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Gastroenterology: Inflammatory Bowel Disease

A novel diagnostic serum protein signature for pediatric inflammatory bowel disease

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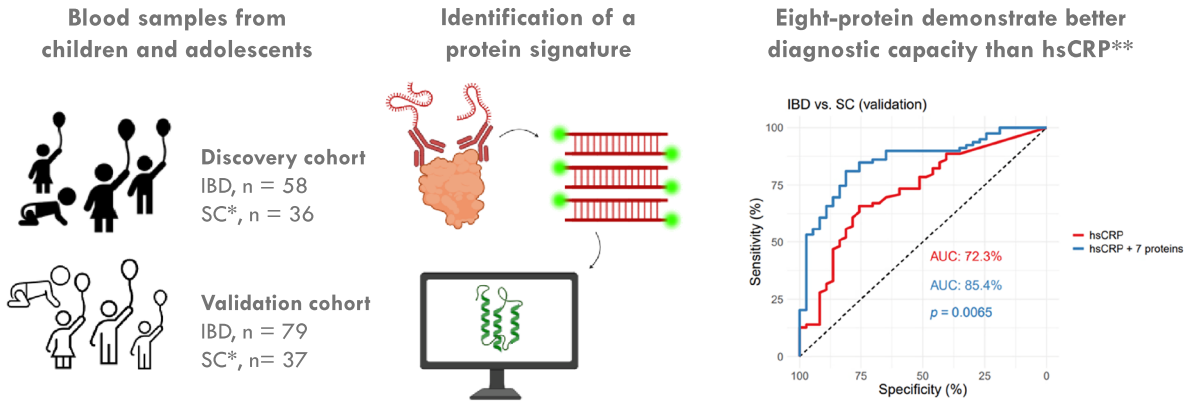
A proteomic signature may aid diagnostic workup of inflammatory bowel disease in children



* Symptomatic controls: Patients referred for suspected IBD but who did not meet ESPGHAN/Porto criteria for IBD **high sensitive C-reactive protein

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Charlotte Bache-Wiig Mathisen and Niklas Nyström contributed equally to this study. Jonas Halfvarson and Marte Lie Høivik are joint senior authors.

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Abstract

Objectives: Diagnostic delay is common in pediatric inflammatory bowel disease (PIBD), and fecal calprotectin (FCP) is often limited by challenges with sample collection. Therefore, we aimed to identify and validate a blood-based diagnostic protein signature of PIBD.

Methods: Proteins were analyzed using proximity extension assay in plasma samples from treatment-naïve pediatric patients in a Swedish inception cohort referred for suspected inflammatory bowel disease (IBD) and validated in an independent Norwegian population-based pediatric inception cohort. Diagnostic performance was estimated by the area under the curve (AUC) with 95% confidence intervals (CIs).

Results: The discovery cohort included 58 patients with PIBD and 36 symptomatic controls without evidence of IBD, while the validation cohort consisted of 79 patients with PIBD and 37 symptomatic controls. In total, 154 proteins were examined. Univariable analyses identified 26 differentially regulated proteins for PIBD versus symptomatic controls in the discovery cohort ($q < 0.05$), whereas 29 proteins were differentially regulated in the validation cohort. Using regularized logistic regression, we identified a diagnostic model of 31 proteins that differentiated PIBD from symptomatic controls in the discovery cohort (AUC = 0.83; 95% CI: 0.74–0.90). The protein signature was further reduced to a clinically relevant biomarker consisting of high-sensitivity C-reactive protein (hsCRP) and seven other proteins with diagnostic capacity (AUC = 0.85, 95% CI: 0.78–0.92) outperforming hsCRP in the validation cohort ($p = 0.006$).

Conclusions: We identified and validated a blood-based protein signature for PIBD with superior diagnostic performance compared to hsCRP. Given the challenges of fecal sample collection, further assay development may enable integration of these biomarkers into diagnostic pathways for PIBD.

Trial Registration: ClinicalTrials.gov identifier: NCT02727959.

KEYWORDS

biomarker, Crohn's disease, precision medicine, ulcerative colitis

1 | INTRODUCTION

Onset of pediatric inflammatory bowel disease (PIBD) is associated with a diverse range of symptoms including diarrhea, abdominal pain, weight loss and fatigue, which complicates differentiation from other gastrointestinal diseases, contributing to diagnostic delays.[1] Early diagnosis and timely disease control are crucial to halt disease progression, prevent growth impairment and reduce long-term gastrointestinal damage.[2,3]

