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A Novel Signature of Serum Interleukin 3 in Ankylosing Spondylitis

Baneen Mueen Abd Ali[1] , Inas K. Sharquie PhD[1] , *Faiq I. Gorial FIBMS[2]*
[1]*Department of Microbiology & Immunology, College of Medicine, University of Baghdad, Baghdad, Iraq.*
[2]*Department of Medicine, College of Medicine, University of Baghdad, Iraq.*

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Abstract:

One of the chronic autoimmune diseases with complex pathogenesis is ankylosing spondylitis (AS), which is regulated by numerous cytokines. Interleukin-3 (IL-3) involvement as a biomarker for AS has remained unexplored. Therefore, this study aims to assess serum IL-3 levels to improve the accuracy of AS diagnostics and enhance targeted therapies while exploring the role of human leukocyte antigen (HLA-B27) in influencing IL-3. The study examined the IL-3 serum levels of 88 patients with AS and 44 healthy controls recruited from the Baghdad Teaching Hospital. Patients with AS disease were further divided into two subcategories according to AS status: inactive (n = 44) and active (n = 44). Serum IL-3 titers were quantified using enzyme-linked immunosorbent assay (ELISA). Clinical parameters like complete medical histories, body mass index, sex, age, and disease duration were also recorded. Additionally, laboratory disease parameters, such as HLA-B27, C-reactive protein, and rheumatoid factor, were analyzed. IL-3 levels were higher in the AS patients (456.63 ± 237.118 pg/ml) than in the healthy controls (178.21 ± 16.015 pg/ml, $p < 0.01$). The mean IL-3 levels were highest in patients with active AS (663.27 ± 156.293 pg/ml) and became incrementally lower with reduced disease severity, inactive, 250 ± 44.148 pg/ml. Serum IL-3 exhibited strong diagnostic for AS, demonstrating a cut-off value of ≥ 193 pg/ml as well as an area under the curve score of 0.997. Sensitivity, accuracy, in addition to specificity were 98.9%, 91.67%, and 77.3%, respectively. In conclusion, serum IL-3 levels were significantly elevated in AS patients and correlated to the disease's activity. This suggests that IL-3 could be valuable in predicting disease severity and monitoring AS progression.

Keywords: Interleukin-3, Ankylosing Spondylitis, Disease Activity, HLA-B27, Autoimmune Disease.

رابط جديد لمستويات انترلوكين IL-3 في مصل الدم لدى مرضى التهاب الفقار المقسط

بنين معين عبد علي [1] ، إيناس خليفة الشرقي[1]، فائق ايشو كوريال[2]
[1] فرع الأحياء المجهرية والمناعة، كلية الطب، جامعة بغداد، بغداد، العراق
[2] فرع الطب ، كلية الطب ، جامعة بغداد ، العراق

الخلاصة:

يُعد التهاب الفقار اللاصق أحد أمراض المناعة الذاتية المزمنة ذات التسبب المعقد، والذي تُنظمه العديد من السيتوكينات. ولم يُستكشف بعد دور الإنترلوكين-3 (IL-3)كعلامة حيوية لالتهاب الفقار المقسط. لذلك، تهدف هذه الدراسة إلى تقييم مستويات الإنترلوكين-3 في المصل لتحسين دقة تشخيص التهاب الفقار

