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Th1/Th17 cytokine signatures correlate with Crohn's disease activity in a 54-week cohort of patients treated with adalimumab

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ABSTRACT

Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract with a relapsing–remitting course. Cytokine dysregulation plays a central role in its pathogenesis, and biological therapies—such as anti-TNFα agents, vedolizumab, and anti-IL-23 monoclonal antibodies—have improved disease control. However, variability in therapeutic response underscores the need for biomarkers to support personalized treatment strategies. A longitudinal study was conducted in 26 patients with clinical-remission/mild-activity CD who were treated with adalimumab over 54 weeks. Serum samples were collected at baseline, week 14, and week 54. Eighteen cytokines were quantified using high-sensitivity Luminex MAP technology. TNFα values were excluded due to drug interference. Clustering and PERMANOVA analyses were used to explore cytokine profiles and their

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association with disease activity. Four cytokine clusters were identified: Th1-like, Th2-like, Th17-like, and a mixed inflammatory profile including IL-1β, IL-2, IL-12, IL-21, and IL-23. Significant reductions in CDAI, C-reactive protein (CRP), and fibrinogen were observed during treatment. IL-2, IL-10, and CCL20 levels increased significantly in patients with active CD (CDAI $\geq$ 150). Th1 and Th17 signatures were positively correlated with disease activity. Serum assessment of Th1 (IL-1β, IL-7) and Th17 (GM-CSF) signatures generated ROC curves with an AUC of 0.795, compared with CRP (AUC = 0.686) and fibrinogen (AUC = 0.774). Serum cytokine profiling reveals immunological heterogeneity in CD patients treated with adalimumab and may serve as an exploratory biomarker of disease activity and therapeutic response; however, these findings are hypothesis generating and require validation in larger, independent cohorts before clinical applicability can be established.

1. Introduction

Crohn's disease (CD) is a chronic autoimmune disorder characterized by transmural inflammation of the gastrointestinal tract, leading to symptoms such as abdominal pain, diarrhea, bowel obstruction, and perianal lesions [1]. The disease typically follows a relapsing–remitting course, alternating between periods of clinical activity and remission [2]. Disease activity is commonly assessed using clinical indices such as the Crohn's Disease Activity Index (CDAI) and Harvey–Bradshaw Index (HBI), or endoscopic scores including CDEIS and SES-CD [3].

Although CD is traditionally more prevalent in developed regions, its increasing incidence and prevalence in many parts of the world have expanded the clinical and economic burden of the disease. In Europe, incidence ranges from 4.1 to 22.78 per 100,000 inhabitants, with prevalence estimates of 61.6–178 per 100,000 [4]. This growing burden further reinforces the need for improved tools to monitor disease activity and optimize therapeutic decisions.

The intestinal immune system plays a central role in maintaining mucosal homeostasis, with cytokines acting as key mediators. Dysregulation of cytokine networks contributes to the pathogenesis of inflammatory bowel disease (IBD) and has driven the development of targeted biological therapies. Anti-TNFα agents such as infliximab, adalimumab, and certolizumab pegol have demonstrated efficacy in inducing and maintaining remission [5]. However, a substantial proportion of patients experience primary non-response or secondary loss of response, highlighting the need for personalized monitoring strategies [6].

In recent years, additional biologics have expanded the therapeutic arsenal [7]. Vedolizumab, a monoclonal antibody targeting α4β7 integrin, selectively inhibits lymphocyte trafficking to the gut and offers a gut-specific mechanism with a favorable safety profile [8]. Similarly, anti-IL-23 agents such as ustekinumab and mirikizumab/risankizumab/guselkumab modulate Th17-related pathways implicated in CD pathogenesis [9–11]. These therapies underscore the importance of understanding immune signatures and cytokine dynamics in guiding treatment decisions.

This study aims to evaluate serum cytokine profiles in CD patients treated with adalimumab and explore their potential as biomarkers of disease activity and therapeutic response. More studies are warranted in patients with low inflammatory activity, where early identification of immune signatures could support timely therapeutic adjustments toward alternative biologics before clinical deterioration occurs.

2. Methods

2.1. Experimental design

A prospective, observational, and analytical cohort study was conducted involving 26 patients diagnosed with CD according to the criteria established by the European Crohn's and Colitis Organisation [12]. The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki (2013). All participants provided written informed consent prior to inclusion. The study was observational in nature, and treatment followed standard clinical protocols. All participants initiated anti-TNF therapy based on clinical indications, as part of the PREDICROHN study (GET-CRO-2010-01), approved by the research division of the Spanish Working Group on Crohn's Disease and Ulcerative Colitis (GETECCU).

Disease activity was evaluated using the Crohn's Disease Activity Index (CDAI), derived from eight clinical parameters integrating symptoms, physical findings, hematocrit, and body weight.

Inclusion and exclusion criteria

Inclusion criteria included:

a) Age $\geq$ 18 years;
b) Confirmed diagnosis of CD based on clinical, radiological, endoscopic, and/or histological findings;
c) Clinical indication for anti-TNF therapy, including patients in clinical remission or with mild disease activity, with indication for biologic treatment due to prior therapeutic failure, intolerance to conventional therapy, or high-risk disease features.
d) Provision of written informed consent.

Exclusion criteria included:

a) Pregnancy or breastfeeding;
b) Prior biological therapy;
c) Diagnosis of ulcerative colitis;
d) Short bowel syndrome;
e) Contraindications to biologics;
f) Presence of an ostomy;
g) Abdominal surgery within the past 6 months;
h) Active hepatitis B or C;
i) HIV infection;
j) Positive tuberculin skin test;
k) Anti-TNF therapy for indications other than CD.

2.2. Treatment protocol and sample collection

Patients received adalimumab subcutaneously: 160 mg at week 0 (baseline), 80 mg at week 2, and 40 mg at week 6, followed by 40 mg every 8 weeks (weeks 14, 22, 30, 38, 46, and 54). Clinical follow-up was maintained throughout the study, but serum samples for cytokine analysis were collected only at baseline (visit 1), week 14 (visit 4), and week 54 (visit 9).

Biochemical markers (serum): albumin, ferritin, C-reactive protein (CRP), and fibrinogen. Hematological markers (whole blood): hemoglobin, leukocyte count, neutrophils, platelets, and erythrocyte sedimentation rate (ESR). Routine laboratory tests were performed at each hospital's clinical laboratory. Serum samples for cytokine profiling were stored at −80 °C until centralized analysis.

2.3. Cytokine and chemokine profiling

Serum concentrations of 18 cytokines and chemokines were measured: IL-1β, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12p70, IL-13, IL-17A, IL-21, IL-23, IFN-γ, GM-CSF, CCL3, CCL4, CCL20, CX3CL1, and CXCL11. Blood was collected in dry tubes and samples were centrifuged at 2000 rpm for 20 min, and aliquots were stored at −80 °C.