The heterogeneous clinical presentation of PIBD underscores the importance of objective diagnostic tools. Currently C-reactive protein (CRP) and fecal calprotectin (FCP) are the most widely used biomarkers to guide referrals for endoscopy.[4] Additionally, hypoalbuminemia, abnormal platelet count and elevated erythrocyte sedimentation rate may provide information of systemic inflammation.[5,6] However, CRP performs poorly in ulcerative colitis (UC), and up to 21% of pediatric patients with Crohn's disease (CD) do not mount a CRP response.[7] Therefore, normal results from existing blood-based tests cannot reliably exclude IBD.[7,8] While FCP is widely used for ruling out IBD in some healthcare systems, false-positive results are common, and fecal sampling is poorly accepted by some pediatric patients.[9,10] Additionally, the diagnostic accuracy of FCP appears lower in children compared to adults.[11]

Identifying novel diagnostic biomarkers in blood is imperative for timely diagnosis and treatment. With advances in high-throughput protein profiling, detection of disease-specific protein signatures has become feasible. Over recent decades, several attempts have been made to identify diagnostic or prognostic proteomic biomarkers for patients with IBD; however, most of these studies comprised small cohorts, and the findings have generally not been validated.[12–14] Based on these observations, we aimed to identify and validate a diagnostic protein signature of PIBD using blood samples from two independent inception cohorts of treatment-naïve patients.

What is Known

- Diagnosis of pediatric inflammatory bowel disease (PIBD) when delayed may have negative influence on growth and maturation.
- C-reactive protein and fecal calprotectin (FCP) are the available biomarkers to guide referral and further diagnostic work-up, but FCP is not available in all healthcare systems and stool sampling can be challenging in children and adolescents.

What is New

- We identified and validated a blood-based protein signature for PIBD, with potential for development into a clinically applicable assay to support diagnostic decision-making.

2 | METHODS

2.1 | Ethics statement

The study was approved by the regional ethical boards (Uppsala University Ethics Committee [2008/395] and the South-Eastern regional Ethical board [2015/946]), and all centers were granted local ethics approval. All patients provided written informed consent before participating in this study.

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2.2 | Study design

We performed a cross-sectional study and assayed plasma proteins in children and adolescents prospectively referred to the Uppsala University Children's Hospital for the suspicion of IBD. By comparing pediatric patients diagnosed with IBD with symptomatic non-IBD controls and applying statistical models, we identified a diagnostic protein signature of IBD. To validate the results, we analyzed serum samples from an independent population-based inception cohort, the IBSEN III cohort from South-Eastern Norway and compared the diagnostic performance of the protein signature with that of the existing biomarker CRP.

2.3 | The discovery cohort

Children aged <18 years with clinically suspected IBD who were referred to the Uppsala University Children's Hospital between 2009 and 2018 were invited to participate. The inclusion criterion was presence of gastrointestinal symptoms indicative of IBD. Exclusion criteria were a previous diagnosis of IBD or other chronic gastrointestinal diseases and treatment with antibiotics within the preceding 3 months. After obtaining written informed consent, all included children and adolescents underwent a routine diagnostic workup for IBD. The diagnosis was based on the European Society for Paediatric Gastroenterology, Hepatology and Nutrition/Porto criteria,[15] including gastroscopy and ileocolonoscopy with serial mucosal biopsies for histology. The Paris classification was used to categorize IBD patients by disease phenotype,[16] and the short pediatric CD activity index (sPCDAI)[17,18] and the pediatric UC activity index (PUCAI)[19,20] were used to assess disease activity.

2.4 | The validation cohort

The IBSEN III inception cohort study was used for validation. The cohort has been described in detail elsewhere.[21] In short, patients with suspected IBD in the South-Eastern Health Region of Norway (HSØ) in the period 2017–2019, were prospectively invited to participate. For the purpose of this study, we only included patients aged <18 years. The criteria for defining the diagnosis of IBD, categorizing the phenotype and assessing clinical disease activity in the IBSEN III cohort were consistent with the criteria used in the discovery cohort. After complete investigation, children and adolescents who did not meet the diagnostic criteria for IBD were included as symptomatic non-IBD controls. Like the discovery cohort, symptomatic non-IBD controls in the validation cohort were defined as patients who presented symptoms similar to IBD but without endoscopic or histological signs of inflammation. Patients with all other causes of acute or chronic bowel inflammation were excluded.

2.5 | Sample collection

In both cohorts, blood and feces samples were collected during the diagnostic workup, that is, before the start of IBD treatment. In the Uppsala cohort, 4 mL BD Vacutainer® CPTTM Cell Preparation Tubes with sodium citrate were used, and blood was processed to plasma within 4 h of sampling by centrifugation at 1800 g for 20 min. All samples were aliquoted and stored at −80°C. In the IBSEN III study, 8 mL Vacuette CAT with Serum Sep Clot Activator were used. Blood was centrifuged at 2000 g for 10 min within 2 h of sampling before serum aliquots were stored at −80°C.

