

Serum Interleukin-22 in Iraqi Patients with Crohn's Disease: Association with Biologic Treatment Status and Clinical Disease Activity

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ABSTRACT

Background: Interleukin-22 (IL-22) is an immunologically relevant cytokine in Crohn's disease (CD), but its circulating clinical significance remains uncertain. **Aims:** This study aims to evaluate serum IL-22 in Iraqi patients with CD and examine its association with biologic treatment status and disease activity. **Methodology:** This case-control study included 120 participants divided into three groups: 30 CD patients receiving biologic therapy, 30 untreated CD patients, and 60 healthy controls. Clinical activity for the patients was assessed using the Harvey–Bradshaw Index (HBI). Serum IL-22 concentrations were compared among the groups together with age and body mass index (BMI). **Results:** Serum IL-22 levels were significantly elevated in CD patients compared with healthy controls ($p = 0.0001$). Within the CD cohort, age did not differ significantly between biologically treated and untreated patients (29.33 ± 12.48 vs. 28.70 ± 9.70 years; $p = 0.820$). In contrast, BMI was significantly higher in the biologic-treatment group (25.44 ± 5.14 vs. 18.52 ± 1.66 kg/m²; $p = 0.0001$). Patients receiving biologic therapy had markedly lower HBI scores than untreated patients (3.03 ± 1.10 vs. 11.47 ± 3.41 ; $p = 0.0001$). Serum IL-22 was also significantly lower in the biologic-treatment group, with median values of 291.38 pg/mL (242.48–367.13) versus 455.36 pg/mL (332.52–562.48) in untreated patients ($p = 0.0001$). **Conclusion:** In this cohort of Iraqi patients with CD, circulating IL-22 levels were significantly higher than in healthy controls. Furthermore, biologic treatment was associated with lower clinical activity and lower circulating IL-22 levels. These findings support a relationship between serum IL-22 and disease state, but they should be interpreted cautiously. Further longitudinal and mechanistic studies are needed to determine whether IL-22 has value as a monitoring biomarker or reflects treatment-modulated inflammatory pathways.

Keywords: Crohn's disease; Interleukin-22; IL-22; Biologic Therapy; Harvey–Bradshaw Index.

Article Information

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INTRODUCTION

Inflammatory bowel disease (IBD) comprises chronic, relapsing, immune-mediated disorders of the gastrointestinal tract, of which Crohn's disease (CD) and ulcerative colitis are the principal phenotypes. CD is distinguished by its ability to involve any segment of the gastrointestinal tract from mouth to anus, typically in a discontinuous pattern, and by its heterogeneous inflammatory, stricturing, and penetrating behavior (1,2). Beyond intestinal symptoms such as abdominal pain, diarrhea, rectal bleeding, and weight loss, IBD exerts a substantial systemic burden through

extraintestinal manifestations and long-term impairment in quality of life (3). Contemporary reviews further emphasize that the global burden of IBD has risen markedly over recent decades; estimates based on Global Burden of Disease 2019 data place the number of affected individuals at approximately 4.9–5 million worldwide, underscoring the need for more precise biological stratification and better tools for monitoring disease activity (4).

The pathogenesis of CD is multifactorial and immunologically complex, resulting from the interplay of host genetics, environmental exposures, gut microbiota, and dysregulated

mucosal immunity (1). Despite the considerable expansion of therapeutic options, no curative treatment is available at the moment and management is still based on the suppression of intestinal inflammation, induction of remission and mucosal healing. Importantly, currently available treatments are limited by incomplete response, loss of response and adverse effects and inter-patient variability in clinical benefit. Thus, the search for circulating and tissue biomarkers that reflect inflammatory pathways more directly than routine clinical assessment remains a high-priority objective in CD research (5).

The interleukin-22 (IL-22) has emerged as a biologically interesting but clinically unsettled cytokine in intestinal inflammation. IL-22 belongs to the IL-10 family of cytokines and has recently been identified as an important regulator of epithelial homeostasis. Mechanistically, IL-22 primarily acts on non-hematopoietic epithelial cells, rather than leukocytes, where it impacts epithelial growth, permeability, mucus production, antimicrobial peptide expression and complement-related host defense programs. These properties are particularly pertinent in CD, given that epithelial barrier dysfunction and defective mucosal healing are central to the perpetuation of the disease. At the same time, IL-22 biology is not unidimensional, and depending on the inflammatory milieu, upstream cytokine networks, and tissue context, IL-22 may be either a compensatory epithelial-repair response or an active inflammatory circuit. This duality is precisely what makes IL-22 attractive as a research target, yet difficult to interpret clinically (6),

Recent interleukin-focused reviews continue to classify IL-22 among the key cytokines implicated in IBD pathobiology, while also making clear that the translational field remains in evolution (7). Interleukin-centered therapeutic research in IBD has advanced rapidly, especially around IL-23-related signaling, but the clinical placement of

IL-22 as a diagnostic, activity-related, or treatment-response biomarker remains far less settled. Thus, IL-22 occupies an important intermediary position between mechanistic plausibility and incomplete clinical validation(6,7).

The available clinical literature illustrates this uncertainty. In an early and influential human study, Schmechel et al. reported that serum IL-22 concentrations were significantly increased in patients with CD compared with healthy controls, were higher in active than inactive disease, and were associated with IL23R genotype status, thereby linking circulating IL-22 to the IL-23/Th17 axis and to disease activity in CD(8). These findings provided a strong rationale for considering IL-22 as a biologically meaningful circulating marker. But subsequent evidence has not been entirely concordant. Later, Farag et al. described a different circulating profile with lower serum IL-22 levels in CD and ulcerative colitis compared to controls and concluded that IL-22 system in circulation in IBD is dysregulated but not well enough understood to support a simple clinical interpretation at this time. Such divergence between cohorts is scientifically interesting as it indicates that serum IL-22 may be influenced by disease phenotype, treatment exposure, timing of sampling, compartment-specific biology or interactions with regulatory molecules such as IL-22 binding protein, rather than being a uniformly directional marker in all IBD populations (9).

