Alimentary Pharmacology & Therapeutics

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ORIGINAL ARTICLE

Anti-Integrin αvβ6 Autoantibodies Distinguish Ulcerative Colitis From Crohn's Disease With High Diagnostic Accuracy and Outperform ANCA and ASCA Status: A Prospective Cross-Sectional Study

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Received: 12 June 2026 | **Revised:** 3 August 2026 | **Accepted:** 5 August 2026

Handling Editor: Sree Subramanian

Keywords: anti-integrin αvβ6 antibodies | anti-neutrophil cytoplasmic antibodies (ANCA) | anti-*Saccharomyces cerevisiae* antibodies (ASCA) | autoantibodies | Crohn's disease | inflammatory bowel disease | ulcerative colitis

ABSTRACT

Background: Reliable biomarkers to distinguish ulcerative colitis (UC) from Crohn's disease (CD) remain limited.

Aims: To assess the diagnostic accuracy of anti-integrin αvβ6 autoantibodies for differentiating UC from CD and to compare their performance with anti-neutrophil cytoplasmic antibodies (ANCA) and anti-*Saccharomyces cerevisiae* antibodies (ASCA).

Methods: In this prospective cross-sectional study in patients with UC and CD, serum anti-integrin αvβ6 autoantibodies, ASCA, and ANCA were measured and clinical data were collected. The primary outcome was the accuracy of anti-integrin αvβ6 antibodies in distinguishing UC from CD. Secondary analyses compared their performance with ASCA and ANCA status and across IBD subgroups.

Results: A total of 224 patients were included (UC: $n = 106$, CD: $n = 118$, male: 63.8%, median age: 43.5 years). Patients with indeterminate colitis were excluded. At a cut-off of 2.44 U/mL, anti-integrin αvβ6 autoantibodies demonstrated a diagnostic accuracy of 86.5%, with a sensitivity of 85.8% and specificity of 87.1%. In a multivariate logistic regression analysis, these autoantibodies were independently associated with UC (OR 36.18, 95% CI 17.0–83.0, $p = 4.86 \times 10^{-19}$) and demonstrated superior diagnostic

Abbreviations: AIC, Akaike information criterion; ANCA, anti-neutrophil cytoplasmic antibodies; ASCA, anti-*Saccharomyces cerevisiae* antibodies; AUC, area under the curve; CD, Crohn's disease; CRP, C-reactive protein; FCP, faecal calprotectin; IBD, inflammatory bowel disease; IQR, interquartile range; PRO-2, patient reported outcomes; PSC, primary sclerosing cholangitis; ROC, receiver operating characteristics; UC, ulcerative colitis; WBC, white blood count.

Samuel Truniger and David Waber have contributed equally to this work.

performance compared to ANCA and ASCA status (Akaike information criterion 184.8 vs. 252.4). Positivity rates followed a gradient across the Montreal classification, increasing from ileal (L1: 7.9%) and ileocolonic (L3: 8.1%) CD towards colonic CD (L2: 43.8%) and UC: 85.8% ($p = 2.04 \times 10^{-31}$).

Conclusions: Anti-integrin αvβ6 autoantibodies demonstrate high diagnostic accuracy and outperform conventional serological markers in distinguishing UC from CD. Importantly, this study provides the first direct comparison with ANCA and ASCA status within the same patient cohort.

1 | Introduction

The inflammatory bowel diseases (IBD) ulcerative colitis (UC) and Crohn's disease (CD) represent a heterogeneous group of chronic disorders causing inflammation of the gastrointestinal tract. The pathogenesis is multifactorial, involving genetic predisposition, environmental factors, epithelial barrier defects, and dysregulated immune responses [1–3].

Despite advances in endoscopic, histological, radiological, and serological diagnostics, distinguishing UC from CD often remains a significant challenge. Both entities share overlapping symptoms, genetic predispositions and immune mechanisms; however, they differ in the extent and distribution of the inflammation, histopathological features, and therapeutic approaches [1, 2, 4, 5]. Accurate differentiation is essential, as the diagnosis directly impacts disease management such as medication choice and surgical decision-making. Nevertheless, current serological markers including anti-*Saccharomyces cerevisiae* antibodies (ASCA) and perinuclear or atypical anti-neutrophil cytoplasmic antibodies (p/aANCA) lack sufficient sensitivity and specificity to reliably discriminate between these two diseases [6–20].

In recent years, anti-integrin αvβ6 has emerged as a potential biomarker to discriminate UC from CD [21, 22]. However, no universal cut-off value exists, as reported thresholds vary substantially depending on the assay platform, unit definition, and reference population [21, 23–29]. Using low cut-offs, UC sensitivities of approximately 75%–95% were consistently observed, whereas specificity against CD ranged from 65% to 80%, reflecting a relevant proportion of anti-integrin αvβ6-positive CD patients. Subsequent studies demonstrated that anti-integrin αvβ6 positivity in CD is not evenly distributed but is enriched in patients with colonic or UC-like CD phenotypes, while patients with ileal CD are predominantly negative [24–26, 30, 31]. Furthermore, a number of studies revealed that anti-integrin αvβ6 positivity correlated with disease activity [21, 23, 26, 30–32]; however, this correlation could not be replicated in other studies [22, 25].

Therefore, this study aimed to define a cut-off value of anti-integrin αvβ6 autoantibody levels, measured using a commercially available enzyme-linked immunosorbent assay (ELISA), with high accuracy for distinguishing UC from CD in a prospectively collected and rigorously characterized IBD patient cohort. Notably, this is the first study to compare the accuracy of anti-integrin αvβ6 levels to distinguish UC from CD with the conventional serological markers p/aANCA+/ASCA− in the same serum samples.

2 | Materials and Methods

2.1 | Study Design

The study is a prospective, cross-sectional, observational study aiming to investigate the prevalence of a/pANCA, ASCA, and anti-integrin αvβ6 positivity in UC and CD and to study whether anti-integrin αvβ6 improves the ability to discriminate UC from CD. Prospective data were collected using a patient questionnaire and via the assessment of blood samples taken at inclusion. Serum samples were stored until analysis at −80°C. Serum samples were analysed centrally (Centre of Laboratory Medicine, St. Gallen, Switzerland). Retrospective data were collected from electronic medical records. The data were recorded using a specifically designed browser-based secuTrial database.

The study protocol was approved by the Ethics Committee of Eastern Switzerland (project-ID 2023-00961).

2.2 | Study Population

Participants of this study were recruited at the IBD outpatient clinic of the Cantonal Hospital St. Gallen, Switzerland, and at the outpatient clinic Rorschach, Switzerland, during hospital visits scheduled for the application of a biologic therapy or for a follow-up consultation. All participants were at least 18 years old. Further inclusion criteria were the diagnoses of UC or CD established based on clinical, laboratory, endoscopic, and histopathological criteria [33].

Exclusion criteria included lack of written informed consent, indeterminate colitis, and age < 18 years.