*Email: inasksharquie@comed.uobaghdad.edu.iq

اللاصق، وتعزيز العلاجات الموجهة، مع استكشاف دور مستضد الكريات البيضاء البشرية ((HLA−B27في التأثير على الإنترلوكين−3. فحصت الدراسة مستويات IL−3 في مصل الدم لمجموعة كبيرة مكونة من 88 مريضًا مصابًا بالتهاب الفقار المقسط و44 من الأشخاص الأصحاء الذين حضروا الى وحدة أمراض الروماتيزم في مستشفى بغداد التعليمي. تم تقسيم مرضى التهاب الفقار المقسط إلى مجموعتين فرعيتين وفقًا لحالة المرض: غير نشط (ع = 44) ونشط (ع = 44). تم قياس مستويات IL−3 في مصل الدم باستخدام تقنية اختبار الممتز المناعي المرتبط بالانزيم. كما تم تسجيل التاريخ الطبي الكامل ومؤشر كتلة الجسم والجنس والعمر ومدة المرض. كما تم قياس معايير المرض المختبرية مثل البروتين المتفاعل −C ومستضد الكريات البيضاء البشرية وعامل الروماتويد. كانت مستويات IL−3 أعلى لدى مرضى التهاب الفقار المقسط (456.63 ± 237.118 بيكوغرام/مل) مقارنة بالأصحاء (178.21 ± 16.015 بيكوغرام / مل، p < 0.01). كانت مستويات IL−3 أعلى لدى المرضى الذين يعانون من التهاب الفقار المقسط النشط (663.27 ± 156.293 بيكوغرام/مل) وانخفضت تدريجيًا مع انخفاض شدة المرض، غير نشط، 250 ± 44.148 بيكوغرام/مل. اظهر IL−3 في مصل الدم صلاحية ممتازة في تشخيص ومتابعة مرضى التهاب الفقار المقسط. مع قيمة الحد الفاصل ≤ 193 بيكوغرام/مل ومساحة تحت المنحنى 0.997، كانت حساسية التشخيص ودقته وخصوصيته 98.9% و91.67% و77.3% على التوالي. وفي الختام، كانت مستويات IL−3 في مصل الدم مرتفعة بشكل ملحوظ لدى المرضى المصابين بالتهاب الفقار المقسط مقارنة بالأصحاء وأظهرت ارتباطًا بنشاط المرض. يمكن أن يكون هذا الإنترلوكين قيمًا في التنبؤ بشدة المرض.

1. Introduction

Ankylosing spondylitis (AS) is a chronic rheumatic inflammatory autoimmune disorder that affects the sacroiliac joints as well as spinal vertebrae, with a prevalence of about 1% worldwide [1] and characterized by progressive inflammation, structural damage, and reduction in the functioning of the sacroiliac joints as the bones gradually fuse. AS Patients usually experience chronic back pain, stiffness, pain, and fatigue, which are associated with a reduction in their quality of life [2]. AS is also associated with a high incidence of other conditions, including inflammatory bowel disease (IBD) and eye inflammation (iritis). At present, no definitive cure exists for AS; therefore, management of this condition focuses on alleviating the symptoms and slowing the disease progression. Treatments include pain management, physiotherapy, and immune-modifying drugs, including disease-modifying antirheumatic drugs (DMARDs) and interleukin-17 (IL-17) inhibitors, such as secukinumab. A combination of therapies may significantly extend the time it takes for AS to progress to spinal fusion [3].

Although there are significant benefits to the early diagnosis of AS in terms of patient outcomes, this can be challenging due to the lack of a definitive test for the condition. Diagnosis is confirmed using an X-ray of the sacroiliac joint, which shows inflammation and potential fusion of the joints in cases of AS. However, this is not apparent until the condition progresses. Therefore, research has focused on the presence of biomarkers that could be used to develop a simple blood test to assess AS risk [4]. The presence of the human leukocyte antigen (HLA-B27) gene is associated with the heritability of AS within families, with around half of the patients with AS and the HLA-B27 gene present passing on the condition to their children [5]. C-reactive protein (CRP) has also been identified as a potential biomarker for AS. However, the elevation of CRP levels due to inflammation in AS is not specific to the disease and, therefore, provides relatively poor diagnostic value. As such, attention has turned to other biomarkers that could have diagnostic uses in AS [5]. A range of novel biomarkers have been explored in AS patients, which could form part of a diagnostic battery. Goral and Hassan [6] found that the red cell distribution width (RDW), which is a measure of the variability of red blood cells within the complete blood count (CBC), was significantly higher in AS patients than in controls without AS and was correlated with the

CRP levels in these patients. A study by Jassim et al. [7] found that patients with AS showed significantly higher levels of serum Epstein–Barr virus (EBV) immunoglobulin G (IgG) antibody than the controls, suggesting that EBV infection could be used as a marker of risk for the development of AS. A variety of other biomarkers have also been explored. These include genetic markers, such as endoplasmic reticulum aminopeptidase 1; markers of bone turnover, such as wnt-3; bone-specific alkaline phosphatase and osteocalcin; and markers of inflammation, such as vascular endothelial growth factor and interleukin-17 [5].
Jaber and Ad'hiah [8] have suggested the potential use of interleukins (IL), a family of cytokines, as biomarkers for AS. The results of this study suggest that further exploration of ILs in AS as potential biomarkers would be a valuable contribution to the development of diagnostic tests. Therefore, the present research will examine the diagnostic and predictive value of IL-3 in AS. Interleukins are commonly used as biomarkers to monitor the progression of the disease and various medical conditions. For instance, in severe cases of COVID-19, IL-6, IL-8, and IL-38 have been identified as valuable biomarkers for determining disease severity and prognosis [9-12]. Additionally, IL-37 may act as a biomarker for diagnosing juvenile arthritis [13], while IL-39 and IL-40 are associated with rheumatoid arthritis (RA), thyroid disease, as well as systemic lupus erythematosus (SLE) [14-17]. IL-6 has also been shown to play a significant role in RA and SLE [18-20].