METHODS

Study design

This case-control study was conducted from 6 November 2025 to 15 March 2026 at Specialized Hospital for Gastroenterology and Hepatology Najaf and Al-Manathira General Hospital, Iraq. Laboratory processing and biomarker analyses were performed at the Middle Euphrates Cancer Research Center, Najaf, Iraq.

Study population and clinical data

A total of 120 participants were enrolled and divided into two main groups: Cases (n=60): Patients diagnosed with Crohn's disease (CD) by gastroenterologists based on integrated clinical, endoscopic, and histological criteria. This group was further stratified into: Group A (n=30): Patients were receiving biologic therapy: Anti-TNF α (Infliximab). Group B (n=30): Patients not receiving biologic therapy. Controls (n=60): Apparently healthy individuals, clinically evaluated and confirmed by a physician to have no history of inflammatory bowel disease or systemic autoimmune disorders, matched with cases by age and sex to serve as a reference for IL-22 baseline levels. Clinical and demographic information was collected using a structured study questionnaire/case-record form completed during patient interview and review of the medical file. The form was used to document at minimum the variables analyzed in the reported results, including age, body mass index (BMI), Harvey-Bradshaw Index (HBI) score, serum IL-22 concentration, and biologic treatment status. Anthropometric assessment was standardized by calculating BMI as weight in kilograms divided by the square of height in meters (kg/m²).

To preserve methodological accuracy, only variables directly supported by the provided results and source materials are described here in operational detail. When treatment-related analyses were performed, biologic therapy was coded as a binary variable according to whether the participant was receiving biologic treatment at the time of evaluation.

Assessment of Crohn's disease activity

The Harvey–Bradshaw Index (HBI) is a streamlined clinical activity score favored in routine assessment and Scientific research because it is fast, intuitive, and no laboratory tests required (Axelrod et al., 2025). HBI comprises four symptom-centric components and grades activity as remission (<5), mild (5–

7), moderate (8–16), or severe (>16) and HBI correlates reasonably with Crohn's Disease Activity Index (CDAI) that considered older and more complex (10).

Blood sampling and serum processing

By 5 mL of venous blood was collected from each participant under aseptic conditions. For the current biomarker study, blood intended for serum-based analyses was collected into Gel tubes. Samples were processed within 15 minute of collection. Serum was separated by centrifugation at 3000 rpm for 10 minutes, transferred into labeled Eppendorf tubes, and stored for 5 months at –20 °C until biochemical test and immunoassay analysis.

All procedures related to phlebotomy, labeling, centrifugation, aliquoting, and storage were standardized across the participating recruitment centers.

Measurement of serum interleukin-22

Serum interleukin-22 (IL-22) concentrations were measured using a commercially available Human Interleukin 22 ELISA Kit from BT Laboratory (Cat. No. E0038Hu), as documented in the kit material provided by the investigators and verified against the manufacturer information. According to the manufacturer, this is a sandwich enzyme-linked immunosorbent assay (ELISA) for quantitative detection of human IL-22 in biological specimens including serum and plasma. The manufacturer-reported standard curve range is 5-1500 ng/L, and the analytical sensitivity is 2.48 ng/L.

Statistical Analysis

Statistical analyses were performed with the IBM SPSS Statistics software, version 26 (IBM Corp., Armonk, NY, USA). Data were entered into a dedicated study database and checked for completeness and internal consistency prior to inferential analysis. Continuous variables were summarised using measures appropriate to their distribution and categorical variables were summarised as counts and percentages. Associations between age, BMI, HBI, and serum IL-22 were assessed

using Spearman's rank correlation coefficient based on the distributional characteristics and correlation output reported in the Results section. Correlation coefficients were interpreted in terms of both magnitude and direction, and significance levels were reported. Diagnostic performance of IL-22 was evaluated by Receiver operating characteristic (ROC) curve analysis, and area under the curve (AUC) with 95% confidence interval (CI) was calculated. The optimal cutoff value was selected according to the maximum Kolmogorov-Smirnov statistic as shown in the reported table and the diagnostic indices, including sensitivity and specificity, were calculated at that cutoff value. A binary logistic regression analysis was performed to examine the association between IL-22 and biologic treatment status, with biologic treatment as the dependent outcome category and No as the reference category in the table reported. Univariable and multivariable models were estimated and results were expressed as odd ratios (ORs) with corresponding 95% CIs and *p* values. All tests were two-sided and p value < 0.05 was considered statistically significant.

RESULTS

Baseline Demographic and Clinical Characteristics

As shown in **Table 1**, patients with Crohn's disease and controls were similar in age, age-group distribution, sex, and residency (all $p > 0.05$). However, CD patients had a significantly lower BMI than controls (21.98 ± 5.15 vs 24.20 ± 2.96 kg/m², $p = 0.004$), and the distribution of BMI classes differed significantly between groups ($p = 0.0001$). Smoking status also differed significantly, with current and former smokers observed only among CD patients ($p = 0.0001$). In addition, serum IL-22 levels were significantly elevated in CD patients compared with controls, both by mean $\pm$ SD and median (IQR) values, and this difference was highly significant ($Z = -5.097$, $p = 0.0001$). Within the CD group, most patients had been diagnosed for less than 3 years, whereas disease location and phenotype distributions were not significantly heterogeneous. The distribution of HBI classes was significant, with remission representing the most frequent category. As illustrated in Figure 1, the increase in IL-22 in the CD group was also visually evident, further supporting the marked difference between patients and controls.

