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ORIGINAL ARTICLE

Interleukin-1 associations in inflammatory bowel disease and the enteropathic seronegative spondylarthritis

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Abstract
Purpose This study aims to investigate any associations of the proinflammatory cytokine IL-1 in treated patients with inflammatory bowel disease (IBD) and the enteropathic seronegative spondylarthritis (eSpA).
Methods Thirty-four patients with Crohn's disease (CD), 26 with ulcerative colitis (UC) and 14 patients with SpA participated in the study. Valid clinical indexes, CRP values and the endoscopic and histologic examination were used for the determination of disease activity. IL-1α, IL-1β, IL-1 receptor antagonist (IL-1Ra) were measured by ELISA. Nonparametric tests were used for continuous and categorical data.
Results Enteropathic SpA diagnosed in 29.4 % CD and 30.8 % UC patients. Active disease had 58.8 % CD (aCD), 76.9 % UC and 50 % SpA patients. Active and inactive CD (iCD) significantly differ on IL-1α levels (11.2 vs. 3.9 pg/ml; $p = 0.034$). Active and inactive UC significantly differ on IL-1β (3.7 vs. 2.3 pg/ml; $p = 0.054$) and IL-1Ra levels (15.9 vs. 12.7 pg/ml; $p = 0.023$). Active and inactive SpA (iSpA) significantly differ on IL-1Ra (16.9 vs. 14.8 pg/ml; $p = 0.033$) and marginally on IL-1α levels (20 vs. 3.9 pg/ml; $p = 0.06$). Patients with aCD/ieSpA exhibited significant differences on IL-1α ($p = 0.022$) compared to those with iCD/ieSpA.
Conclusions IL-1α is associated with CD activity, while IL-1β and IL-1Ra are associated with UC activity in treated patients with IBD. Prominent cytokine in SpAs seems to be IL-1α.

Keywords Crohn's disease · Ulcerative colitis · Cytokines · Extra-intestinal manifestations

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Introduction

Inflammatory bowel disease (IBD) and seronegative spondylarthritis (SpA) share common clinical characteristics and they frequently overlap. Some believe that they represent a different expression of the same disease based on common epidemiological, pathogenetic and clinical features such as the overlap of symptoms on a patient or the presence of different forms of seronegative spondylarthritis among family members [1].

Proinflammatory cytokines, particularly tumor necrosis factor alpha (TNFα) and interleukin-1 (IL-1), are found to play a pivotal role during the acute phase response. Although treatment may modify the cytokine profile, this has not been studied extensively. In IBD, the disease process is divided into three phases—initiation, augmentation and perpetuation [2]. Rheumatologists, on the other hand, prefer to focus on clinical and radiological findings when they follow-up the evolution of SpAs. Despite treatment, there are frequent relapses in both conditions, and we

hypothesize that proinflammatory cytokines have in any occasion the same cornerstone function, although such findings are not consistent [3].

This study is a combined gastroenterology and rheumatology approach aiming to investigate the role of IL-1 in treated patients with IBD and seronegative spondylarthritidis. It is an effort to analyze the prevalence of IL-1 subtypes, interleukin-1α (IL-1α) and interleukin-1β (IL-1β) as well as their antagonist receptor (IL-1Ra) in exacerbation and remission in patients with Crohn's disease (CD), ulcerative colitis (UC) and their associations with the enteropathic spondylarthritis (eSpA).

Materials and methods

Study population

Seventy-four adult patients with IBD and SpA (male/female ratio: 48/26) fulfilled the inclusion criteria and enrolled in the study after informed consent was obtained. The study was approved by the Ethical Committee of the University Hospital of Alexandroupolis.

Inclusion and exclusion criteria

Patients had a definite diagnosis for Crohn's disease or ulcerative colitis according to conventional clinical, endoscopic and histologic criteria. Patients with seronegative spondylarthritis had a diagnosis of SpA according to ASAS criteria [4]. All patients were over 18 years of age, and they underwent an endoscopic evaluation at the time of assessment. This was a standard procedure for patients with IBD during the disease evaluation and follow-up. Individuals with SpA were examined endoscopically after their informed consent as it is known that up to seventy percent of SpA patients have abnormal intestinal pathology [5].