Variables recorded for this study include participants' demographics (age, gender, ethnicity, education, BMI, smoking status, and comorbidities) and IBD-specific information such as disease type (UC or CD), disease extent (maximum since diagnosis) evaluated using the Montreal classification [34], clinical activity (at time of study visit) assessed with the PRO-2 questionnaire [35–38], C-reactive protein (CRP), white blood count (WBC; at the time of study visit), and faecal calprotectin (FCP; within 3 months of the study inclusion). Furthermore, data on extra-intestinal manifestations and medical treatment from time since diagnosis and at time of inclusion were collected. Detailed characteristics of the study participants can be found in Table 1.

TABLE 1 | Patients' baseline characteristics of the study cohort.

Characteristics	CD, $n = 118$	UC, $n = 106$	p-Value
Sex			0.457
Female	40 (34%)	41 (39%)	
Male	78 (66%)	65 (61%)	
Ethnicity			1.000
Asia	5 (4.2%)	4 (3.8%)	
Europe	109 (92%)	99 (93%)	
Other	4 (3.4%)	3 (2.8%)	
Smoking status			**0.002***
Current smoker	38 (32%)	13 (12%)	
Ex-smoker	43 (36%)	46 (43%)	
Non-smoker	37 (31%)	47 (44%)	
Age at sampling (years)	45.0 (31.0, 62.0)	41.5 (32.0, 57.0)	0.527
Age at onset (years)	26.0 (20.0, 39.0)	29.0 (22.0, 47.0)	0.262
Disease duration (years)	10.5 (5.0, 22.0)	8.0 (5.0, 16.0)	0.128
Montreal classification (UC)			
E1 proctitis		8 (7.5%)	
E2 left-sided		43 (41%)	
E3 extensive		55 (52%)	
Montreal location (CD)			
L1 ileal	38 (32%)		
L2 colonic	16 (14%)		
L3 ileocolonic	64 (54%)		
Montreal behaviour (CD)			
B1 non-stricturing	46 (39%)		
B2 stricturing	31 (26%)		
B3 penetrating	41 (35%)		
Perianal involvement (p)	30 (25%)		
Extraintestinal manifestations	37 (31%)	30 (28%)	0.618
PRO-2 category (UC)			
Remission (0)		52 (50%)	
Mild (1, 2)		40 (38%)	
Moderate (3, 4)		8 (7.6%)	
Severe (5, 6)		5 (4.8%)	
PRO-2 category (CD)			
Remission (< 8)	85 (72%)		
Mild (8–13)	18 (15%)		
Moderate (14–33)	14 (12%)		
Severe (34+)	1 (0.8%)		

(Continues)

TABLE 1 | (Continued)

Characteristics	CD, $n=118$	UC, $n=106$	p-Value
WBC (G/L)	7.1 (6.0, 8.9)	7.0 (5.9, 8.2)	0.771
CRP (mg/L)	2.0 (1.0, 5.0)	2.0 (1.0, 4.0)	0.477
Calprotectin (μg/g)	55.0 (20.0, 154.0)	49.0 (10.0, 254.0)	0.838
Calprotectin < 50 μg/g			0.746
< 50	57 (48%)	53 (50%)	
≥ 50	61 (52%)	52 (50%)	
Calprotectin < 100 μg/g			0.357
< 100	80 (68%)	65 (62%)	
≥ 100	38 (32%)	40 (38%)	
No treatment	6 (5.1%)	2 (1.9%)	0.286
5-ASA	16 (14%)	77 (73%)	**3.25×10^{-19}***
Corticosteroids	8 (6.8%)	19 (18%)	**0.011***
Immunomodulators	1 (0.8%)	2 (1.9%)	0.604
Anti-TNF therapy	72 (61%)	28 (26%)	**1.98×10^{-7}***
Non-anti-TNF biologics	35 (30%)	48 (45%)	**0.016***
JAK inhibitors	1 (0.8%)	4 (3.8%)	0.192
IBD-related surgery (incl. colectomy)	6 (5.1%)	6 (5.7%)	

Note: Continuous variables are reported as median (IQR); categorical variables as *n* (%). *p*-values are calculated using the Wilcoxon rank-sum test for continuous data and Fisher's exact test for categorical data. *Significant *p*-values ($p<0.05$) are highlighted in bold. Non-anti-TNF biologics: vedolizumab, ustekinumab.

2.3 | Study Outcomes

Our primary outcome was to determine a cut-off value of anti-integrin αvβ6 antibodies with an optimal accuracy to distinguish UC from CD. We further performed a direct comparison between this novel biomarker (anti-integrin αvβ6) and conventional serological markers (pANCA, aANCA, ASCA IgA, ASCA IgG). In addition, we evaluated the relationship between anti-integrin αvβ6 antibodies and disease activity as well as disease location. Multivariate logistic regression was used to assess the independent predictive value of anti-integrin αvβ6 for distinguishing UC from CD, adjusted for age, sex, smoking status, and disease duration.

2.4 | Serological Analysis

2.4.1 | Anti-Integrin αvβ6 Autoantibodies

Anti-integrin αvβ6 antibodies were quantified using a commercially available ELISA kit (MBL Medical & Biological Laboratories Co. Ltd., Tokyo, Japan). The ELISA was performed according to the manufacturer's instructions. Details on the methodology are presented in the Supporting Information.

2.4.2 | Anti-Neutrophil Cytoplasmic Antibodies

Anti-neutrophil cytoplasmic antibodies (ANCA) were detected using NOVA Lite ANCA indirect immunofluorescence assay slides (Inova Diagnostics, Carrollton, TX, USA). Ethanol- and formaldehyde-fixed human neutrophil granulocytes were incubated with patient serum using a PhD lx Workstation (Bio-Rad Laboratories, Cressier, Switzerland). Slides were evaluated using a fluorescence microscope (ZEISS Axio Scope A1, Carl Zeiss Microscopy GmbH, Jena, Germany). Two experienced laboratory technicians independently assessed the samples and classified results as 'negative', 'cANCA', 'pANCA', or 'atypical ANCA'.

Positive control samples for both cANCA and pANCA were included in each analytical batch. The cut-off titre for a positive result was set at 1:20 in accordance with the manufacturer's recommendations.

2.4.3 | Anti-*Saccharomyces cerevisiae* Antibodies

Anti-*Saccharomyces cerevisiae* antibodies (ASCA; IgG and IgA) were measured using the automated EliA Phadia 250 system (Thermo Fisher Scientific, Waltham, MA, USA). According to the manufacturer's recommendations, values < 7 U/mL were considered as negative, values > 10 U/mL as positive, and values between 7 and 10 U/mL as indeterminate.

2.4.4 | C-Reactive Protein

C-reactive protein (CRP) serum levels were quantified using a Beckman Coulter AU5800 analyser with a latex-enhanced

immunoturbidimetric assay (Beckman Coulter, Brea, CA, USA). An in-house-validated cut-off value of 8 mg/L was used to distinguish physiological from pathological levels.