IL-3, a hematopoietic growth factor, has been associated with increased inflammation in conditions such as sepsis and reduced disease severity in patients with collagen-induced arthritis [21, 22]. Considering the encouraging outcomes of inhibiting certain interleukins in inflammatory disorders, including AS, a comprehensive exploration of pro- and anti-inflammatory cytokines is warranted [23, 24]. Therefore, the evaluation of serum IL-3 could potentially contribute to our understanding of AS pathogenesis and the development of practical diagnostic tools.

IL-3, a hematopoietic cytokine, is one of the growth factors for hematopoietic bone marrow cells. This 28 kDa glycoprotein consistently stimulates myelopoiesis and is primarily produced by bone marrow cells and antigen- or mitogen-activated T cells. It also influences the development of dendritic cells in terms of their antiviral or antitumor reactivity and bone marrow hematopoiesis, indicating its potential role in immune response modulation, a key aspect of AS pathogenesis.
While there is no direct information on the role of IL-3 in AS, it is known that macrophages and monocytes play a role in the pathogenesis of spondyloarthritis, including AS. Therefore, IL-3 may play a role in the differentiation of macrophages and monocytes, which are involved in the pathogenesis of AS. However, it is crucial to emphasize that more research is urgently needed to determine the exact role of IL-3 and its interaction with HLA-B27 in AS. This is a pressing and significant area of study.

2. Materials and Methods

Patients and controls

Blood samples were collected from 88 AS patients diagnosed who visited the Rheumatology Department at Baghdad Teaching Hospital, along with 44 control subjects, between November 2023 and January 2024. The patients were selected based on the classification criteria established by the Assessment of Spondylarthritis International Society (ASAS). The AS group was further categorised into two subgroups based on the severity of their disease (44 inactive and 44 active severe). The patients' ages ranged between 18 and 57. Forty-four apparently healthy individuals aged between 18 and 55 were selected as control subjects. Patients with overlapping autoimmune and inflammatory diseases, like RA, chronic

inflammatory disorders of the intestine, and psoriasis, who were pregnant, had comorbidities or declined participation in the study were excluded.
With the guidance of a rheumatologist, each patient completed an information sheet and a consent form. Collected data encompassed duration of the disease, BMI, sex, age, therapy, erythrocyte sedimentation rate (ESR), and smoking status. Disease activity was evaluated using the Bath-Ankylosing Spondylitis Disease Activity Index (BASDAI), and the functional impairment of AS was measured using the Bath-Ankylosing Spondylitis Functional Index (BASFI). CRP, HLA-B27, and rheumatoid factor (RF) were laboratory parameters that correlated to the AS disease.
All participants were thoroughly informed about the study's objectives and procedures, and their formal consent was obtained before commencing the study. The Scientific Ethics Committee of the University of Baghdad College of Medicine approved the study. The immunological tests were performed at the Research and Development International Centre.

Immune assays
Five ml of blood samples were obtained from AS patients and controls. Samples were centrifuged at 3000 rpm for 15 minutes and stored at -20 degrees Celsius.
The enzyme-linked immunosorbent assay (ELISA) (Cloud-Clone Corp., USA), an exact method, was used to measure IL-3 and CRP levels. In contrast, HLA-B27 and RF were measured using the Elabscience Company and FineTest Company. The testing process was conducted with the utmost adherence to the manufacturer's instructions, ensuring the accuracy of the measurements. The absorbance at 450 nm was measured using a plate reader.

Statistical analysis
Statistical analysis was conducted using SPSS software and Microsoft Office Excel (2010). Data following a normal distribution were presented as mean ± standard deviation (SD). In comparisons of quantitative measures (age/year, BMI, IL-3 pg/ml, etc.), across the studied groups, the Independent Samples t-test (Student Test), analysis of variance test (ANOVA), as well as the less significant difference (LSD) test were implemented. The Pearson chi-square test ($\chi 2$) was used to compare the qualitative variables between the studied groups (age categories, BMI groups, and smoking status). The Pearson correlation test detected the correlation between IL-3 levels and parameters, including age/year, BMI, AS duration, ESR, CRP, HLA-B27, and BASDI, in addition to BASFI. Tests validity was assessed with a ROC curve analysis and calculation of the threshold value, area under the curve (AUC), sensitivity, specificity, overall accuracy, as well as positive and negative predictive value (PPV and NPV). A *P*-value> 0.05 was considered a non-significant difference (NS). In contrast, a *P*-value < 0.05 was considered a significant difference (S), and a P-value < 0.01 was considered a highly significant difference (HS).