To eliminate any possible confounding factors on cytokine levels, patients were excluded if they had (1) an infectious disease prior to the evaluation month; (2) other immune-mediated diseases (bronchial asthma, allergies, etc.); (3) history of malignancy; (4) history of surgical intervention during the past 3 months and (5) presence of any other than arthritis extra-intestinal manifestation.

Disease definitions

Crohn's Disease Activity Index (CDAI) for CD, Simple Colitis Clinical Activity Index (SCCAI) for UC and the Bath Ankylosing Spondylitis Activity Index (BASDAI) for SpAs were used among with C-reactive protein and the endoscopic and histologic examination of intestinal biopsies for the estimation of disease activity.

Active disease for CD and UC is considered if (1) CDAI >150 and SCCAI ≥2, respectively, (2) presence of endoscopic activity (3) treatment with steroids >5 mg prednisone or equivalent steroid medication. If steroids were used under this level in stable or on tapered dose due to clinical and endoscopic remission, then IBD was considered inactive.

For patients with SpA, the activity was defined by the presence of BASDAI ≥ 4 or active peripheral arthritis on clinical examination by rheumatologist (PV).

Laboratory investigation

Serum samples were collected on the day of evaluation, before the endoscopic procedure and stored at −40 °C until measurement. Interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-1 receptor antagonist (IL-1Ra) and TNFα were measured by ELISA using the FIDIS Human Cytokine 10-plex kit (BMD Biomedical Diagnostics, Marne la Vallee, France). C-reactive protein (CRP) was measured by ELISA (normal values <5 mg/l), along with other routine laboratory parameters.

Statistics

The SPSS standard version 17.0 for Windows (SPSS Inc., USA) was used for the statistical analyses. The median values among with the 5th and 95th percentile position were estimated for variables in every group and subgroup of the study. Nonparametric tests were used for comparisons. The Mann–Whitney nonparametric test and the Fisher's exact test were used for comparisons between groups for continuous and categorical data, respectively. For comparisons of categorical data in more than two groups, the Kruskal–Wallis analysis of variance (ANOVA) was applied. The level of statistical significance was set to 0.05.

Results

Baseline characteristics

CD was diagnosed in 34 patients, UC in 26 and SpA in 14 patients. All of them were in treatment with disease modifying drugs. The demographic and clinical data of patients are shown in Table 1.

According to endoscopy findings, the localization of intestinal disease in CD patients was characterized as small bowel involvement, large bowel involvement or mixed small and large bowel involvement. Ten patients (29.4 %) had small bowel, 8 patients (23.5 %) had colon and 16 patients (47.1 %) had combined small and large bowel

Table 1 Demographic and clinical data of patients

	Crohn's disease	Ulcerative colitis	Arthritis (SpA)
Patients (N)	34	26	14
Sex (M/F)	22/12	15/11	11/3
Age (years)			
Median [range]	32.5 [18–61]	42.5 [23–70]	34.5 [23–65]
BMI			
Median [range]	23.2 [18–34]	25.7 [19–31]	24.9 [18–28]
Disease duration (years)			
Median [range]	2 [0.1–20]	3 [0.1–30]	4 [0.2–20]
Disease localization			
Small bowel	10		3
Large bowel	8	26	2
Small and large bowel	16		2
IBD activity			
Active	20	20	–
Inactive	14	6	7
Arthritis	10	8	
Active	5	4	10
Inactive	5	4	4
Medications			
ASA	24	25	3
Corticosteroids	26	10	5
AZA	13	5	0
MTX	0	0	4
Anti-TNF	17	1	4
Combi medication (%)	28 (82)	13 (50)	2 (14)

SpA Seronegative spondyloarthritis, *ASA* aminosalicylates, *AZA* azathioprine, *MTX* methotrexate, *Anti-TNF* anti-tumor necrosis factor alpha treatment

involvement. Patients with UC had solely involvement of the colon (no backwash ileitis). Seven out of 14 (50 %) patients with SpA had nonspecific histologic findings in intestinal biopsies from small and large bowel. Patients with SpA who were diagnosed with CD or UC automatically belonged to the IBD group, as enteropathic SpA.