Quantification was performed using Luminex MAP technology with a

high-sensitivity multiplex assay kit (Milliplex MAP, HSTCMAG-28SK-21; Merck, Darmstadt, Germany). Microspheres encoded with distinct fluorescence ratios captured specific cytokines. Samples were incubated for 16–18 h in 96-well plates, followed by biotinylated detection antibodies and streptavidin-phycoerythrin (Strep-PE) conjugate. Fluorescence was measured using the MAGpix system (Merck), and concentrations were calculated using Analyst software based on standard curves and mean fluorescence intensity (MFI). Detection limits were defined according to the manufacturer's protocol.

2.4. Graphical analysis

Cytokine expression and interrelationships were visualized using R (version 4.3.3). All boxplots and heatmaps were generated with the ggplot2 package, while hierarchical clustering dendrograms were created using factoextra. Circular correlation networks (Circos plots), representing pairwise cytokine associations, were constructed with the igraph and ggraph packages. For these visualizations, data were filtered to include correlation coefficients greater than 0.7, thereby highlighting biologically relevant relationships across patient subgroups. Finally, receiver operating characteristic (ROC) curves were generated using the pROC package.

2.5. Statistical analysis

All statistical analyses were performed using R (version 4.3.3). Longitudinal changes in hematological and biochemical parameters were evaluated using repeated-measures ANOVA for normally distributed variables or the Friedman test otherwise, based on the Shapiro–Wilk normality test, followed by Bonferroni-corrected paired post-hoc comparisons. This same approach was applied to evaluate longitudinal changes in individual cytokine and chemokine levels throughout the study. Subsequently, these data were also analyzed at the multivariate level using tests for homogeneity of multivariate dispersion, followed by permutational multivariate analysis of variance (PERMANOVA).

Associations between cytokine profiles and disease activity status (active vs. remission) were evaluated at the individual level using the Shapiro-Wilk test, followed by either the *Student's t*-test or Mann-Whitney *U* test, as appropriate. At the multivariate level, dispersion tests and PERMANOVA were again applied.

Statistical significance was defined as $p < 0.05$ (*), and $p < 0.01$ (**).

Finally, combinations of predictors were evaluated by fitting logistic regression models in which the cytokines of interest were entered as independent variables in all possible two- and three-marker combinations. Disease activity served as the dependent dichotomous outcome, defined as CDAI >150 (active disease) versus CDAI ≤ 150 (remission). To assess the internal validity of the final composite models and classical inflammatory markers (CRP and fibrinogen), bootstrap resampling (1000 iterations) was performed to obtain bias-corrected AUC estimates and to quantify model optimism.

3. Results

3.1. Baseline sociodemographic and clinical values of the study population

This study describes the baseline clinical, biochemical, and hematological characteristics of 26 patients with Crohn's disease (CD) who received adalimumab treatment over a 54-week period (Table 1). The cohort corresponded to a nested subpopulation of the PREDICROHN study (GET-CRO-2010-01), designed to explore immunological markers associated with disease activity and therapeutic response. At baseline, the cohort exhibited predominantly low-activity CD, reflecting the clinical profile of patients selected for biomarker discovery. Specifically, 15/26 patients were in clinical remission (CDAI <150), 9/26 had mild activity (150–220), and 2/26 had moderate activity (221–450); none presented severe activity (>450). Overall, 24/26 patients (92.3%) were in remission or mild activity at study entry. The predominance of remission and mild activity at baseline provided a homogeneous low-activity profile for evaluating longitudinal cytokine changes at weeks 14 and 54. Regarding concomitant treatments during follow up, 21 out of 26 patients (81%) received azathioprine concomitantly throughout the study period, and 8 patients (31%) required rescue corticosteroids at one or more time points, as detailed in Table 1.

Table 1
Baseline characteristics of the study cohort.

	Baseline characteristics 0 Weeks (n = 26)
Age, years. Median (IQR)	42.5 (36.3–50)
Sex	
– Men. N (%)	11 (42%)
– Women. N (%)	15 (58%)
Smoking habit. N (%)	8 (31%)
Weight, kg. Median (IQR)	68 (57−82)
Height, cm. Median (IQR)	164.5 (159–172.3)
BMI, kg/m². Median (IQR)	24.2 (21.5–27.8)
Clinical parameters	
– CDAI. Median (IQR)	134.5 (74–185.3)
– HBI. Median (IQR)	5 (4−7)
Concomitant medications during follow-up	
– **Azathioprine. N (%)**	21 (81%)
– **Rescue corticosteroids at any time point. N (%)**	8 (31%)
Analytical parameters. Median (IQR)	
Hematology	
– Leukocytes (10^3/mm³)	5.8 (4.9–8.4)
– Neutrophils (10^3/mm³)	4.1 (3.1–6.1)
– Platelets (10^3/mm³)	304 (241.8–350.8)
– ESR (mm/h)	22.5 (9–31.5)
– Hemoglobin (g/dL)	13.1 (12.1–14.2)
Biochemistry	
– Albumin (g/dL)	4.2 (4–4.4)
– Ferritin (ng/mL)	37.4 (17.8–121)
– CRP (mg/L)	0.8 (0.4–6.5)
– Fibrinogen (mg/dL)	436 (391−505)

Abbreviations: IQR, Interquartile Range; BMI, Body Mass Index; CDAI, Crohn's Disease Activity Index; HBI, Harvey-Bradshaw Index; ESR, Erythrocyte Sedimentation Rate; CRP, C-Reactive Protein.

3.2. Evolution of clinical, hematological, and biochemical parameters in patients with Crohn's disease during adalimumab treatment

Treatment with adalimumab led to a significant reduction in CDAI scores starting in week 14, which was sustained through week 54 (Fig. 1). Similarly, serum levels of CRP and fibrinogen showed a significant decrease at the same time points.

In contrast, no significant changes were observed in other biochemical parameters, such as albumin and ferritin, or in hematological parameters, including leukocyte, neutrophil, platelet, hemoglobin and ESR values (Fig. 1).

