ustekinumab in UC is still ongoing.

Of note, the increasing evidences demonstrating a critical role for IL-23 in mucosal inflammation, lead to the development of selective IL-23 inhibitors. Risankizumab is a humanized monoclonal antibody against p19 subunit, which is specific for IL-23, thus preventing the binding with its receptor. The phase 2 trial including moderate-to-severe CD patients showed that this drug is more effective than placebo in inducing clinical remission after 12 weeks[57]. The open label extension study showed that risankizumab is effective in increasing clinical response and remission rates at week 26, and provides a sustained response at week 52[58]; Phase 3 trials, both in UC and CD, are still ongoing. Brazikumab, another anti IL-23 p19 human IgG2 monoclonal antibody, showed higher rates of

clinical response, compared to placebo, in a randomized phase 2b trial in moderate to severe CD patients who had failed at least one anti-TNF drug, with no differences in terms of safety[59]. Other selective anti IL-23 antibodies, such as mirikizumab and guselkumab, approved in 2017 for the treatment of moderate to severe psoriasis, have shown encouraging results in phase 2 studies performed both in CD and UC patients, and are now on phase 3 testing.

As indicated in the previous paragraph, another potential target in IBD treatment is IL-6, a pleiotropic cytokine that plays a central role as a regulator of the adaptive immune response in IBD[60]. In phase I trial, tocilizumab, a monoclonal antibody anti-IL-6R, showed its efficacy in inducing clinical remission in active CD at week 8 and 12, despite no difference was observed in endoscopic and histological response[61]. In a randomized phase 2 trial performed in moderate-to-severe CD patients, PF-04236921, a fully human Ig-G2 monoclonal antibody that binds IL-6, has been studied. This drug showed to elicit a significant clinical improvement compared to those treated with placebo at week 8 and week 12, [62].

IL-13 has emerged as an important cytokine effective in UC and fistulizing CD, thus representing another attractive target for the development of a novel potential therapeutic option in IBD[63]. Anrukinzumab, a humanized IgG1 antibody that binds IL-13 and inhibits binding of IL-13 to IL-4Rα, has been evaluated in a phase IIa study. In this context, 152 patients with mild to moderate UC were randomized to receive placebo or anrukinzumab at different doses. At week 14, there were no statistically significant differences between the actively treated group at any dose compared to placebo, suggesting that IL-13 is not an effective therapeutic target in UC.[64]

Another promising target for IBD management is Interleukin-17 (IL-17). Indeed, in the intestine this pro-inflammatory cytokine, stimulates the secretion of IL-6 and IL-8 from epithelial cells and myofibroblasts, and the upregulation of intercellular adhesion molecule-1 (ICAM-1) on intestinal endothelial cells[65].

Secukinumab, a fully human anti-IL-17 monoclonal antibody, was tested on 59 CD patients in a randomized, double-blind, placebo-controlled trial, showing no significant clinical benefit, and displaying higher rate of adverse events compared to placebo[66]. Brodalumab, another human monoclonal antibody against IL-17RA, was evaluated in a phase II trial in moderate to severe CD patients. This study was early suspended because a significant worsening of CD was observed in patients actively treated with the drug compared to placebo, with no evidence of efficacy[67].

Another therapeutic approach in the treatment of IBD was focused on the promotion of the activities of the regulatory cytokines, such TGF-β or IL-10. At present, the only therapeutic option tested in

IBD to activate TGF-β activity is represented by Morgensen,[68] an oral antisense oligonucleotide, but it is not a monoclonal antibody and, therefore, it is not relevant for the purposes of this review. When evaluating the effect of a human recombinant IL-10 in CD patients, SCH 52000, despite the encouraging results in the first studies[69], a further double-blind, placebo-controlled trial did not show any benefit in preventing endoscopic recurrence after 12 weeks[70].

All the cytokines-based therapies proposed are illustrated in Table 1.

Cytokines as biomarkers of treatment response

A key feature of the mucosal cytokine network is its dynamic fluidity, and it is likely that the blockade of a single cytokine in patients with IBD may lead to the establishment of alternative compensatory pro-inflammatory cytokine pathways.[4] This is probably the reason why available therapies in IBD have a high rate of loss of response over time. When considering the biologic drugs currently available for medical prescription, it has been described a loss of response rate ranging between 23 and 46% for anti-TNF drugs,[71] 39% for VDZ[72], and 36% for ustekinumab[73]. Based on these findings, the design of selective targeted therapy for individual patients with IBD would decrease the number of patients that lose the response to biological drugs.