2.4.5 | White Blood Cell Count

White blood cell (WBC) counts were determined using a Sysmex SX-1500 haematology analyser (Sysmex Corporation, Kobe, Japan) based on fluorescence flow cytometry and laser light scattering. A reference range of 4.0–10.0 G/L was considered physiological for adult patients.

2.4.6 | Faecal Calprotectin

Faecal calprotectin was extracted according to the manufacturer's instructions and quantified using a fully automated chemiluminescence immunoassay (CLIA) on a LIAISON XL instrument (DiaSorin, Saluggia, Italy) with the LIAISON Calprotectin assay. A cut-off value of 100 µg/g stool was used to differentiate physiological from pathological samples.

2.5 | Statistical Analysis

Continuous variables are reported as median (IQR) and compared using Wilcoxon rank-sum tests; categorical variables are reported as *n* (%) and compared using Fisher's exact tests. The diagnostic accuracy of anti-integrin αvβ6 was assessed by receiver operating characteristic (ROC) analysis, with the optimal cut-off determined by the Youden index. Spearman correlation coefficients were computed to evaluate the relationship between anti-integrin αvβ6 and classical serological markers (pANCA, aANCA, ASCA IgA, ASCA IgG) as well as systemic inflammatory markers (WBC, CRP, faecal calprotectin). Comparative diagnostic accuracy was evaluated by fitting logistic regression models for anti-integrin αvβ6 (continuous titre), conventional serology (ANCA+/ASCA−), and a combined model (conventional serology + anti-integrin αvβ6), and comparing the resulting ROC curves. AUCs are reported with 95% DeLong confidence intervals, and sensitivity/specificity at the Youden-optimal threshold with 95% bootstrap confidence intervals (2000 replicates). The anti-integrin αvβ6 positivity rates across UC and CD locations based on the Montreal classification [34] were compared using Fisher's exact tests. Patient-reported outcomes were assessed using the PRO-2 scoring system, with disease-specific symptom components: liquid stool frequency and abdominal pain for CD, and stool frequency and rectal bleeding for UC. Anti-integrin αvβ6 titre distributions across PRO-2 severity categories were compared using Wilcoxon rank-sum tests (for each subgroup; severe vs. others for CD stool frequency and abdominal pain; severe vs. others for UC liquid stool and moderate vs. others for UC rectal bleeding). Anti-integrin αvβ6 titres across more than two groups (CD Montreal locations and UC, and the PRO-2 severity categories) were compared with the Kruskal–Wallis test with Holm-corrected pairwise Wilcoxon post hoc comparisons (Supporting Information). Multivariate logistic regression was used to assess the independent association of anti-integrin αvβ6 with UC versus CD, adjusted for age, sex, smoking status, and disease duration. Model fit was compared using the Akaike Information Criterion (AIC) and the likelihood ratio test (LRT). All analyses were performed in R.

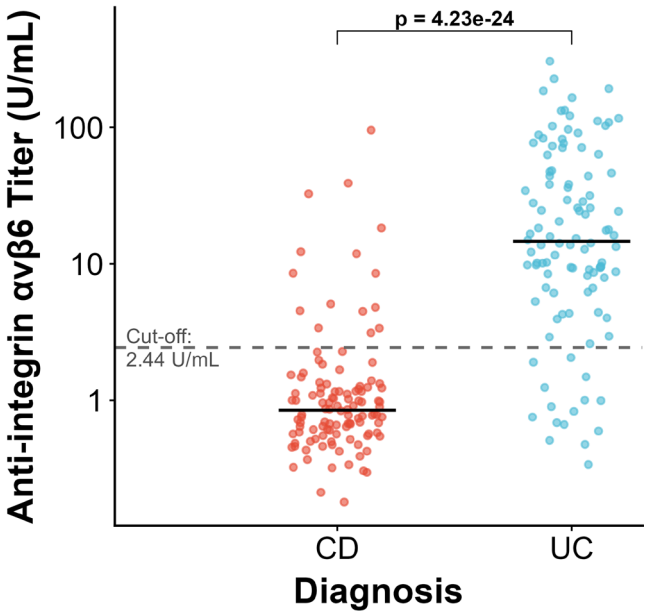

FIGURE 1 | Distribution of anti-integrin αvβ6 antibody titres in UC and CD. The dashed horizontal line indicates the Youden-optimized diagnostic cut-off (2.44 U/mL). *p*-values indicate significant separation between populations (Wilcoxon rank-sum test).

3 | Results

3.1 | Characteristics of Patients

Between January 8, 2023 and August 6, 2024, a total of 224 patients (UC: $n = 106$, CD: $n = 118$) were recruited. 52% of UC patients had pancolitis and 35% of CD patients had penetrating disease. Nearly one third of patients (UC: 28%, CD: 31%) had extraintestinal manifestations. 75% of patients with UC and 92% of patients with CD were on advanced therapies such as biologics or small molecules; however, most patients were in remission or had only mild disease activity on advanced therapy (patients with complete remission or only mild disease activity: UC: 88%, CD: 87%). As vedolizumab was more frequently used as a first-line biologic in UC than in CD, significant differences in the proportions of patients using anti-TNF therapy and non-anti-TNF biologics were observed between the two IBD subtypes. Furthermore, 5-aminosalicylic acids were rarely used in CD due to their limited efficacy. Detailed characteristics of the study population are summarized in Table 1.

3.2 | Anti-Integrin αvβ6 Autoantibodies Have a High Accuracy to Distinguish UC From CD

Anti-integrin αvβ6 titres were markedly higher in UC than in CD (median 14.6 vs. 0.85 U/mL, $p = 4.23 \times 10^{-24}$). ROC analysis demonstrated excellent diagnostic performance, with an AUC of 0.897 (CI: 0.853–0.942). At an optimal cut-off of 2.44 U/mL, sensitivity was 85.8%, specificity 87.1%, positive predictive value 85.8%, and negative predictive value 87.1% for distinguishing UC from CD (Figures 1 and 2).