Table 1: Baseline demographic, clinical, and laboratory characteristics of patients with Crohn's disease and healthy controls.

Variable	Category	CD Patients (n=60)	Controls (n=60)	Test Statistic	p-value
Age (years)	Mean $\pm$ SD	29.02 $\pm$ 11.08	31.00 $\pm$ 11.43	t = -0.97	0.337
Age groups	17–26	30 (50.0%)	23 (38.3%)	$\chi^2 = 2.20$	0.333
	27–36	22 (36.7%)	24 (40.0%)		
	>36	8 (13.3%)	13 (21.7%)		
Sex	Male	32 (53.3%)	32 (53.3%)	$\chi^2 = 0.00$	1.00
	Female	28 (46.7%)	28 (46.7%)		
BMI (kg/m ²)	Mean $\pm$ SD	21.98 $\pm$ 5.15	24.20 $\pm$ 2.96	t = -2.90	0.004*
BMI class	Underweight	19 (31.7%)	0 (0.0%)	$\chi^2 = 22.97$	0.0001*
	Normal weight	26 (43.3%)	41 (68.3%)		
	Overweight	11 (18.3%)	15 (25.0%)		
	Obese	4 (6.7%)	4 (6.7%)		
Smoking status	Current	12 (20.0%)	0 (0.0%)	$\chi^2 = 21.176$	0.0001*
	Former	6 (10.0%)	0 (0.0%)		

Variable	Category	CD Patients (n=60)	Controls (n=60)	Test Statistic	p-value
	Never	42 (70.0%)	60 (100.0%)		
Residency	Rural	19 (31.7%)	24 (40.0%)	$\chi^2 = 0.906$	0.341
	Urban	41 (68.3%)	36 (60.0%)		
Date of diagnosis†	< 3 years	45 (75.0%)	—	$\chi^2 = 15.00$	0.0001*
	> 3 years	15 (25.0%)			
Disease location†	Ileal	28 (46.7%)	—	$\chi^2 = 5.70$	0.058
	Colonic	19 (31.7%)			
	Ileocolonic	13 (21.7%)			
Disease phenotype†	Stricturing	25 (41.7%)	—	$\chi^2 = 2.50$	0.287
	Inflammatory	20 (33.3%)			
	Fistulizing	15 (25.0%)			
HBI score	Mean $\pm$ SD Median (q1-q3)	7.25 $\pm$ 4.94; 5.0 (3.00–11.75)			
HBI class†	Remission	30 (50.0%)	—	$\chi^2 = 21.90$	0.0001*
	Moderate	27 (45.0%)			
	Mild	3 (5.0%)			
Biologic treatment†	Yes	30 (50.0%)	—	$\chi^2 = 0.00$	1.000
	No	30 (50.0%)			
IL-22 (pg/mL)	mean $\pm$ SD and median (IQR).	408.38 $\pm$ 236.79; 343.22 (291.12–464.90)	266.95 $\pm$ 53.52; 261.52 (223.30–312.69)	Z= -5.097	0.0001*

Data are presented as mean $\pm$ SD or n (%). Independent t-test and χ^2 test were used for comparisons. A p-value < 0.05 was considered significant (*). Variables marked (†) were analyzed in CD patients only.

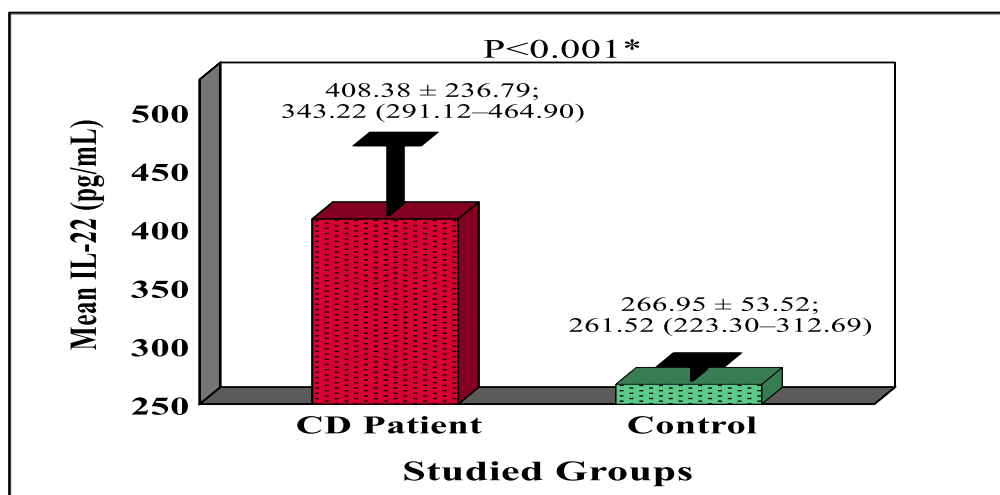


Figure 1: Comparison of serum IL-22 concentrations between patients with Crohn's disease and healthy controls.

Subgroup Analysis by Disease Location and Phenotype

As presented in Table 2, subgroup analysis of Crohn's disease patients according to disease location and phenotype revealed no

statistically significant differences in age, BMI, disease activity, or IL-22 levels (all $p > 0.05$). Across disease-location categories, age, BMI, HBI, and IL-22 were comparable among colonic, ileal, and ileocolonic disease. Likewise,

no significant differences were identified among Stricturing, inflammatory, and fistulizing phenotypes. Although some numerical variation

was observed, particularly for IL-22 levels across subgroups, these differences were not statistically significant.