2.6 | High-sensitivity CRP and FCP

Samples from both cohorts were assayed for high-sensitivity CRP (hsCRP) with a particle-enhanced immunoturbidimetric hsCRP assay (cardiac C-reactive protein [Latex] high sensitive, Roche diagnostics) on a Roche Cobas c501 at Uppsala BioLab, Uppsala Clinical Research Center (UCR), Uppsala, Sweden.

Fecal samples from the validation cohort were extracted in a single batch after inclusion of all patients, and concentrations were analyzed by fCAL ELISA Calprotectin assay (Bühlmann Laboratories AG) at Unger Vetlesen Institute, Lovisenberg Diaconal Hospital, Oslo, Norway.

2.7 | Protein profiling

Proteins were measured using the proximity extension assay (PEA) technology and two different immunoassays (Olink Proteomics, Proseek® Multiplex Inflammation I and Oncology II 96 × 96). The methodology enables the analysis of 92 protein biomarkers across 96 samples per panel and has been described in detail elsewhere.[22]

2.8 | Statistical analysis

Univariable two-group comparisons were performed by the Wilcoxon rank-sum test for IBD and subtypes (CD and UC) and Benjamini–Hochberg false discovery rate (FDR) was used for multiple comparison adjustment.[23] Proteins were considered statistically significantly differentially regulated with an absolute fold change >1.2 and a q-value < 0.05.

In multivariable analyses, regularized logistic regression with the smoothly clipped absolute deviation (SCAD) penalty was used to create a diagnostic protein signature discriminating patients with IBD from symptomatic controls.[24] In addition to SCAD logistic regression, random forest regression models were fitted.[25] The number of proteins in the diagnostic signature was further reduced using stepwise backward elimination with Bayesian information criterion (BIC), mandating hsCRP inclusion.

Diagnostic performance was assessed by testing the models developed with the discovery cohort on an independent validation cohort. Performance metrics included receiver operating characteristic (ROC) curves with area under the curve (AUC).[26] Youden index determined optimal cutoffs for calculating sensitivity, specificity, and positive and negative likelihood ratios (LR+, LR−). Net reclassification index (NRI) and integrated discrimination improvement (IDI) were also calculated. All statistical

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analyses and data processing were primarily performed using R version 4.2.3 and STATA 16.1. For further details on proteomics data preprocessing and statistical analyses, see Supporting Information S1: File S1.

3 | RESULTS

3.1 | Patient cohorts

Blood samples were available from 95 children and adolescents in the discovery cohort and 125 in the validation cohort. During PEA analyses and subsequent data control, one sample from the discovery cohort and nine from the validation cohort were excluded due to technical failures. As a result, the final discovery cohort used to identify the diagnostic protein signature comprised 58 patients with IBD (CD, $n = 44$; UC, $n = 12$; and IBD-U, $n = 2$) and 36 symptomatic non-IBD controls. The validation cohort included 79 children with IBD (CD, $n = 53$; UC, $n = 20$; IBD-U, $n = 6$) and 37 symptomatic non-IBD controls. As shown in Table 1, age, sex and disease phenotypes, according to the Paris classification, were comparable between the two cohorts. Seventy-six patients in the validation cohort provided FCP samples, and these patients did not differ in baseline characteristics from the full cohort (Supporting Information S1: Table S1).

3.2 | Differentially regulated proteins

In total, 184 proteins were analyzed, six of which were measured across both panels. Twenty-four proteins were excluded from the analysis due to their quantified levels falling below the limit of detection (LOD) in more than 50% of pediatric IBD patients as well as symptomatic controls. Consequently, 154 proteins were retained for inclusion in the final analysis.

To identify differentially regulated proteins in treatment-naïve children with incident IBD compared to symptomatic non-IBD controls, we first conducted univariable analyses. As shown in Figure 1A, 26 proteins were identified as differentially regulated in pediatric patients with IBD within the discovery cohort, whereas 29 proteins were differentially regulated in the validation cohort (Figure 1B). Across both cohorts, 10 of the proteins consistently showed higher relative levels in pediatric patients with incident IBD compared to symptomatic controls, while levels of carboxypeptidase E (CPE), Delta and Notch-like epidermal growth factor related receptor (DNER), Seizure 6-like protein (SEZ6L) and stem cell factor (SCF) were consistently lower. Among proteins that differed most between PIBD and symptomatic controls were C-X-C motif chemokine 9 (CXCL9), Interleukin-8 (IL-8), matrix metalloproteinase-10 (MMP-10), and interleukin 17-A (IL-17A). Subtype-specific analyses for CD and UC are shown in Supporting Information S1: Figure S1.

