Inflammatory bowel disease-related arthritis – clinical evaluation and possible role of cytokines

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Abstract

Objectives: In inflammatory bowel disease (IBD), characterized by chronic mucosal inflammation, rheumatic abnormalities ranging from arthralgia to spondyloarthritis (SpA) are the most common extraintestinal manifestations. The pathogenesis of IBD-related arthritis is unclear. In this study, we search for clinical and immunological differences between patients with IBD-associated spondyloarthritis and IBD patients without SpA symptoms.

Material and methods: Patients with an established diagnosis of IBD, suffering from Leśniowski-Crohn disease (L-CD, $n = 24$) or ulcerative colitis (UC, $n = 27$), were enrolled in the study. Clinical evaluation of patients, based on medical history, blood tests, physical and radiological examinations, allowed two subgroups of patients to be established. One subgroup comprised patients fulfilling criteria for both IBD and SpA (IBD + SpA, $n = 29$), while the other included IBD patients with arthralgia only (IBD, $n = 22$). Serum concentrations of interleukins (IL-6, IL-10, IL-21, IL-22, IL-23) and interferon γ (IFN-γ) were measured by specific enzyme-linked immunosorbent assays (ELISA).

Results: Patients with IBD + SpA were characterized by shorter disease duration (3 vs. 9 years), higher frequency of HLA-B27 positivity (60.7% vs. 4.5%) and uveitis (20.7% vs. 0%), compared with the IBD subgroup. The serum concentrations of C-reactive protein (CRP) and tested cytokines did not differ between IBD + SpA and IBD patients, or between L-CD and UC groups. However, in the IBD + SpA subgroup there was weak to moderate positive correlation between serum concentrations of CRP and several cytokines (IL-6, IL-21, IFN-γ), and additional moderate positive correlation between serum concentrations of IL-23 and clinical activity of SpA. By contrast, in IBD subgroup a strong inverse correlation between serum concentrations of Interleukin 23 and CRP was found.

Conclusions: IBD-related spondyloarthritis occurs relatively early, affects mostly HLA-B27(+) individuals, and is often accompanied by ocular involvement. In these patients several circulating cytokines are associated with systemic inflammation. IL-23 seems to be protective in IBD while detrimental in IBD-related spondyloarthritis.

Key words: inflammatory bowel disease, proinflammatory cytokines, spondyloarthritis.

Introduction

The two major forms of inflammatory bowel disease (IBD), Leśniowski-Crohn disease (L-CD) and ulcerative colitis (UC), are accompanied by a variety of extra-intestinal manifestations. The most common is an articular involvement, which occurs in 17% to 30% of IBD patients [1, 2]. Peripheral arthritis occurs in the forms of both pauciarticular and polyarticular disease and its prevalence in IBD is of a wide range (from 0.4% to 34.6%) [3]. Axial involvement ranges from isolated inflammatory back pain to ankylosing spondylitis, and is reported to be present in 2–16% of IBD patients, with a higher prev-

alence in L-CD than UC patients [1]. IBD-related arthritis is classified as seronegative spondyloarthritis (SpA) [3].

The aetiology of both types of IBD, i.e. L-CD and UC, is still largely unknown. The pathogenesis of these diseases seems to results from a combination of environmental, genetic and immunological factors that altogether lead to inflammation of the gut mucosa in genetically predisposed individuals. First of all, it is commonly accepted that IBD originates from a dysregulated immune response to enteric bacteria. Many clinical and histological studies have shown that gut involvement is also a prominent feature of patients suffering from spondyloarthritis [4–6]. Numerous ileocoloscopic examinations have demonstrated gut involvement in 25% to 75% of patients with spondyloarthritis [6, 7]. This observation has been confirmed by recent results obtained using abdominal scintigraphy with labelled leucocytes. Application of this method has revealed signs of intestinal inflammation in 50% of patients suffering from spondyloarthritis [8], which is the first level of evidence about the existence of a gut-joint axis.