3.3. Interrelationships among circulating cytokines and chemokines in Crohn's disease patients

Heat map of pairwise correlations among circulating cytokines and chemokines in Crohn's disease is shown in Fig. 2A. Cytokines and chemokines were ordered and grouped based on previously published data. Their classification considered the expression profiles associated with Th1-Type and innate immune signature (Th1-like), Treg-associated cytokine profile (Treg), Th2-Type and humoral immune signature (Th2-like), Th17-associated immune signature (Th17-like), as well as chemokines associated with immune cell trafficking (other chemokines). The numerical values appearing in each of the boxes correspond to the ρ

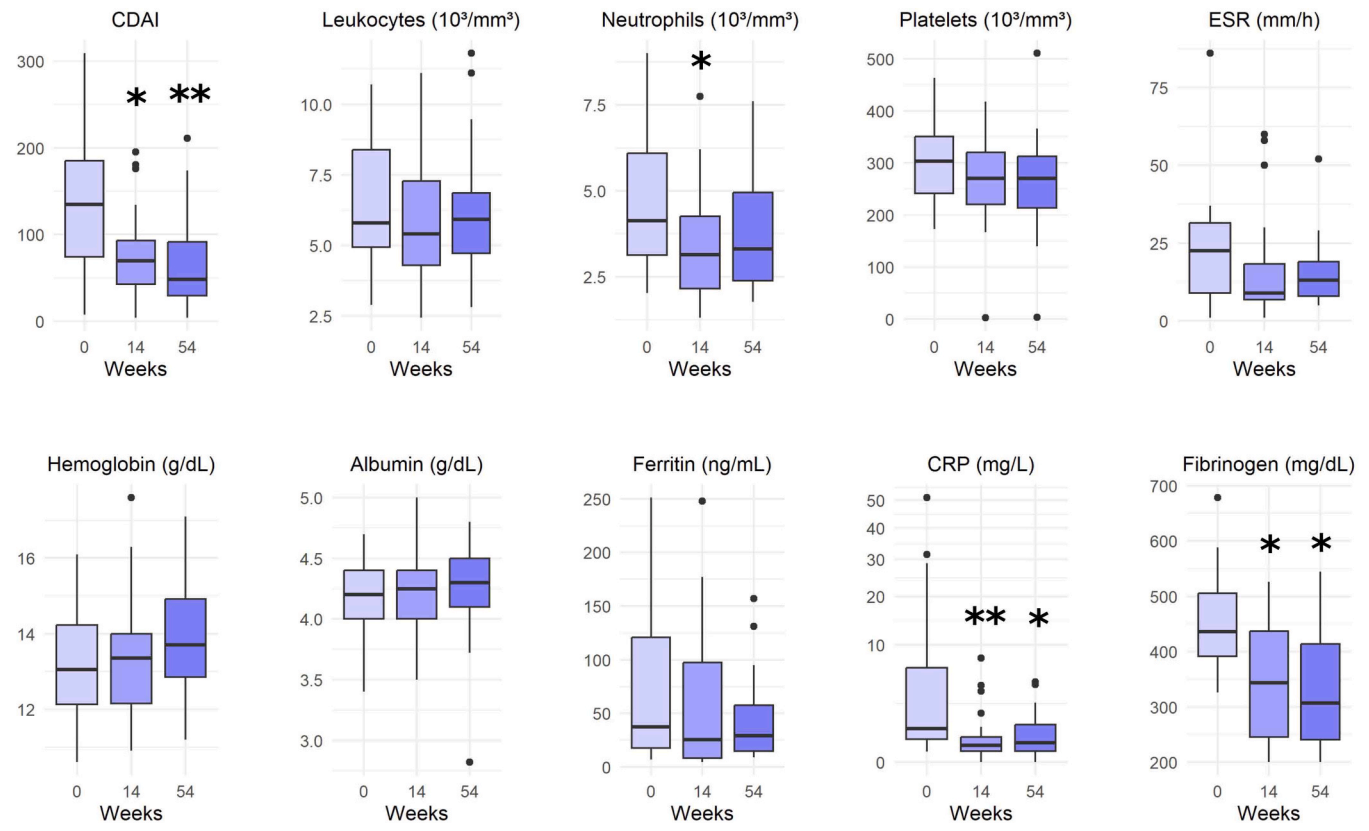


Fig. 1. Evolution of analytical parameters in the study cohort during adalimumab treatment. Statistical significance: $p < 0.05$ (*) and $p < 0.01$ (**).

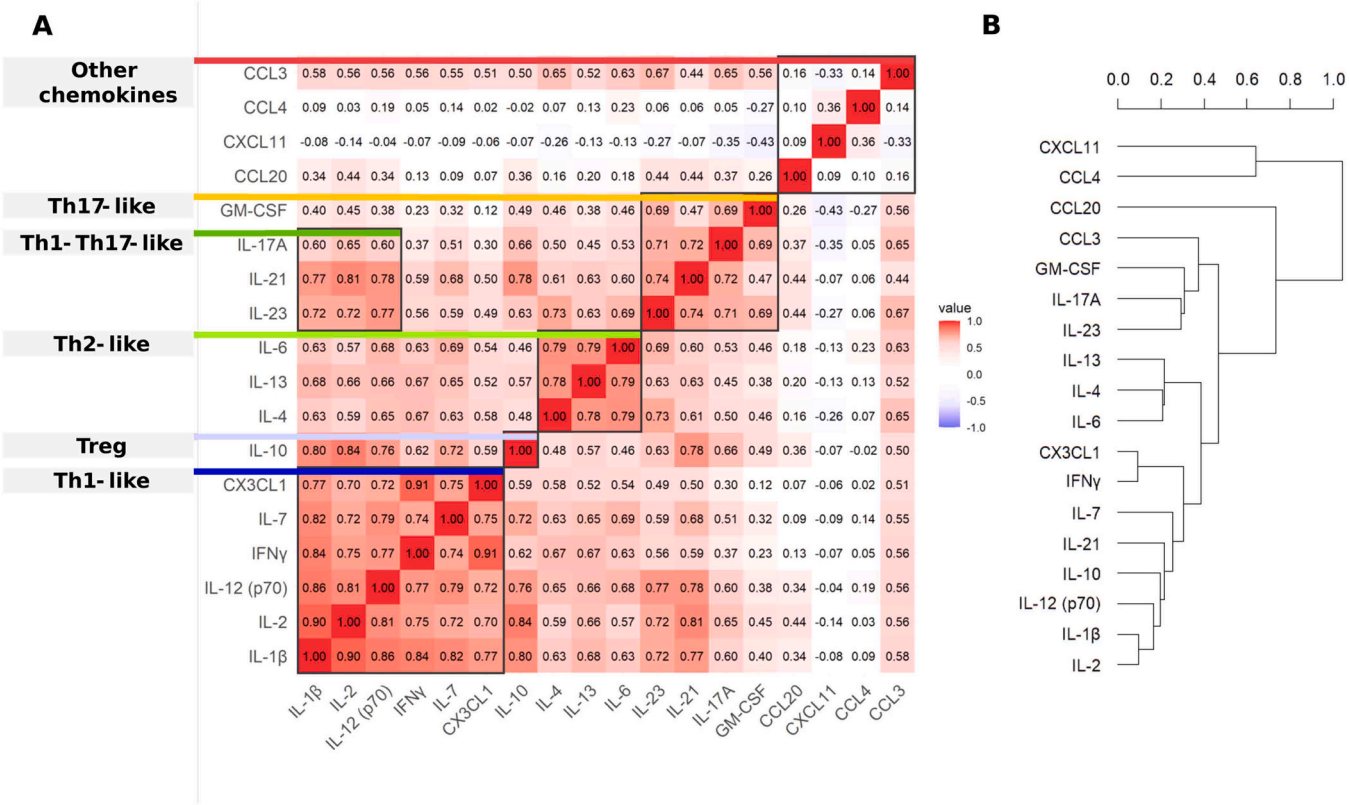


Fig. 2. A. Spearman's correlation matrix (ρ) for the study cohort. B. Hierarchical dendrogram illustrating Spearman's correlation strength; the upper scale represents the dissimilarity index, where higher values indicate lower correlation between variables.