Results and Discussion
Characteristics of patients
Eighty-eight AS patients aged between 18 and 57 were divided according to their severity into two groups (44 inactive and 44 active). Moreover, forty-four apparently healthy individuals were used as control subjects, their ages ranging from 18 to 55 years.

A statistically non-significant difference at $P > 0.05$ was observed when AS patients and their controls were compared for all demographic parameters, as follows:
The 31–40 age group was the most highly represented among the groups, with 19 controls (43.2%) and 30 AS patients (34.1%), followed by the 18–30 age group, consisting of 19

controls (43.2%) and 27 AS patients (30.7%). The 41–50 age group was less represented, with 4 controls (9.09%) and 26 AS patients (29.5%), ($P = 0.059$).
Males were predominant in both the studied groups, with 57 AS patients (64.8%) and 29 controls (65.91%). Females were fewer, with 31 AS patients (35.2%) and 15 controls (34.09%), ($P = 0.656$).
Among the AS patients, 32 (36.4%) were obese, 25 were overweight (28.4%), and 31 (35.2%) were of normal weight. Among the controls, 22 (50%) had a normal weight, 16 (36.4%) were overweight, and 6 (13.6%) were obese ($P = 0.214$). Among the AS patients, 58 (65.9%) were non-smokers and 30 (34.1%) were smokers. Among the controls, there were 22 (50%) smokers and 22 (50%) non-smokers ($P = 0.076$).

The mean age/years of the control group was (32.19 ± 9.179), and for the AS patients, it was (36.25 ± 9.33), ($P = 0.021$). Finally, for the mean of the BMI, the AS patients showed (28.1086 ± 5.59267), more than for the control group (25.7351 ± 4.36213), which was statistically a non-significant difference ($P = 0.016$).
Table 1 illustrates comparisons between the AS patients (inactive and active) and the controls for all the demographic parameters under study.
The age group (31–40) was more highly represented among the active AS, at 19 (43.2%), and among the inactive AS patients, there were 19 (43.2%) within the 18–30 age group. Among the controls, there were 19 (43.2%) individuals in both the 18–30 and 31–40 age groups, $P = 0.018$ at $P < 0.05$.

Males were predominant among both the inactive AS patients and controls, at 35 (79.5%) and 19 (43.2%), respectively. Among the active AS patients, there were 22 (50%) males and 22 (50%) females ($P = 0.015$).
Among the active AS patients, 17 (38.6%) were obese, while 16 (36.4%) were overweight. Twenty-two (50%) controls and 20 (45.5%) inactive AS patients were of normal weight, $P = 0.011$ at $P < 0.05$. Among the AS patients, 58 (65.9%) were non-smokers, and 30 (34.1%) were smokers, while among the controls, there were 22 (50%) smokers and 22 (50%) non-smokers ($P = 0.182$).

There was a similarity in the mean age between the control group (32.19 ± 9.179), inactive AS patients (34.43 ± 10.301), and active AS patients (38.07 ± 7.952), with a statistically non-significant difference, with the exception of the comparison between the healthy controls and the active AS, ($P = 0.0003$).

Again, there was a similarity in the mean of the BMI Kg/m^2 between the controls (25.7351 ± 4.36213), inactive AS patients (27.2042 ± 5.53374), and active (29.013 ± 5.56661), with a non-significant difference, while for the comparison between the active AS and controls, $P = 0.004$ at $P < 0.01$.