At present, this approach is difficult, since it needs one or more reliable biomarkers in order to identify the modification of inflammatory pathways. The most used biomarker in IBD practice is represented by fecal calprotectin, but it reflects only a non-specific anti-inflammatory response.[74, 75] For this reason, an analysis of cytokine levels, in serum or in the inflamed mucosa, could be useful to predict the pharmacological response to biological drugs.

Crohn's disease

Niederau et al.[76] firstly proposed the role of cytokines in monitoring clinical activity of IBD, and demonstrated that a reduction of serum IL-6 during conventional medical therapy could reliably predict therapeutic efficacy in CD patients. However, only few studies in CD included the assessment of serum IL-6 as a biomarker of therapeutic response to biologic therapy. An interesting study by Billiet et al.[77] performed on a large cohort of CD patients, compared the serum levels of IFNγ, TNF and IL-6 in primary non-responders and responders to IFX therapy at the end of the induction. Serum TNF levels increased significantly after each drug infusion and this increase resulted more pronounced from week 0 to week 14 in responders. On the other hand, IFNγ and IL-6 concentrations decreased significantly at week 2 and week 6 in responders compared to primary non-responders. Similarly, a prospective study by Ogawa et al.[78] demonstrated that serum levels of TNF were significantly higher both at week 2 and 6 compared to baseline in CD patients treated with IFX, regardless of therapeutic outcome. In the same study, the authors demonstrated that higher levels of IL-12, IL-17, and IL-23, at baseline might be predictive markers for poor therapeutic response to IFX treatment. With regard for IL-6, a small study by Song et al.[79] showed a decrease of this cytokine after 12 weeks of treatment with anti-TNF, which was more pronounced in responders than in non-responders, in line with the findings of Billiet et al.[77]

Defendenti et al.[80], following the evaluation of a panel of serum cytokines in CD and UC patients, demonstrated a significant correlation between IL-9 level and disease severity in CD, but not in UC. In this perspective, a recent study by Feng et al.[81] showed that serum IL-9 level declined in patients with CD with clinical response after 30 weeks of IFX therapy. Interestingly, IL-9 level at week 14 could predict therapeutic outcome at week 30. Moreover, the cutoff values for IL-9 were incrementally lower in the prediction of clinical remission and mucosal healing. The decrease in the level of this cytokine seem to reflect directly the increasing level of inflammatory suppression required to achieve these outcomes in clinical practice. For this reason, serum IL-9 represents a putative reliable biomarker of therapeutic response to IFX, and it would be interesting to evaluate its role during adalimumab or even VDZ treatment.

Fluorescently labelled TNF-specific antibodies have been used to determine the abundance of immune cells that express membrane-bound TNF in the mucosa of patients with CD, obtaining a kind of "in-vivo imaging".[82] The presence of high levels of immune cells expressing membrane-bound TNF predicted a positive clinical response to subsequent therapy with anti-TNF drugs. Conversely, the presence of few immune cells expressing membrane-bound TNF was prognostic of a scarce response to this type of therapy, suggesting that gut inflammation was TNF-independent. Although this approach does not represent a real assessment of cytokines as a biomarker of biologic therapy, it is conceivable that "in-vivo imaging" should be improved and more extensively used in monitoring therapies in the next future.

Lastly, it is worthy to mention the possible role of IL-17 as putative biomarker, although great expectations have been disappointed. In CD, a Th_{17}-dependent disease, an increased IL-17 level in the inflamed mucosa and serum of IBD patients was reported.[83] At present, no studies have been aimed to monitor serum IL-17 during biologic therapy. However, a recent study performed during the Phase 2 trial performed on CD patients treated with Risankizumab, highlighted that this monoclonal antibody targeting the IL-23 p19 subunit could modify transcriptomic expression of IL-17-dependent genes in colonic mucosa only in drug responders.[84] On the other hand, Katz et al.[85] demonstrated that anti-TNF therapy did not have a significant impact on the expression of IL-17 in CD4 lymphocytes of CD patients. Of note, a tissue and serum assessment of IL-17 during biologic therapy could be useful to clarify whether this cytokine might be considered as a possible biomarker of therapeutic response.