3.3 | Identification and validation of a diagnostic protein signature of IBD

Following the demonstration of diagnostic potential of the proteins in the univariable analyses, we applied a multivariable approach to identify a diagnostic protein signature. Using SCAD-regularized logistic regression, we constructed models to differentiate patients with PIBD from symptomatic controls in the discovery cohort. A total of 31 diagnostic proteins were selected by the model for separating patients with PIBD from symptomatic controls (Supporting Information S1: Figure S2A). The predictive potential of the protein models was assessed by the AUC of the ROC curve for the validation cohort. The regularized regression model with 31 proteins achieved an AUC of 83.0% (95% confidence interval [CI]: 74.8%–90.2%), significantly better ($p = 0.015$) than hsCRP (AUC = 72.3%, 95% CI: 61.5%–81.6%) (Supporting Information S1: Figure S2B). A model for separating CD and UC selected 29 proteins, yielding an AUC of 70.8% (95% CI: 57.7%–82.5%) not significantly better than hsCRP (AUC = 66.1%, 95% CI: 52.3%–78.6%, $p = 0.59$) (Supporting Information S1: Figure S2C,D). Our SCAD-regularized regression approach outperformed random forest regression in terms of diagnostic capacity, but both approaches identified similar protein signatures (Supporting Information S1: Figure S3A,B).

3.4 | Identification and validation of a shorter protein signature

A signature comprising many proteins may limit the feasibility for assay development and clinical implementation. To address this, we returned to the discovery cohort and applied backward logistic regression to develop a smaller model capable of differentiating PIBD from symptomatic controls. Starting from hsCRP and the full protein signature with 31 proteins, proteins were removed one by one until only hsCRP and seven other proteins remained (Figure 2A). This eight-protein signature comprised hsCRP, CXCL9, Eotaxin (CCL11), Eukaryotic initiation factor 4-E-binding protein 1 (4EBP1), Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM 1), Monocyte chemoattractant protein-1 (MCP1), Granzyme H (GZMH), and SEZ6L.

When applied to the validation cohort the eight-protein model yielded a similar diagnostic capacity (AUC of 85.4%, 95% CI: 78.2%–92.0%) as the full model for separating PIBD from symptomatic controls. Using the optimal cutoff value, we found a sensitivity of 81.0% and a specificity of 81.1% for the eight-protein model, corresponding to an LR+ of 4.3 and an LR− of 0.23 (Table 2). The diagnostic capacity of hsCRP alone was significantly lower ($p = 0.006$) than that of the eight-protein model (Figure 2B), with a sensitivity of 65.8% and a specificity of 75.7% at the optimal cutoff, corresponding to an LR+ of 2.7 and an LR− of 0.45 (Table 2). We also stratified the analysis by IBD subtype to examine the diagnostic accuracy of the eight-protein model, which was trained to separate PIBD from symptomatic controls regardless of subtype, in pediatric CD and UC separately. The eight-protein model achieved AUCs of 84.8% (95% CI: 76.1%–91-9%) and 86.7% (95% CI: 75.5%–94.7%) for CD and UC, respectively. The improvement in diagnostic capacity compared to hsCRP was significant for UC ($p = 0.0007$) but not for CD ($p = 0.10$) (Figure 2C,D).

To establish the clinical relevance of the eight-protein model, we further calculated its ability to reclassify patients with PIBD and symptomatic controls in the validation cohort compared to hsCRP alone. Adding the seven proteins to

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TABLE 1 Demographics and clinical characteristics of the discovery and the validation pediatric IBD cohorts.