3.3 | Anti-Integrin αvβ6 Autoantibodies Have a Better Accuracy Than the ANCA+/ASCA− Status to Distinguish UC From CD

Given that p/aANCA are established biomarkers of UC and ASCA are biomarkers of CD, we expected a positive correlation of anti-integrin αvβ6 antibodies with ANCAs and a negative correlation with ASCAs [10, 15, 16, 18]. Indeed, in our cohort, anti-integrin αvβ6 antibodies were positively associated with pANCA (ρ (rho) = 0.16, $p = 0.014$) and aANCA ($\rho = 0.25$, $p = 1.95 \times 10^{-4}$), and negatively associated with ASCA IgA ($\rho = -0.32$, $p = 9.14 \times 10^{-7}$) and ASCA IgG ($\rho = -0.30$, $p = 8.52 \times 10^{-6}$) (Figure 3). However, these trends were weak, likely reflecting the relatively low number of ANCA-positive patients in our cohort, which may be explained by the high proportion of UC patients in remission or with only mild disease activity (88%) [11, 39]. Notably, the continuous anti-integrin αvβ6 model demonstrated superior discriminatory performance for distinguishing UC from CD (AUC 0.897) compared with conventional ANCA/ASCA-based serological definitions (AUC 0.736 for p/aANCA+/ASCA IgG− and 0.704 for p/aANCA+/ASCA IgA-). Addition of anti-integrin αvβ6 autoantibodies to the p/aANCA+/ASCA IgG− model had almost the same AUC (0.902) as the anti-integrin αvβ6-only model (AUC: 0.897) but a lower accuracy than the anti-integrin αvβ6-only model (0.842 vs. 0.865), suggesting that addition of the conventional serology (p/aANCA+/ASCA−) to the anti-integrin αvβ6 model does not improve the accuracy of the anti-integrin αvβ6 model (Figure 4, Table 2).

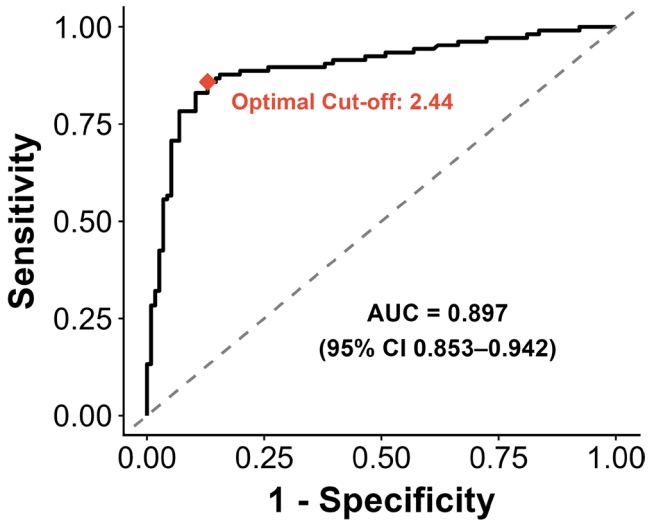

FIGURE 2 | The receiver operating characteristic (ROC) curve is quantifying the ability of anti-integrin αvβ6 titres to discriminate UC from CD. The optimal cut-off (Youden Index) and area under the curve (AUC) are annotated.

3.4 | Anti-Integrin αvβ6 Autoantibodies Are More Frequent in Colonic CD Than Ileal and Ileocolonic CD

Comparing anti-integrin αvβ6 titres across IBD location based on the Montreal classification [34] showed a significant difference between UC and L1 ileal CD ($p = 1.90 \times 10^{-13}$), a significant difference between UC and L3 ileocolonic CD ($p = 7.64 \times 10^{-17}$),

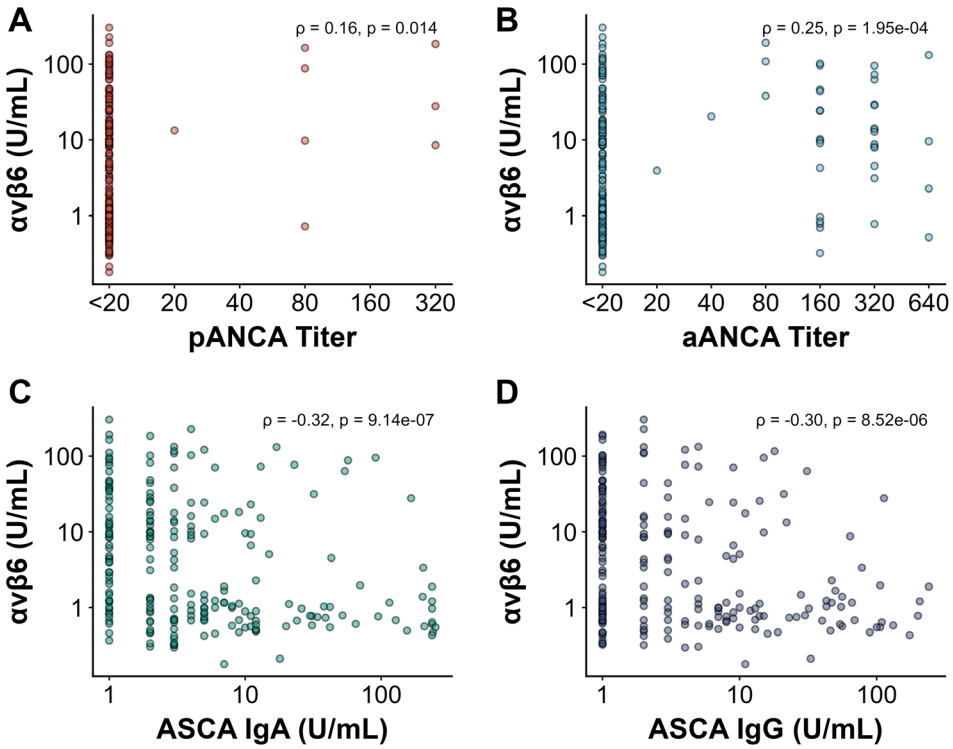

FIGURE 3 | Spearman correlation of log-transformed anti-integrin αvβ6 titres with classic serological markers: (A) pANCA, (B) aANCA, (C) ASCA IgA, and (D) ASCA IgG. Rho and p-values are derived from Spearman correlation tests. Shown are the correlation coefficient (Rho = ρ) and p-values (p).