Twenty patients (58.8 %) had active CD (aCD) and 20 (76.9 %) had active UC (aUC) at the time of the evaluation. Fifty percent of IBD patients with enteropathic SpA ($n = 18$) had active arthritis (aeSpA), 5 in CD and 4 in UC groups. Nine SpA patients (64.3 %) had radiologically established ankylosing spondylitis, and 5 patients (35.7 %) had early spondylarthritis as defined by MRI plus peripheral arthritis.

All patients were on treatment while a large proportion (58.1 %) was in a combination of medications. Aminosalicylates were the main treatment in CD (76 %) and in UC (96.2 %) and less favorable in arthritis patients (21.4 %). Medications included mesalazine (median 3 g daily, range 2–4) or sulphasalazine (2 g daily) and enema formulations in twelve active UC patients. Corticosteroids (prednisone or equivalent medication, median 20 mg/day, range 2.5–40 mg/day) were used in 76.5 % CD patients, 38.5 % UC and 35.7 % SpA patients; 28.6 % of the later were also treated with methotrexate (median 10 mg/week, range 7.5–12.5). Azathioprine received 18 (24.3 %) patients in a median dose 100 mg/day, range 100–200 mg/day. Anti-TNF treatment was applied more often to CD patients (50 %) and less commonly in arthritis (28.6 %) and UC (3.8 %) patients (Tables 1, 2). Those in anti-TNF treatment received infliximab 5 mg/Kg per body weight in bimonthly intravenous infusions or adalimumab 40 mg subcutaneously every other week. Two patients in active CD received adalimumab in weekly injections. The median treatment duration was 14 months (range 2–90) for CD patients, 11.5 months (range 2–240) for UC patients and 13.5 months (range 3–48) for spondylarthritis patients.

Activity in IBD and arthritis

One of the main points of attention of this study is the overlap of IBD and arthritis in relation to clinical activity of both conditions. Patients were categorized according to disease activity in order to avoid misinterpretations in cytokine levels. A careful evaluation implementing clinical and endoscopic findings divided IBD patients in the groups that are shown in Table 2. The lower than the determined CDAI and SCCAI scores in some patients with active CD and UC were due to endoscopic activity.

The nonparametric Mann–Whitney test was used for comparisons between groups. Patients did not differ in age within groups except for patients with aUC and inactive UC (iUC, $p = 0.055$). There was statistical difference between aCD and inactive CD (iCD) in relation to CDAI (153.5 vs. 75.5; $p = 0.024$) and CRP (7.1 vs. 0.9 mg/l; $p = 0.014$). There was also a significant difference between aUC and iUC in relation to SCCAI (4.5 vs. 1; $p = 0.003$) and CRP (6.1 vs. 0.5 mg/l; $p = 0.011$). In case of spondylarthritis, there was a statistical difference in the BASDAI between active SpA (aSpA) and inactive SpA (iSpA) (4.7 vs. 3.5; $p = 0.005$) but not in CRP values (6.1 vs. 4.3 mg/l; $p = 0.67$).

Comparison of cytokines

Initially, we evaluated the active and inactive forms of each disease. Due to the overlap of IBD and arthritis, we analyzed these conditions further having as primary criterion their activity. Distribution of these patients is shown on Table 3, and the comparisons that performed between with statistical results are shown in Table 4.

Between active ($n = 20$) and inactive CD ($n = 14$), there was statistical difference on IL-1α levels (11.2 vs.

