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Original Article

Blood proteomic signatures associated with disease activity in inflammatory bowel diseases

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Abstract

Background and Aims: Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), remain heterogeneous disorders with variable response to biologics. Post-operative recurrence in CD is common despite surgery and prophylactic biotherapies. Understanding the inflammatory mediators associated with recurrence and treatment response could pave the way for personalized strategies.

Methods: We analyzed serum inflammatory protein signatures using proteomics in two prospective cohorts. The REMIND cohort included post-operative CD patients undergoing ileocecal resection with endoscopic assessment at 6 months (M6). Serum samples were collected at surgery and 6 months later. The ELYP cohort consisted of active IBD patients starting new biotherapies (anti-tumor necrosis factor [anti-TNF], ustekinumab, or vedolizumab). Serum samples were collected pre- and post-treatment (Weeks 14 and 52).

Results: In the REMIND cohort, proteomic analysis revealed elevated levels of IFN-γ, CXCL9, and MMP-10 in patients with recurrence, with concentrations associated with recurrence severity. Preoperative MMP-10 levels predicted severe recurrence (AUC = 0.70). Under biotherapies, treatment-specific proteins were associated with recurrence: CXCL9 for anti-TNF and OSM/TGFα modules for ustekinumab. In the ELYP cohort, IFN-γ and CXCL9 were significantly elevated in CD compared to UC and associated with disease activity. Early response to anti-TNF treatment (Week 14) was associated with reductions in CXCL9, MMP-10, and OSM, while deep remission (Week 52) correlated with decreases in CXCL9 and OSM.

Conclusion: Our findings reveal inflammatory blood proteomic signatures associated with post-operative recurrence and biologic treatment failure in IBD. Several key biomarkers were identified. These results support the rationale for personalized approaches, including combination therapies targeting multiple pathways.

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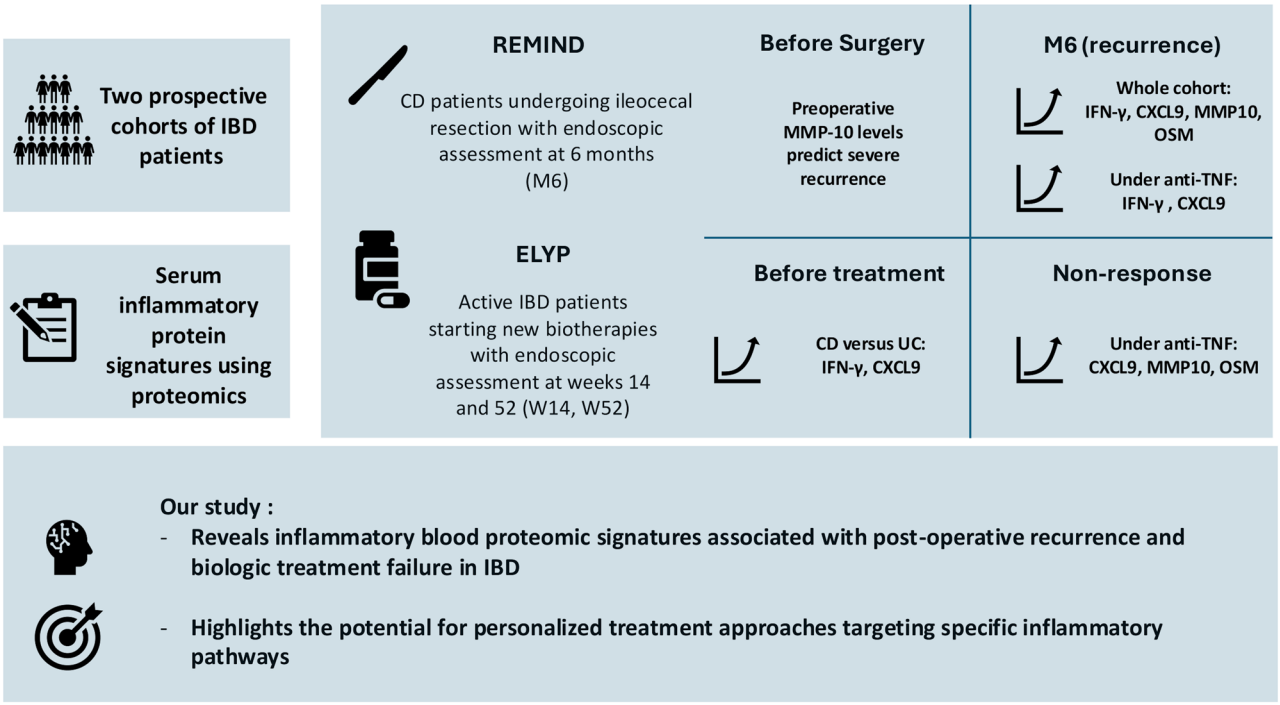
Key words: inflammatory bowel diseases; proteomics; biomarkers.

Graphical Abstract



1. Introduction

The treatment of inflammatory bowel diseases (IBD) has greatly evolved in the last 30 years with the use of biologics.[1,2] Monoclonal antibodies against tumor necrosis factor alpha (TNF) have been used to successfully treat Crohn's disease (CD) and ulcerative colitis (UC) patients. However, only 30% of patients experience a long-lasting remission under this treatment.[3,4] In many cases, the reasons behind treatment failure are poorly understood. In the ABIRISK study, immunosuppressants and antibiotics were associated with decreased risk of immunogenicity, whereas tobacco smoking and infections during the study were associated with increased risk.[5]

Monoclonal antibodies targeting the p40 subunit common to interleukin (IL)12 and IL23 have shown efficacy in both CD and UC.[6,7] In addition, the anti-integrin antibodies targeting alpha4beta7 (a4b7) have also been approved for the treatment of IBD.[8,9] Unfortunately, these treatments show a similar rate of treatment failures as compared with anti-TNF.

Despite improvements in the medical management of CD, surgery remains highly probable during the long-term evolution of CD.[10–12] Furthermore, intestinal resection is not curative as the disease recurs in many patients. The mechanisms underlying this recurrence are heterogeneous and remain partially understood. We recently demonstrated, through a transcriptomic study, that the upregulation of certain pathways, such as JAK–STAT, is associated with post-operative recurrence.[13]

Given the heterogeneity observed in IBD and to avoid over- or undertreatment, personalized treatment strategies tailored to the specific inflammatory pathways involved may enhance treatment response rates. Recent studies have highlighted associations between the expression of specific cytokines and responses to anti-TNF.[14–16] To go further, there is a need for prospective cohort studies.

The REMIND-POP cohort is a prospective cohort of patients undergoing surgery for the treatment of ileal CD. Clinical parameters have been associated with post-operative recurrence, such as previous surgery and smoking. Therefore, some patients receive prophylactic biologic treatments after surgery.[17]

The ELYP cohort is a prospective cohort including active IBD patients starting a new biologic treatment,[18] aimed at deciphering clinical and biological parameters associated with treatment outcomes.

Understanding the inflammatory mediators associated with disease activity, post-operative recurrence, and treatment response could pave the way for personalized strategies. Proteomic analysis is an interesting way to assess the pathways involved.

In this study, we evaluated changes in serum inflammatory mediators of patients in two different clinical situations with the aim to identify blood proteomic signatures associated with disease activity, treatment responses, and potential predictive markers of treatment efficacy. We show that patients exhibiting disease activity at inclusion, recurrence after surgery, and non-reponse to biologic therapies show an elevated inflammatory signal in the serum mainly driven by IFN-γ and CXCL9. Components of the inflammatory protein signature in the blood are different between patients under anti-TNF compared to anti-IL12/23.