Ulcerative colitis

Olsen et al.[86] demonstrated that IFX treatment was less effective in UC patients with high mucosal expression of TNF mRNA at baseline. In addition, the same authors pointed out that after 10 weeks of treatment with IFX the tissue expression of TNF and IFNγ mRNA underwent a more pronounced decrease in therapy responders than in non-responders, in terms of clinical and endoscopic improvement.[87] Accordingly, Hassan et al.[88] showed that tissue TNF down-regulation in UC patients responding to the induction of IFX therapy was strictly associated with a dramatic regression of the inflammation evaluating the histological activity.

Similar findings were reported in studies evaluating serum levels of TNF. Magnusson et al.[89] demonstrated that serum levels of IL-5, IL-8 and TNF decreased after 2 weeks of treatment with IFX in UC patients responding to therapy, but not in therapy failures.

The therapeutic effects of IFX in UC patients are likely to arise not only from a direct suppression of tissue expression of TNF, but also from an indirect suppression of other Th_1 cytokines, such as IFN-γ. In this regard, a study by Dahlen et al.[90] evaluated both serum and mucosal expression of a panel of cytokines in UC patients treated with anti-TNF. At baseline, IFX responders had lower mucosal mRNA expression for IL-1β, IL-17A, IL-6 and IFN-γ than non-responders. Fourteen weeks after baseline, mucosal IL-1β and IL-6 were down-regulated in therapy responders but not in non-responders, and serum levels of IL-6 were decreased only in responders. By contrast IFNγ and IL-12 p70 were increased in non-responders. On the other hand, a study by Rismo et al.[91] performed in the same subset of patients, showed that high mRNA expression of mucosal IFN-γ and IL-17A at baseline was associated with therapeutic response.

The serum IL-6 levels seem to be the most important biomarker in predicting therapeutic efficacy to IFX. Accordingly to Dahlen et al.[90], Sato et al.[92] displayed that, after 8 weeks of treatment, serum IL-6 concentration was significantly lower in responders than in non-responders at week 26. Likewise, Nishida et al.[93] demonstrated that lower levels of IL-6 at baseline are associated with clinical response to IFX induction. The reason about this correlation is still poor defined. In this regard, it has been hypothesized that, one of the mechanisms of action of IFX therapy is related to its ability also to suppress the IL-6 expression. For this reason higher levels of this cytokine might not be sufficiently suppressed, and this might determine a failure in drug response. The study by Sato et al.[92] evaluated a panel of 17 cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, granulocyte macrophage colony-stimulating factor (GM-CSF), IFN-γ, TNF, granulocyte colony-stimulating factor (G-CSF), macrophage inflammatory protein (MIP)-1β, and monocyte chemotactic protein (MCP)-1). This study pointed out that only IL-6 showed a correlation with clinical response to IFX. In parallel IL-8 and MIP-1β decreased significantly after 8 weeks of treatment. Not significant

changes was observed in the serum concentration of other cytokines, suggesting that, in UC patients, the use of serum cytokines as biomarkers of therapeutic response to IFX is still limited.

Another challenge for the next future is the identification of biomarkers useful to predict the response to VDZ, an interesting new therapeutic option for the treatment of patients with moderate-to-severe UC and CD that are refractory or intolerant to either conventional treatments or anti-TNF agents. In this regard, Battat et al.[94] reported that serum TNF decreased more rapidly in responders than in non-responders, during the first 14 weeks of VDZ treatment. This result is in line with which previously reported during anti-TNF therapy, suggesting that the decrease in TNF is probably an effect of immunosuppression induced by both therapies, even if the mechanism of action of VDZ is not completely understood.[95]

The serum IL-6 level was evaluated as putative therapeutic marker even in VDZ-treated patients[96]. The study demonstrated that IL-6 levels were higher in non-responders than in responders, in line to what observed in the study by Nishida et al.[93] taking into consideration the IFX-treated patients. It is worth to note that, this study included both CD and UC patients, an element which can represents a critical bias, since it has been widely recognized that IL-6 was significantly higher in CD than in UC.[35]