Table 2: Serum IL-22, BMI, and clinical disease activity according to disease location and phenotype in Crohn's disease.

Analysis domain	Subgroup	n	Age, years mean $\pm$ SD	BMI, kg/m ² mean $\pm$ SD	HBI mean $\pm$ SD	HBI median (IQR)	IL-22, pg/mL mean $\pm$ SD	IL-22, pg/mL median (IQR)	Overall, between-group
Disease location	Colonic	19	29.05 $\pm$ 11.35	22.64 $\pm$ 6.10	7.16 $\pm$ 4.87	6.00 (3.00–11.00)	419.95 $\pm$ 316.55	309.65 (290.85–481.27)	Age: F = 1.152; p = 0.323 BMI: F = 1.109; p = 0.337 HBI: H = 0.007; p = 0.996 IL-22: H = 1.908; p = 0.385
	Ileal	28	30.79 $\pm$ 12.25	20.95 $\pm$ 4.18	7.32 $\pm$ 4.82	7.00 (3.00–11.50)	423.49 $\pm$ 209.34	380.17 (297.48–489.11)	
	Ileocolonic	13	25.15 $\pm$ 7.05	23.23 $\pm$ 5.50	7.23 $\pm$ 5.66	4.00 (3.50–12.50)	358.91 $\pm$ 152.85	316.76 (255.32–440.50)	
Disease phenotype	Stricturing	25	30.68 $\pm$ 10.92	22.06 $\pm$ 5.66	7.48 $\pm$ 4.87	7.00 (3.00–12.00)	467.89 $\pm$ 319.96	364.37 (305.18–476.03)	Age: F = 1.047; p = 0.358 BMI: F = 0.006; p = 0.994 HBI: H = 0.098; p = 0.952 IL-22: H = 1.645; p = 0.439
	Inflammatory	20	29.55 $\pm$ 13.53	21.96 $\pm$ 5.11	7.50 $\pm$ 5.49	5.00 (3.00–12.75)	366.32 $\pm$ 147.75	345.03 (250.17–438.67)	
	Fistulizing	15	25.53 $\pm$ 6.81	21.87 $\pm$ 4.61	6.53 $\pm$ 4.52	4.00 (4.00–10.00)	365.26 $\pm$ 142.56	321.32 (232.60–466.61)	

Abbreviations: BMI, body mass index; CD, Crohn's disease; HBI, Harvey–Bradshaw Index; IL-22, interleukin-22; IQR, interquartile range; SD, standard deviation. Data are presented as mean $\pm$ SD and median (IQR). One-way analysis of variance (ANOVA) is reported as F for normally distributed variables; Kruskal–Wallis testing is reported as H for non-parametric variables. P values represent overall comparisons among disease-location subgroups or disease-phenotype subgroups within each analysis domain. Statistical significance was defined as $p < 0.05$.

Clinical Activity (HBI) and Biomarker Variations

As presented in **Table 3**, stratification by HBI class revealed no significant difference in age among the moderate, mild, and remission groups ($p = 0.760$). However, BMI differed significantly across groups ($p = 0.0001$), with the remission group showing significantly higher BMI than both the mild and moderate groups. HBI values also differed significantly across all classes (overall $p = 0.0001$), and all

pairwise comparisons remained significant. Similarly, IL-22 levels varied significantly by disease activity class ($p = 0.0001$), with the highest levels observed in the moderate group and the lowest in the remission group. Pairwise comparisons demonstrated significantly lower IL-22 levels in remission versus both mild and moderate disease, whereas the difference between mild and moderate disease was not statistically significant.

Table 3: Serum IL-22 and body mass index across Harvey–Bradshaw Index disease-activity categories in Crohn’s disease.

Variable	Moderate (n=27)	Mild (n=3)	Remission (n=30)	Test	p-value
Age (years)	29.19 ± 9.90	24.33 ± 7.57	29.33 ± 12.48	F = 0.28	0.760
Pairwise	Mod vs Mild: 0.758	Mild vs Rem: 0.743	Mod vs Rem: 0.999		
BMI (kg/m ²)	18.38 ± 1.67	19.77 ± 1.10	25.44 ± 5.14	F = 24.50	0.0001*
Pairwise	Mod vs Mild: 0.825	Mild vs Rem: 0.046*	Mod vs Rem: 0.0001*		
HBI	12.04 ± 3.09	6.33 ± 0.58	3.03 ± 1.10		
	12.00 (9.00–15.00)	6.00 (6.00–7.00)	3.00 (2.00–4.00)	H = 47.02	0.0001*
Pairwise	Mod vs Mild: 0.0001*	Mild vs Rem: 0.0001*	Mod vs Rem: 0.0001*		
IL-22 (pg/mL)	530.75 ± 300.49	419.49 ± 56.66	297.13 ± 78.35		
	459.77 (326.36–591.49)	388.64 (384.95–484.89)	291.38 (242.48–367.13)	H = 25.88	0.0001*
Pairwise	Mod vs Mild: 0.795	Mild vs Rem: 0.025*	Mod vs Rem: 0.0001*		

Data are presented as mean ± SD and median (IQR). For the Mild group (n=3), IQR is represented using minimum–maximum values. One-way ANOVA (F) and Kruskal–Wallis test (H) were used as appropriate. Pairwise comparisons were conducted using Tukey or Mann–Whitney U tests. A p-value < 0.05 was considered statistically significant (*).