2. Patients and methods

2.1. Cohort selection

Blood samples were collected from patients included in two different cohorts.

The REMIND study is a prospective study performed in 14 centers of the REMIND group. All patients who had surgery

for ileal CD were eligible. Inclusion criteria were adult patients (age ≥18 years), a confirmed diagnosis of CD, with ileal involvement, and an indication of CD-related intestinal surgery (ileocolonic resection). Patients in whom surgery consisted of ileal resection with ileal–ileal anastomosis leaving an intact ileocecal valve, a sub-total or near sub-total colonic resection were excluded. Patients who had a suspicion of dysplasia or cancer were also excluded. The REMIND postoperative cohort is associated with an extensive biobanking at time of surgery and endoscopy. Blood samples for proteomic analyses were taken before surgery and endoscopy. The study was approved by Agence Française de Sécurité Sanitaire et des Produits de Santé (AFSSAPS) (IDRCB: 2009-A00205-52) and the French Ethic Committee—Hôpital Saint-Louis (CPP 2009/17) and registered on ClinicalTrials.gov (NCT03458195).

The ELYP cohort is a prospective single-center study including patients with IBD who have started a biotherapy for active disease. Blood samples were taken before treatment initiation (Week 0: W0), and over time at W14 and W52. The ELYP cohort study was approved by the French Ethic Committee—Hôpital Saint-Louis (CPP 2016-01-05 RBM) and registered on ClinicalTrials.gov (NCT02693340).

2.2. Data collection

In both cohorts, demographic and clinical data, as well as past medical history, were recorded at inclusion, before surgery or treatment initiation. Demographic and clinical data included: age at diagnosis, gender, smoking status (never-smoker, ex-smoker, active smoker), disease duration between diagnosis and index surgery, prior intestinal resection, disease phenotype and behavior (cumulative assessment from diagnosis to surgery) according to the Montréal classification, surgical indication, presence of granuloma on endoscopic biopsies and on surgical specimens (as indicated in the pathological reports) from diagnosis to surgery or treatment initiation, body weight and body mass index (BMI), and perianal disease (past history or active). In the REMIND cohort, prophylaxis given after surgery, name, and route and date of the first dose were registered at the follow-up visit after surgery. Smoking status and Harvey–Bradshaw index were collected at the time of postoperative endoscopy.

2.3. Outcomes

In the REMIND cohort, a colonoscopy was proposed within 1 year, ideally 6 months after surgery. In case of a temporary stoma, endoscopy was performed 6 months after stoma closure. Patients who had a colonoscopy beyond 1 year were excluded from the analysis. Detailed endoscopic lesions were reported in all segments, including the anastomotic region and the neo-terminal ileum, and registered in the electronic case report form (eCRF). The modified Rutgeerts score was evaluated for each patient by the endoscopist, with the following grades: i0 (no lesions—normal); i1 [≤5 aphthous lesions]; i2a (ulcerated lesions confined to the anastomosis); i2b (>5 aphthous lesions with normal intervening mucosa or skip areas between larger lesions on the neoterminal ileum); i3 (diffuse aphthous ileitis with diffusely inflamed mucosa); or i4 (diffuse inflammation with severe ulceration, nodules, or stricture). All scores were reviewed by two endoscopic experts, and compared to the description of elementary lesions. Endoscopic recurrence was defined as a Rutgeerts score ≥i2b. Severe endoscopic recurrence was defined as a Rutgeerts score ≥i3.

For ELYP patients, subsequent endoscopies were performed at W14 and W52 after biotherapy initiation. The Crohn's Disease Endoscopic Index Score (CDEIS) and the Ulcerative Colitis Endoscopic Index Score (UCEIS) were respectively assessed at both endpoints for CD and UC patients. For CD, response was defined by a 50% decrease of the CDEIS score and remission by a CDEIS score <3. For UC, response was defined by a 2-point decrease of the UCEIS and remission by a UCEIS equal to 0. Primary outcomes used in this study were endoscopic response at W14 and endoscopic remission at W52.

2.4. Protein quantification and quality controls

Proteins were measured using an Olink® target 92 inflammation panel (ie, 92 proteins tested). The same panel was used for all analyses and samples were processed at the same timepoint. The technology used on the array was a proximity extension assay (PEA), in which pairs of oligonucleotide-labeled antibodies bind to target proteins. Upon dual binding, the oligos hybridize and are amplified by quantitative PCR, allowing highly sensitive and specific multiplex detection of 92 inflammation-related proteins from minimal serum volume. Briefly, a tube of blood without anticoagulant (dry tube) was collected. After centrifugation (3000 *g* for 10 min at room temperature), serum was collected within 2 h of collection, and stored at −80 °C without delay at the "Centre de Ressources Biologiques Biobank Lariboisière (BB-0033-00064), Hôpital Lariboisière, Paris, France." A serum volume of 40 µL was plated into 96-well qPCR full skirted randomized plates. Paired samples from a given individual were placed on the same plate. Samples not passing internal QC were removed from the analysis. Assays having less than 30% of the samples with a Normalized Protein Expression (NPX) equal to or above the detection limit (assay specific) were removed, leaving 72 proteins to analyze. Subsequent analyses were made on the subset of proteins varying the most in the studied sample set, defined as assays with a standard deviation above or equal to $\log_2(1.5)$.

2.5. Statistical analysis

All statistical analysis were performed in R [4.4.1].

2.5.1. Differential analysis

Differentially expressed protein analyses were performed by regressing a logistic model, containing the tested variable in addition to clinical features with an uneven distribution between the two tested groups (Student's t test for continuous variables and χ^2 test for categorical variables, with limma [3.50.3]). Clinical variables were included in the models to account for potential confounding and to improve the accuracy of the protein-level associations. *P*-values were calculated by an empirical Bayes moderated t-statistics test and adjusted with the Benjamini–Hochberg procedure. Two-way ANOVA and post-hoc Tukey's honest significant difference tests for multiple comparisons were performed, with rstatix [0.7.2], to identify differentially expressed proteins when more than two groups of patients were studied. Before applying this test, variance equality was verified with a Leneve test (car [3.1-3]), and data normality with a Wilcoxon test.

2.5.2. Classification

A logistic regression model was fitted to the data for classification purposes. Features, clinical criteria, or proteins were

selected by applying a stepwise procedure using the Akaike Information Criterion as the decisive metric (MASS [7.3-61]). To evaluate the model performance, 10-fold cross-validation was applied. The absence of multi-collinearity was verified using the variance inflation factor. Area under the curves (AUCs) were estimated with a 95% confidence with 200 bootstrap samples using ROCit [2.1.2]. To test gain of performance between two models, the AUCs were compared using DeLong's test (pROC [1.18.5]).

2.5.3. Modules of correlated proteins

For each group of treatment (anti-TNF, anti-IL12, and no treatment), correlation between each pair of proteins was calculated using a two-sided Pearson correlation test with a 95% CI. To create modules, assays were clustered by applying the Louvain algorithm, with igraph [2.1.1], on the pairs of proteins for which the correlation coefficient was ≥0.7, and the *P*-value adjusted with the Benjamini–Hochberg procedure was ≤.05. The correlation score was used as weight. For each patient, considering a module m composed of the ensemble of assays $N_m=\{1 \dots n\}$, and μ_n the mean abundance of assay n in patients with no recurrence (I_0) who received the same treatment, a score S_m was calculated using the following formula:

$$S_m = \frac{\sum_{n=1}^{N_m} \frac{NPX_n}{\mu_n}}{N_m}$$

Differential analysis was performed by applying a Wilcoxon test after verifying the non-normality of the module score distributions with a Shapiro test.