Table 2 Clinical and demographic data of patients in relation to disease activity

	Crohn's disease ($n = 34$)		Ulcerative colitis ($n = 26$)		SpA ($n = 14$)	
	Active	Inactive	Active	Inactive	Active	Inactive
Patients	20	14	20	6	10	4
Sex (M/F)	14/6	8/6	11/9	4/2	7/3	4/0
Age (years)						
Median [range]	28 [18–61]	36.5 [18–56]	35 [23–65]	65 [23–70]	32 [23–65]	38.5 [23–46]
Disease duration (years)						
Median [range]	2.5 [0.2–20]	2 [0.1–11]	2 [0.1–20]	7.5 [0.4–30]	2.5 [0.2–20]	7 [2–15]
BMI						
Median [range]	22.9 [19–28]	23.8 [18–34]	25.3 [19–31]	26.1 [20–27]	25.1 [18–28]	24.2 [24–26]
CDAI						
Median [range]	153.5* [12–400]	75.5 [20–150]	–	–	–	–
SCCAI						
Median [range]	–	–	4.5* [1–8]	1 [0–2]	–	–
BASDAI	($n = 6$)	($n = 4$)	($n = 6$)	($n = 2$)		
Median [range]	3.2 [1.2–5.2]	3 [1.8–3.8]	4 [1.2–6.4]	1.1 [0.8–1.4]	4.7* [4.1–8]	3.5 [1.7–3.8]
CRP						
Median [range]	7.1* [0.2–82.4]	0.9 [0.18–27]	6.1* [0.3–145]	0.5 [0.46–2.6]	6.1 [0.4–27.4]	4.3 [2–5.3]
Enteropathic spondylarthritis	6 (30 %)	4 (28.6 %)	6 (30 %)	2 (33.3 %)	–	–
Cytokine levels (pg/ml)						
IL-1$^{\alpha}$						
Median [range]	11.2* [3.9–250]	3.9 [3.9–250]	3.9 [3.9–250]	3.9 [3.9–7.63]	20 [3.9–250]	3.9
IL-1β						
Median [range]	3.9 [2.2–17.1]	4.2 [2.5–27.7]	3.7* [1.8–244.7]	2.3 [1.8–3.7]	3.3 [1.8–210]	3.4 [2.8–38]
IL-1Ra						
Median [range]	19 [10.6–186]	16.9 [12.7–119.9]	15.9* [10.6–1,671]	12.7 [12.7–16.9]	16.9* [14.8–47]	14.8 [12.3–14.8]
TNFα						
Median [range]	4.4 [0–49]	4.4 [0–22.4]	3.8 [0–17.1]	3.9 [0–5.1]	8.6 [0.2–20.8]	5.3 [0–12.3]
Medications						
ASA	14	10	20	5	2	1
Corticosteroids	16	10	7	3	4	1
AZA	8	5	5	0	0	0
MTX	0	0	0	0	2	2
Anti-TNF	12	5	0	1	2	2

* $p < 0.05$

SpA Seronegative spondyloarthritis, *ASA* aminosalicylates, *AZA* azathioprine, *MTX* methotrexate, *Anti-TNF* anti-TNF alpha treatment with infliximab or adalimumab

3.9 pg/ml; $p = 0.034$), while there was no difference in other cytokines (IL-1β, $p = 0.28$; IL-1Ra, $p = 0.75$; TNFα, $p = 0{,}94$) (Table 2). In contrast, between active ($n = 20$) and inactive ($n = 6$) UC, there was no difference on IL-1α levels ($p = 0.28$) but significant differences were observed on IL-1β (3.7 vs. 2.3 pg/ml; $p = 0.054$) and IL-1Ra (15.9 vs. 12.7 pg/ml; $p = 0.023$). Interestingly, noteworthy variations were not found on TNFα levels ($p = 0.58$). Regarding arthritis, there was nearly statistical significance on IL-1α levels between active ($n = 10$) and inactive ($n = 4$) forms of SpA (20 vs. 3.9 pg/ml; $p = 0.06$) and significant difference on IL-1Ra (16.9 vs. 14.8 pg/ml; $p = 0.033$). No differences were found on IL-1β ($p = 0.62$) and TNFα ($p = 0.43$) (Table 2). It is apparent that the activity of CD determines the cytokine pattern regardless of the presence of arthritis. IL-1α has a cornerstone function at this point (Tables 2, 3, 4).