In this regard, our group conducted a study in UC patients treated with VDZ, demonstrating that serum IL-8 at baseline was significantly higher in patients who achieved mucosal healing at week 54.[97] Moreover, also the decrease over the first 6 weeks of treatment of the serum levels of IL-8 and IL-6 were correlated to therapeutic response in terms of mucosal healing.[97] Therefore, an early evaluation of serum levels of these two cytokines seemed to predict a one-year therapeutic outcome of VDZ treatment. Nevertheless, larger studies are needed to evaluate if serum or either tissue levels of cytokines could be used in the management of VDZ treatment.

At present, no data are currently available regarding the evaluation of cytokines as possible biomarkers for the therapeutic response to ustekinumab.

Conclusions

Many studies have been conducted to identify the role of cytokines in the pathogenesis of IBD, highlighting their central role. For this reason, most of the biological therapies available for IBD patients are aimed to counteract their pro-inflammatory effect. However, only few studies evaluated cytokines as predictors of therapeutic efficacy, in particular if therapeutic response was assessed in terms of mucosal healing, which is currently the gold standard.

Even if promising data are available, especially as regards TNF and IL-6 during anti-TNF treatment, at the moment there is no enough evidence to suggest a regular monitoring of cytokines during biologic therapy in IBD patients. Large real-life prospective studies are needed, because many patients still experience loss of response during biologic therapy, and cytokines monitoring could have a pathophysiologic rationale.

References

1. de Souza HS, Fiocchi C. Immunopathogenesis of IBD: current state of the art. Nat Rev Gastroenterol Hepatol 2016;13:13-27.
2. Bevivino G, Monteleone G. Advances in understanding the role of cytokines in inflammatory bowel disease. Expert Rev Gastroenterol Hepatol 2018;12:907-915.
3. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007;448:427-34.
4. Neurath MF. Cytokines in inflammatory bowel disease. Nat Rev Immunol 2014;14:329-42.
5. Xu XR, Liu CQ, Feng BS, et al. Dysregulation of mucosal immune response in pathogenesis of inflammatory bowel disease. World J Gastroenterol 2014;20:3255-64.
6. Schett G, Neurath MF. Resolution of chronic inflammatory disease: universal and tissue-specific concepts. Nat Commun 2018;9:3261.
7. Friedrich M, Pohin M, Powrie F. Cytokine Networks in the Pathophysiology of Inflammatory Bowel Disease. Immunity 2019;50:992-1006.
8. Liongue C, Sertori R, Ward AC. Evolution of Cytokine Receptor Signaling. J Immunol 2016;197:11-8.
9. Rutgeerts P, Van Assche G, Vermeire S. Optimizing anti-TNF treatment in inflammatory bowel disease. Gastroenterology 2004;126:1593-610.
10. Reimund JM, Wittersheim C, Dumont S, et al. Increased production of tumour necrosis factor-alpha interleukin-1 beta, and interleukin-6 by morphologically normal intestinal biopsies from patients with Crohn's disease. Gut 1996;39:684-9.
11. Slevin SM, Egan LJ. New Insights into the Mechanisms of Action of Anti-Tumor Necrosis Factor-alpha Monoclonal Antibodies in Inflammatory Bowel Disease. Inflamm Bowel Dis 2015;21:2909-20.
12. Billmeier U, Dieterich W, Neurath MF, et al. Molecular mechanism of action of anti-tumor necrosis factor antibodies in inflammatory bowel diseases. World J Gastroenterol 2016;22:9300-9313.
13. Lichtenstein GR. Comprehensive review: antitumor necrosis factor agents in inflammatory bowel disease and factors implicated in treatment response. Therap Adv Gastroenterol 2013;6:269-93.
14. Vignali DA, Kuchroo VK. IL-12 family cytokines: immunological playmakers. Nat Immunol 2012;13:722-8.
15. Ma X, Trinchieri G. Regulation of interleukin-12 production in antigen-presenting cells. Adv Immunol 2001;79:55-92.