3. Results

3.1. REMIND cohort dataset and quality control

At least one serum sample was available for 416 patients: 231 at both inclusion (M0) and 6 months after surgery (M6), 164 at M0 only, and 21 at M6 only. We hence had 395 serum samples at M0 and 252 at M6. Seven samples at M0 and two at M6 did not pass OLink quality controls. Clinical data were available at M0 for 304 patients (Table S1). At M6, clinical data were not available for 12 patients leaving 238 samples to analyze at this timepoint.

3.2. Inflammation-related proteins are increased in the serum of patients and are associated with endoscopic recurrence

We investigated the correlation between cytokine serum levels at 6 months after surgery (M6) and endoscopic recurrence. Serum proteomic analysis was available for 238 patients 6 months after surgery. Among these, 117 patients (49%) did not receive postoperative prophylaxis, while 61 (26%) were treated with anti-TNF therapy and 20 (8%) with ustekinumab. Serum analysis revealed that TNF levels were significantly elevated in patients treated with anti-TNF after surgery as compared to patients who did not receive the drug, showing a positive correlation with serum drug levels. Similarly, IL12b levels were significantly increased in patients treated with ustekinumab (Figure 1) as compared to patients who did not receive the drug. Results were similar in the ELYP cohort, probably associated with the drug's mechanism of action. These findings may also reflect the pharmacokinetic and immunological print of therapeutic binding and sequestration, rather than active inflammatory signaling. Hence, to avoid treatment-related bias, TNF and IL12b were excluded from subsequent proteomic analyses.

Endoscopic recurrence was observed in 101 patients (42%) (45 patients on postoperative prophylaxis [19 anti-TNF, 14 immunosuppressors, 10 ustekinumab and 2 vedolizumab]) (Figure 2A). Several proteins, including IFN-γ (Fold Change (FC) = 1.66, False Discovery Rate (FDR) = 3.69E-4), CXCL9 (FC = 1.42, FDR = 2.66E-3), OSM (oncostatin M) (FC = 1.36, FDR = 8.03E-3), and MMP-10 (FC = 1.24, FDR = 8.03E-3), were significantly elevated at M6 in patients with endoscopic recurrence (Table S2A). Notably, these proteins exhibited a concentration gradient, increasing with the severity of recurrence. (Figure 2B). Patients with severe recurrence demonstrated an additional 13 differentially expressed proteins as compared with those with mild or moderate recurrence (Table S2B).

A classifier combining these proteins (ie, CXCL9, IFN-γ, HGF, MMP-10, IL6, OSM, and TGFα) to clinical criteria (AUC = 0.81) significantly outperformed a model based on clinical features (ie, sex, smoking, previous resection, post-operative treatment, perineal manifestations, and extraintestinal manifestations) only (AUC = 0.70) in predicting recurrence (*P* = 3.83E-4). However, the gain of accuracy observed when comparing the models containing one or the other type of features was not significant (*P* = 0.27). A simplified model using only IFN-γ, HGF, and MMP-10 achieved comparable accuracy (AUC = 0.75, improving to 0.81 with clinical factors [Figure 2C and E]). Hence, according to this model, 74% of patients were correctly classified (Figure 2E). Moreover, the combination of these three proteins to clinical factors remained significantly better than the clinical classifier (*P* = 4.14E-3). The best correlation model between serum proteins and severe endoscopic recurrence at M6 was obtained with IFN-γ, MMP-10, HGF, CXCL9, CXCL6, and CXCL11. The predicted value of these proteins (AUC = 0.80) was significantly superior to the one of clinical criteria (AUC = 0.63), combined (*P* = 6.38E-4) or not (*P* = 1.85E-3) to clinical factors (Figure 2D and F), reflecting that serum proteomics was better associated with endoscopic recurrence than clinical factors alone. According to this model, 71% of patients were correctly classified (Figure 2F).

3.3. Specific biomarkers are associated with recurrence under anti-TNF or ustekinumab

We assessed whether several biomarkers were associated with post-operative treatment and recurrence at M6 depending on the post-operative treatment strategy. Four proteins (or group(s) of correlated proteins referred as module(s)) were significantly over-abundant in the serum of patients with endoscopic recurrence who received a biologic after surgery (Figure 3). Regardless of the treatment group, IFN-γ was consistently higher in patients with recurrence as compared to patients without. CXCL9 was significantly increased in anti-TNF-treated patients with recurrence (FC = 0.49, FDR = 2.98E-2) but not ustekinumab as compared to patients without significant endoscopic lesions. ST1A1 (FC = 0.36, FDR = 2.98E-2) and the module OSM/TGFα/TNFSF14/HGF/EN-RAGE (FC = 0.6, FDR = 2.98E-2) were over-abundant in patients with recurrence who received ustekinumab (Figure 3A and B). CXCL9, IFN-γ,

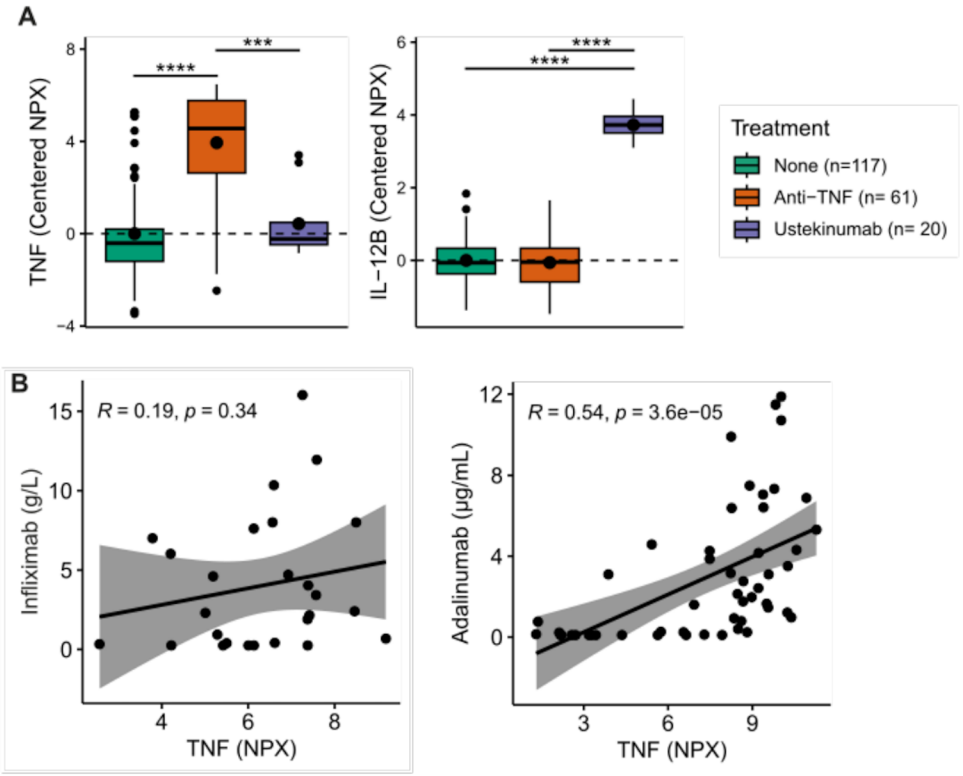


Figure 1. TNF is increased in the serum of patients receiving anti-TNF treatment, while IL12b is increased in the serum of patients receiving ustekinumab. TNF and IL12B levels were measured by multiplex immunoassay in the serum of CD patients 6 months after surgery. (A) Protein relative abundance (NPX) is centered on its mean value in patients who did not receive treatment post-surgery. *P*-values were adjusted with the Benjamini–Hochberg procedure (FDR); ****FDR < 0.0001 and ***FDR < 0.001. (B) Infliximab and adalimumab drug levels were measured in the serum of CD patients who received an anti-TNF therapy in prevention of post-operative recurrence. Pearson's correlation coefficient (*R*) and two-tailed *P*-value are presented on top of the plots

and the module OSM/TGFα/TNFSF14/HGF/EN-RAGE were also overabundant in patients with recurrence without post-operative treatment, reflecting the broad activation of inflammatory pathways that may lead to a global upregulation of multiple cytokines.