Discussion

Following initial reports 25 years ago [6], there is enough evidence today that IL-1 is together with TNFα the promoting cytokines of the inflammatory procedure in IBD and autoimmune arthritis [7, 8]. Seronegative spondylarthritis and the IBD share common features and they frequently overlap. Their correlation on cytokine level and specifically on IL-1 level has not yet been studied extensively.

Interleukin-1 exists in two forms, alpha and beta, and it is not clear if any of these contribute to a different extent to the phenotype of IBD including the presence of seronegative SpA. Interleukin-1 receptor antagonist is required to minimize and balance the effects of active IL-1 forms.

We studied three groups of patients, the CD, UC and pure arthritis (SpA) group. We subdivided IBD patients according to the presence of arthritis and finally all groups according to disease activity in order to identify which cytokine from the IL-1 complex is involved in the disease exacerbation. All patients were on treatment, thus to be specific, the applied investigation refers to the phase of disease augmentation or perpetuation of IBD [2]. We adopted a rheumatology perception for disease remission where stable minimal doses of steroids considered as part of the disease modifying treatment and not just an anti-inflammatory regimen, if full resolution of clinical, endoscopic and histologic findings was achieved.

We compared the groups without using controls of normal subjects because this has already been performed in other studies showing the impact of IL-1 and TNF in inflammatory procedure [6, 9, 10]. Furthermore, participants should undergo a full colonoscopy as a prerequisite for the enrolment in the study, thus an ethical dilemma for a group of normal subjects was deterrent.

In our cohort, the prevalence of arthritis in CD patients was 29.4 % and in UC patients 30.8 %. Abnormal non-specific intestinal pathology in patients with SpA was 50 %. CD was twice more common in males, and equal percentages on both sexes were found in UC. In arthritis group, there was a 3:1 ratio between males and females. The above demographic data of patients in our study are similar to those reported in medical literature.

Cytokine levels differ during disease activity, and this confirms their role as orchestrators of acute phase response. The innovation of this study is that it provides insights into role of the forms of IL-1 in both IBD and seronegative spondylarthropathies. Distinctive disease-specific cytokine profiles were identified.

In Crohn's disease, IL-1α mediates the exacerbations during treatment. Levels of IL-1α were significantly higher in patients on active disease compared to remission (11.2 vs. 3.9 pg/ml; $p = 0.034$), while no differences were observed on other cytokines.

The reverse phenomenon was observed in UC patients where IL-1β and IL-1Ra are the profound cytokines during exacerbations (3.7 vs. 2.3 pg/ml; $p = 0.054$ and 15.9 vs. 12.7 pg/ml; $p = 0.023$, respectively).

Patients with seronegative spondylarthropathy have a similar to the above both conditions pattern with IL-1α and IL-1Ra playing a key role on disease exacerbations (20 vs. 3.9 pg/ml; $p = 0.06$ and 16.9 vs. 14.8 pg/ml; $p = 0.033$, respectively).

The two forms of IL-1 are on different transcriptional control, and this may explain the variances that were observed in CD and UC [11, 12]. Ludwiczek et al. [13] reported increased IL-1Ra plasma levels but not IL-1α and IL-1β levels in unselected patients with CD and UC compared to healthy control subjects. In their study, colonic explant cultures exhibited increased levels of IL-1α and IL-1Ra in non-lesional and lesional CD, lesional UC but not in non-lesional UC. They also found that IL-1β was elevated in lesional UC and CD but not in non-lesional CD. Thus, a subtle trend toward IL-1α in CD and IL-1β in UC was noted. Leon et al. [14] reported that IL-1β levels are higher in affected areas compared to unaffected ones in UC patients but not in CD patients.