16. Hunter CA. New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. Nat Rev Immunol 2005;5:521-31.
17. Wills-Karp M. IL-12/IL-13 axis in allergic asthma. J Allergy Clin Immunol 2001;107:9-18.
18. Caspi RR. IL-12 in autoimmunity. Clin Immunol Immunopathol 1998;88:4-13.
19. Bettelli E, Carrier Y, Gao W, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006;441:235-8.
20. Ahluwalia B, Moraes L, Magnusson MK, et al. Immunopathogenesis of inflammatory bowel disease and mechanisms of biological therapies. Scand J Gastroenterol 2018;53:379-389.
21. Weaver CT, Hatton RD, Mangan PR, et al. IL-17 family cytokines and the expanding diversity of effector T cell lineages. Annu Rev Immunol 2007;25:821-52.
22. Pender SL, Braegger C, Gunther U, et al. Matrix metalloproteinases in necrotising enterocolitis. Pediatr Res 2003;54:160-4.
23. Siakavellas SI, Bamias G. Role of the IL-23/IL-17 axis in Crohn's disease. Discov Med 2012;14:253-62.
24. Rengarajan J, Szabo SJ, Glimcher LH. Transcriptional regulation of Th1/Th2 polarization. Immunol Today 2000;21:479-83.
25. Hering NA, Fromm M, Schulzke JD. Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics. J Physiol 2012;590:1035-44.
26. Seyfizadeh N, Seyfizadeh N, Gharibi T, et al. Interleukin-13 as an important cytokine: A review on its roles in some human diseases. Acta Microbiol Immunol Hung 2015;62:341-78.
27. Newcomb DC, Boswell MG, Huckabee MM, et al. IL-13 regulates Th17 secretion of IL-17A in an IL-10-dependent manner. J Immunol 2012;188:1027-35.
28. Heller F, Florian P, Bojarski C, et al. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. Gastroenterology 2005;129:550-64.
29. Fuss IJ, Heller F, Boirivant M, et al. Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. J Clin Invest 2004;113:1490-7.
30. Rosen MJ, Frey MR, Washington MK, et al. STAT6 activation in ulcerative colitis: a new target for prevention of IL-13-induced colon epithelial cell dysfunction. Inflamm Bowel Dis 2011;17:2224-34.
31. Okada M, Kitahara M, Kishimoto S, et al. IL-6/BSF-2 functions as a killer helper factor in the in vitro induction of cytotoxic T cells. J Immunol 1988;141:1543-9.

32. Kimura A, Kishimoto T. IL-6: regulator of Treg/Th17 balance. Eur J Immunol 2010;40:1830-5.
33. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol 2014;6:a016295.
34. Korn T, Bettelli E, Oukka M, et al. IL-17 and Th17 Cells. Annu Rev Immunol 2009;27:485-517.
35. Gross V, Andus T, Caesar I, et al. Evidence for continuous stimulation of interleukin-6 production in Crohn's disease. Gastroenterology 1992;102:514-9.
36. O'Garra A, Vieira P. Regulatory T cells and mechanisms of immune system control. Nat Med 2004;10:801-5.
37. Yamada A, Arakaki R, Saito M, et al. Role of regulatory T cell in the pathogenesis of inflammatory bowel disease. World J Gastroenterol 2016;22:2195-205.
38. Schmidt A, Oberle N, Krammer PH. Molecular mechanisms of treg-mediated T cell suppression. Front Immunol 2012;3:51.
39. Babyatsky MW, Rossiter G, Podolsky DK. Expression of transforming growth factors alpha and beta in colonic mucosa in inflammatory bowel disease. Gastroenterology 1996;110:975-84.
40. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. Annu Rev Immunol 1998;16:137-61.
41. Kashiwagi I, Morita R, Schichita T, et al. Smad2 and Smad3 Inversely Regulate TGF-beta Autoinduction in Clostridium butyricum-Activated Dendritic Cells. Immunity 2015;43:65-79.
42. Torchinsky MB, Garaude J, Martin AP, et al. Innate immune recognition of infected apoptotic cells directs T(H)17 cell differentiation. Nature 2009;458:78-82.
43. Ihara S, Hirata Y, Koike K. TGF-beta in inflammatory bowel disease: a key regulator of immune cells, epithelium, and the intestinal microbiota. J Gastroenterol 2017;52:777-787.
44. Sabat R, Grutz G, Warszawska K, et al. Biology of interleukin-10. Cytokine Growth Factor Rev 2010;21:331-44.
45. Ding Y, Qin L, Zamarin D, et al. Differential IL-10R1 expression plays a critical role in IL-10-mediated immune regulation. J Immunol 2001;167:6884-92.
46. El Kasmi KC, Smith AM, Williams L, et al. Cutting edge: A transcriptional repressor and corepressor induced by the STAT3-regulated anti-inflammatory signaling pathway. J Immunol 2007;179:7215-9.