Impact of Biologic Therapy

As presented in Table 4, age did not differ significantly between patients receiving biologic treatment and those not receiving biologics (p = 0.820). However, the biologic treatment group had a significantly higher BMI (25.44 ± 5.14 vs. 18.52 ± 1.66 kg/m²; p = 0.0001), markedly lower HBI scores (3.03 ±

1.10 vs. 11.47 ± 3.41; p = 0.0001), and significantly lower IL-22 levels (median 291.38 vs. 455.36 pg/mL; p = 0.0001). These between-group differences are consistent with Figure 2, which illustrates significantly reduced mean HBI and IL-22 values in patients receiving biologic therapy (p < 0.001 for both).

Table 4: Clinical and biomarker differences according to biologic treatment status in patients with Crohn’s disease.

Variable	Yes (n=30)	No (n=30)	Test	p-value
Age (years)	29.33 ± 12.48	28.70 ± 9.70	t = 0.23	0.820
BMI (kg/m ²)	25.44 ± 5.14	18.52 ± 1.66	t = 6.93	0.0001*
HBI	3.03 ± 1.10	11.47 ± 3.41	t = -12.89	0.0001*
	3.00 (2.00–4.00)	11.50 (8.75–14.25)		
IL-22 (pg/mL)	297.13 ± 78.35	519.62 ± 286.93		
	291.38 (242.48–367.13)	455.36 (332.52–562.48)	Z = -5.09	0.0001*

Data are presented as mean ± SD and median (IQR). Independent samples t-test was used for normally distributed variables and Mann–Whitney U test for non-parametric variables. A p-value < 0.05 was considered statistically significant (*).

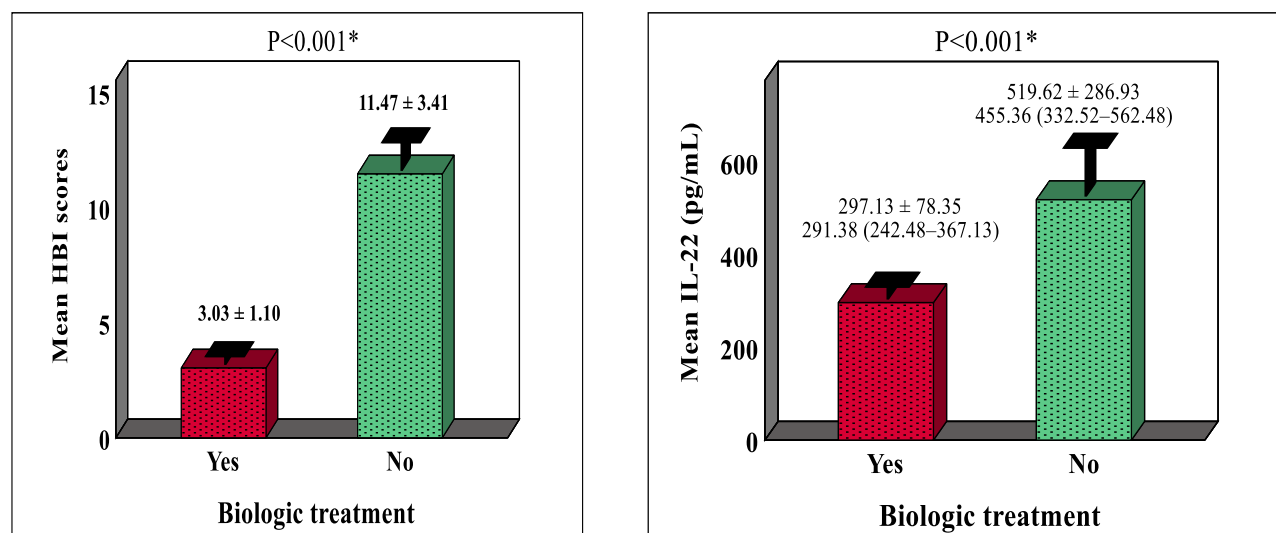


Figure 2: Comparison of Harvey-Bradshaw Index score and serum IL-22 concentration according to biologic treatment status.

Nutritional Status (BMI) and Inflammatory Correlation

Table 5 demonstrates significant variation in clinical and biomarker characteristics across BMI classes in patients with Crohn's disease. Age showed a modest overall difference between groups ($F = 3.04$, $p = 0.036$), although post hoc pairwise comparisons were not significant. BMI differed markedly across all categories ($F = 202.01$, $p = 0.0001$), with significant differences in all pairwise

comparisons. HBI scores also differed significantly among BMI classes ($H = 24.06$, $p = 0.0001$), being highest in underweight patients and lowest in overweight/obese patients; all pairwise comparisons were significant. Similarly, IL-22 levels varied significantly across groups ($H = 18.45$, $p = 0.0001$), with the highest concentrations observed in underweight patients and the lowest in overweight/obese patients, and significant differences detected between all subgroup pairs.

Table 5: Clinical disease activity and serum IL-22 according to body mass index category in patients with Crohn's disease.