Correlating the clinical features of CD and UC with the pattern of IL-1 forms, we could anticipate that IL-1α may be a contributing factor to the fibrosing and granulosing procedure that is observed in CD, while IL-1β acts as a more potent immunoreactive cytokine. The latter is supported by studies which have shown that IL-1β mRNA in activated monocytes is over-expressed 25–50 times than IL-1α [15]. Furthermore, the half-life of intracellular IL-1β is 15 h versus 2.5 h of IL-1α [16]. The significantly higher levels of the IL-1Ra that we observed in patients with active UC are required to control and minimize the effects

Table 3 Distribution of CD and UC patients in relation to arthritis and their activity

	aCD/aeSpA	aCD/ieSpA	iCD/aeSpA	iCD/ieSpA	aUC/aeSpA	aUC/ieSpA	iUC/aeSpA	iUC/ieSpA
Patients (N)	5	15	0	14	4	16	0	6
Sex (M/F)	4/1	10/5	0	8/6	4/0	7/9	–	4/2
Age								
Median [range]	35 [20–61]	27 [18–46]	–	36.5 [18–56]	34 [30–63]	37.5 [23–65]	–	65 [23–70]
Disease duration								
Median [range]	3 [2–6]	2 [0.2–20]	–	2 [0.1–11]	4.5 [1–10]	2 [0.1–20]	–	7.5 [0.4–30]
CDAI								
Median [range]	200 [130–237]	100 [12–400]	–	75.5 [20–150]	5.5 [4–8]	4 [1–7]	–	1 [0–2]
BASDAI								
Median [range]	4.7 [1.2–5.2]	2.4 [1.6–3.2]	–	3 [1.8–3.8]	4 [3.7–6.4]	1.2	–	1.1 [0.8–1.4]
CRP								
Median [range]	8.3 [6–32]	6.8 [0.2–82.4]	–	0.9 [0.18–27]	10.3 [4–145]	5.9 [0.3–14.6]	–	0.5 [0.46–2.6]
Cytokines (pg/ml)								
IL-1α								
Median [range]	3.9 [3.9–118.4]	14.6 [3.9-250]	–	3.9 [3.9–250]	3.9 [3.9–3.9]	9.1 [3.9–250]	–	3.9 [3.9–7.63]
IL-1β								
Median [range]	3.7 [2.5–15.3]	4 [2.2–17.1]	–	4.1 [2.5–27.7]	36.4 [3.7–58]	3.2 [1.8–244]	–	2.3 [1.8–3.7]
IL-1Ra								
Median [range]	21.1 [12.7–186]	16.9 [10.6–70.8]	–	16.9 [12.7–119.9]	91.3 [16.9–260]	14.8 [10.6–1,671]	–	12.7 [12.7–16.9]
TNFα								
Median [range]	8 [0.8–49]	3 [0–13]	–	4.3 [0–22.4]	2.2 [0–10.8]	4.3 [0–17.1]	–	3.9 [0–5.1]

aCD active Crohn's disease, *iCD* inactive Crohn's disease, *aUC* active ulcerative colitis, *iUC* inactive ulcerative colitis, *aeSpA* active enteropathic spondylarthritis, *ieSpA* inactive enteropathic spondylarthritis

Table 4 Comparisons of cytokine median values (Tables 2, 3) between IBD and SpA groups. (p refers to Mann–Whitney U test)

Comparing groups			IL-1α	IL-1β	IL-1Ra	TNFα
[1]aCD ($n = 20$)	vs	iCD ($n = 10$)	$p = 0.034$	NS	NS	NS
[1]aUC ($n = 20$)	vs	iUC ($n = 6$)	NS	$p = 0.054$	$P = 0.023$	NS
[1]aSpA (n= 10)	vs	iSpA ($n = 4$)	$p = 0.06$	NS	$P = 0.033$	NS
[2]aCD/aeSpA (n= 5)	vs	aCD/ieSpA ($n = 15$)	NS	NS	NS	NS
[2]aCD/ieSpA (n= 15)	vs	iCD/ieSpA ($n = 14$)	$p = 0.022$	NS	NS	NS
[1]aSpA (n= 10)	vs	[3]iSpA + ieSpA (n= 24)	$p = 0.01$	NS	NS	NS
[4]aeSpA sum ($n = 9$)	vs	[1]aSpA ($n = 10$)	NS	NS	NS	NS