Table 1: Distribution of demographics and other parameters among the studied groups (AS patients and controls)

Parameters		Severity of AS			*P*-value	
		Control	Inactive	Active		
Age groups	18–30	19 (43.2%)	19 (43.2%)	8 (18.2%)	*P* = 0.018	
	31–40	19 (43.2%)	11 (25%)	19 (43.2%)		
	41–50	4 (9.09 %)	12 (27.3%)	14 (31.8%)		
	51–60	2 (4.51%)	2 (4.5%)	3 (6.8%)		
Sex	Male	29 (65.91%)	35 (79.5%)	22 (50%)	*P* = 0.015	
	Female	15 (34.09%)	9 (20.5%)	22 (50%)		
BMI groups	Normal weight	22 (50%)	20 (45.5%)	11 (25%)	*P* = 0.011	
	Overweight	16 (36.4%)	9 (20.5%)	16 (36.4%)		
	Obese	6 (13.6%)	15 (34.1%)	17 (38.6%)		
Smoking	Smokers	22 (50%)	17 (38.6%)	13 (29.5%)	*P* = 0.182	
	Non-smokers	22 (50%)	27 (61.4%)	31 (70.5%)		
Age/Year	Mean	32.19	34.43	38.07	A	*P* = 0.257
	Standard Deviation	9.179	10.301	7.952	B	*P* = 0.003
	Standard Error	1.4	1.553	1.199	C	*P* = 0.066
	ANOVA test, *P* = 0.012					
BMI	Mean	25.7351	27.2042	29.013	A	*P* = 0.189
	Standard Deviation	4.36213	5.53374	5.56661	B	*P* = 0.004
	Standard Error	0.66522	0.83424	0.8392	C	*P* = 0.105
	ANOVA test, *P* = 0.015					

Note: A: is defined as the control group vs. inactive group, B: is defined as the control group vs. active group, and C: represents inactive group vs. active group.

Table 2 indicates a highly significant difference in most comparisons of the severity of AS disease, except for the duration/years and rheumatoid factor (RF), IU/ml, with a statistically non-significant difference and with a mean ± SD similarity.

The level of disease activity was lower in inactive patients [BASDI (2.366 ± 1.0828) and BASFI (2.632 ± 1.2251)] than in active patients [BASDI (5.121 ± 0.9721), and BASFI (5.266 ± 1.2826)]. The higher ESR mm/hour mean was observed in the blood of the active AS patients (22.695 ± 13.7981) than in the inactive AS patients (15.045 ± 10.4969), and it decreased in the control group (6.023 ± 3.5623). The mean of CRP mg/l was (2.46 ± 0.323) in the controls, (2.27 ± 0.364) in the inactive AS patients, and (1.93 ± 0.292) in the active AS patients.

Table 2: Mean distributions of parameters within AS groups

Parameters		AS patients groups	N	Mean	Standard Deviation	Standard Error	P-Value	
Disease duration /Year		Inactive	44	7.57	4.552	0.686	$P = 0.805$	
		Active	44	7.32	4.888	0.737		
		Total	88					
Disease activity	BASDI	Inactive	44	2.366	1.0828	0.1632	$P = 0.006$	
		Active	44	5.121	0.9721	0.1465		
		Total	88					
	BASFI	Inactive	44	2.632	1.2251	0.1847	$P = 0.001$	
		Active	44	5.266	1.2826	0.1934		
		Total	88					
ESR (mm/hour)		Control	44	6.023	3.5623	0.5432	A	$P = 0.005$
		Inactive	44	15.045	10.4969	1.5825	B	$P = 0.002$
		Active	44	22.695	13.7981	2.0801	C	$P = 0.001$
		Total	132	ANOVA test, $P = 0.003$				
CRP (mg/l)		Control	44	2.46	0.323	0.049	A	$P = 0.006$
		Inactive	44	2.27	0.364	0.055	B	$P = 0.004$
		Active	44	1.93	0.292	0.044	C	$P = 0.005$
		Total	132	ANOVA test, $P = 0.009$				
RF (IU/ml)		Control	44	27.95	5.306	0.809	A	$P = 0.085$
		Inactive	44	25.66	5.747	0.866	B	$P = 0.148$
		Active	44	26.03	7.163	1.081	C	$P = 0.776$
		Total	132	ANOVA test, $P = 0.503$				
HLA-B27 (ng/ml)		Control	44	4.596	1.0574	0.1613	A	$P = 0.003$
		Inactive	44	6.232	1.0319	0.1556	B	$P = 0.007$
		Active	44	9.539	2.3467	0.3538	C	$P = 0.005$
		Total	132	ANOVA test, $P = 0.009$				

Note: A: is defined as the control group vs. inactive group, B: is defined as the control group vs. active group, and C: represents inactive group vs. active group.