3.4. Preoperative MMP-10 levels predict severe recurrence

We assessed the predictive value of serum proteomics at the time of surgery (ie, M0) for severe recurrence at M6. MMP-10 levels were significantly elevated in patients who later developed severe post-operative recurrence as compared to patients who did not. (Figure 4A). A model including serum MMP-10 level at surgery and clinical factors (smoking habits and previous resection) better predicted severe post-operative recurrence as compared to each of these criteria taken alone (AUC 0.70 [0.63-0.76]) with a strong negative predictive value (0.86) (Figure 4B).

3.5. ELYP cohort dataset and quality control

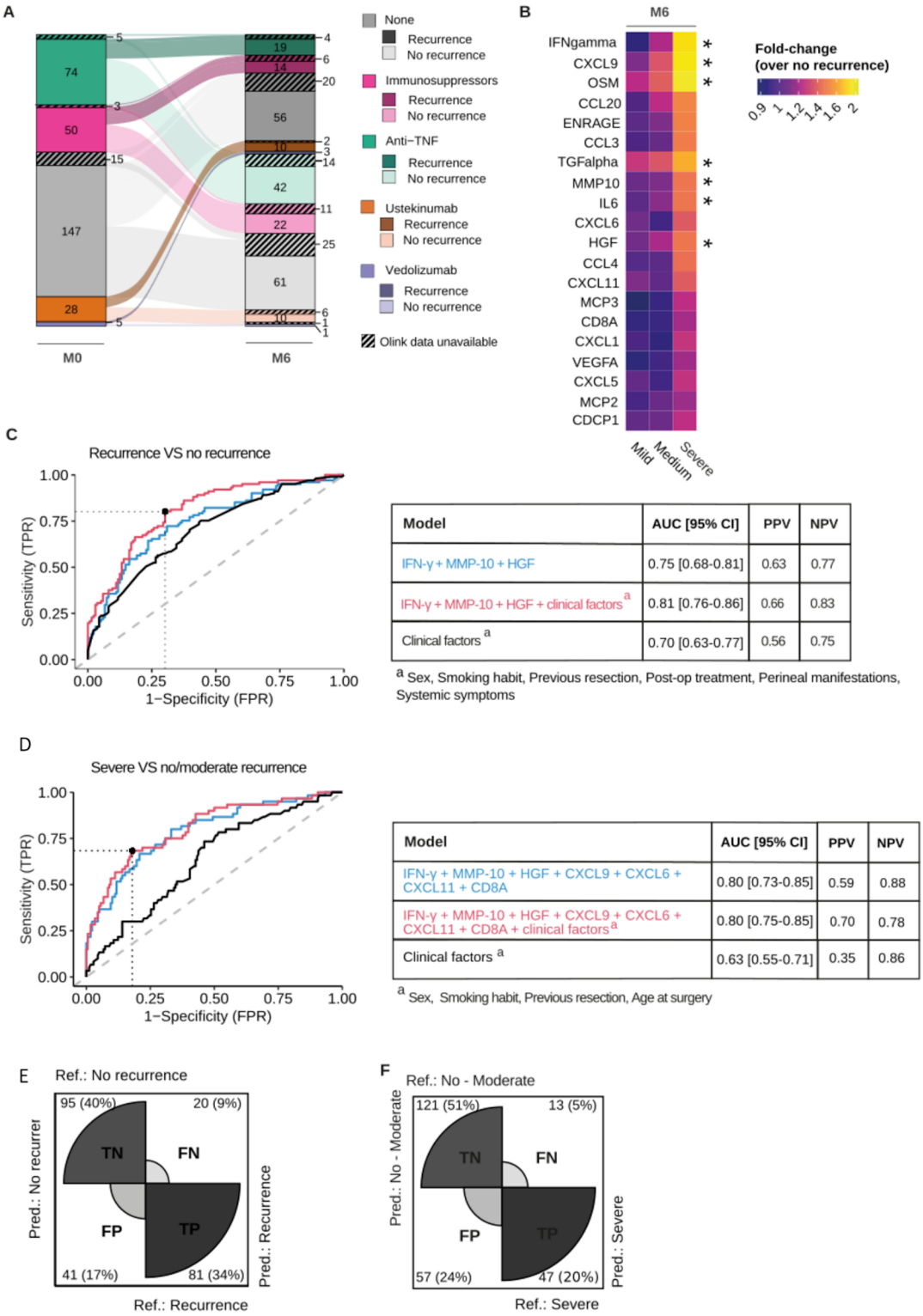
At least one serum sample was available for 96 patients: 95 at inclusion (W0), and 84 and 65 respectively at W14 and W52 after inclusion. After quality controls, 92 samples were remaining at W0, 81 at W14, and 64 at W52. Seventy-nine patients had samples for both W0 and W14 timepoints, 63 at both W0 and W52 timepoints, and 10 at W0 only. Clinical data were available for 92 patients (Table S3).

3.6. Distinct proteomic signatures are correlated with disease type and severity

From the ELYP cohort, a model of active IBD patients receiving biotherapies, we evaluated the relationship between active disease and serum proteomics. Serum samples for 41 patients with CD, 45 patients with UC, and six patients with pouchitis were available. Fifty-three received an anti-TNF, 21 ustekinumab, and 18 vedolizumab. Data on response to biotherapy were available (Figure 5A). Several proteins were significantly differentially expressed in patients with CD as compared to UC such as CXCL9, IFN-γ and ST1A1 (Figure 5B). Interestingly, these three proteins were also associated with post-operative recurrence in ileal CD. We then assessed whether proteins were specifically associated with ileal-dominant CD and the only protein found to be significantly different in abundance as compared to UC or colonic-dominant CD was FGF-19, which was reduced in patients with ileal CD (Figure 5C). Six proteins were finally found to be overabundant in patients with a high level of C-reactive protein (CRP) (OSM, HGF, IFN-γ, TNFS14, CXCL10, and IL6) (Figure S1).