IBD Inflammatory bowel disease, *aCD* active Crohn's disease, *aUC* active ulcerative colitis, *iCD* inactive Crohn's disease, *iUC* inactive ulcerative colitis, *SpA* seronegative spondylarthritis, *eSpA* spondylarthritis related to inflammatory bowel disease (enteropathic), *aeSpA* active enteropathic spondylarthritis, *ieSpA* inactive enteropathic spondylarthritis, *NS* not significant

[1] Groups and median values in Table 2

[2] Groups and median values in Table 3

[3] Groups and median values in Tables 2 and 3 (sum of inactive SpA ($n = 4$), iCD/ieSpA ($n = 14$) and iUC/ieSpA ($n = 6$)

[4] Groups and median values in Table 3 (sum of aCD/aSpA and aUC/aSpA)

of IL-1β. The colon rather promotes a forceful immunostimulatory reaction, induced by IL-1β, due to its microbial burden. Libby et al. [17] reported a maximal increase in IL-1 beta mRNA after exposure of human vascular smooth muscle cells to bacterial endotoxin. Animal models demonstrate the absence of colitis, gastritis and arthritis in a sterile environment, while both pathogenic and normal enteric microflora can induce and perpetuate chronic intestinal inflammation [18]. Quantitative changes as well as the functional activity of *microbiota* seem to be important in disease states [19]. The release of Th1-related proinflammatory cytokines IL-1β and TNFα has also been correlated with other chronic auto-immune and microbial-linked conditions [20, 21].

We noted nearly significant difference on IL-1α levels in active and inactive SpAs ($p = 0.06$), and we assume that a greater number of patients would be clearly able to clarify this observation. There is recent evidence that IL-1α gene polymorphisms are associated with susceptibility to ankylosing spondylitis [22]. Romero-Sanchez et al. [23] found that among twenty-two cytokines, IL-1α was able to distinguish responders and non-responders of anti-TNF treatment. It would be very likely that ankylosis of the spine and in general the fibrosing properties of IL-1 exhibited via its alpha form. There is also enough knowledge to disassociate SpAs from IL-1β. Gratacos et al. [24] were of the first groups that reported the lack of association of IL-1β to ankylosing spondylitis. Studies correlate IL-1β with rheumatoid arthritis rather than with SpA patients [25].

We did not find alterations on TNFα levels during exacerbations in both IBD and SpAs, and these could be attributed to the effects of medication and the stage of perpetuation that were the majority of patients. Similar findings have been reported in medical literature [3, 26]. The above suggest that IL-1 may act as an escape phenomenon of the inflammatory procedure during treatment and IL-1 forms mediate different pathways in IBD.

One of the limitations of this study is the relatively small cohort. A higher number of patients would be probably able to provide more striking differences, especially where a borderline insignificance was found. We considered that the primary criterion is the disease remission regardless of the type of medication. The number of patients did not allowed further study of the effects of treatment on cytokine profile. Another limitation mainly due to financial reasons is the omission of examining the IL-1RI and IL-1RII receptors. This could further enhance our knowledge on IL-1 cytokines profile that is involved in IBD and SpAs.

The multidisciplinary approach of a "many faces" disease and the evaluation of patients in a custom clinical setting are among the strengths of the study. We enhanced the observations which declare that the two forms of IL-1 are probably differently acting cytokines.

In conclusion, the present study demonstrated that the alpha form of IL-1 is related to exacerbations in Crohn's disease, while the beta form of IL-1 is related to ulcerative colitis flares, during treatment of IBD patients. Seronegative spondylarthritis and enteropathic spondylarthritis correlate with the rise of IL-1 alpha and its receptor antagonist. IL-1α may be responsible for the fibrosing skeletal and intestinal manifestations of seronegative spondylarthritis and Crohn's disease.

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Conflict of interest Periklis Vounotrypidis, Georgios Kouklakis, Konstantinos Anagnostopoulos, Petros Zezos, Alexandros Polychronidis,

Efstratios Maltezos, Eleni Efremidou, Michael Pitiakoudis, Nikolaos Lyratzopoulos declare that they have no conflict of interest.

Informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

Animal studies No animal studies were carried out by the authors for this article.

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