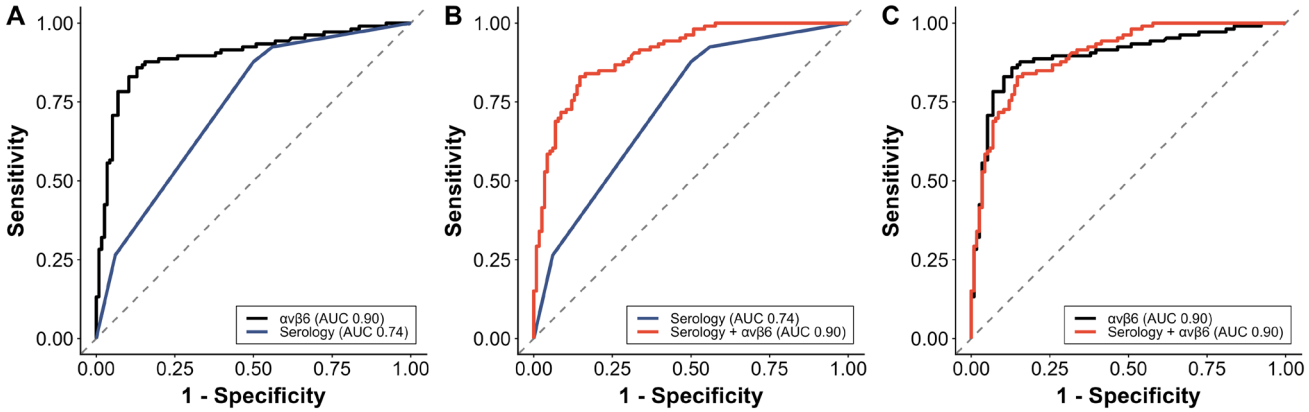

FIGURE 4 | Comparative diagnostic accuracy (ROC curves). (A) anti-integrin αvβ6+ vs. conventional serology (p/aANCA+/ASCA IgG−), compared by the DeLong test ($p = 7.04 \times 10^{-6}$). (B) Conventional serology (p/aANCA+/ASCA IgG−) vs. the combined model (conventional serology + anti-integrin αvβ6+), compared by a likelihood ratio test ($p = 7.60 \times 10^{-16}$). (C) Anti-integrin αvβ6+ vs. the combined model, compared by a likelihood ratio test ($p = 5.24 \times 10^{-6}$). Curve labels give the AUC; the corresponding 95% DeLong confidence intervals are as follows: Anti-integrin αvβ6+: 0.897 (0.853–0.942); conventional serology (p/aANCA+/ASCA IgG−): 0.736 (0.679–0.794); and combined (conventional serology + anti-integrin αvβ6+): 0.902 (0.863–0.941). Anti-integrin αvβ6+ is modelled as the continuous titre throughout.

TABLE 2 | Comparison of diagnostic accuracy metrics with 95% confidence intervals for the anti-integrin αvβ6+, conventional serology (p/aANCA+/ASCA IgG−), and combined models (p/aANCA+/ASCA IgG− and anti-integrin αvβ6+).

Model	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV
anti-integrin αvβ6+	0.897 (0.853–0.942)	0.858 (0.755–0.925)	0.871 (0.819–0.957)	0.865	0.858	0.871
p/aANCA+/ASCA IgG−	0.736 (0.679–0.794)	0.877 (0.830–0.962)	0.500 (0.379–0.586)	0.680	0.616	0.817
p/aANCA+/ASCA IgG− and anti-integrin αvβ6+	0.902 (0.863–0.941)	0.830 (0.717–0.906)	0.853 (0.784–0.948)	0.842	0.838	0.846

Note: AUC 95% CI by the DeLong method; sensitivity/specificity 95% CI by 2000 bootstrap replicates, at the Youden-optimal threshold.
PPV; positive predictive value, NPV; negative predictive value.

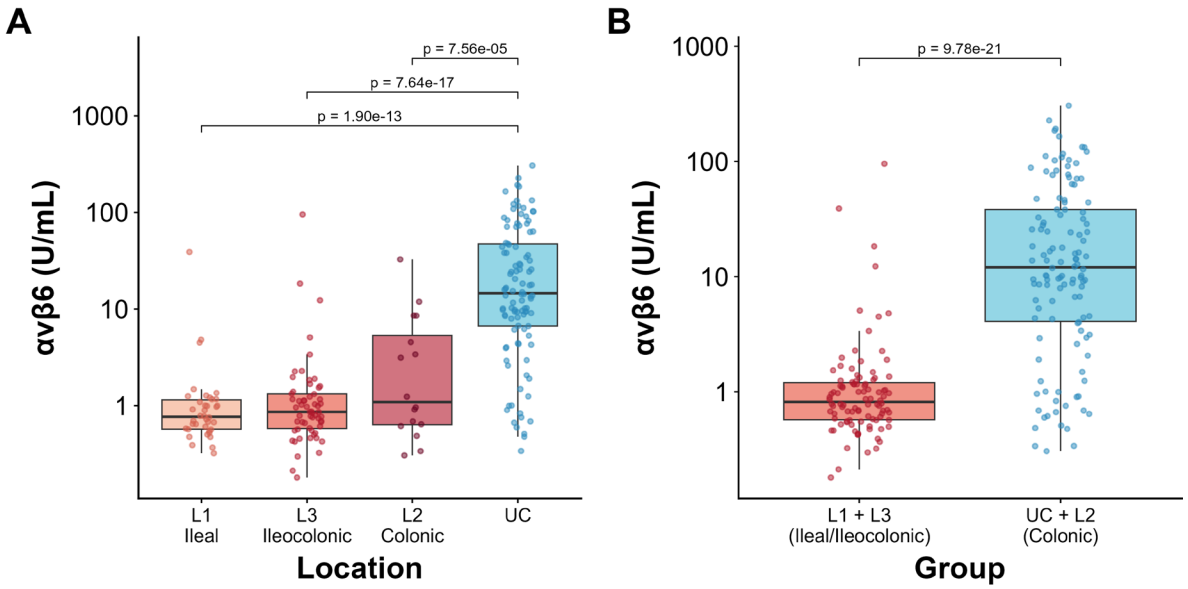

FIGURE 5 | (A) Anti-integrin αvβ6 titres across CD locations based on Montreal classification (L1–L3) and UC. (B) Grouped comparison: L1 ileal + L3 ileocolonic CD vs. UC + L2 colonic CD. Anti-integrin αvβ6 titres were compared with the Kruskal–Wallis omnibus test followed by pairwise Wilcoxon rank-sum (Mann–Whitney *U*) tests with Holm correction for multiple comparisons.

and a significant difference between UC and L2 colonic CD ($p = 7.56 \times 10^{-5}$). The grouped comparison—colonic (UC + L2) vs. ileal/ileocolonic (L1 + L3)—showed a significant difference ($p = 9.78 \times 10^{-21}$) (Figure 5, Table S3). Anti-Integrin αvβ6 positivity rates followed the same gradient (Fisher's exact test, $p = 2.04 \times 10^{-31}$; Figure 6).

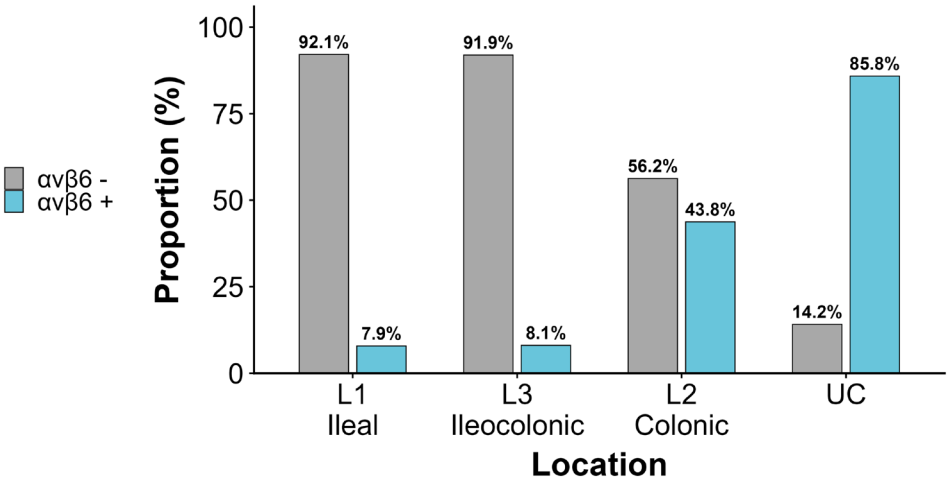

FIGURE 6 | Anti-integrin αvβ6 positivity rate by CD location based on Montreal classification (L1: ileal, L3: ileocolonic, L2: colonic) and UC. Groups were compared by Fisher's exact test. Differences between subgroups were analysed using Freeman–Halton extension (4×2 table) which tests whether anti-integrin αvβ6 positivity and disease location are independent. As shown in this figure, anti-integrin αvβ6 positivity is associated with colonic disease location ($p = 2.04 \times 10^{-31}$).