	Discovery cohort ($n = 94$)		Validation cohort ($n = 116$)	
	IBD ($n = 58$)	Symptomatic controls ($n = 36$)	IBD ($n = 79$)	Symptomatic controls ($n = 37$)
Age, median (IQR 1–3)	15 (12–16)	12 (9–16)	14 (10–16)	12 (7–14)
Males, n (%)	35 (60)	16 (44)	45 (57)	23 (62)
hsCRP, median (IQR 1–3)	5 (0.8–12.0)	0.52 (0.2–1.9)	4 (1–11.8)	0.6 (0–2.5)
Fecal samples (%)	57 (98)	31 (86)	56 (71)	20 (54)
Fecal calprotectin, median (IQR 1–3)	1334 (739–2112)	69 (41–408)	355 (131–1801)	87 (49–131)
Subtype IBD (%)				
CD	44 (76)	NA	53 (67)	NA
UC	12 (20)	NA	20 (25)	NA
IBD-U	2 (3)	NA	6 (8)	NA
Age at CD diagnosis, *n* (%)				
A1a (<10 years)	6 (13)	NA	7 (13)	NA
A1b (10-16 years)	32 (73)	NA	39 (74)	NA
A2 (≥17 years)	6 (13)	NA	7 (13)	NA
Location of CD, *n* (%)				
L1 (terminal ileum)	12 (27)	NA	8 (15)	NA
L2 (colon)	17 (39)	NA	12 (23)	NA
L3 (ileocolon)	15 (34)	NA	27 (51)	NA
L4 A[a]	10 (22)	NA	5 (9)	NA
L4 B[b]	0 (0)	NA	1 (2)	NA
Behavior of CD, *n* (%)				
B1 (nonstricturing, nonpenetrating)	41 (93)	NA	44 (83)	NA
B2 (stricturing)	3 (7)	NA	8 (15)	NA
B3 (penetrating)	0 (0)	NA	1 (2)	NA
p (perianal disease)	9 (20)	NA	8 (15)	NA
				NA
PCDAI, median (IQR 1–3)	40 (29–56)	NA	15 (10–25)	NA
Age at UC diagnosis, *n* (%)				
A1a (<10 years)	0 (0)	NA	5 (25)	NA
A1b (10–16 years)	12 (86)	NA	10 (50)	NA
A2 (≥17 years)	2 (14)	NA	5 (25)	NA
Extent of UC, *n* (%)				
E1 (proctitis)	2 (17)	NA	6 (30)	NA
E2 (left sided)	2 (17)	NA	3 (15)	NA
E3 (extensive)	7 (58)	NA	3 (15)	NA
E4 (pancolitis)	1 (8)	NA	8 (40)	NA
PUCAI, median (IQR 1–3)	43 (30–49)	NA	25 (15–40)	NA

Abbreviations: CD, Crohn's disease; GI, gastrointestinal; hsCRP, high-sensitivity C-reactive protein; IBD, inflammatory bowel disease; IBD-U: inflammatory bowel disease unclassified; IQR, interquartile range; NA, not available; PCDAI, pediatric Crohn's disease activity index; PUCAI, pediatric ulcerative colitis activity index; UC, ulcerative colitis.

[a]Upper GI tract prox. Treitz.

[b]Upper GI tract distal of Treitz.

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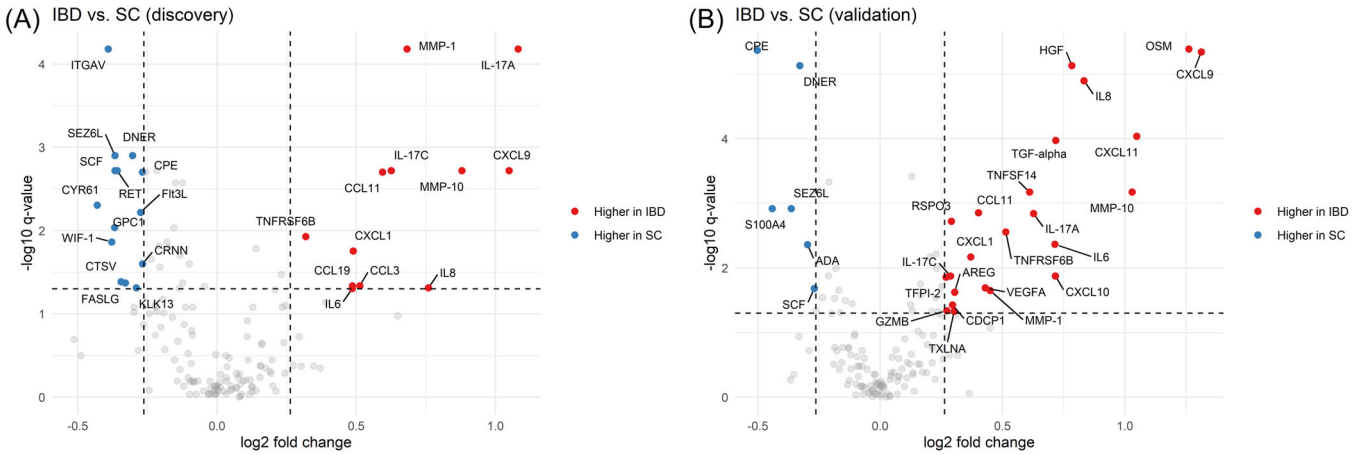

FIGURE 1 Univariable analyses were performed by Wilcoxon rank-sum test and revealed 26 differentially regulated proteins in PIBD patients ($n = 58$) of the discovery cohort (A), and 14 of these were rediscovered in the validation cohort ($n = 79$) (B). The volcano plot represents the fold change (log2) on the *x*-axis and the corresponding –log10 (*q*-value) on the *y*-axis. CPE, carboxypeptidase E; CXCL9, C-X-C motif chemokine 9; DNER, Delta and Notch-like epidermal growth factor related receptor; IL-17A, interleukin 17-A; IL-8, interleukin-8; MMP-10, matrix metalloproteinase-10; PIBD, pediatric inflammatory bowel disease; SC, subcutaneous; SCF, stem cell factor; SEZ6L, seizure 6-like protein.