Variable	Overweight/Obese (n=15)	Underweight (n=19)	Normal weight (n=26)	Test	p-value
Age (years)	32.13 ± 12.99	27.21 ± 11.65	28.54 ± 9.44	$F = 3.04$	0.036*
Pairwise	OW/OB vs UW: 0.999	UW vs NW: 0.975	OW/OB vs NW: 0.997		
BMI (kg/m ²)	29.31 ± 4.16	17.36 ± 1.10	21.12 ± 1.65	$F = 202.01$	0.0001*
Pairwise	OW/OB vs UW: 0.0001*	UW vs NW: 0.0001*	OW/OB vs NW: 0.0001*		
HBI	2.80 ± 1.21	10.79 ± 4.40	7.23 ± 4.62		
	3.00 (2.00–4.00)	10.00 (8.00–15.00)	6.00 (3.00–12.00)	$H = 24.06$	0.0001*
Pairwise	OW/OB vs UW: 0.0001*	UW vs NW: 0.012*	OW/OB vs NW: 0.002*		
IL-22 (pg/mL)	280.41 ± 78.22	552.31 ± 347.78	377.02 ± 123.35		
	291.91 (207.40–307.00)	466.61 (323.46–549.27)	349.13 (260.89–453.15)	$H = 18.45$	0.0001*
Pairwise	OW/OB vs UW: 0.0001*	UW vs NW: 0.022*	OW/OB vs NW: 0.005*		

OW/OB, Overweight/Obese; NW, Normal weight; UW, Underweight, Values are presented as mean ± SD and median (IQR). ANOVA (F) was used for parametric variables; Kruskal–Wallis (H) with Mann–Whitney U for pairwise comparisons for non-parametric variables. $p < 0.05$.

Figure 3 shows distinct correlation patterns among age, BMI, HBI, and IL-22. Age was only weakly correlated with the other variables, indicating no meaningful association with disease activity or IL-22 levels. In contrast, BMI showed significant negative correlations with both HBI ($r = -0.742$, $p < 0.01$) and IL-22 ($r = -0.628$, $p < 0.01$), indicating that lower BMI was associated with greater disease activity and higher IL-22 concentrations. A significant positive correlation was also observed between HBI and IL-22 ($r = 0.607$, $p < 0.01$), suggesting that increased clinical disease activity was associated with higher IL-22 levels, as illustrated in Figure 3.

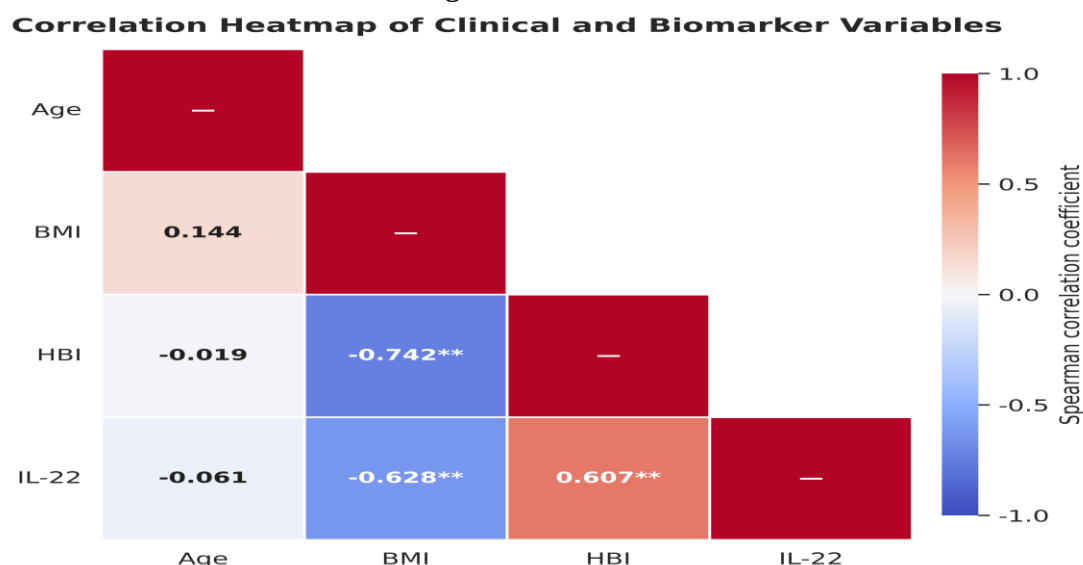


Figure 3: Heatmap of correlations among Age, BMI, HBI, and IL-22. Color intensity represents the magnitude and direction of the Spearman correlation coefficient. Positive correlations are shown in red and negative correlations in blue. Double asterisks indicate statistical significance * $p < 0.05$, ** $p < 0.01$. p -values are reported to three decimal places, with very small values expressed as 0.0001.

Diagnostic Performance and Logistic Regression

As presented in Table 6 and Figure 4, serum IL-22 demonstrated statistically significant discriminatory ability in the ROC curve analysis. The area under the curve was 0.77 with a 95% confidence interval of 0.69–

0.86 and a p -value of 0.0001, indicating that IL-22 performed significantly better than chance in distinguishing the studied outcome. According to the cutoff derived from the maximum K–S statistic, the optimal threshold for IL-22 was 362.35 pg/m.

Table 6: Diagnostic performance of serum IL-22 in ROC analysis for discrimination of the studied outcome.