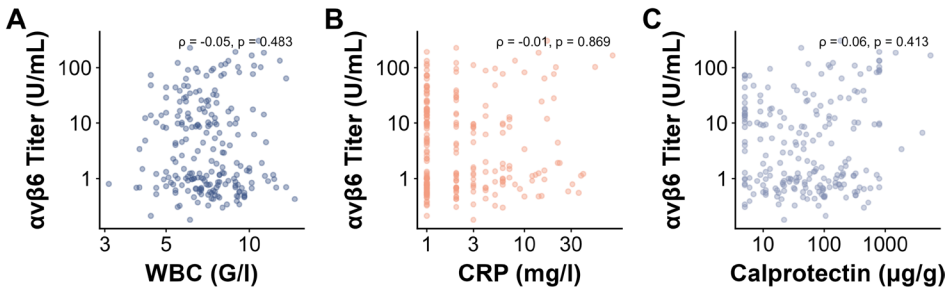

FIGURE 7 | Correlation of anti-integrin αvβ6 titre with inflammatory markers: (A) WBC, (B) CRP and (C) faecal calprotectin (µg/g). Rho and *p*-values are derived from Spearman correlation tests. Shown are the correlation coefficient (Rho = ρ) and *p*-values (p).

Disease behaviour in CD (stricturing, penetrating disease) was not associated with anti-integrin αvβ6 positivity (Table S2).

3.5 | Correlation of Anti-Integrin αvβ6 Autoantibody Positivity and Disease Activity

There were no significant correlations of anti-integrin αvβ6 with WBC (ρ (rho) = −0.05, $p = 0.483$), CRP ($\rho = -0.01$, $p = 0.869$), and faecal calprotectin ($\rho = 0.06$, $p = 0.413$) (Figure 7). However, this should be interpreted with caution, as most patients (88%) were in remission or had only mild disease activity.

In the exploratory PRO-2 analysis, no significant difference was observed in the comparison of anti-integrin αvβ6 with each subgroup (non vs. mild, mild vs. moderate, moderate vs. severe) (Figures 8 and 9). Furthermore, no association was observed for liquid stool frequency (severe vs. others: $p = 0.265$), while abdominal pain (severe vs. others) showed a significant relationship with anti-integrin αvβ6 positivity ($p = 0.012$) in CD (Table S4). In UC, stool frequency (severe vs. others: $p = 0.009$) and rectal bleeding (moderate vs. others: $p = 0.035$) were associated with anti-integrin αvβ6 positivity (Table S4).

In UC, there was a trend towards a higher prevalence of extraintestinal manifestations (EIM) in anti-integrin αvβ6-positive patients compared with anti-integrin αvβ6-negative patients (31% vs. 13%), whereas in CD, the occurrence of extraintestinal manifestations was similar regardless of anti-integrin αvβ6 status (33% vs. 32%) (Tables S1 and S2).

3.6 | Multivariable Modelling Confirmed That Anti-Integrin αvβ6 Positivity Was Independently Associated With UC

In multivariate logistic regression modelling, anti-integrin αvβ6 positivity was independently associated with UC (OR 36.18, 95% CI 17–83, $p = 4.86 \times 10^{-19}$). Given that the anti-integrin αvβ6-only and conventional-serology models are non-nested, their relative fit was compared by AIC (184.8 vs. 252.4). Adding anti-integrin αvβ6 to conventional serology (nested combined model) improved model fit by the likelihood ratio test ($p < 0.001$), and adding conventional serology to the anti-integrin αvβ6-only model also improved fit ($p < 0.001$) (Tables 3–5). However, as shown above, the addition of anti-integrin αvβ6 autoantibodies to the p/aANCA+/ASCA IgG- model had only a minimal effect on the AUC (0.902) compared to the anti-integrin αvβ6-only

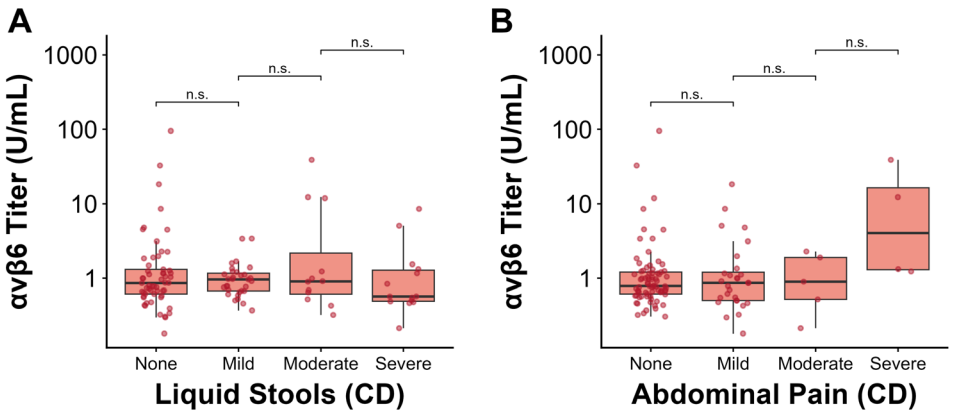

FIGURE 8 | Anti-integrin αvβ6 titre distribution stratified by symptomatic PRO-2 components (stool frequency and abdominal pain) for CD patients. Anti-integrin αvβ6 titres were compared with the Kruskal–Wallis omnibus test followed by pairwise Wilcoxon rank-sum (Mann–Whitney *U*) tests with Holm correction for multiple comparisons. While there were no significant differences in the comparison of the subgroups shown in this figure, comparison of the 'severe abdominal pain' group with the combined other subgroups demonstrated statistically significant differences. Details of the comparison are shown in Table S4.