Among the sample of patients with active AS (n = 44), inactive AS (n = 44), and the controls (n = 44), there was no significant difference in the mean age ($p = 0.257$) and BMI ($p = 0.189$) of all three groups, although there were some significant between-group differences found that could potentially act as confounders. These included significant differences in the age profiles of the control and disease groups ($p = 0.018$) and a significant difference in the BMI of the active AS and control groups ($p = 0.0110$). As in previous studies, the severity of AS was found to be associated with disease activity (BASDI/BASFI), ESR, CRP concentration, RF, and HLA-B27 levels [25-29]. These results confirm the previous findings that inflammatory markers are raised in active AS and that function is reduced.

IL-3 serum levels

This study showed a mean of IL-3 pg/ml in the sera of AS patients (456.63 ± 237.118), which was more than in the control group (178.21 ± 16.015), utilizing a statistically significant difference, $P = 0.001$. The mean of IL-3 pg/ml in the sera was the largest in the

active AS patients (663.27 ± 156.293), followed by that of the inactive AS patients (250 ± 44.148), with the smallest mean found in the control group (178.21 ± 16.015), as shown in Table 3.

Table 3: Mean distributions of interleukin assays among the severity levels of AS disease

IL-3 (pg/ml)						
Severity of AS	N	Mean	Standard Deviation	Standard Error	LSD test *P*-Value	
Control	44	178.21	16.015	2.442	A	$P = 0.001$
Inactive	44	250	44.148	6.656	B	$P = 0.009$
Active	44	663.27	156.293	23.562	C	$P = 0.006$
Total	132	ANOVA, $P = 0.0002$				

Note: A: is defined as the control group vs. inactive group, B: is defined as the control group vs. active group, and C: represents inactive group vs. active group.

The main findings of the present study were that IL-3 is significantly elevated in AS patients compared with the control group and that this was true both for inactive AS and active AS patients. This suggests that IL-3 could play an important role in the diagnosis and monitoring of AS. A further finding of the research was that IL-3 serum concentration was associated with disease severity, with higher concentrations present in more severe cases of AS.

Previous research by Jaber and Ad'hiah [8] found that the levels of other interleukins such as IL-36α, IL-37, IL-38, IL-39, and IL-40 were significantly higher in AS patients than in controls, but this is the first study to show that this is also true for IL-3. It has been proposed that interleukins are upregulated in AS as the inflammatory response they are involved in has a causative role in AS development [30]. Although research into the mechanistic role of interleukins within AS is in its early stages, this presents an opportunity for utilize of IL-3 both as a marker and as a target for treatment. Current diagnostic examination using X-rays of the sacroiliac joints does not provide a clear diagnosis until later stages of AS [31], so the ability to use IL-3 as a blood biomarker prior to this diagnosis could form part of risk assessment and early diagnosis [32]. The association between disease severity and IL-3 serum level in the present study is particularly promising, as it suggests that IL-3 could be used to monitor disease risk and progression over time in a single patient, which has been the goal of studies in AS biomarker identification [33].

It can be observed from this study (Table 4) that etanercept (Enbrel) was predominant in both inactive AS patients, at 31 (70.5%), and active patients, at 30 (68.2%). Subsequently, adalimumab (Amgevita) was used for 7 (15.9%) inactive AS patients and 6 (13.6%) active patients. $P = 0.158$.

A non-significant difference was found in most medication intake comparisons between inactive and active patients, while there was a significant difference in sulfasalazine intake. Table 4 demonstrates the likeness of frequency.

Table 4: Distributions of medication intake in AS patient groups

Medication intake		Severity				*P*-Value
		Inactive		Active		
		Intake	NONE	Intake	NONE	
Sulfasalazine	N	0	44	4	40	*P* = 0.041
	%	0%	100%	9.1%	90.9%	
Methotrexate	N	0	44	1	43	*P* = 0.315
	%	0%	100%	2.3%	97.7%	
Adalimumab (Humera)	N	0	44	1	43	*P* = 0.315
	%	0%	100%	2.3%	97.7%	
Etanercept (Enbrel)	N	31	13	30	14	*P* = 0.817
	%	70.5%	29.5%	68.2%	31.8%	
Adalimumab (Amgevita)	N	7	37	6	38	*P* = 0.764
	%	15.9%	84.1%	13.6%	86.4%	
Infliximab (Ixifi)	N	3	41	0	44	*P* = 0.078
	%	6.8%	93.2%	0%	100%	
Infliximab (Remsima)	N	3	41	2	42	*P* = 0.645
	%	6.8%	93.2%	4.5%	95.5%	

Table 5 shows the correlations between the AS patients' parameters, as follows: For the serum IL-3 pg/ml level and other parameters, there was an inverse (negative) relationship, with a highly significant difference with CRP mg/l (r = -0.404, *P* = 0.0001).