47. Braun DA, Fribourg M, Sealfon SC. Cytokine response is determined by duration of receptor and signal transducers and activators of transcription 3 (STAT3) activation. J Biol Chem 2013;288:2986-93.
48. Lang R, Patel D, Morris JJ, et al. Shaping gene expression in activated and resting primary macrophages by IL-10. J Immunol 2002;169:2253-63.
49. Cassatella MA, Tamassia N, Crepaldi L, et al. Lipopolysaccharide primes neutrophils for a rapid response to IL-10. Eur J Immunol 2005;35:1877-85.
50. Itoh K, Hirohata S. The role of IL-10 in human B cell activation, proliferation, and differentiation. J Immunol 1995;154:4341-50.
51. Hutchins AP, Diez D, Miranda-Saavedra D. The IL-10/STAT3-mediated anti-inflammatory response: recent developments and future challenges. Brief Funct Genomics 2013;12:489-98.
52. Rutgeerts P, D'Haens G, Targan S, et al. Efficacy and safety of retreatment with anti-tumor necrosis factor antibody (infliximab) to maintain remission in Crohn's disease. Gastroenterology 1999;117:761-9.
53. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. Aliment Pharmacol Ther 2011;33:987-95.
54. Niederreiter L, Adolph TE, Kaser A. Anti-IL-12/23 in Crohn's disease: bench and bedside. Curr Drug Targets 2013;14:1379-84.
55. Feagan BG, Sandborn WJ, Gasink C, et al. Ustekinumab as Induction and Maintenance Therapy for Crohn's Disease. N Engl J Med 2016;375:1946-1960.
56. Hanauer SB, Sandborn WJ, Feagan BG, et al. IM-UNITI: 3 Year Efficacy, Safety, and Immunogenicity of Ustekinumab Treatment of Crohn's Disease. J Crohns Colitis 2019.
57. Feagan BG, Sandborn WJ, D'Haens G, et al. Induction therapy with the selective interleukin-23 inhibitor risankizumab in patients with moderate-to-severe Crohn's disease: a randomised, double-blind, placebo-controlled phase 2 study. Lancet 2017;389:1699-1709.
58. Feagan BG, Panes J, Ferrante M, et al. Risankizumab in patients with moderate to severe Crohn's disease: an open-label extension study. Lancet Gastroenterol Hepatol 2018;3:671-680.
59. Sands BE, Chen J, Feagan BG, et al. Efficacy and Safety of MEDI2070, an Antibody Against Interleukin 23, in Patients With Moderate to Severe Crohn's Disease: A Phase 2a Study. Gastroenterology 2017;153:77-86 e6.
60. Waldner MJ, Neurath MF. Master regulator of intestinal disease: IL-6 in chronic inflammation and cancer development. Semin Immunol 2014;26:75-9.