Recent evidence suggests that also immunological disorders, especially cytokine network abnormalities, may critically contribute to the pathogenesis of both IBD and spondyloarthritis. Leśniowski-Crohn disease is thought to be driven by type 1 T helper (Th1) cells, because elevated levels of interleukin 18 (IL-18) and interferon γ (IFN-γ) are detected in patients sera, especially in clinically active disease [5, 9, 10]. By contrast, ulcerative colitis is regarded as a type 2 T helper (Th2)-like disease, for it is accompanied by the elevation of cytokines associated mostly with Th2 cell differentiation pathway, e.g. IL-4, IL-5, IL-13 [5]. Moreover, it has recently been proposed that also the IL-23/IL-17 cytokine axis plays a key role in pathogenesis of both IBD and spondyloarthritis. This axis includes not only IL-23 and IL-17 but also IL-21 and IL-22 produced by T lymphocytes and other cell types. By exerting either anti- or pro-inflammatory properties, all cytokines of IL-23/IL-17 axis are implicated in the regulation of gut homeostasis. Therefore, dysregulation of these cytokines balance is believed to represent a key factor related to the development of gut inflammation [11–15]. Resent studies shows evidently that in the joint inflammation cytokines of IL-23/IL-17 axis play detrimental role [16, 17]. Moreover, another cytokines, including IL-6 endowed with pro-inflammatory and anti-inflammatory properties and immunosuppressive IL-10, are implicated in setting of inflammation intensity [18, 19]. The above data suggests that disturbances of cytokine network contribute to IBD pathogenesis and IBD-related arthritis development, but deeper understanding of these abnormalities is required.

In this study, we analysed whether: (i) the circulating concentrations of cytokines related to pathogenesis of both IBD and spondyloarthritis, i.e. IL-6, IL-10, IL-21, IL-22, IL-23, and IFN-γ differ between patients suffering from IBD and IBD-related spondyloarthritis, and (ii) whether in these groups of patients the cytokines concentrations show any association with demographic data, clinical manifestations, disease activity, and laboratory parameters.

Material and methods

Fifty-one patients with an established diagnosis of IBD were enrolled in the study. Among them 24 patients suffered from L-CD and 27 from UC. They were recruited from patients admitted to the Early Arthritis Diagnostic Department of the National Institute of Geriatrics, Rheumatology, and Rehabilitation (NIGRR), as a part of the routine diagnostic procedures for musculoskeletal complaints. When recruiting participants we used the following exclusion criteria: pregnancy or breastfeeding; clinically significant impairment of hepatic and renal function; alcohol abuse; active hepatotropic viral infection; treatment-resistant infection; ongoing history of cancer if no remission was achieved; uncontrolled diabetes. This study meets all criteria contained in the Declaration of Helsinki and was approved by the Ethics Committee of the NIGRR. Written informed consent was obtained from all participants before they entered the study.

Clinical evaluation of patients was based on medical history, physical examination, blood tests (HLA-B27 typing), and laboratory tests evaluating inflammatory activity, i.e. the measurement of C-reactive protein (CRP) serum concentration, as well as on recommended radiological examinations (X-rays, magnetic resonance or ultrasonography). The diagnosis of spondyloarthritis (SpA) was established according to the ASAS (Assessment of SpondyloArthritis International Society) criteria [20]. Disease activity was assessed using BASDAI (Bath Ankylosing Spondylitis Disease Activity Index). Patients were classified into two groups based on the results of diagnostic process. One subgroup comprised patients fulfilling criteria for both IBD and spondyloarthritis (IBD + SpA, $n = 29$), while the other included IBD patients with arthralgia only (IBD, $n = 22$).

Serum concentrations of IL-6, IL-10, IL-21, IL-22, IL-23 and IFN-γ were determined with commercially available enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, San Diego, CA, USA). The expression of HLA-B27 antigen was detected on erythrocyte-lysed whole blood using HLA-B27 kit (BD Bioscience, San Jose, CA, USA) and immunofluorescence method. Data were analysed using Statistica 10 software (StatSoft Inc., Tulsa, OK, USA). The Mann-Whitney U test or Fisher's exact test were used for intergroup comparison of continuous or discrete

variables, respectively. Correlation was assessed using a Spearman's rank test (*R* value is shown). *P* values < 0.05 were considered significant.