3.7. CXCL9 and OSM are associated with early endoscopic response and deep remission in patients treated with anti-TNF at W14 and W52

We finally evaluated the association between serum proteomics and treatment response at W14 and remission at W52. Among the 53 patients treated with anti-TNF, whatever the disease



Model	AUC [95% CI]	PPV	NPV
IFN-γ + MMP-10 + HGF	0.75 [0.68-0.81]	0.63	0.77
IFN-γ + MMP-10 + HGF + clinical factors [a]	0.81 [0.76-0.86]	0.66	0.83
Clinical factors [a]	0.70 [0.63-0.77]	0.56	0.75

[a] Sex, Smoking habit, Previous resection, Post-op treatment, Perineal manifestations, Systemic symptoms

Model	AUC [95% CI]	PPV	NPV
IFN-γ + MMP-10 + HGF + CXCL9 + CXCL6 + CXCL11 + CD8A	0.80 [0.73-0.85]	0.59	0.88
IFN-γ + MMP-10 + HGF + CXCL9 + CXCL6 + CXCL11 + CD8A + clinical factors [a]	0.80 [0.75-0.85]	0.70	0.78
Clinical factors [a]	0.63 [0.55-0.71]	0.35	0.86

[a] Sex, Smoking habit, Previous resection, Age at surgery

Figure 2. Association between inflammation-related proteins in the serum and post-operative endoscopic recurrence. (A) Alluvial diagram describing the number of patients per treatment and recurrence status at M0 and M6. (B) Over-abundant proteins (FDR < 0.05) in the serum of CD patients with moderate (I2b) and severe endoscopic recurrence (I3 or I4) are marked by an asterisk. The remaining proteins are over-abundant (FDR < 0.05) in patients with severe endoscopic recurrence only. The heatmap represents the fold-change of abundance of CD patients with mild (I1 or I2a), moderate (I2b), or severe (I3 or I4) recurrence as compared to patients with no recurrence (I0). (C and E) ROC curves for models identifying patients in moderate to severe endoscopic (C) or severe recurrence (E) from others. Three groups of models were tested: including clinical variables only (black), including proteins only (blue), and including proteins and clinical variables (red). The dot represents the optimal cutting-point (Youden index) for the best model. (D and F) Four-fold plot summarizing the total number of TP (bottom right), TN (upper left), FP (bottom left), and FN (upper right) obtained from (C) and (E) respectively. NPV, negative predictive value; PPV, positive predictive value; TP, true positive; TN, true negative; FP, false positive; FN, false negative. *P*-values were calculated by an empirical Bayes moderated t-statistics test and adjusted with the Benjamini—Hochberg procedure (FDR). Extraintestinal manifestations in the joint(s), eye(s), and/or skin were grouped under the term "systemic symptoms."

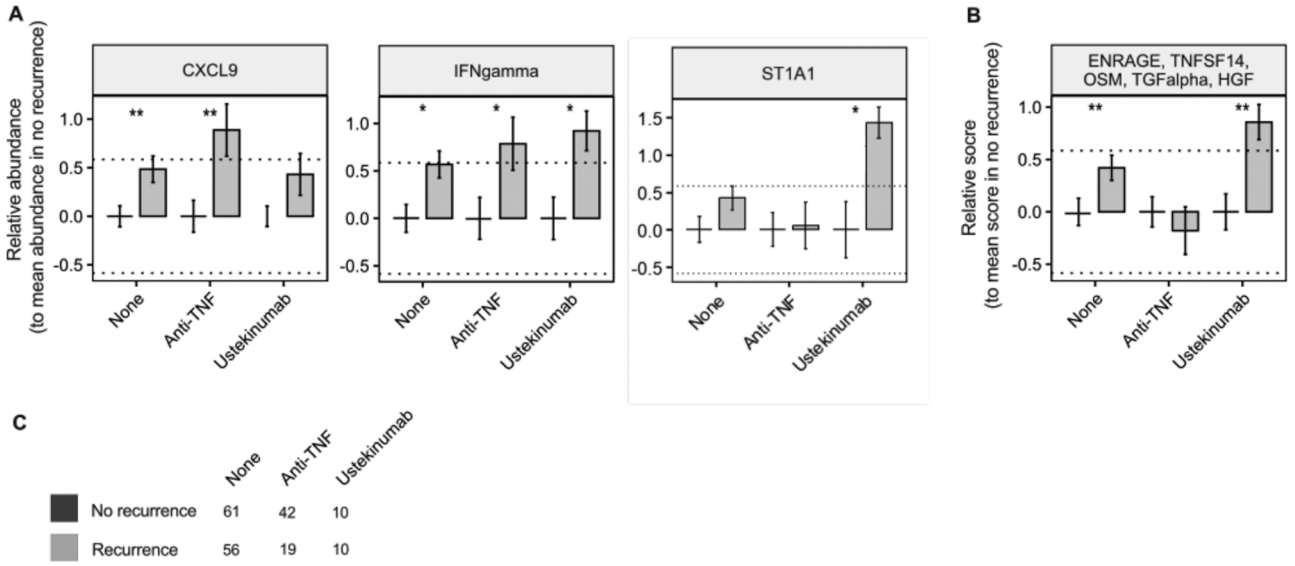


Figure 3. Proteins varying under anti-TNF or ustekinumab treatments. (A) Protein abundance, grouped by treatment and recurrence status, divided by the mean abundance in the no/mild recurrence group. (B) Mean relative abundance of correlated proteins by treatment and recurrence status. In (A) and (B), the relative abundance is the ratio between the abundance (expressed in NPX) in the patient serum and the mean abundance in the group of patients experiencing no, or mild, recurrence. (C) Table recapitulating the number of patients in each studied group. In (A) and (B), the vertical line represents the mean abundance standard error. FDR-adjusted *P*-values were calculated by the Wilcoxon ranksum test and adjusted with the Benjamini–Hochberg procedure (FDR); *FDR < 0.05.

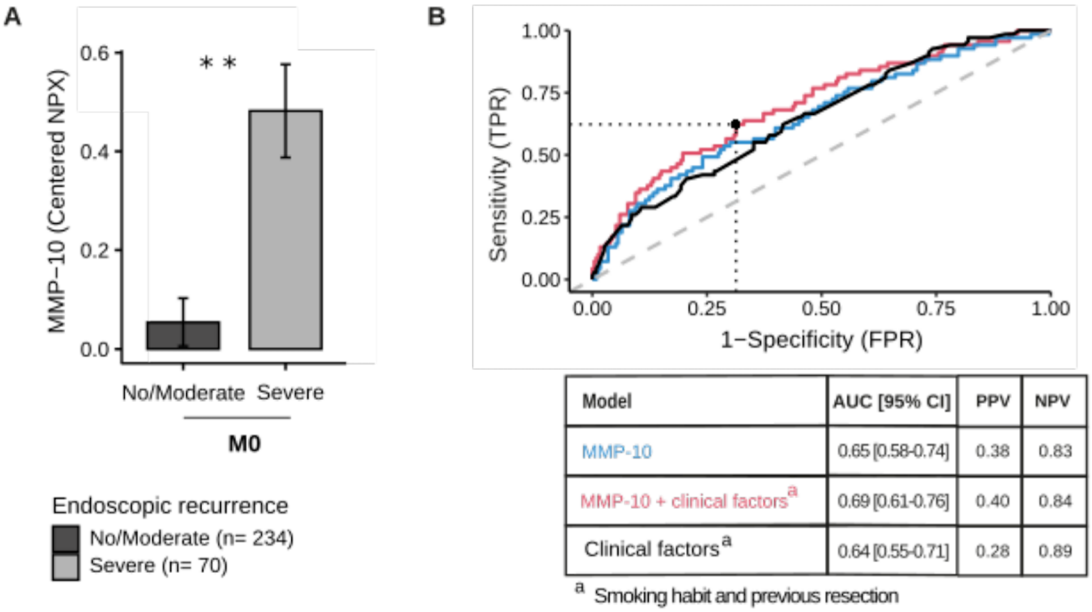


Figure 4. Increased MMP-10 levels in the serum at time of surgery predicts severe endoscopic recurrence 6 months after surgery. (A) Comparison of mean abundance of MMP-10 at time of surgery in patients who developed no/mild and severe post-operative endoscopic recurrence. (B) ROC curves of the models including clinical variables only (black), MMP-10 only (blue), and MMP-10 and clinical variables (red). The dot represents the optimal cutting-point (Youden index) for the best model (MMP-10 + clinical factors). *P*-values were calculated by an empirical Bayes moderated t-statistics test and adjusted with the Benjamini–Hochberg procedure (FDR); **FDR < 0.01. NPV: negative predictive value; PPV: positive predictive value.

considered, clinical data and serum samples were available for 45 at W14 and for 36 at W52. Decreases in CXCL20, CXCL9, HGF, MMP-10, and OSM were significantly associated with anti-TNF response at W14 (Figure 6A and B). Decreases in 12 proteins including CCL4, CXCL9, IL6, IL8, OSM, and TGF-alpha were significantly associated with remission under anti-TNF treatment at W52 (Figure 6C and D). Such associations were not found with ustekinumab probably due to a lack of power (Figure S2). Hence, analyzing data from the REMIND and ELYP cohorts, CXCL9 and MMP-10 were the two cytokines most strongly associated with disease activity and response to treatment.