A positive correlation with highly significant differences at $P < 0.01$ was observed between IL-3 and HLA-B27 (ng/ml) levels (r = 0.715, P = 0.0007), BASD (r = 0.696, *P* = 0.0002), and BASFI (r = 0.661, P = 0.0004), and a significant difference at $P < 0.05$ with erythrocyte sedimentation rate (ESR) mm/hour (r = 0.261, *P* = 0.014). There was a weak positive relation for the other parameters, presenting a non-significant difference.

Lastly, the HLA-B27 (ng/ml) level and other parameters showed a positive relation, with significant differences, and in relation to age/year (r = 0.239, *P* = 0.025).

Furthermore, a positive relation, with highly significant differences at $P < 0.01$, was observed between HLA-B27 and both BASDI (r = 0.545, *P* = 0.0006) as well as BASFI (r = 0.578, *P* = 0.0009). There was a weak positive relation for all the other correlations, showing a non-significant difference.

The association between IL-3 concentration and disease severity in the present study also suggests that IL-3 and its receptors could be a target for drug therapies in AS. Recent systematic review evidence has found that the use of IL-17A inhibitors in patients with AS improved symptoms significantly in randomized controlled trials [34]. The proposed mechanism of this effect is the disruption of the IL-23/IL-17 axis, which is involved in immune dysfunction and activated autoimmune inflammation. However, the study also found that IL-17A inhibitors were associated with a significantly higher rate of adverse events (AEs) [8]. It is likely that further understanding of the complex interplay of ILs and other factors, such as the presence of the *HLA-B27* gene, will be needed before more targeted IL-focused drug interventions can be developed [35]. In the case of IL-3, the design of the present study shows a strong association between IL-3 levels and AS severity but does not allow conclusions to be drawn about whether IL-3 is causative in AS or is raised as a result of AS.

Table 5: Correlation study between IL-3 levels, HLA-B27, and other parameters of AS patients

Pearson Correlation (AS patients)		IL-3 (pg/ml)	HLA-B27 (ng/ml)
HLA-B27 (ng/ml)	r	0.715	
	P-value	0.0007	
	Significance	HS	
Age / Year	r	0.167	0.239
	P-value	0.121	0.025
	Significance	NS	S
BMI (Kg/m^2)	r	0.155	0.158
	P-value	0.149	0.141
	Significance	NS	NS
Duration / Year	r	0.113	0.002
	P-value	0.294	0.989
	Significance	NS	NS
BASDI (disease activity)	r	0.696	0.545
	P-value	0.0002	0.0006
	Significance	HS	HS
BASFI (disease activity)	r	0.661	0.578
	P-value	0.0004	0.0009
	Significance	HS	HS
ESR (mm/ hour)	r	0.261	0.114
	P-value	0.014	0.291
	Significance	S	NS
CRP (mg/l)	r	-.404	-.304
	P-value	0.0001	0.004
	Significance	HS	HS
RF (IU/ml)	r	0.106	0.140
	P-value	0.327	0.195
	Significance	NS	NS

The present study also investigated the correlation between IL-3 levels and other parameters, including disease activity and the level of other serum compounds. Medication use was found to be significantly higher in active AS patients for sulfasalazine and infliximab (ixifi) but not for other medications. No significant difference was found between those taking and those not taking any of the medications in the level of IL-3. Patients taking infliximab, an inhibitor of interleukin 1beta (IL-1beta) and IL-6 gene expression [36], showed a marked reduction in IL-3 versus those not taking the drug, but this decrease was insignificant. This suggests that none of these medications has a significant impact on the level of IL-3 as a biomarker of AS, although it was beyond the scope of the present research to explore this relationship further. There are very few previous studies investigating drug interactions with IL-3 levels with which to compare these results [37].

ROC curve analysis

Figure 1 shows that serum IL-3 pg /ml had excellent validity in the diagnosis and monitoring AS patients, demonstrating a threshold (up to 193 pg/ml) and AUC (0.997). The sensitivity was at a very high level (98.9%), specificity was good (77.3%), with PPV (89.7%), NPV (97.1%), in addition to accuracy (91.67%), showing a highly significant difference ($p < 0.00$).