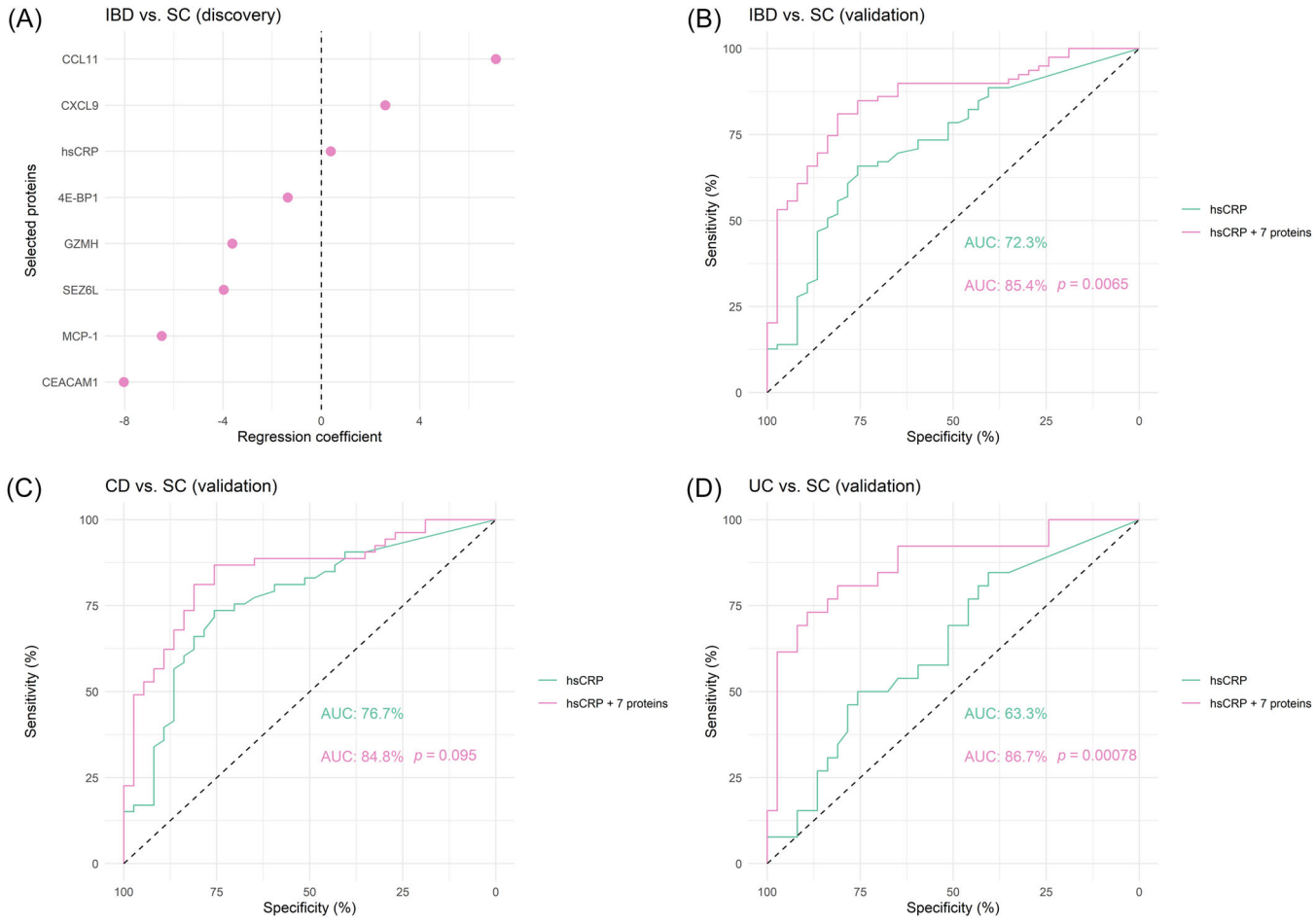

FIGURE 2 (A–D) Employing backward regression, we derived a shorter protein model consisting of hsCRP and seven other proteins (A). Panels (B–D) shows the seven-protein model performance compared to hsCRP alone for IBD versus SC, CD versus SC and UC versus SC, respectively. AUC, area under the curve; CD, Crohn's disease; CXCL9, C-X-C motif chemokine 9; hsCRP, high-sensitivity C-reactive protein; IBD, inflammatory bowel disease; SC, subcutaneous; SEZ6L, seizure 6-like protein; UC, ulcerative colitis.