value of the Spearman correlation coefficient. Fig. 2B shows an unsupervised clustering dendrogram illustrating the associations among serum cytokines and chemokines in patients with Crohn's disease. The upper scale represents the dissimilarity index, where higher values indicate lower correlation between variables. The strongest correlation was observed between IFN-γ and CX3CL1, with a ρ value of 0.91.

The results of the predefined cluster classification were largely consistent with those obtained through unsupervised clustering, with a few exceptions. For instance, IL21 was initially assigned to (Th17-like) in the literature-based grouping but was positioned differently in the unsupervised analysis (Figs. 2A and 2B). In subsequent analyses, we applied supervised clustering techniques to serum cytokine and chemokine data in order to identify immunological patterns associated with disease activity and treatment response.

3.4. Longitudinal analysis of serum cytokines and chemokines during adalimumab treatment in Crohn's disease

Throughout the 54-week adalimumab treatment period, no significant changes from baseline were observed in the serum levels of any individual cytokine or chemokine in patients with Crohn's disease (Fig. 3). Grouped cytokines and chemokines, as defined in Fig. 2A, were later assessed at the multivariate level using PERMANOVA, which analyses differences throughout the treatment period not for a single analyte, but for the entire profile of variables integrated within each cluster. Prior to this, tests for homogeneity of multivariate dispersion showed non-significant results in all cases ($p > 0.05$), thus confirming that the results of subsequent comparisons were not confounded by unequal dispersion. According to the PERMANOVA analysis conducted over the 54-week adalimumab treatment period, no statistically significant changes were observed in the combined serum levels of cytokines and chemokines across the Th1-like, Th2-like, Th17-like, and other immunological profiles (Fig. 3).

3.5. Study of serum cytokine and chemokine levels in patients in clinical remission (CDAI ≤ 150) and with active (CDAI > 150) Crohn's disease

Fig. 4 summarizes the comparison of serum cytokine and chemokine levels between patients in clinical remission and those with active Crohn's disease. Serum levels of IL-2, IL-10, and CCL20 were significantly higher in patients with active CD than in those in remission. In contrast, the remaining analytes—including IL-1β, IL-12, IFN-γ, TNF-α, IL-6, IL-7, IL-17A, IL-21, IL-23, CX3CL1, and other chemokines—did not exhibit significant differences between groups.

Therefore, we proceeded to compare cytokine profiles grouped according to the clusters described in Fig. 2A. Prior to PERMANOVA, homogeneity of multivariate dispersion was assessed using PERMDISP. For the Th1-like, Th17-like, and other chemokine groups, dispersion tests yielded non-significant results ($p > 0.05$), indicating comparable variances between remission and active disease and ensuring that PERMANOVA outcomes were not affected by unequal dispersion. In contrast, the Th2-like cluster showed a significant dispersion result ($p = 0.024$), reflecting unequal variability between groups; therefore, PERMANOVA was not interpreted for this cluster. Using the PERMANOVA statistical test, increased cytokine levels were found in patients with active Crohn's disease compared to those in remission, across Th1-like and Th17-like groups (Fig. 4).

The above results were compared with nonspecific inflammatory markers, CRP and fibrinogen, obtained from the same patients (Fig. 4). Both markers were significantly higher in active Crohn's disease than in clinical remission, aligning with the cytokine-based findings.