Results

Clinical characteristics of the patients with IBD and IBD-related spondyloarthritis are shown in Table I. Most of the enrolled patients 88.2% (*n* = 45) received anti-inflammatory and immunosuppressive drugs: sulfasalazine (*n* = 20), mesalazine (*n* = 11), azathioprine (*n* = 10), methotrexate (*n* = 2), 6-mercaptopurine (*n* = 2). Only a few patients have been treated in the past with tumor necrosis factor inhibitors, only four patients receiving systemic glucocorticosteroids. There were no significant differences between these groups in the patients age, proportion of males and females, serum CRP concentration, as well as disease activity evaluated by BASDAI. Nevertheless, patients with IBD-related spondyloarthritis were characterized by shorter disease duration, significantly more frequent HLA-B27 positivity and uveitis noted in more than half and almost in quarter of them, respectively. However the serum concentrations of tested cytokines did not differ significantly between IBD + SpA and IBD patients (Fig. 1). Similarly, no differences in serum concentration of analysed cytokines were found between the patients with L-CD and UC (Fig. 1).

Despite this, an association of various cytokines with the serum CRP levels were stated in both IBD and IBD-related arthritis groups. In the patients with IBD + SpA there was positive, weak to moderate, correlation between CRP levels and serum concentrations of three cytokines, i.e. IL-6 (*R* = 0.394), IL-21 (*R* = 0.494) and IFN-γ (*R* = 0.52) (Fig. 2). By contrast, in patients with IBD the level of CRP was strongly but inversely (*R* = –0.641) correlated with IL-23 serum concentration (Fig. 2). In addition, in the group of patients with IBD-related spondyloarthritis serum IL-23 concentration positively and rather strongly (*R* = 0.57) correlated with spondyloarthritis clinical activity assessed by BASDAI (Fig. 3). As CRP is a marker of systemic inflammation, these observations suggest contribution of several cytokines (IL-6, IL-21, and IFN-γ) to systemic inflammation intensity in IBD-related spondyloarthritis, but not in IBD. Moreover, present results may suggest opposite role of IL-23 in IBD and IBD-related spondyloarthritis.

Discussion

In the search for biological markers discriminating between IBD and IBD-related spondyloarthritis, we focused at measuring the concentrations of circulating cytokines, endowed with regulatory, pro- and anti-inflammatory activities, that are thought to contribute to these diseases pathogenesis. Unfortunately, we failed to find strong differences in serum cytokine levels between tested patients groups. This may result from various reasons. First, we compared a relatively small cohort

Table I. Baseline characteristics of the study patients

	IBD (*n* = 22)	IBD + SpA (*n* = 29)	*p* value
Demographics			
Age, median (IQR), years	45.5 (41)	42.3 (40)	0.49
Gender, male/female, %	41/59	48.3/51.7	1.0
Disease duration, median (IQR), years	9 (9)	3 (8.5)	**0.04**
IBD type			
UC, %	54.5	51.7	0.5
L-CD, %	45.5	48.3	0.5
Clinical and laboratory data			
HLA-B27 antigen positivity, %	4.5	60.7	**< 0.0001**
Sacroiliitis, %	0	75	**< 0.0001**
Arthritis, %	0	44.8	**0.00018**
Uveitis, %	0	24.1	**0.014**
BASDAI, median (IQR)	3.2 (2)	6.85 (1.8)	0.075
CRP, median (IQR) mg/l	8.5 (13)	12.5 (19)	0.24