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TABLE 2 Youden index, sensitivity, specificity, LR+ and LR− of hsCRP were evaluated in comparison to the eight-protein signature for predicting pediatric inflammatory bowel disease in the validation cohort.

Model	Youden index (J)	Sensitivity (%)	Specificity (%)	LR (+)	LR (−)
hsCRP	0.41	65.8	75.7	2.7	0.45
hsCRP + 7 proteins	0.62	81.0	81.1	4.3	0.23
hsCRP	NA	90	30.8	1.3	0.32
hsCRP + 7 proteins	NA	90	35.1	1.4	0.28
hsCRP	NA	28.7	90	2.9	0.79
hsCRP + 7 proteins	NA	60.7	90	6.1	0.44

Note: The first two rows display the diagnostic test statistics derived from the optimal Youden index. The rows below represent statistics calculated with a fixed sensitivity of 90% and a fixed specificity of 90%.

Abbreviations: hsCRP, high sensitivity C-reactive protein, LR(+), likelihood ratio for positive test result; LR(−), likelihood ratio for a negative test result; NA, not available.

hsCRP significantly improved reclassification, as demonstrated by an NRI of 20.6% (95% CI: 2.2%–39.0%; $p = 0.029$) and an IDI of 41.8% (95% CI: 28.5%–54.5%; $p < 10^{-5}$). Compared to hsCRP alone, the eight-protein model achieved net reclassification of 15.2% for PIBD and 5.4% for symptomatic controls (Supporting Information S1: Table S1).

Comparisons between protein signatures and FCP were constrained by the fact that more than one-third (40/116) of the pediatric patients in the validation cohort did not provide a fecal sample. Among patients providing a fecal sample ($n = 76$), the AUC of FCP was 93.1% (95% CI: 86.9%–98.0%), whereas the AUC of the eight-protein model was 85.0% (95% CI: 75.5%–92.7%) in this subgroup of patients ($p = 0.053$).

4 | DISCUSSION

We identified a diagnostic protein signature comprising 31 proteins in a cohort of treatment-naïve, newly diagnosed children and adolescents with IBD and validated its performance in an independent population-based inception cohort. The model outperformed hsCRP (AUC of 83.1% vs. 72.3%) in the validation cohort. Employing backwards elimination, we derived a shorter model consisting of hsCRP and seven additional proteins, which demonstrated statistically significantly superior diagnostic performance compared to hsCRP (AUC 85.4% vs. 72.3%), and an improved reclassification of 15.2%. In the patients with IBD who provided a stool sample, the diagnostic performance of the shorter protein model was not materially different from the performance of FCP.

Previous proteomic studies have revealed dysregulation of the proteome in patients with IBD,[12,13] although most studies explored patients with longstanding disease. Fabian et al. reviewed 31 proteomic papers on adult and PIBD and found that most proteins were identified in biopsies,[12] whereas only one pediatric study had examined blood samples and also validated the findings in an independent cohort.[27] In a recent study including multiple adult IBD cohorts across Europe, patients were included within 6 months of diagnosis. Using the PEA technique in serum samples, the researchers identified 66 proteins that were significantly differentially expressed in patients with IBD compared to symptomatic controls.[28] In our study, 12 of these 66 proteins were also found to be differentially expressed in the discovery cohort, 16 in the validation cohort and 10 proteins found in both cohorts. The fact that the top four enriched proteins in our univariable analysis (IL-17A, IL-8, MMP10, and CXCL9) were also increased in the European multicohort study of adult IBD strengthens our results.

Our eight-protein model comprised hsCRP, CXCL9, CCL11, 4EBP1, CEACAM 1, MCP1, GZMH, and SEZ6L. CXCL9 is a well-known chemoattractant of both the innate and adaptive immune system.[29] Previous studies investigating preclinical UC and CD have consistently reported elevated CXCL9 even in the preclinical phase, i.e. before the diagnosis.[30,31] Elevated levels of chemoattractant protein CCL11 have been demonstrated in both serum and tissue specimens from patients with established IBD,[32] and increased serum levels have also been reported several years before the diagnosis of UC.[30] The protein 4EBP1, a repressor of mRNA translation, was a negative contributor in our eight-protein model, suggesting poor performance as a single biomarker for IBD. However, in a recent genome-wide association study, genetically predicted elevated plasma levels of 4EBP1 were associated with an increased risk of IBD, displaying conflicting results.[33] CEACAM 1 serves as an important regulator of T cell functions and may modulate the immune response by inhibiting the production of inflammatory cytokines.[34] Elevated expression of several CEACAMs has been shown in in vitro studies from colonic biopsies from adult and pediatric IBD patients.[35] Our findings support the hypothesis that CEACAM1 is involved in the dysregulated immune response in IBD.