To characterize interactions among immune mediators, correlations

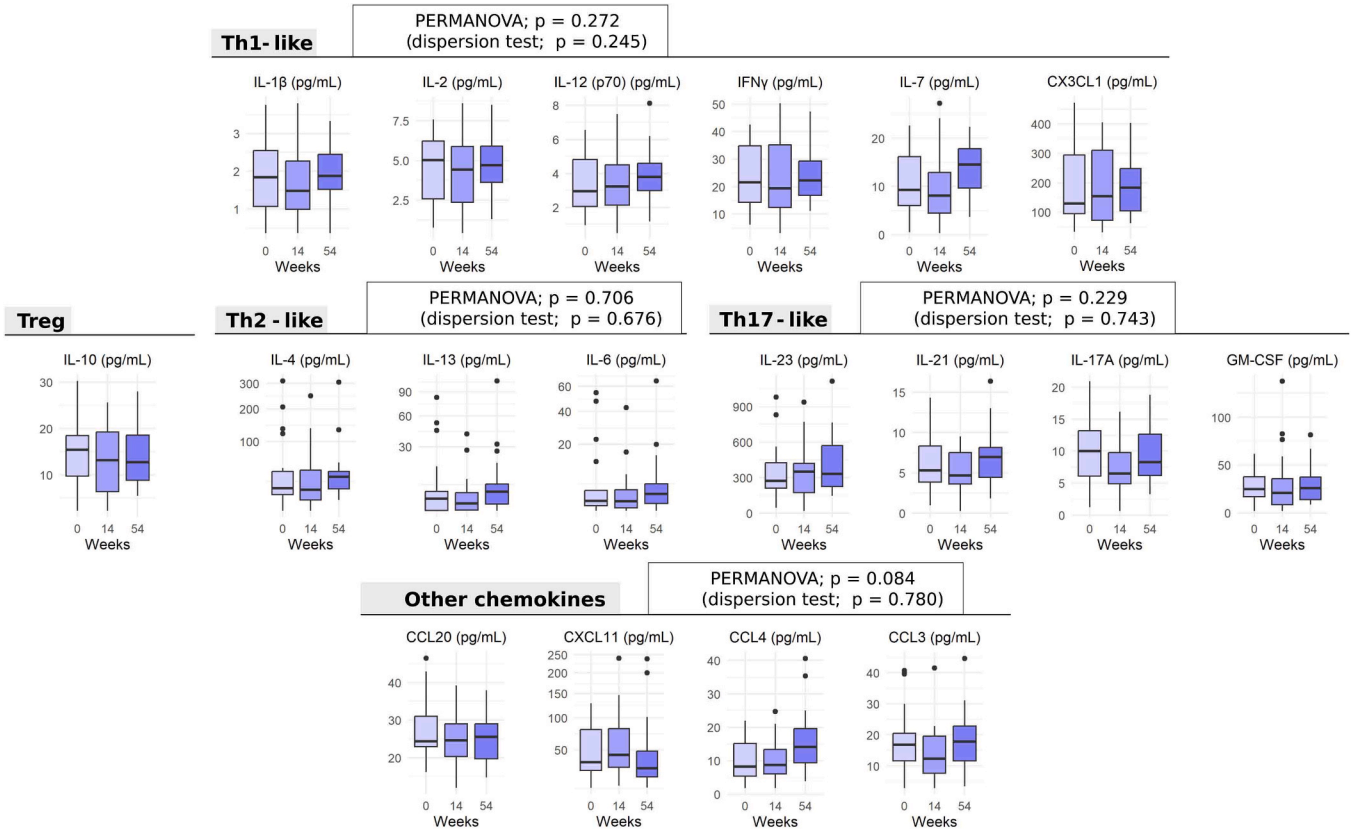


Fig. 3. Evolution of cytokine and chemokine levels in the study cohort throughout the trial. Boxplots are grouped by immune response categories: Th1-like, Treg, Th2-like, Th17-like, and Other chemokines. Multivariate statistical comparisons were performed using dispersion tests followed by PERMANOVA; p-values for both are reported for each group.

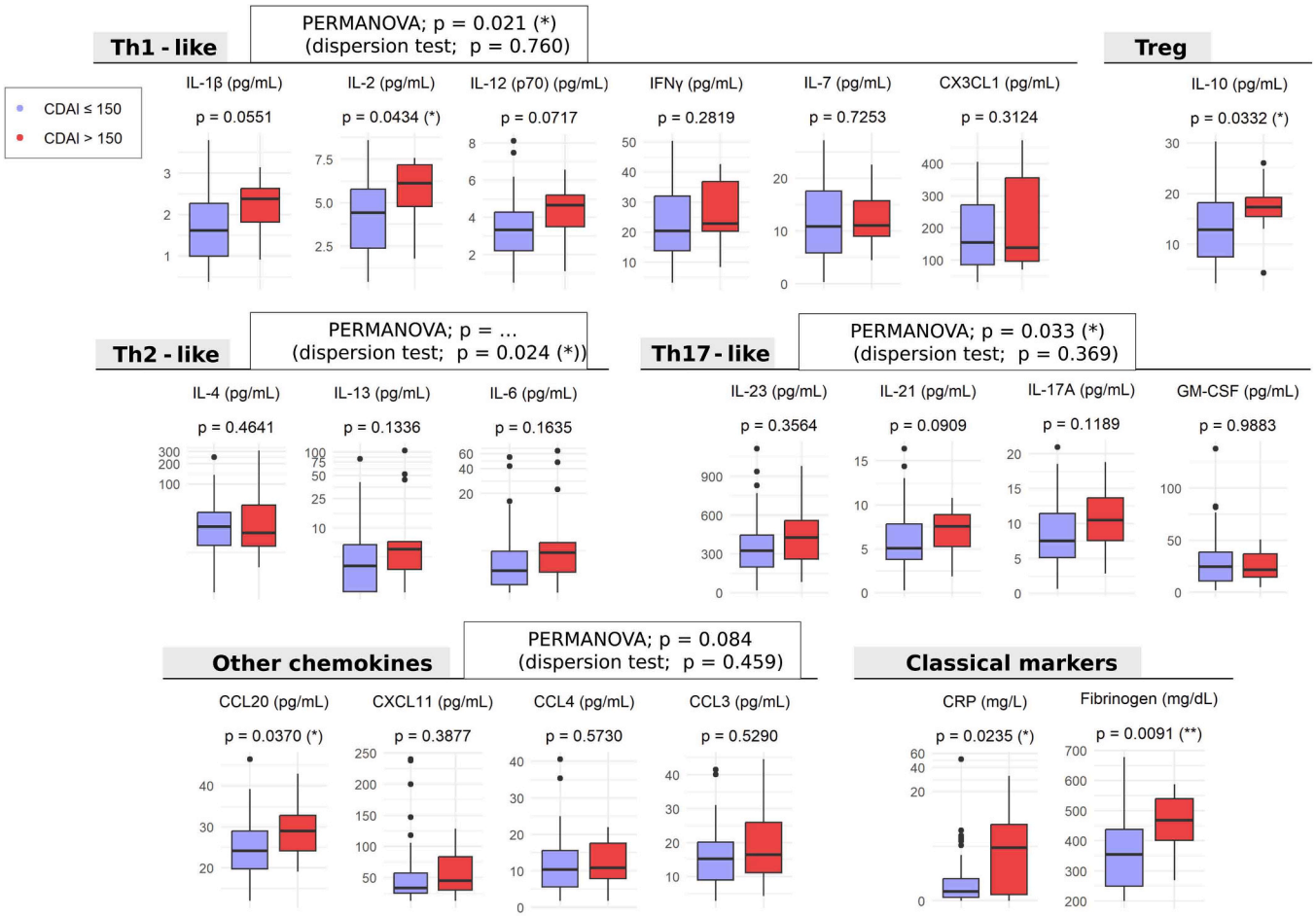


Fig. 4. Serum cytokines, chemokines and classical markers in patients in remission (CDAI ≤ 150) and active disease (CDAI > 150). Boxplots are grouped by categories: Th1-like, Treg, Th2-like, Th17-like, other chemokines, and classical markers. Differences at the single-analyte level were tested using the Mann-Whitney *U* test; p-values are displayed below each analyte name. Multivariate comparisons were assessed by dispersion tests followed by PERMANOVA; p-values for both are reported for each group in the upper white boxes. PERMANOVA was not performed in the group with a significant result in the dispersion test.
Statistical significance: $p < 0.05$ (*) and $p < 0.01$ (**).