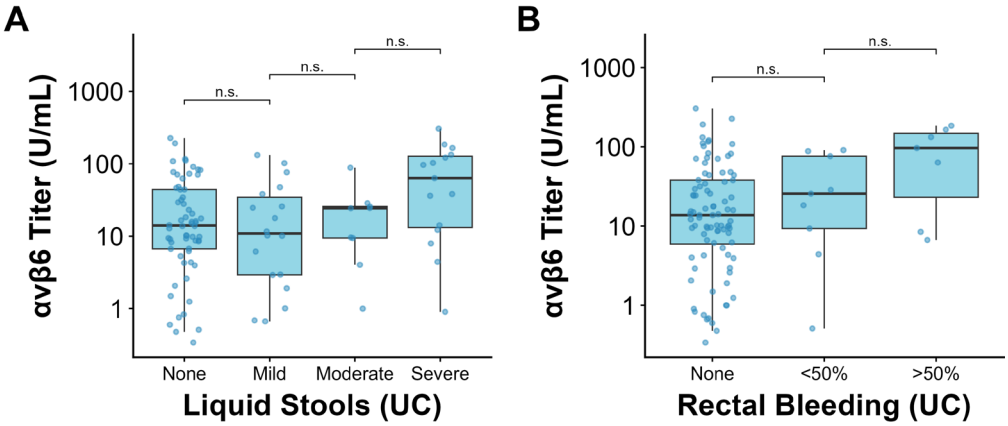

FIGURE 9 | Anti-integrin αvβ6 titre distribution stratified by symptomatic PRO-2 components (stool frequency and rectal bleeding) for UC patients. Avβ6 titres were compared with the Kruskal–Wallis omnibus test followed by pairwise Wilcoxon rank-sum (Mann–Whitney *U*) tests with Holm correction for multiple comparisons. While there were no significant differences in the comparison of the subgroups shown in this figure, comparison of the 'severe liquid stool' group and the 'severe rectal bleeding' group with the combined other subgroups demonstrated statistical significant differences. Details of the comparison are shown in Table S4.

TABLE 3 | Model 1: Multivariate logistic regression predicting the IBD type (UC vs. CD).

Characteristics	OR	95% CI	*p*-value
Anti-integrin αvβ6 > 2.44	**36.2**	17.0, 83.0	**4.86×10^{-19}***
Sex (female)	0.78	0.34, 1.80	0.557
Age (sample)	1.01	0.98, 1.03	0.715
Smoking			
Current smoker	—	—	
Ex-smoker	2.55	0.86, 7.84	0.095
Non-smoker	2.04	0.70, 5.99	0.191
Duration (years)	0.97	0.93, 1.01	0.152

Note: Model includes anti-integrin αvβ6 status adjusted for age, sex, smoking, and disease duration. *$p < 0.05$. Significant *p*-values are highlighted in bold letters.
Abbreviations: CI, confidence interval; OR, odds ratio.

model (AUC: 0.897), and the accuracy in the combined model was even lower than that of the anti-integrin αvβ6-only model (0.842 vs. 0.865).

4 | Discussion

Anti-integrin αvβ6 autoantibodies are promising serological biomarkers in IBD, particularly in UC, with evidence supporting their utility in early disease detection [40, 41], the discrimination of CD and their different disease phenotypes from UC, and even risk stratification [21, 23, 25, 26, 30–32].

Notably, their diagnostic accuracy in distinguishing UC from CD appears to exceed that of conventional serological markers such as ASCA and ANCA [21, 26].

However, to our best knowledge, this is the first study which prospectively compared the accuracy of anti-integrin αvβ6 with

TABLE 4 | Model 2: Multivariate logistic regression predicting the IBD type (UC vs. CD) using classic serology definitions (ANCA and ASCA).

Characteristics	OR	95% CI	*p*-value
p/aANCA+	**3.78**	1.71, 8.83	**0.001***
ASCA IgG+	**0.12**	0.06, 0.26	**7.07×10^{-8}***
Sex (female)	0.80	0.41, 1.56	0.509
Age (sample)	0.99	0.97, 1.01	0.505
Smoking			
Current smoker	—	—	
Ex-smoker	4.31	1.82, 10.7	**0.001***
Non-smoker	4.02	1.74, 9.73	**0.001***
Duration (years)	0.96	0.93, 1.0	**0.024***

Note: Model adjusted for the same demographic and treatment covariates as Model 1 in Table 3. *$p < 0.05$. Significant *p*-values are highlighted in bold letters. Abbreviations: CI, confidence interval; OR, odds ratio.

TABLE 5 | Comparison of model fit.

Model	AIC	LRT *p*-value (vs. combined)
αvβ6 (+ covariates)	184.8	**7.046×10^{-4}***
Serology (+ covariates)	252.4	**3.566×10^{-19}***
Serology + αvβ6 (+ covariates)	174.3	— (reference)

Note: AIC compares all three (non-nested) models; the likelihood ratio test (LRT) tests each simpler model against the nested combined model (conventional serology + anti-integrin αvβ6). The anti-integrin αvβ6-only vs. conventional serology-only comparison is non-nested and is therefore assessed by AIC only. 'Serology' represents the conventional serology (ANCA+/ASCA−). 'αvβ6' represents positivity for anti-integrin αvβ6. *$p < 0.05$. Significant *p*-values are highlighted in bold letters.

that of the conventional serological markers p/aANCA+/ASCA− to distinguish UC from CD using the same serum samples.

First, we demonstrate, that anti-integrin αvβ6 autoantibodies have a very high accuracy to distinguish UC from CD achieving a sensitivity, specificity, PPV, and NPV of more than 85% with an optimal cut-off value of 2.44 U/mL. This cut-off value should be considered as a candidate threshold and should be confirmed in further replication studies. However, the cut-off is in line with other cohorts, with reported sensitivities ranging from approximately 79% to 94% and specificities from 82% to 95% across diverse populations, albeit with variability depending on cohort characteristics and disease phenotypes [24, 26, 30, 31]. Across studies, the diagnostic threshold for anti-integrin αvβ6 autoantibodies has varied substantially depending on assay methodology and comparator group, with many early studies applying predefined cut-offs derived from healthy controls (e.g., mean + 3 standard deviations or the 95th percentile) for discrimination of UC from both CD and non-IBD controls [21, 23–25], whereas more recent analyses have employed ROC-based thresholds [30, 31], typically ranging from ~1.1 to 2.1 U/mL for UC versus CD discrimination and ~1.6–1.7 U/mL for UC versus non-UC comparisons. Higher cut-offs (e.g., 13 U/mL) were proposed to maximize specificity in clinical settings [31]. These values are similar to our cut-off value of 2.44 U/mL, which was determined by the Youden index. A Swedish cohort used a different test (in-house fluorescence enzyme immunoassay based on EliA technology); therefore, the result of this study cannot be compared directly with our result [26].