Biomarker	AUC	95% CI	p-value	Cutoff	Sensitivity	Specificity
IL-22 (pg/mL)	0.77	0.69–0.86	0.0001*	362.35	0.48	0.98

AUC = area under the ROC curve; CI = confidence interval. Cutoff values were selected based on maximum K–S statistic. A p -value < 0.05 was considered statistically significant

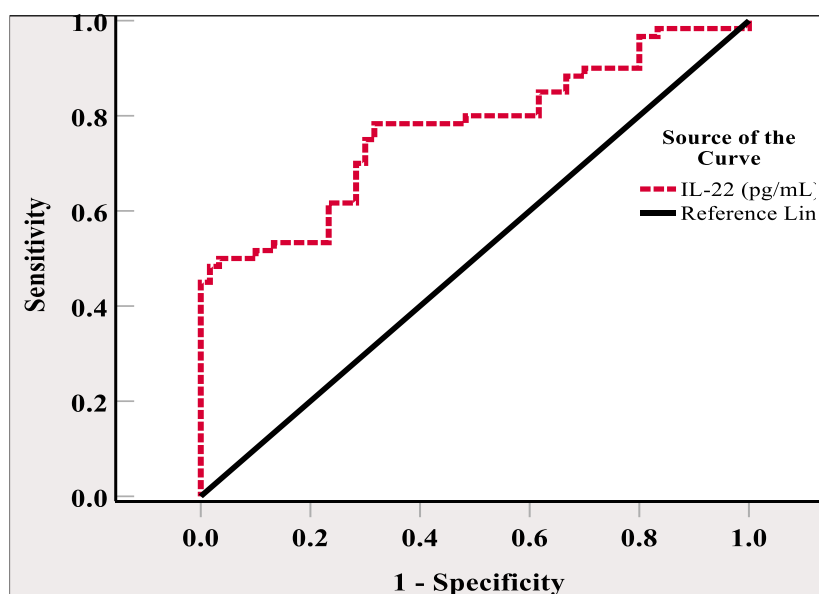


Figure 4: Receiver operating characteristic curve of serum IL-22 for between Crohn's disease patients and healthy controls.

As shown in Table 7, logistic regression analysis identified serum IL-22 as a significant factor associated with biologic treatment status, using no biologic treatment as the reference category. In the univariate model, IL-22 was significantly associated with biologic treatment, with an odds ratio (OR) of 0.98 (95% CI: 0.98–0.99, $p = 0.0001$). This finding indicates that increasing IL-22 levels were associated with lower odds of belonging to the biologic treatment group relative to the reference category.

This association remained statistically significant after multivariable adjustment, as demonstrated in Table 7. In the multivariate model, IL-22 retained an independent

association with biologic treatment status, with an adjusted OR of 0.98 (95% CI: 0.96–1.00, $p = 0.023$). Although the adjusted effect size was modest, the persistence of statistical significance suggests that IL-22 remained an independent predictor after accounting for other covariates included in the model.

The reference category for the dependent variable was no biologic treatment. In the multivariate model, adjusted estimates reflect the covariates entered into the final regression model. OR denotes odds ratio, CI denotes confidence interval, and IL-22 concentrations are expressed in pg/mL. A p value < 0.05 was considered statistically significant.

Table 7: Logistic regression analysis of factors associated with biologic treatment status.

Variable	Univariate OR (95% CI)	p-value	Multivariate OR (95% CI)	p-value
IL-22 (pg/mL)	0.98 (0.98–0.99)	0.0001*	0.98 (0.96–1.00)	0.023*

Logistic regression analyses for biologic treatment status. Odds ratios are presented with 95% confidence intervals.

DISCUSSION

The present study demonstrated a highly significant elevation in systemic Interleukin-22 (IL-22) levels among patients with Crohn's disease (CD) compared to healthy controls ($p=0.0001$). In addition to this immunological change, the CD cohort had significantly lower Body Mass Index (BMI) and different smoking profile whereas demographic factors such as age and sex were statistically comparable thus reducing potential demographic confounding. This prominent systemic elevation of IL-22 is consistent with the findings of Bai-Sui et al. reporting upregulated concentrations of this cytokine in inflammatory bowel disease (IBD) cohorts.

This noted elevation could mechanistically be explained by severe dysregulation of the mucosal immune response, i.e., hyperactivation of the Th17 axis. In CD pathogenesis, continuous exposure to luminal antigens results in overproduction of IL-22 by T helper 17 (Th17) cells and type 3 innate lymphoid cells (ILC3s). IL-22 essentially serves as a mucosal protective factor for barrier restitution, but its sustained spillover in the systemic circulation indicates a severe, unresolved inflammatory burden (11). However, although it was noted that there was a systemic increase in the overall analysis, the subgroup analysis showed that IL-22 levels and clinical disease activity (HBI) and BMI did not differ significantly between the different locations of the disease (colonic, ileal and ileocolonic) and phenotypes (Stricturing, inflammatory and fistulizing) ($p > 0.05$). This homogeneity is in agreement with observations by Wenyu Jiang et al., Jarrod A. Dudakov et al., that peripheral Th17-related cytokine levels reflect a generalized, cumulative inflammatory load rather than specific indicators of anatomical involvement. When the mucosal barrier is sufficiently compromised, localized concentration gradients are diluted in the bloodstream to a similar systemic threshold,

regardless of the anatomical segment. In addition, the presence of patients on active biologic therapy in the current cohort leads to a systematic dampening of the Th17 axis, which might obscure subtle cytokine differences that are phenotype-specific and would otherwise be present in purely treatment-naïve populations. The level of systemic IL-22 was found to be profoundly dictated by the degree of continuous clinical disease activity and the nutritional status of the patient rather than by anatomical location (12,13). These interdependencies were further consolidated by Spearman correlation analysis showing a strong positive correlation of systemic IL-22 with HBI ($r= 0.607$) and a significant negative correlation with BMI ($r= -0.628$) forming a robust, interconnected triad. HBI stratification confirmed this step-wise association with IL-22 peaking in moderate disease and dropping significantly in clinical remission.