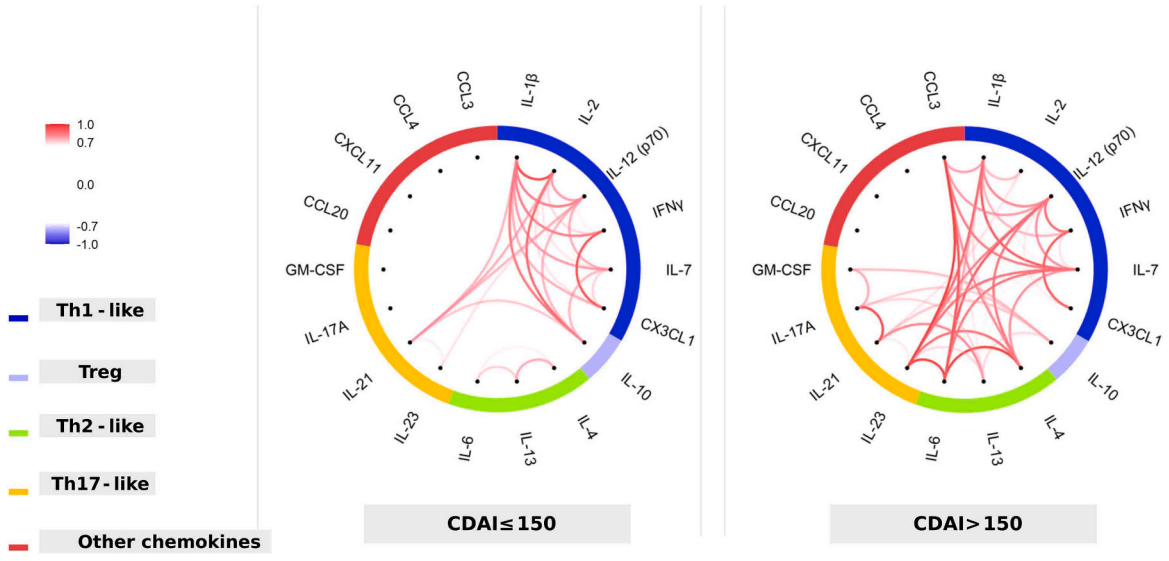


Fig. 5. Circular correlation network of serum cytokines and chemokines in patients in clinical remission (CDAI ≤ 150) and those with active disease (CDAI > 150), showing correlations greater than $\rho = 0.7$. Each node represents an individual analyte, grouped by immunological clusters as defined in Fig. 2A. The remission and active disease networks include 60 and 18 observations, respectively, pooled across the three study time points (baseline, week 14, week 54) from the 26 study participants. Line color intensity reflects the strength of the correlation. Abbreviations: CDAI, Crohn's Disease Activity Index.

between cytokines and chemokines were examined (Fig. 5). Circular diagrams display Spearman correlations ($\rho \geq 0.7$) within Th1-like, Th2-like, Th17-like, Treg-associated, and other chemokine groups during remission and active disease. Active disease showed increased co-association among Th1-like, Th2-like, and Th17-like mediators, indicating enhanced coordination within pro-inflammatory networks.

In remission, IL-10 exhibited strong positive correlations with Th1-associated cytokines. During active disease, these associations weakened and shifted toward IL-17A and IL-21, suggesting changes in regulatory dynamics consistent with increased Th17-related activity.

3.6. Discriminative performance of cytokines for Crohn's disease severity: ROC curve analysis and comparison with traditional biomarkers

We evaluated the discriminative performance of cytokines for assessing CD severity using ROC curve analysis (Fig. 6). Their accuracy was compared with that of conventional biomarkers, including CRP and fibrinogen (Fig. 6A). The AUC was calculated for each marker, and detailed performance metrics are summarized in Table 2. For each analyte or combination of analytes, the AUC with its 95% CI is reported, along with the optimal threshold, Youden's index, and the corresponding specificity and sensitivity values.

IL-2 within the Th1-like cluster (Fig. 6B) showed a discriminative performance comparable to CRP and only slightly lower than fibrinogen. Additional Th1-like cytokines were then incorporated. ROC analyses showed that combining IL-2 with other Th1-like mediators improved discrimination relative to IL-2 alone.

For Th2-associated cytokines such as IL-4 and IL-13, the AUC values were low, and adding IL-6 did not improve performance (Fig. 6C).

For Th17-associated cytokines, IL-17A yielded an AUC of 0.640 (Fig. 6D). Adding GM-CSF increased the AUC to 0.725, and the combination of IL-17A, GM-CSF, and IL-23 reached an AUC of 0.743.

The highest discriminative performance was obtained by combining IL-1β and IL-7 with GM-CSF (AUC = 0.795; Fig. 6E). CRP ($J = 0.449$) and fibrinogen ($J = 0.461$) showed only modest discriminative capacity (Table 2). In contrast, the composite IL-1β + IL-7 + GM-CSF yielded the highest Youden index (0.558), reflecting a favorable balance between sensitivity and specificity (Table 2).

4. Discussion

Identifying reliable biomarkers of disease reactivation in Crohn's disease (CD) remains a major clinical challenge, particularly in patients with quiescent–low activity, in whom conventional inflammatory markers often fail to detect early immune shifts [13]. In this study, we investigated the immunological dynamics of 26 patients with quiescent–low activity CD (median CDAI = 134.5) treated with adalimumab over 54 weeks. Clinical and biochemical parameters (CDAI, CRP, fibrinogen) significantly improved, providing a clinically stable framework in which to explore immunological associations rather than definitive biomarkers.

Studying patients with minimal symptoms may appear counterintuitive; however, this setting is particularly valuable for the exploratory identification of immune alterations that may precede clinical relapse and remain undetected by standard markers. Rather than predicting individual outcomes, this transitional phase may help characterize subtle immune fluctuations associated with impending disease activity [14].

Unsupervised clustering revealed four immunological profiles: Th1-like, Th2-like, Th17-like, and a mixed inflammatory cluster (IL-1β, IL-2, IL-12, IL-21, IL-23). This hybrid profile likely reflects overlapping inflammatory signals rather than a discrete immune phenotype, highlighting the plastic and dynamic nature of the immune environment beyond classical T-helper polarization [15].

Circular correlation analysis comparing remission–low activity and active states revealed distinct cytokine co-expression patterns. In remission, correlations suggested coordinated immune regulation, while in active disease, simultaneous activation of Th1 and Th17 pathways predominated. Regulatory cytokines such as IL-10 showed stronger coordination with Th1-associated cytokines among patients in remission, suggesting a comparatively balanced immune state, which may be disrupted during inflammatory reactivation rather than serving as a stable protective signature. These findings support the concept of context-dependent cytokine interaction modules, whose dysregulation may