IBD – inflammatory bowel disease; SpA – spondyloarthritis; UC – ulcerative colitis; L-CD – Leśniowski-Crohn disease; BASDAI – Bath Ankylosing Spondylitis Disease Activity Index; CRP – C-reactive protein.
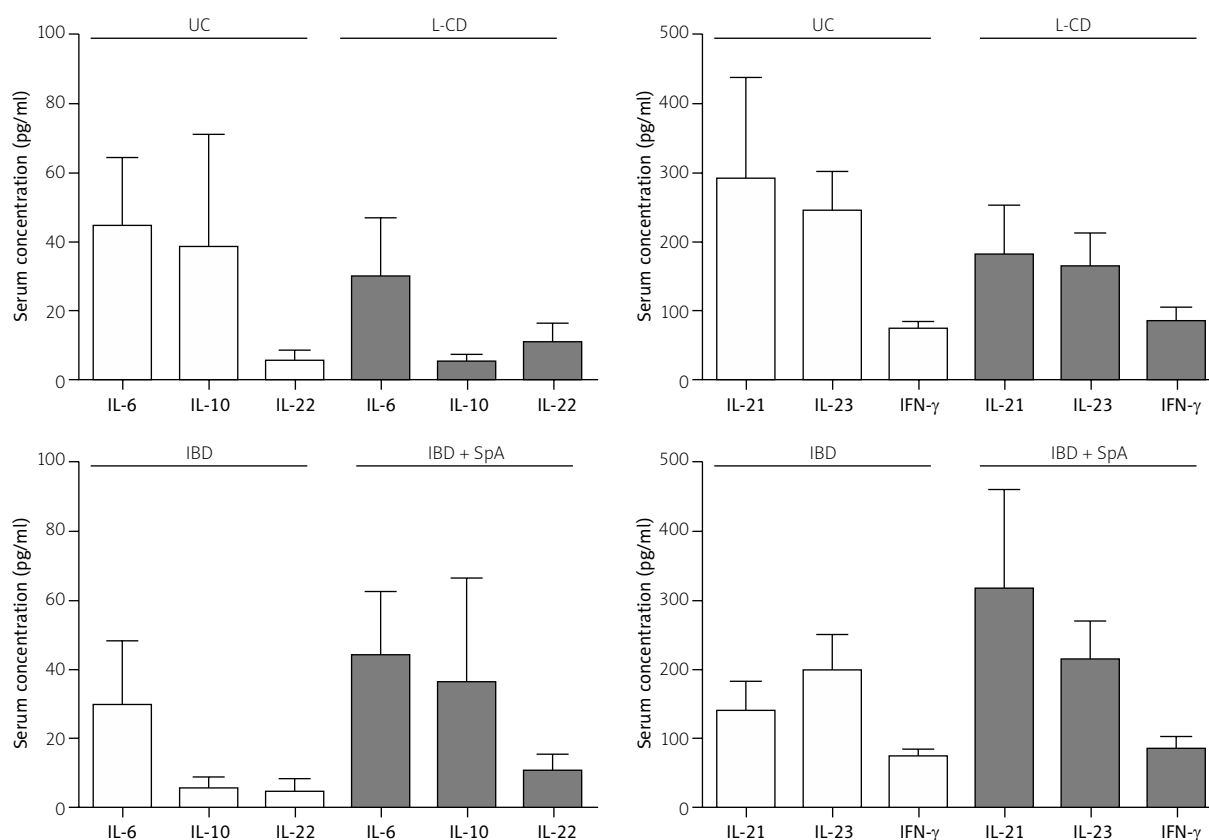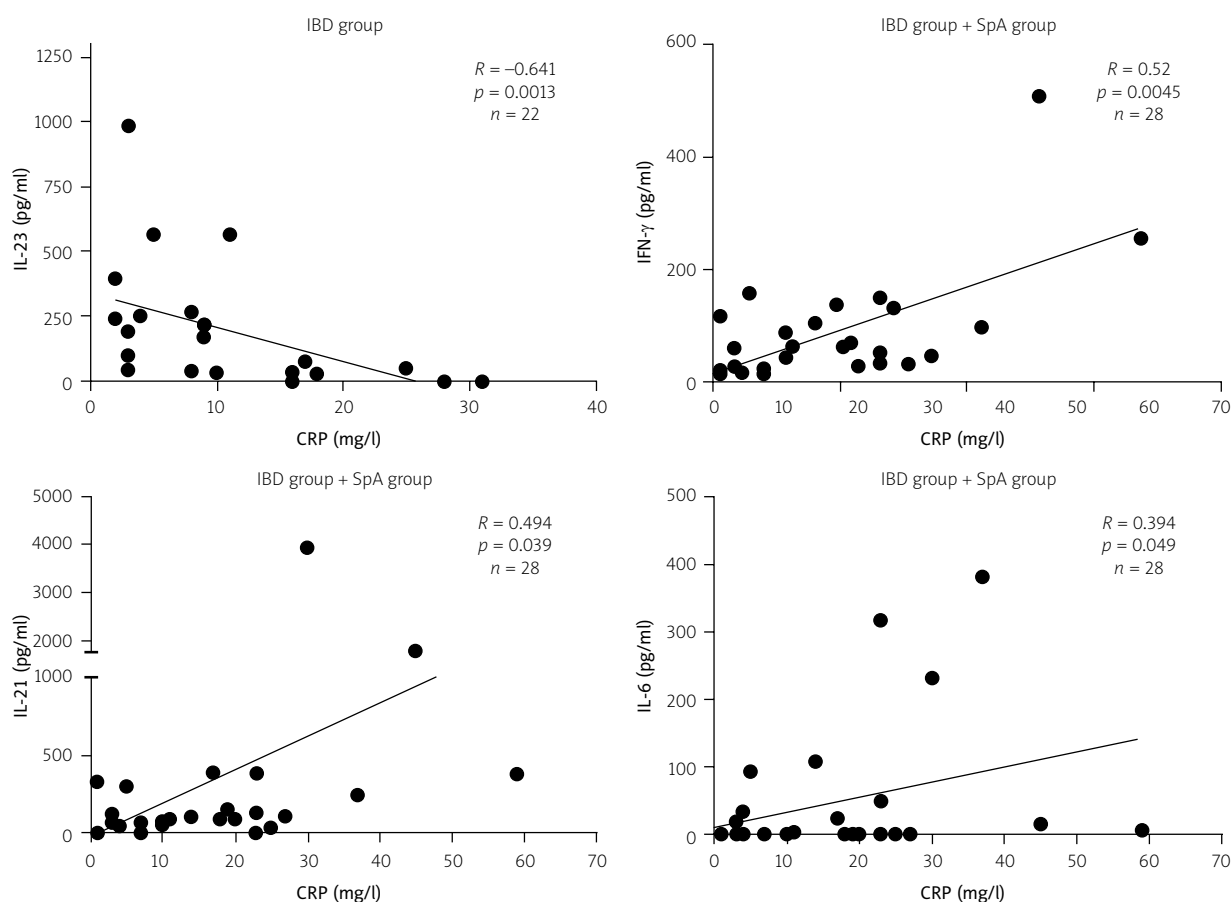
Fig. 1. Serum concentrations of cytokines in patients subgroups. No significant differences were found between patients suffering from ulcerative colitis (UC, *n* = 27) vs. Leśniowski-Crohn disease (L-CD, *n* = 24) and with inflammatory bowel disease (IBD, *n* = 22) vs. IBD-related arthritis (IBD + SpA, *n* = 29). Data are expressed as the mean ± SEM.