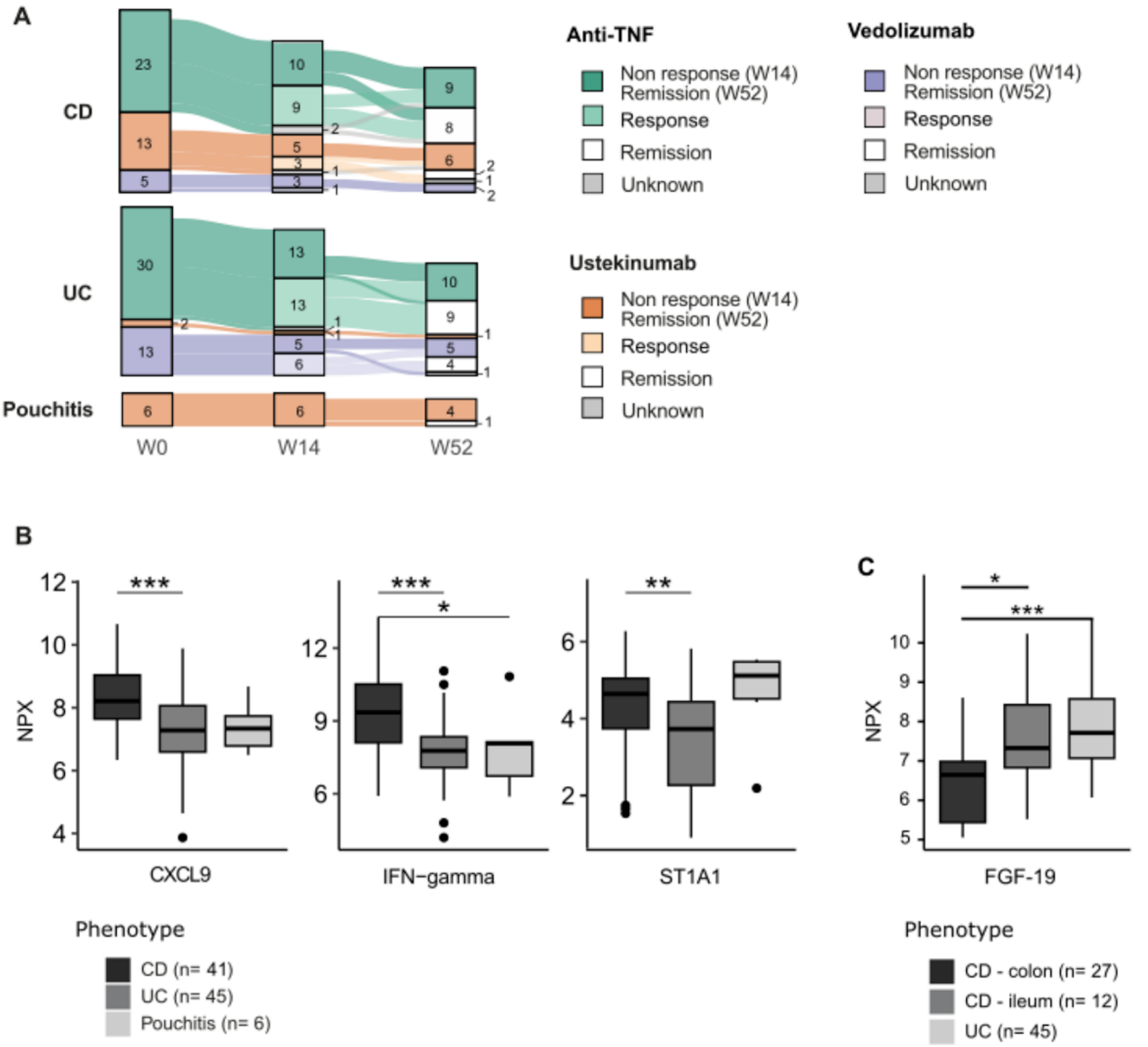


Figure 5. Description of the ELYP cohort and active IBD patients at W0. (A) Alluvial diagram describing the number of patients per treatment and status at W14 (response) and W52 (remission). (B and C) Boxplots representing the abundance (in NPX) of proteins significantly different between CD, UC, and/or pouchitis patients (B), UC or CD affecting the colon, and CD affecting the ileum (C). *P*-values were calculated by Tukey post-hoc tests (B and C), and adjusted with the Benjamini–Hochberg procedure (FDR); *FDR < 0.05, **FDR < 0.01, and ***FDR < 0.001.

4. Discussion

In this study, we demonstrated that specific inflammatory protein signatures were associated with post-operative recurrence in CD and response to biologic treatments in IBD. Using proteomic analyses from two well-characterized cohorts, REMIND-POP (post-operative CD patients) and ELYP (active IBD patients starting biologics), we identified IFN-γ, CXCL9, MMP-10, and OSM as key biomarkers of disease recurrence and response to therapy. Notably, we found consistent results in both cohorts with cytokines correlated with disease activity in patients in different clinical situations.

Our findings add to recent studies highlighting the role of IFN-γ and CXCL9 in intestinal inflammation. IFN-γ, a pro-inflammatory cytokine, is a central driver of Th1-mediated immune responses, which are well-established contributors to CD pathophysiology.[19,20] CXCL9, a chemokine induced by IFN-γ, plays a pivotal role in T-cell recruitment to inflamed tissues.[21,22] In a recent study, the GEM Consortium aimed to assess associations of serum proteins with future CD onset and with other biomarkers predicting CD risk in a healthy at-risk cohort. CXCL9 had the highest odds ratio (OR) with future risk of CD showing the importance of this pathway in the initiation of inflammation.[20] Our results also corroborate earlier work that associated elevated levels of CXCL9 and its ligand CXCR3 with post-operative recurrence in CD.[23]

Interestingly, our study also highlights the relevance of MMP-10 as a predictor of severe post-operative recurrence. Elevated pre-operative MMP-10 levels were strongly associated with endoscopic recurrence at 6 months, suggesting its potential utility as a predictive biomarker. Interestingly, in the ELYP cohort, MMP10 was also associated with anti-TNF response at W14. This metalloproteinase is involved in tissue remodeling