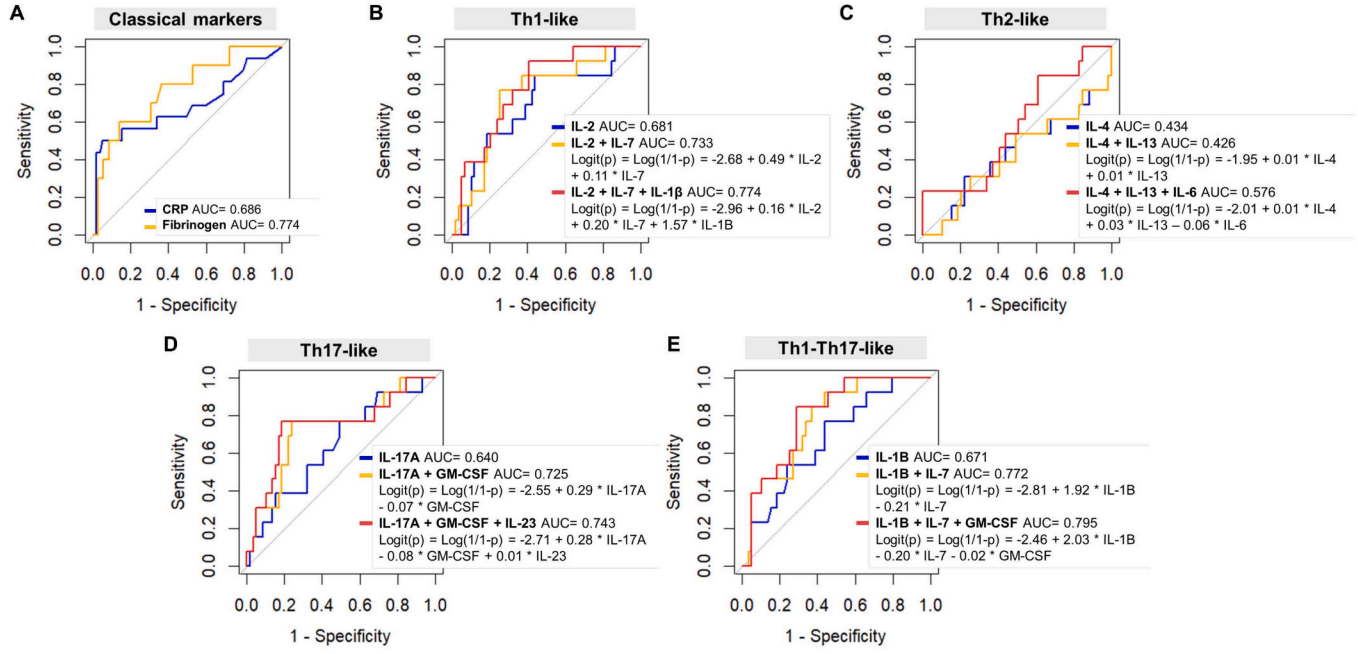


Fig. 6. ROC curves for distinguishing active disease (CDAI > 150) in the study cohort. Logistic regression models were used to combine multiple predictors within each cluster as defined in Fig. 2A. Bias-corrected AUC values are reported in parentheses for the final composite model (IL-1β + IL-7 + GM-CSF) and the classical inflammatory markers (CRP and fibrinogen). Abbreviations: ROC, Receiver Operating Characteristic; AUC, Area Under the Curve; CRP, C-Reactive Protein.

Table 2
Diagnostic performance of selected serum biomarkers for distinguishing active disease (CDAI > 150) in the study cohort. Thresholds represent optimal cutoffs based on the Youden's index. For single biomarkers, thresholds represent raw concentration values (mg/L for CRP, mg/dL for fibrinogen, pg/mL for cytokines). For multivariable logistic regression models, thresholds correspond to predicted probabilities (unitless, ranging from 0 to 1) derived from the corresponding model.

	Thr	Youden's index	AUC (95% CI)	Bias-corrected AUC (95% CI)	Se	Sp
Classical markers						
CRP (mg/L)	4.95	0.449	0.686 (0.507–0.865)	0.693 (0.436–0.878)	0.949	0.500
Fibrinogen (mg/dL)	461	0.461	0.774 (0.604–0.943)	0.773 (0.589–0.930)	0.861	0.600
Th1-like						
IL-2 (pg/mL)	4.618	0.405	0.681 (0.516–0.846)	0.680 (0.490–0.851)	0.559	0.846
IL-2 + IL-7	0.202	0.515	0.733 (0.587–0.879)	0.703 (0.558–0.839)	0.746	0.770
IL-2 + IL-7 + IL-1β	0.148	0.516	0.774 (0.651–0.898)	0.727 (0.631–0.860)	0.593	0.923
Th2-like						
IL-4 (pg/mL)	11.463	0.087	0.434 (0.237–0.631)	0.291 (0.268–0.619)	0.780	0.308
IL-4 + IL-13	0.136	0.053	0.426 (0.234–0.619)	0.338 (0.305–0.751)	0.746	0.308
IL-4 + IL-13 + IL-6	0.134	0.236	0.576 (0.402–0.750)	0.487 (0.333–0.778)	0.390	0.846
Th17-like						
IL-17A (pg/mL)	7.538	0.278	0.640 (0.471–0.808)	0.643 (0.476–0.813)	0.508	0.769
IL-17A + GM-CSF	0.202	0.532	0.725 (0.561–0.889)	0.699 (0.536–0.850)	0.763	0.769
IL-17A + GM-CSF + IL-23	0.232	0.583	0.743 (0.572–0.914)	0.690 (0.551–0.861)	0.814	0.769
Th1-Th17-like						
IL-1β (pg/mL)	1.803	0.329	0.671 (0.517–0.826)	0.673 (0.472–0.823)	0.559	0.769
IL-1β + IL-7	0.139	0.482	0.772 (0.648–0.896)	0.748 (0.632–0.864)	0.559	0.923
IL-1β + IL-7 + GM-CSF	0.182	0.558	0.795 (0.680–0.910)	0.760 (0.650–0.877)	0.712	0.846

Abbreviations: AUC, Area Under the Curve; CI, Confidence Interval; CRP, C-Reactive Protein; Thr, Threshold; Se, sensitivity; Sp, specificity.

accompany, rather than predict, disease reactivation [16,17].

Longitudinal changes in cytokine clusters were assessed using PERMANOVA. No significant differences were observed over time. However, Th1 and Th17 cytokines were significantly elevated in patients with active disease compared to those in remission, indicating a stronger cross-sectional association with inflammatory activity than with long-term therapeutic modulation. This pattern is consistent with the acute phase responsiveness and temporal variability of these mediators [18].

Despite clinical improvement with adalimumab, pro-inflammatory cytokine levels remained stable over time. This suggests that systemic cytokine suppression is not a consistent longitudinal hallmark of anti-TNF therapy, as previously described [19], particularly in patients with low baseline inflammatory burden. Instead, cytokine elevations appear episodic and flare-dependent, reflecting transient immune activation during exacerbations. Accordingly, cytokine profiling may preferentially reflect episodic inflammatory activity.

Most patients received concomitant azathioprine, and some required rescue corticosteroids; adjustment for these effects was limited by sample size and exploratory design.

ROC curve analysis was performed to distinguish between clinical remission (CDAI ≤ 150) and active (CDAI > 150) states. IL-2 showed moderate discriminative performance (AUC = 0.681), which increased when IL-7 was added to the model (AUC = 0.733), and IL-1β (AUC = 0.774), highlighting the value of exploratory combinatorial cytokine signatures over individual markers [20]. Discriminative estimates were internally stabilized using bootstrap resampling, showing close agreement with estimates derived from the original data and mitigating small-sample variability. IL 2 promotes T cell proliferation, IL 7 supports T cell survival, and IL 1β is a key mediator of innate immune activation [16,17], together reflecting convergent inflammatory processes associated with disease activity rather than a defined pre-relapse signature.