This association was also reflected in nutritional stratification where the highest clinical activity scores and the most markedly increased IL-22 concentrations were seen in underweight patients ($p = 0.0001$). Biologically, this is a reflection of the intense hypermetabolic and catabolic state driven by chronic cytokine overproduction. Recognizing that circulating IL-22 rises in response to active mucosal ulceration, it actively interferes with neuroendocrine appetite regulation, increases resting energy expenditure, and promotes systemic catabolism, resulting in a progressive decline in body mass that mathematically correlates with the escalating inflammatory burden. This phenomenon is highly consistent with studies on IBD-driven cachexia conducted by Claudia Cabrera et al. and Atsushi Mizoguchi et al. The Th17/IL-22 axis plays a critical role in the observed clinical and nutritional decline, best demonstrated by the cohort's response to pharmacological intervention. Patients on biologic treatment had significantly lower systemic IL-22 levels, significantly lower HBI

scores and higher BMI compared to those not on biologic treatment ($p = 0.0001$). The parallel reduction of clinical symptoms and systemic inflammatory burden shows the direct translation of targeted immunological suppression into tangible deep mucosal healing. Biologics deeply starve the Th17/ILC3 axis by blocking upstream inflammatory signals (e.g. anti-TNF α or anti-IL-12/23 agents) that inhibit cytokine-induced catabolism and restore intestinal absorptive capacity (14,15). Successful biological therapy plays a pivotal role in promoting systemic mucosal healing by neutralizing upstream pro-inflammatory cytokines (e.g., TNF- α). This targeted blockade halts the chronic inflammatory cascade, thereby allowing the intestinal epithelial barrier to repair and restore its integrity. Consequently, the resolution of mucosal ulceration drastically diminishes the systemic leakage of localized inflammatory mediators, which clinically manifests as deep remission and biochemically as a marked reduction in circulating biomarkers like IL-22.

Importantly, multivariable logistic regression analysis showed that systemic IL-22 level is a strong and independent predictor of therapeutic status (Adjusted OR = 0.98, $p = 0.023$). This suggests that the suppression of IL-22 is not merely a by-product of clinical improvement, but a direct, independent molecular footprint of the biologic drug successfully maintaining immunological blockade, echoing precision medicine models proposed by previous research. The translation of these pathophysiological and therapeutic insights into clinical practice led to the assessment of the diagnostic utility of serum IL-22. Receiver operating characteristic (ROC) curve analysis showed an Area Under the Curve (AUC) of 0.77 ($p = 0.0001$). At the optimal threshold of 362.35 pg/mL, IL-22 had an extremely high specificity of 98% and a relatively low sensitivity of 48%. In line with

the observations by Bertin et al. and Roblin et al., this profile indicates that although increased systemic IL-22 is a very good rule-in confirmatory marker for active Th17-mediated mucosal inflammation (with low false positive rates), it is not ideal as a sole screening tool. The lowered sensitivity is a direct biological consequence of the extreme therapeutic heterogeneity of the cohort: effective biologic treatments and periods of clinical remission actively suppress the Th17 axis and pull a large fraction of true-positive patients below the diagnostic threshold. Ultimately, this study supports the clinical use of IL-22 in a multi-biomarker panel to better understand the full and dynamic continuum of Crohn's disease (16,17).

However, the current study findings and pathophysiological insights should be interpreted with caution because of some methodological limitations. First, although the overall sample size was sufficient to detect primary between-group differences, the division of the Crohn's disease cohort into smaller clinical subgroups (e.g. specific Montreal phenotypes or the mild activity class, $n=3$) greatly diminished the statistical power. This limitation heightens the vulnerability to Type II errors that might conceal subtle immunological differences within these particular subgroups. Second, the cross-sectional design of the present study does not allow to clearly establish temporal or causal relationships between systemic biomarker changes, nutritional decline (BMI) and therapeutic intervention. Longitudinal tracking of IL-22 and other parameters during biologic induction and maintenance phases would be required to validate their predictive kinetics. Third, the immunological evaluation was performed only in peripheral serum samples. Serum biomarkers are a good representation of the total systemic inflammatory burden; however, they are indirect surrogate measures and may not be an accurate reflection of the localized immune

microenvironment and cytokine gradients within the intestinal mucosa. Fourth, clinical disease activity was assessed using the Harvey–Bradshaw Index (HBI), which relies heavily on subjective patient-reported symptoms. The lack of concurrent endoscopic, cross-sectional imaging or histological validation (i.e. SES-CD scoring) may have resulted in misclassification of deep subclinical mucosal inflammation in patients otherwise in symptom-based clinical remission. Finally, the therapeutic heterogeneity of the CD cohort, while providing a valuable comparative dimension in terms of the efficacy of biologic agents, introduces major immunological confounding. Some of the intra-group variance seen in the systemic cytokine profiles may be due to the fact that the specific types, dosages and durations of biologic therapies were not uniformly standardized.

CONCLUSION

In conclusion, systemic serum IL-22 is a highly specific biomarker for active Crohn's disease that accurately reflects mucosal inflammation, clinical severity, and subsequent nutritional decline, regardless of the disease's location or phenotype. Furthermore, because successful biologic therapy effectively halts this inflammatory cascade and dramatically reduces IL-22 levels, this cytokine serves as a strong independent predictor of treatment success and a valuable molecular footprint for monitoring deep remission. Although its sensitivity is lowered in therapeutically suppressed patients—limiting its use as a standalone screening tool—its remarkable specificity makes IL-22 an excellent confirmatory diagnostic adjunct within a comprehensive multi-biomarker panel for managing and monitoring the disease.

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Ethical approval

The present study which is conducted by authors (Ghaith Adnan and Khawlah Abdallah Salman.) was approved by the local Department of Najaf Health Directorate and the Committee on Publication Ethics, College of Medicine, University of Kufa committee.

Statement of Permission and Conflict of Interests

The authors declare that there is no conflict of interest.

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