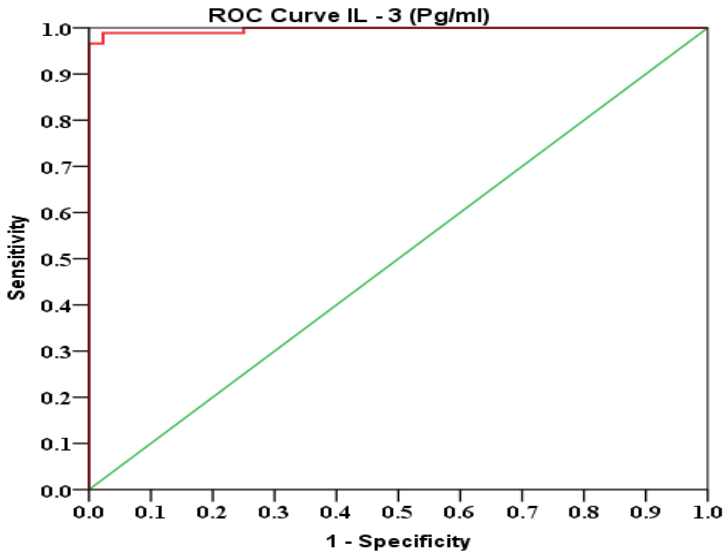

Figure 1: Validity tests of interleukin 3 (pg/ml) using the ROC test in the sera of ankylosing spondylitis patients and the control group

Figure 2 shows that a serum HLA-B27 (ng/ml) had excellent diagnostic performance, achieving a cut-off value (up to 5.2) and AUC (0.953). The sensitivity was elevated (95.5%), with good specificity (70.5%), PPV (86.6%), NPV (f 88.6%), and accuracy (67.12%), with a statistically highly significant difference ($p < 0.007$).

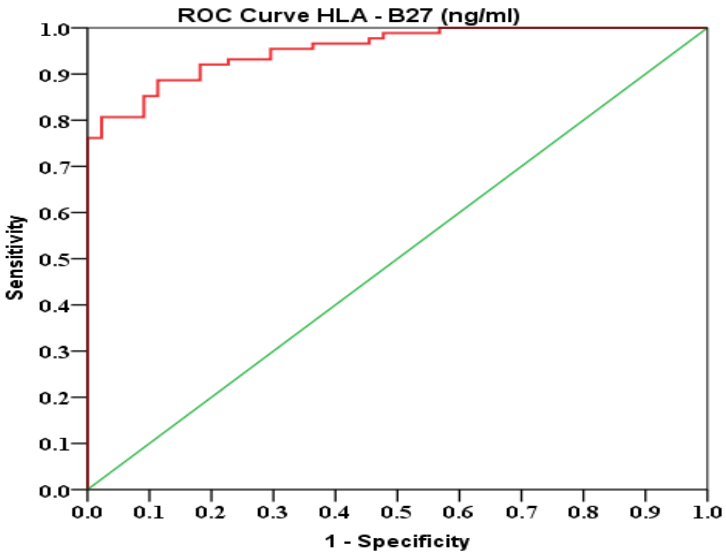

Figure 2: Validity tests of HLA-B27 (ng/ml) using the ROC test in the sera of ankylosing spondylitis patients and the control group

Finally, the study investigated the relationship between IL-3 levels and other parameters. Significant positive correlations were found between IL-3 and HLA-B27, BASDI, BASFI, and ESR, while a significant negative correlation was found with CRP. These results link the concentration of IL-3 to markers that have been found to be associated with AS in previous research [25-29, 38] and strongly suggest that IL-3 is a promising biomarker for AS. This was confirmed through tests of sensitivity (98.9%), specificity (77.3%), PPV (89.7%), NPV (97.1%), and accuracy (91.67%), which compared favourably with other proposed diagnostic tests in the literature, such as polygenic risk scores and machine learning diagnosis [38, 39].

3. Conclusion

In this study, IL-3 was found to be a useful biomarker for AS. In the sample of patients with active and inactive AS, the IL-3 level was found to be significantly higher than in non-disease controls, and it appears to have excellent predictive value, sensitivity, and good specificity for AS. Future research should investigate the use of IL-3 prospectively in the early detection of AS in patients who have not yet been diagnosed in order to determine how well it performs as a diagnostic biomarker compared with other biomarkers, genetic risk scores, and machine learning techniques as methods of providing early diagnosis for AS patients.

Ethical Clearance: This study received approval from the Scientific Ethics Committee of the College of Medicine, University of Baghdad. It was also endorsed by Iraq's Ministry of Health and the Ministry of Higher Education and Scientific Research. The Ethical approval number is: 0240
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Conflict of Interest: No conflict of interest is to be declared.

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