Increased serum levels of MCP-1, a chemokine regulating monocyte chemotaxis, have been associated with adult CD and proposed as a candidate biomarker of the disease.[36] In our univariable results, this protein was neither significantly over- nor under-expressed, indicating limited use as a solitary biomarker of IBD, yet contributes positively to the eight-protein model. Neither GZMH, an extracellular granzyme, nor SEZ6L, a transmembrane protein involved in neurologic disorders and proposed as a biomarker for ovarian cancer, has been reported in previous IBD studies, although extracellular granzymes are elevated in the blood of patients with autoimmune disease and SEZ6L is known to act as a regulator in the complement system.[37–39]

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A major strength of this study is the prospective collection of samples from two independent, well-characterized, population-based pediatric inception cohorts, both of which used standardized criteria to confirm the diagnosis or rule out IBD and to collect blood samples before treatment initiation. To identify a diagnostic protein signature and evaluate its accuracy, we used SCAD-regularized regression and tested the model, which was developed using the discovery cohort, on the validation dataset. This strict methodological approach increases the generalizability of our findings. The inclusion of non-IBD controls with gastrointestinal symptoms further strengthens the potential clinical utility of our findings.

However, we could not match patients with IBD and symptomatic non-IBD controls by sex, age, and date of sampling, as patients with suspicion of IBD were prospectively included. Therefore, we cannot rule out the influence of bias due to potential demographic differences. Due to limited cohort size analyses on Paris classification subtypes could not be performed. In addition, plasma samples were examined in the discovery cohort and serum samples in the validation cohort. While this difference may introduce variability, it also supports the robustness of our findings.

5 | CONCLUSIONS

In conclusion, our study identified and validated an eight-protein signature, which includes hsCRP that discriminated PIBD from symptomatic controls, that is, children and adolescents with symptoms suggestive of IBD but lacking diagnostic evidence. In two independent cohorts, this protein model outperformed hsCRP, the most widely used blood-based marker, and demonstrated superior reclassification performance. Although its accuracy was numerically lower than that of FCP, the protein signature may offer greater clinical utility given the challenges with feces collection. However, further validation in larger cohorts also including children and adolescents with other inflammatory conditions and assay development are needed before the signature can be translated into a scalable test to support clinical decision-making.

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CONFLICT OF INTEREST STATEMENT

Niklas Nyström has served as speaker and/or advisory board member for Abigo, Baxter, Ferring, Fresenius-Kabi, Mylan/Meda, Nutricia, Shire, Takeda, Thermo Fisher Scientific, Tillotts Pharma, and Viatris. Vendel Ailin Kristensen has served as speaker, consultant, and advisory member for or has received investigator-initiated research funding from Takeda, Janssen, Ferring and Tillotts Pharma. Mare Carlson has received speaker's fees from ViforPharma and AbbVie. She is the national PI for clinical trials for AstraZeneca. None of these activities have any relation to the present study. Charlotte R. H. Hedin has received speaker fees from Takeda, Ferring, AbbVie, and Janssen, and consultancy fees from Pfizer, has acted as local principal investigator for clinical trials for Janssen and GlaxoSmithKline and has received project grants from Takeda and Tillotts Pharma. Maria K. Magnusson has received speaker fees from Takeda, and Janssen. Lena Öhman has received financial support for research from Genetic Analysis AS, Biocodex, Danone Research and AstraZeneca and served as Consultant/Advisory Board member for Genetic Analysis AS, and as a speaker for Biocodex, Ferring Pharmaceuticals, Takeda, AbbVie, and MEDA. Jonas Halfvarson has received consulting and/or advisory board fees from: AbbVie,

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Alfasigma, Aqilion, Bristol Myers Squibb, Celgene, Celltrion, Eli Lilly, Ferring, Galapagos, Gilead, Hospira, Index Pharma, Janssen, Johnson & Johnson, MEDA, Medivir, Medtronic, Merck, Merck Sharp & Dohme, Novartis, Pfizer, Prometheus Laboratories Inc., Sandoz, Shire, STADA, Takeda, Thermo Fisher Scientific, Tillotts Pharma, Vifor Pharma, UCB; and speaker's fees from: AbbVie, Alfasigma, Bristol Myers Squibb, Celgene, Eli Lilly, Ferring, Galapagos, Gilead, Hospira, Janssen, Johnson & Johnson, Merck Sharp & Dohme, Novartis, Pfizer, Shire, Takeda, Thermo Fisher Scientific, Tillotts Pharma; and research grant support from Janssen, Merck Sharp & Dohme and Takeda. Marte Lie Høivik has served as speaker, consultant, and advisory member for or has received investigator-initiated research funding from MSD, Abbvie, Pfizer, Takeda, Janssen, Gilead/Galapagos, Ferring, and Tillotts Pharma. The remaining authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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