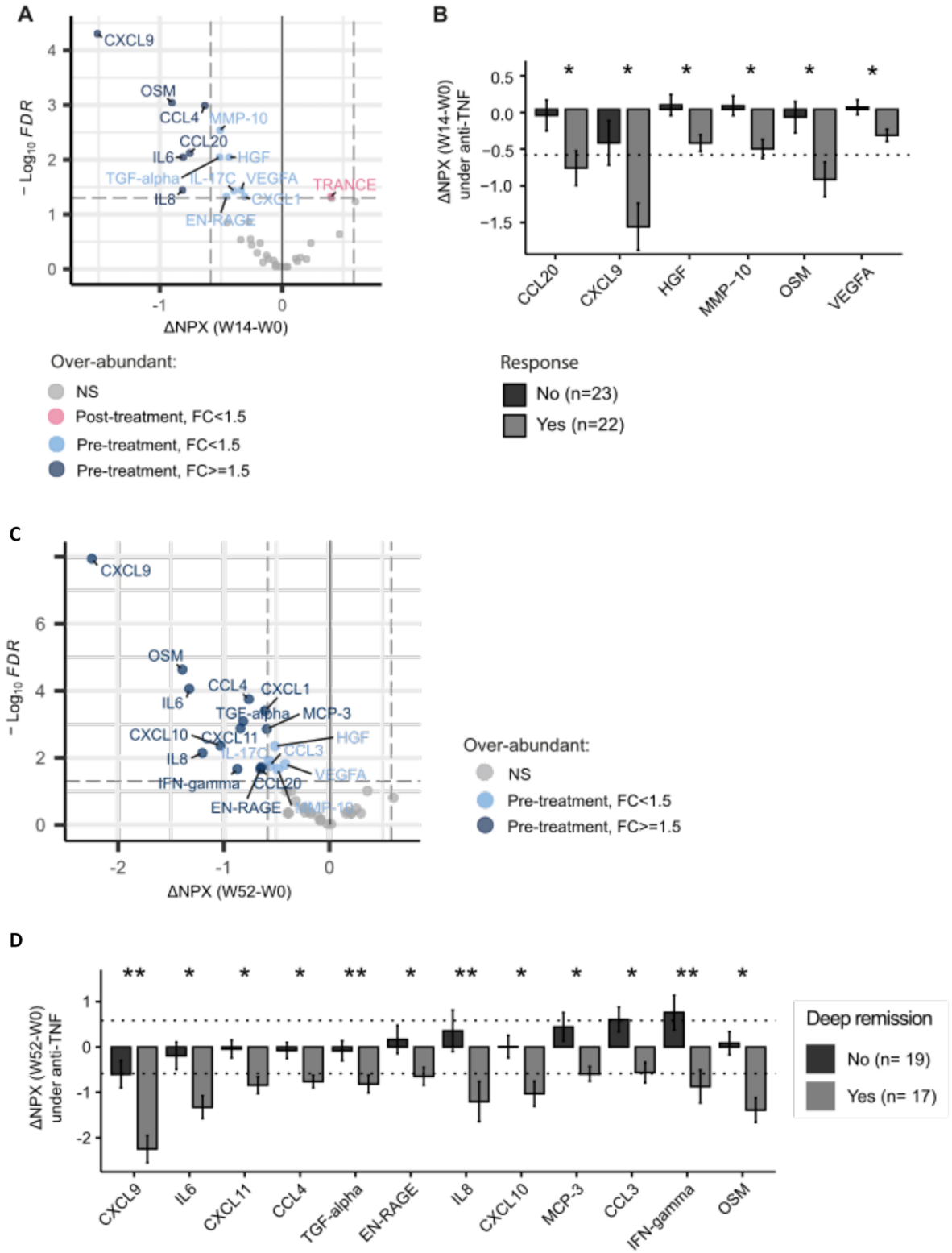


Figure 6. Variation of inflammation-related proteins in the serum of IBD patients responding to anti-TNF at week 14 and in remission under anti-TNF at week 52. (A) Volcano plot displaying the proteins that vary significantly between W0 and W14 in the serum of patients responding to anti-TNF. The vertical dashed line represents the fold-change thresholds FC = 1.5 or FC = 0.5, and the horizontal line the FDR threshold equal to 0.05. (B) Barplots representing the protein abundance mean variation between W0 and W14 in the serum of patients who responded to treatment (light gray) or not (dark gray), considering patients under anti-TNF. (C) Volcano plot displaying the proteins that vary significantly between W0 and W52 in serum of patients responding to anti-TNF. The vertical dashed line represents the fold-change thresholds FC = 1.5 or FC = 0.5, and the horizontal line the FDR threshold equal to 0.05. (D) Barplots representing the protein abundance mean variation between inclusion and W52 in patients who were responding to treatment (light gray) or not (dark gray), considering patients under anti-TNF. The vertical line represents the mean abundance standard error. *P*-values were calculated with empirical Bayes moderated t-statistics tests and adjusted by applying the Benjamini–Hochberg procedure (FDR); *FDR < 0.05, and ns FDR > 0.05.

and fibrosis in various clinical situations and organs.[24–26] This finding could be due to impaired healing processes in IBD and expands on scarce but existing prior research showing that MMPs contribute to tissue remodeling and inflammation in IBD in both pre-clinical models and patients.[27–29]

Our study also highlights the role of OSM as a key inflammatory mediator associated with disease activity and treatment response in IBD. Decreased OSM levels were highly significantly associated with anti-TNF response at W14 and remission at W52. Interestingly, these findings are in line with the study from West et al in which OSM has been shown to correlate with anti-TNF failure at the transcriptomic level, suggesting it may contribute to primary non-response.[15] In our study, elevated OSM levels were also observed in patients with post-operative recurrence, suggesting a broader role for OSM across different stages and phenotypic expressions of disease activity in IBD.

One of the main findings of this study is that inflammatory signatures vary depending on the biologic therapy received.[30] For instance, patients treated with anti-TNF agents displayed elevated serum TNF levels, while those under ustekinumab exhibited increased IL12 levels.[24,25] This is, to our knowledge, the first study to show such an association. However, the elevated TNF levels observed in patients treated with anti-TNF may reflect cytokine sequestration through the presence of TNF–anti-TNF immune complexes rather than indicating increased inflammatory activity. This is also suggested by the pharmacokinetic association found. Similarly, the higher IL12 levels in ustekinumab-treated patients may result from differential sequestration dynamics involving the p40 subunit, to which ustekinumab binds. Additionally, specific biomarkers were associated with recurrence under distinct treatments. For example, CXCL9 was significantly elevated in anti-TNF-treated patients with recurrence, whereas ST1A1 and OSM/TGFα/TNFSF14 modules were associated with recurrence under ustekinumab. In the ELYP cohort, the sample size for ustekinumab-treated patients was limited, which reduced the statistical power to detect such associations. These findings reinforce the rational for combination or sequential therapies to target multiple inflammatory pathways and personalized combination strategies guided by proteomic signatures, which could be the future of IBD treatment.[31,32] Further clinical trials are warranted to evaluate the efficacy, safety, and cost-effectiveness of such approaches in IBD management. They also highlight the complexity of post-operative recurrence mechanisms and suggest that personalized monitoring strategies may improve outcomes.[33]

Several of our findings are in line with previous omics studies in IBD. For instance, the role of OSM in predicting anti-TNF failure has been well established at the transcriptomic level,[15] and we previously reported IFN-γ as a marker of post-operative recurrence based on transcriptomic analyses.[13] While proteomics offers valuable insights into active disease biology, integration with other omics layers, such as metabolomics and genomics, would likely enhance pathophysiological interpretation. Such multi-omics integration is currently being developed within the REMIND project.