Th2-associated cytokines showed poor discriminative power. IL-4 alone yielded an AUC of 0.567, and its combination with IL-13 and IL-6 did not reach robust discriminative capacity, reinforcing the limited role of Th2 pathways in CD reactivation under anti-TNF therapy [21].

Within the Th17-like cluster, IL-17A alone showed moderate performance (AUC = 0.650), which improved with GM-CSF (AUC = 0.725) and IL-23 (AUC = 0.743) [22]. GM-CSF amplifies Th17 pathogenicity by activating myeloid cells and promoting cytokine release, sustaining inflammatory feedback loops [23]. GM-CSF-targeted therapy has demonstrated greater efficacy than IL-17 inhibition in some models of CD [24,25], suggesting it may represent a non-redundant effector within Th17-driven inflammatory contexts, such as CD.

The highest discriminative performance was achieved with the combination of IL 1β, IL 7, and GM CSF (AUC = 0.795), integrating innate immune activation, T cell homeostasis, and Th17 effector mechanisms [16,17]. Notably, IL 7—traditionally not linked to Th1/Th17 pathways—has emerging relevance in chronic immune activation [26]. CRP ($J = 0.449$) and fibrinogen ($J = 0.461$) showed modest and partially overlapping discriminative capacity, whereas the cytokine composite achieved the highest Youden index ($J = 0.558$), reflecting a comparatively more favourable balance between sensitivity and specificity.

Taken together, these findings support immune based composite signatures as complementary, exploratory tools for characterizing inflammatory activity beyond conventional markers rather than as standalone clinical biomarkers.

4.1. Clinical implications

A composite cytokine signature may complement current monitoring strategies by helping identify patients in remission–low activity who are at increased risk of inflammatory reactivation. This approach could guide more proactive stool testing, therapeutic review, or early endoscopy, and it aligns particularly well with combining cytokine panels with fecal calprotectin in future work.

4.2. Limitations

This study has several limitations. The cohort was small (n = 26), limiting statistical power. Fecal calprotectin—an essential non-invasive marker of mucosal inflammation—was not available for comparison, preventing assessment of incremental value. Endoscopic indices such as SES-CD were not collected, precluding correlation with mucosal healing. Anti-TNF pharmacokinetics (trough levels and antibodies) were not measured and may influence inflammatory profiles. TNF values could not be interpreted due to methodological interference by adalimumab [19]. Although patients were recruited across several hospitals, the absence of an external, independently recruited validation cohort still limits the generalizability of the findings.

4.3. Strengths

The study has important strengths. The 54-week prospective design

is uncommon in cytokine-centered CD research. Comprehensive profiling of 18 cytokines enabled clustering, network analyses, and multivariable discrimination. The focus on remission–low activity is clinically relevant and rarely explored, offering insight into early immune deviation. Most notably, the identification of a biologically coherent composite signature (IL-1β + IL-7 + GM-CSF) provides a mechanistically plausible biomarker candidate for future comparison with fecal calprotectin.

4.4. Future directions

Future studies should directly compare cytokine signatures with fecal calprotectin, integrate endoscopic activity measures, evaluate anti-TNF pharmacokinetics, validate the biomarker model in larger multicenter cohorts, and apply reclassification methods to quantify clinical utility.

5. Conclusions

This study provides preliminary, hypothesis-generating evidence that multidimensional cytokine signatures may contribute to exploratory risk stratification in Crohn's disease. Among these, the IL-1β + IL-7 + GM-CSF composite showed the highest discriminative capacity within the present cohort; however, it should be regarded as an exploratory signature rather than a validated biomarker, requiring prospective validation. Further evaluation against established markers such as fecal calprotectin and mucosal outcomes, particularly in patients with remission–low disease activity, will be necessary to clarify its potential clinical relevance.

Authorship contribution statement

P.C.M: Conceptualization; Methodology; Investigation; Formal analysis; Data curation; Visualization, Writing – original draft. **L.G.G**: Conceptualization; Supervision; Project administration; Coordination of the multicenter project; Validation; Data interpretation; Writing – review and editing. **J.M**: Methodology; Data curation; Formal analysis; Visualization. **M.C**: Resources; Clinical investigation; Data acquisition. **I.G**: Resources; Clinical data acquisition; Investigation. **M.I**: Resources; Clinical investigation; Data acquisition. **J.L.C**: Resources; Clinical investigation; Data acquisition. **L.B**: Resources; Clinical investigation; Data acquisition. **C.A**: Resources; Clinical investigation; Data acquisition. **V.G.S**: Resources; Clinical investigation; Data acquisition. **I.M.J**: Resources; Clinical investigation; Data acquisition. **M.B.A**: Resources; Clinical investigation; Data acquisition. **I.V**: Resources; Clinical investigation; Data acquisition. **M.D.M.A**: Resources; Clinical investigation; Data acquisition. **F.M**: Resources; Clinical investigation; Data acquisition. **L.S**: Resources; Clinical investigation; Data acquisition. **F.G**: Resources; Clinical investigation; Data acquisition. **J.H**: Resources; Clinical investigation; Data acquisition. **A.G.L**: Data curation; Investigation; Methodological support. **C.G.M**: Formal analysis; visualization; technical support. **O.F.M**: Formal analysis; visualization; technical support. **D.L.B**: Formal analysis; visualization; technical support. **M.A.M**: Supervision; Conceptual support. **M.A.O**: Methodology; Visualization; Critical manuscript review. **J.P.G**: Conceptualization; Resources; Supervision; Coordination of the multicenter project; Data acquisition; Data interpretation; Critical manuscript review.

Consent for publication

The study does not include individual patient data, images, or any other personal identifiable material. Therefore, specific consent for publication was not required. All participants provided written informed consent to participate in the study.

Ethics approval and consent to participate

This study was conducted in full accordance with the Declaration of Helsinki (1975 and later amendments). Ethical approval was obtained from the Institutional Ethics Committees of all participating hospitals under the PREDICROHN initiative (Approval code: GET-CRO-2010-01; Approval date: 23 September 2010). All patients provided written informed consent prior to inclusion in the study.

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Declaration of Competing Interest

M. Chaparro has served as a speaker or received research funding from MSD, AbbVie, Takeda, and Shire Pharmaceuticals.

I. Guerra has served as a consultant for Kern Pharma and as a speaker for Takeda.

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M.D. Martín-Arranz has served as a speaker for MSD, AbbVie, Takeda, Shire Pharmaceuticals, Faes, and Ferring; and as an advisory board member for MSD and AbbVie.

L. Sempere has served as a speaker for MSD, AbbVie, and Otsuka.

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Martínez, D. L. Boaru, M. Álvarez-Mon and M. A. Ortega declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request and subject to institutional and ethical restrictions.

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