61. Ito H, Takazoe M, Fukuda Y, et al. A pilot randomized trial of a human anti-interleukin-6 receptor monoclonal antibody in active Crohn's disease. Gastroenterology 2004;126:989-96; discussion 947.
62. Danese S, Vermeire S, Hellstern P, et al. Randomised trial and open-label extension study of an anti-interleukin-6 antibody in Crohn's disease (ANDANTE I and II). Gut 2019;68:40-48.
63. Jovani M, Fiorino G, Danese S. Anti-IL-13 in inflammatory bowel disease: from the bench to the bedside. Curr Drug Targets 2013;14:1444-52.
64. Reinisch W, Panes J, Khurana S, et al. Anrukinzumab, an anti-interleukin 13 monoclonal antibody, in active UC: efficacy and safety from a phase IIa randomised multicentre study. Gut 2015;64:894-900.
65. Hohenberger M, Cardwell LA, Oussedik E, et al. Interleukin-17 inhibition: role in psoriasis and inflammatory bowel disease. J Dermatolog Treat 2018;29:13-18.
66. Hueber W, Sands BE, Lewitzky S, et al. Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. Gut 2012;61:1693-700.
67. Targan SR, Feagan B, Vermeire S, et al. A Randomized, Double-Blind, Placebo-Controlled Phase 2 Study of Brodalumab in Patients With Moderate-to-Severe Crohn's Disease. Am J Gastroenterol 2016;111:1599-1607.
68. Monteleone G, Neurath MF, Ardizzone S, et al. Mongersen, an oral SMAD7 antisense oligonucleotide, and Crohn's disease. N Engl J Med 2015;372:1104-13.
69. van Deventer SJ, Elson CO, Fedorak RN. Multiple doses of intravenous interleukin 10 in steroid-refractory Crohn's disease. Crohn's Disease Study Group. Gastroenterology 1997;113:383-9.
70. Colombel JF, Rutgeerts P, Malchow H, et al. Interleukin 10 (Tenovil) in the prevention of postoperative recurrence of Crohn's disease. Gut 2001;49:42-6.
71. Ben-Horin S, Kopylov U, Chowers Y. Optimizing anti-TNF treatments in inflammatory bowel disease. Autoimmun Rev 2014;13:24-30.
72. Amiot A, Serrero M, Peyrin-Biroulet L, et al. One-year effectiveness and safety of vedolizumab therapy for inflammatory bowel disease: a prospective multicentre cohort study. Aliment Pharmacol Ther 2017;46:310-321.
73. Wils P, Bouhnik Y, Michetti P, et al. Long-term efficacy and safety of ustekinumab in 122 refractory Crohn's disease patients: a multicentre experience. Aliment Pharmacol Ther 2018;47:588-595.

74. Mumolo MG, Bertani L, Ceccarelli L, et al. From bench to bedside: Fecal calprotectin in inflammatory bowel diseases clinical setting. World J Gastroenterol 2018;24:3681-3694.
75. Caviglia GP, Ribaldone DG, Rosso C, et al. Fecal calprotectin: beyond intestinal organic diseases. Panminerva Med 2018;60:29-34.
76. Niederau C, Backmerhoff F, Schumacher B, et al. Inflammatory mediators and acute phase proteins in patients with Crohn's disease and ulcerative colitis. Hepatogastroenterology 1997;44:90-107.
77. Billiet T, Cleynen I, Ballet V, et al. Evolution of cytokines and inflammatory biomarkers during infliximab induction therapy and the impact of inflammatory burden on primary response in patients with Crohn's disease. Scand J Gastroenterol 2017;52:1086-1092.
78. Ogawa K, Matsumoto T, Esaki M, et al. Profiles of circulating cytokines in patients with Crohn's disease under maintenance therapy with infliximab. J Crohns Colitis 2012;6:529-35.
79. Song L, Hanlon DW, Chang L, et al. Single molecule measurements of tumor necrosis factor alpha and interleukin-6 in the plasma of patients with Crohn's disease. J Immunol Methods 2011;372:177-86.
80. Defendenti C, Sarzi-Puttini P, Saibeni S, et al. Significance of serum Il-9 levels in inflammatory bowel disease. Int J Immunopathol Pharmacol 2015;28:569-75.
81. Feng T, Chen B, Li L, et al. Serum Interleukin 9 Levels Predict Disease Severity and the Clinical Efficacy of Infliximab in Patients with Crohn's Disease. Inflamm Bowel Dis 2017;23:1817-1824.
82. Atreya R, Neumann H, Neufert C, et al. In vivo imaging using fluorescent antibodies to tumor necrosis factor predicts therapeutic response in Crohn's disease. Nat Med 2014;20:313-8.
83. Fujino S, Andoh A, Bamba S, et al. Increased expression of interleukin 17 in inflammatory bowel disease. Gut 2003;52:65-70.
84. Visvanathan S, Baum P, Salas A, et al. Selective IL-23 Inhibition by Risankizumab Modulates the Molecular Profile in the Colon and Ileum of Patients With Active Crohn's Disease: Results From a Randomised Phase II Biopsy Sub-study. J Crohns Colitis 2018;12:1170-1179.
85. Katz LH, Kopylov U, Fudim E, et al. Expression of IL-2, IL-17 and TNF-alpha in patients with Crohn's disease treated with anti-TNF antibodies. Clin Res Hepatol Gastroenterol 2014;38:491-8.