of patients (IBD + SpA, *n* = 29, and IBD, *n* = 22). Second, our patients group included individuals with L-CD and UC and although the majority of them were in clinical remission, an endoscopic evaluation has not been done. Thus, we cannot exclude that the patients may differ in IBD activity. It is likely, that these reasons may account also for the lack of significant differences in the serum cytokine levels between L-CD and UC patients (Fig. 1), observed also by others [21]. On the other hand, we did not include a healthy volunteers group, because the intention of our present preliminary study was to search for any dissimilarities between patients with IBD and IBD-related spondyloarthritis.

Despite these limitation, we managed to observed striking differences in the frequency of HLA-B27 positivity between IBD and IBD + SpA subgroups. Namely, the expression of HLA-B27 antigen was detected in 60.7% of patients with IBD-related spondyloarthritis, which is consistent with other data reporting the presence of HLA-B27 in 30–80% patients with IBD-related arthritis [3]. By contrast, we detected HLA-B27 antigen only in 4.5% of IBD patients (Table I). Thus, our results support

an important role of HLA-B27 in the pathogenesis of spondyloarthritis, irrespective whether this disease accompanies IBD or occurs separately [22].

It is believed, that concentrations of circulating cytokines reflect, to a certain extent, the intensity of local inflammation response. In this context, another interesting finding of our study was the positive correlation between serum concentrations of several cytokines (i.e. IL-6, IL-21 and IFN-γ) and CRP observed in IBD-related spondyloarthritis group only (Fig. 2). Interleukin 6 is a pleiotropic cytokine playing immunoregulatory role in the acute inflammatory response but exerting pro-inflammatory effects in the chronic inflammation, and IFN-γ is a typical Th1 cytokine [19]. Both IL-6 and IFN-γ are thought to be engaged in the pathogenesis of L-CD and their neutralization by biological drugs was evaluated in clinical trials. However, anti-IFN-γ therapy did not results in major improvement and the clinical efficacy of anti-IL-6 therapy needs further confirmation [23]. As for spondyloarthritis, the role of IL-6 in disease development and/or progression is not clear, although up-regulated serum level of this cytokine related to systemic

Fig. 2. Correlation between serum concentrations of cytokines and C-reactive protein (CRP) in patients with inflammatory bowel disease (IBD) and IBD-related spondyloarthritis (IBD + SpA). The correlation was assessed using Spearman's rank test; R and p values are shown; n – number of patients. Note that although Spearman's rank correlations were performed, regression lines were used for graphic purposes only.

inflammatory markers has been reported [24]. Accumulating evidence shows that in both IBD and spondyloarthritis atypical Th cells of mixed (Th17/Th1) phenotype play pathogenic role [16, 23]. Interleukin 21 acts as an autocrine factor which promotes and sustains Th17 lineage commitment. In the inflamed intestine of IBD patients this cytokine is produced in excess, originates from various Th cell subsets and is suggested to expand and support the ongoing mucosal inflammation [25]. A similar pro-inflammatory role is attributed to IL-21 in joint inflammation, characteristic for rheumatoid arthritis and spondyloarthritis [16, 17]. Our observations suggest that circulating IL-6, IL-21 and IFN-γ levels reflect inflammation occurring in affected joints rather than in the gut, as the concentrations of these cytokines correlated with CRP levels only in the IBD + SpA group (Fig. 2).

Besides IL-21 also several other cytokines are associated with the development and functional activity of Th17 cells. These cytokines belong to so called IL-23/IL-17 cytokine axis which, according to recent accumulat-

ing data, is thought to play a key role in pathogenesis of both IBD and spondyloarthritis [11–17]. In humans, differentiation of Th lymphocytes into Th17 lineage is controlled by cytokines, which contribute to initiation (IL-6, IL-21) and amplification (IL-21) of this differentiation pathway, or support expansion of Th17 cell pool (IL-23). Interleukin 17, released from Th17 lymphocytes and other cells (e.g. neutrophils, mast cells), plays an important role in the protection of the host against various bacterial and fungal infections, particularly at mucosal surfaces. This cytokine enhances recruitment and facilitates activation of neutrophils, as well as stimulates the production of defensins by epithelial cells [22]. In normal condition anti-bacterial response is controlled by immunomodulatory mechanisms, especially by regulatory T cells (Tregs), which synthesize cytokines, such as IL-10 and transforming growth factor β, endowed with immunosuppressive activities. Disorders of these immunoregulatory pathways lead to the excessive activation of IL-23/IL-17 axis and in consequence to the chronic in-

flammation of not only intestinal mucosa but also joint tissues [14, 16].