This study benefits from the use of two large prospective cohorts with robust clinical and proteomic datasets. The inclusion of both post-operative and active disease models enhances the generalizability of our findings across diverse IBD contexts. However, several limitations must be acknowledged. First, if proteomic analyses provide valuable insights into inflammatory pathways, further validation in independent cohorts and mechanistic studies are required to confirm these findings. Second, we used an inflammation-specific panel of only 92 proteins and, as such, our study was not "assumption free" like genetic or other -omic approaches. Third, several biological data (ie, CRP levels in the REMIND cohort or matching M0 and M6 serum samples) were missing due to complex sample collection logistics inherent to the nature of large-scale multicenter cohorts. However, obtaining such a high number of matched samples under these conditions is already a significant achievement. Finally, as this was not the aim of the study, we did not perform proteomic analysis of mucosal biopsies. Nevertheless, the strong associations observed with known inflammatory markers and pathways suggest that the identified proteomic signatures are biologically meaningful and may still reflect relevant immune processes occurring in the mucosa.

5. Conclusion

Our study identifies IFN-γ, CXCL9, MMP-10 and OSM as critical biomarkers associated with post-operative recurrence in CD and non-response to biologic treatments in IBD. The results of our study underscore the complexity of inflammatory pathways involved in IBD and highlight the limitations of monotherapies in achieving long-term remission. These findings provide new insights into the heterogeneity of IBD inflammation and highlight the potential for personalized treatment approaches targeting specific inflammatory pathways. Future studies should explore whether combining biologics targeting distinct inflammatory pathways (eg, anti-TNF with anti-IL12/23) could improve outcomes in patients with a high inflammatory burden.

Author contributions

M.V. analyzed the data and interpreted the results. N.H. helped with data interpretation and wrote the manuscript. D.H. collected data, performed recruitment and revised the manuscript. V.C. collected samples and critically reviewed the manuscript. J.B., M.L.T.M., C.B., J.M.G., P.S., S.N., F.C., X.T., M.N., A.B., X.H., M.S., M.F., E.L., P.B., and L.P.B. included patients and critically reviewed the manuscript. M.B. supervised the study. V.S. analyzed the data and critically reviewed the manuscript. L.L.B. interpreted the results and supervised the study. M.A. designed the REMIND and the ELYP study, acquired data, interpreted the results, and supervised the study. All authors read and approved submission of the manuscript.

Supplementary material

Supplementary material is available at *ECCO-JCC* online.

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Conflicts of interest

N.H. has served as a consultant/advisory board member to Abbvie, Celltrion, Fresenius Kabi, Janssen, and Lilly and as speaker for Abbvie, Galapagos, and Takeda. C.B. received fees from Abbvie, Amgen, Celltrion, and Takeda. J.M.G. has been a speaker and/advisory board member for Abbvie, Amgen, Celltrion, Takeda, Janssen, and Sanofi Genzyme. P.S. received consulting fees from Takeda, Abbvie, Merck-MSD, Biocodex, Janssen, Amgen, Astellas, and Pfizer and grants from Biocodex and Janssen. S.N. has served as a consultant/advisory board member and speaker for Abbvie, Celltrion, Fresenius Kabi, Janssen, Lilly, Takeda Amgen, and Biogen. F.C. received speaker fees from Abbvie, Biogen, Ferring, Janssen, MSD, Pfizer, Pileje, and Takeda; and participated in advisory boards of Amgen, Arena, Celltrion, Enterome, Ferring, Janssen, Medtronic, Pfizer, Pharmacosmos, Roche, and Tillotts. X.T. declares counselling, boards, or fees for AbbVie, Celltrion, Lilly, Galapagos, Janssen, Takeda, Amgen, and Tillotts. P.W. received board membership, consultancy, or lecture fees from Abbvie, Amgen, Celltrion, Ferring, Janssen, and Takeda. A.Bu. declares consulting fees from Abbvie, Amgen, Arena, Biogen, Celltrion Healthcare, CTMA, Galapagos, Janssen, MSD, Nexbiome, Pfizer, Roche, Takeda, and Tillotts; lecture fees for Abbvie, Amgen, Biogen, Galapagos, Janssen, Mayoli-Spindler, MSD, Norgine, Pfizer, Roche, Takeda, Tillotts, and Vifor Pharma; research grants from Abbvie, Celltrion Healthcare Janssen, Lilly, Pfizer, and Takeda. A.Bo. reports consulting/lecture fees for AbbVie, Lilly, Ferring, MSD, Mauna Kea, Medtronic, J&J, Takeda, Medac, Tillotts, Alfasigma, OSE immunotherapeutics, and Pfizer. X.H. receveived fees for advisory boards from Abbvie, Biogen, Celltrion, Fresenius-Kabi, J&J, Lilly, and Pfizer; educational activities from Abbvie, Amgen, Celltrion, Ferring, Fresenius-Kabi, J&J, Lilly, MSD, Nestlé Health Sciences, Pfizer, Takeda, and Tillots, and clinical research from Abbvie, Abivax, Amgen, Lilly, Fresenius-Kabi, GSK, J&J, InDex Pharmaceuticals, Pfizer, MSD, Roche, Takeda, and Teva. M.S. received boarding or lecture fees from Abbvie, Alfasigma, Amgen, Biogen, Celltrion, Johnson and Johnson, MSD, and Takeda. M.F. reports consulting/lecture fees for Abbvie, Amgen, Arena, Biogen, Celltrion, CTMA, Galapagos, AlfaSigma, Janssen, Fresenius-Kabi, MSD, Pfizer, Takeda, Tillotts, MSD, Nordic Pharma, Lilly, Gilead, Celgene, Sandoz, and Ferring. E.L.: research grant: Janssen, Pfizer, Ferring, Falk, Abbvie, and Takeda; educational grant: AbbVie, Janssen, Fresenius-Kabi, and Takeda; speaker fees: Abbvie, Falk, Ferring, Janssen, Pfizer, Galapagos, BMS, and Takeda; advisory board: Abbvie, Celgene, Ferring, Janssen, BMS, Pfizer, Takeda, Galapagos, Arena, and Elli Lilly; consultant: Abbvie, Biokuris, Aboleris, and Thabor. P.B. has received payment for lectures from Abbvie, Pfizer, Tillotts, Fresenius Kabi, and Gilead. L.P.B. has received lecture/consultant fees fromAbbvie, Abivax, Adacyte, Alimentiv, Amgen, Applied MolecularTransport, Arena, Banook, Biogen, BMS, Celltrion, Connect Biopharm, Cytoki Pharma, Enthera, Ferring, Fresenius Kabi, Galapagos, Genentech, Gilead, Gossamer Bio, GSK, IAC Image Analysis, IndexPharmaceuticals, Inotrem, Janssen, Lilly, Medac, Mopac, Morphic, MSD, Nordic Pharma, Novartis, Oncodesign Precision Medicine, ONO Pharma, OSE Immunotherapeuthics, Pandion Therapeuthics, Par' Immune, Pfizer, Prometheus, Protagonist, Roche, Samsung, Sandoz, Sanofi, Satisfay, Takeda, Telavant, Theravance, ThermoFischer, Tigenix, Tillots, Viatris, Vectivbio, Ventyx, and Ysopia. V.S. is currently a full-time employee at Owkin, France. M.A. received financial support for research from Janssen, Takeda, and Genentech/Roche; consultant fees from Abbvie, Amgen, Astra Zeneca, Biogen, Boehringer-Ingelheim, Bristol Myers Squibb, Celgene, Celsius, Celltrion, Egle Therapeutics, Endpoint health, Ferring, Galapagos, Genentech, IQVIA, Janssen, Lilly, MSD, Novartis, Owkin, Pfizer, Roche, Takeda, and Tillots; and speakers fees from Abbvie, Galapagos, Genentech, Janssen, Pfizer, Takeda, and Tillots. M.V., L.L.B., D.H., J.B., M.L.T.M., V.C., and M.B. have no disclosures to declare.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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