86. Olsen T, Goll R, Cui G, et al. TNF-alpha gene expression in colorectal mucosa as a predictor of remission after induction therapy with infliximab in ulcerative colitis. Cytokine 2009;46:222-7.
87. Olsen T, Cui G, Goll R, et al. Infliximab therapy decreases the levels of TNF-alpha and IFN-gamma mRNA in colonic mucosa of ulcerative colitis. Scand J Gastroenterol 2009;44:727-35.
88. Hassan C, Ierardi E, Burattini O, et al. Tumour necrosis factor alpha down-regulation parallels inflammatory regression in ulcerative colitis patients treated with infliximab. Dig Liver Dis 2007;39:811-7.
89. Magnusson MK, Dahlen R, Strid H, et al. CD25 and TNF receptor II reflect early primary response to infliximab therapy in patients with ulcerative colitis. United European Gastroenterol J 2013;1:467-76.
90. Dahlen R, Magnusson MK, Bajor A, et al. Global mucosal and serum cytokine profile in patients with ulcerative colitis undergoing anti-TNF therapy. Scandinavian journal of gastroenterology 2015;50:1118-26.
91. Rismo R, Olsen T, Cui G, et al. Mucosal cytokine gene expression profiles as biomarkers of response to infliximab in ulcerative colitis. Scand J Gastroenterol 2012;47:538-47.
92. Sato S, Chiba T, Nakamura S, et al. Changes in cytokine profile may predict therapeutic efficacy of infliximab in patients with ulcerative colitis. J Gastroenterol Hepatol 2015;30:1467-72.
93. Nishida Y, Hosomi S, Watanabe K, et al. Serum interleukin-6 level is associated with response to infliximab in ulcerative colitis. Scand J Gastroenterol 2018;53:579-585.
94. Battat R, Dulai PS, Vande Casteele N, et al. Biomarkers Are Associated With Clinical and Endoscopic Outcomes With Vedolizumab Treatment in Ulcerative Colitis. Inflamm Bowel Dis 2018.
95. Zeissig S, Rosati E, Dowds CM, et al. Vedolizumab is associated with changes in innate rather than adaptive immunity in patients with inflammatory bowel disease. Gut 2018.
96. Soendergaard C, Seidelin JB, Steenholdt C, et al. Putative biomarkers of vedolizumab resistance and underlying inflammatory pathways involved in IBD. BMJ Open Gastroenterol 2018;5:e000208.
97. Bertani L, Antonioli L, Baglietto L, et al. Early Measurement of Serum Cytokines As Predictor of Clinical and Endoscopic Outcome to Vedolizumab in Patients with Ulcerative Colitis. Gastroenterology 2019;156:654.

NOTES

Conflicts of interest: None of the authors have potential conflicts of interest to declare

Funding: This paper has not required any source of fundind

Authors' contributions: All authors equally contributed to the manuscript writing and approved the final version

TABLES

Table 1: Anti-cytokines drugs currently available or in study in inflammatory bowel diseases

Drug	**Mechanism**	**Route**	**Clinical Trials**	
			UC	**CD**
Infliximab	Anti-TNF	i.v.	Approved	Approved
Adalimumab	Anti-TNF	s.c.	Approved	Approved
Golimumab	Anti-TNF	s.c.	Approved	Failed
Ustekinumab	Anti-p40 IL-12/23	i.v./s.c.	Phase 3	Approved
Risankizumab	Anti-p19 IL-23	i.v./s.c.	Phase 2	Phase 2
Mirikizumab			Phase 3	Phase 2
Brazikumab			Phase 3	Phase 2
Guselkumab			Phase 2	Phase 2
PF-04236921	Anti-IL-6	s.c.	-	Phase 2
Anrukizumab	Anti-IL-13	i.v.	Failed	-
Secukinumab	Anti-IL-17A	i.v.	-	Failed
Brodalumab	Anti-IL-17RA	i.v.	-	Failed
SCH 52000	rh-IL-10	i.v.	-	Failed