Potential contribution of the IL-23/IL-17 axis to IBD and spondyloarthritis pathology is supported by recent observations, reporting significantly increased concentrations and/or enhanced expression of the IL-23/IL-17 axis-associated cytokines in the sera [26] and synovial fluids of patients with spondyloarthritis as well as in the intestinal mucosa of IBD patients [11, 12]. There is, however, discrepancy concerning association of IL-23 levels with clinical activity of spondyloarthritis [24, 27]. Despite this, some authors have shown that serum IL-23 levels were significantly higher in patients suffering from spondyloarthritis then in healthy volunteers and correlated with disease activity measured by BASDAI scores [27]. It is consistent with our results in the group of patients with IBD-related spondyloarthritis, as in this group serum IL-23 concentration positively and rather strongly ($R = 0.57$) correlated with spondyloarthritis clinical activity assessed by BASDAI (Fig. 3).

Unexpectedly, in patients with IBD the level of CRP was strongly but inversely ($R = -0.641$) correlated with IL-23 serum concentration (Fig. 2). It should be underlined that in the gut IL-23 plays dual role, mediating both protective as well as pathologic functions. Under homeostatic conditions, IL-23 is produced in low amounts and contributes to support intestinal barrier function and anti-bacterial immune response via triggering IL-17 and IL-22 cytokines.
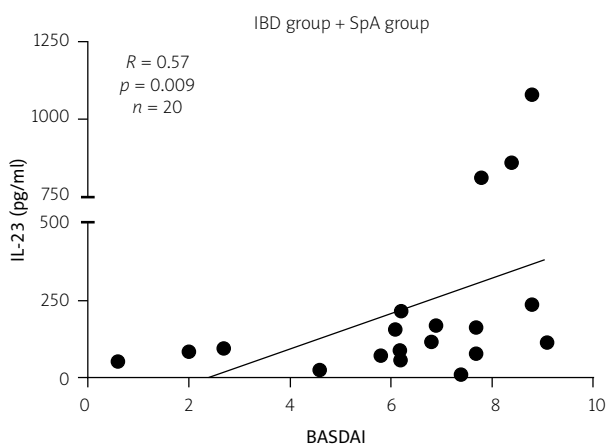
Breakdown of intestinal immune homeostasis by strong inflammatory stimuli up-regulates local IL-23 synthesis, leading to inhibition of Tregs lymphocytes and expansion of Th1 and Th17 cell subsets, innate immune activation and development of chronic mucosal inflammation [12]. Interestingly, in some animal colitis models IL-23 acts as an anti-inflammatory cytokine by suppressing IL-12-driven pathology [28]. Moreover, in acute model of colitis systemic symptoms are dependent on IL-12 whereas IL-23 plays a non-redundant role in gut inflammation [12].

The role of these cytokines in the development of systemic symptoms in human IBD is largely unexplored. Clinical trials with a monoclonal antibody directed against the common p40 subunit of IL-23 and IL-12 have allowed to be accepted this biological drug for the treatment of psoriatic arthritis patients, having shown some clinical response in L-CD and beneficial effect in axial ankylosing spondylitis [29, 30]. It is expected that planned therapeutic application of monoclonal antibody directed to IL-23 specific p19 subunit will clarify the role of IL-12 and IL-23 in human IBD.

Conclusions

1. IBD-related spondyloarthritis can occur in relatively early period of underlying disease, affects mostly HLA-B27(+) individuals and is more frequently accompanied by ocular involvement, compared with IBD.
2. In IBD-related spondyloarthritis patients circulating IL-6, IL-21 and IFN-γ are associated with the intensity of systemic inflammation.
3. Present results may suggest opposite role of IL-23 in IBD and IBD-related spondyloarthritis (beneficial vs. detrimental, respectively).

Fig. 3. Correlation between serum concentrations of interleukin 23 (IL-23) and spondyloarthritis activity in patients with inflammatory bowel disease-related spondyloarthritis (IBD + SpA). Disease activity was evaluated by Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). The correlation was assessed using Spearman's Rank test; *R* and *p* values are shown; *n* – number of patients. Note that although Spearman's Rank correlations were performed, regression lines were used for graphic purposes only.

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