Second, we showed that anti-integrin αvβ6 autoantibodies have a better accuracy than the ANCA+/ASCA− status to distinguish UC from CD. The AUC of anti-integrin αvβ6 (0.897) to distinguish UC from CD was superior to that of p/aANCA+/ASCA IgG− (0.736) and p/aANCA+/ASCA IgA− (0.704), respectively. Addition of anti-integrin αvβ6 autoantibodies to the conventional p/aANCA+/ASCA IgG− model had a very similar AUC (0.902) but a lower accuracy (0.842) than anti-integrin αvβ6 antibodies only (accuracy: 0.865). These findings align with growing evidence indicating that anti-integrin αvβ6 autoantibodies outperform conventional serological markers such as ASCA and p/aANCA in diagnostic accuracy; however, prior data have largely relied on cross-study comparisons rather than direct head-to-head analyses within the same patient cohorts [21, 26]. Notably, the present study provides the first direct evidence demonstrating the superiority of anti-integrin αvβ6 over combined p/aANCA+/ASCA IgG− or IgA− profiles through parallel assessment in identical serum samples. Using the same serum sample for such comparisons is important, given that a very recent analysis demonstrated that anti-integrin αvβ6 antibody levels may change over time [42]. In that study, a significant drop of anti-integrin αvβ6 antibody levels ('serological remission') predicted long-term clinical remission of UC [42].

Third, we expected higher anti-integrin αvβ6 seropositivity in UC and colonic CD, since anti-integrin αvβ6 antibodies are upregulated in injured intestinal epithelium and contribute to TGF-β activation during mucosal repair, a pathway particularly relevant in chronic colonic epithelial inflammation [21, 40]. Indeed, we found that anti-integrin αvβ6 autoantibodies are more frequent in UC and colonic CD than in ileal and ileocolonic CD ($p = 9.78\times10^{-21}$). Anti-integrin αvβ6 positivity rates (L1 (ileal CD): 7.9%, L3 (ileocolonic CD): 8.1%, L2 (colonic CD): 43.8%, UC 85.8%) followed the same gradient towards the highest colonic involvement. Positive anti-integrin αvβ6 reactivity in CD appears to be closely linked to colonic disease location. Several studies have consistently demonstrated higher antibody levels and positivity rates in colonic CD compared to ileal CD, with some cohorts reporting absent reactivity in isolated ileal disease [26, 27]. This pattern is likely explained by the preferential expression of anti-integrin αvβ6 in colonic, but not ileal epithelium, resulting in limited antigen exposure in ileal disease [43]. In addition, colonic CD has been proposed to represent a phenotype that is genetically and immunologically fairly similar to UC, and is frequently characterized by UC-like mucosal inflammation [30, 44–46]. Accordingly, anti-integrin αvβ6 positivity in CD has been associated with colonic involvement and UC-like endoscopic and histological features, suggesting that these so-called false-positive results may instead reflect a shared colonic inflammatory pathway rather than true diagnostic misclassification. Despite the high sensitivity and specificity of anti-integrin αvβ6 for distinguishing UC from CD, the relatively high positivity rate observed in patients with colonic CD

(43.8%) limits its diagnostic utility for differentiating colonic CD from UC. This finding is consistent with preliminary data from other groups, currently available only in abstract form, demonstrating substantial anti-integrin αvβ6 expression in colonic CD [47]. Furthermore, it is well recognized that colonic CD shares several clinical and pathological features with UC [46]. Our findings therefore add to the growing body of immunological and genetic evidence suggesting that IBD comprises a spectrum of distinct disease entities and not a binary classification of IBD into UC and CD only [42, 48].

Fourth, we examined the correlation of anti-integrin αvβ6 autoantibody positivity and disease activity. In this study cohort, there was no significant correlation of anti-integrin αvβ6 autoantibody positivity with WBC, CRP, and faecal calprotectin levels. This may, in part, be attributable to the high proportion of patients in remission or with only mild disease activity under advanced therapy (88% in UC and 87% in CD). Notably, with faecal calprotectin measurements available in almost all patients, our study provides one of the most comprehensive data sets reported to date. In the exploratory PRO-2 analysis, there was a trend of anti-integrin αvβ6 titres correlating with abdominal pain in CD, as well as stool frequency and rectal bleeding in UC. Given the small subgroup sizes and the multiple comparisons performed, the findings of the PRO-2 analyses should be considered preliminary and exploratory and require confirmation in larger, prospective follow-up studies. Correlation analyses across other studies indicate that anti-integrin αvβ6 autoantibody levels show at most modest associations with inflammatory markers, with Rydell et al. [23] demonstrating a correlation with CRP ($r = 0.467$, $p < 0.001$) and faecal calprotectin ($r = 0.342$, $p = 0.0175$) in UC but not in CD. In contrast, other studies reported no or only weak correlations with faecal calprotectin and no association with systemic inflammatory markers, including CRP [25, 26, 30, 31]. Although patient-reported outcome measures, including PRO-2 components, have been collected in recent studies [26], their association with anti-integrin αvβ6 autoantibody levels has not been extensively explored. In the present study, a trend towards a higher prevalence of extraintestinal manifestations was observed in anti-integrin αvβ6-positive patients with UC, whereas no association was evident in CD. As only a single study has reported extraintestinal manifestations descriptively to date [26], this potential association remains insufficiently characterized and may be of interest for further investigation. Taken together, these findings suggest that anti-integrin αvβ6 reflects disease-specific immune and epithelial processes rather than general inflammatory burden or symptom severity, highlighting an important area for future research.

Fifth, in multivariate logistic regression, anti-integrin αvβ6 positivity was independently associated with UC (OR 36.18, 95% CI 17–83, $p = 4.86 \times 10^{-19}$) and clearly outperformed conventional serological tests. Notably, studies performing multivariate analyses on αvβ6 expression remain scarce, with only two studies [26, 30] applying regression modelling to account for potential confounders, highlighting an important gap in the current evidence base.

This study has several limitations. Current endoscopic activity data were not available, and the data were collected at only two centres. In addition, no validated independent control cohort was included. Furthermore, cases with indeterminate colitis were excluded from this study. However, indeterminate colitis accounts only for about 6% of adult cases of IBD [49], therefore requiring very large patient cohorts to analyse the value of biomarkers for re-classifying cases with indeterminate colitis into UC or CD. For example, assuming an estimated prevalence of indeterminate colitis of 6% within an adult IBD cohort [49], approximately 2000 patients with IBD would be required to identify a cohort of patients with indeterminate colitis comparable in size to the 118 patients with CD included in the present study. Similar to our study, the very recently published IBDome project, one of the largest projects to define UC and CD using large-scale omic datasets, excluded patients with indeterminate colitis from its analyses to enable clear disease classification within the predictive models developed in that study [48]. However, the prospective design, the very well-characterized cohort with severe cases from a tertiary hospital, and the direct comparison with the ASCA/ANCA status in the same serum sample strengthen the findings and support the relevance of this study in improving diagnostic accuracy for distinguishing UC from CD.

Finally, B cells are increasingly recognized as contributors to the pathogenesis of IBD and are potential therapeutic targets, particularly in very treatment-refractory UC patients. Our study, highlighting the excellent accuracy of anti-integrin αvβ6 autoantibodies to differentiate UC from CD, adds to the growing body of evidence of the important role of B cells in the pathogenesis of UC, resulting in novel treatment options [45–59]. Furthermore, preclinical IBD detection initiatives such as INTERCEPT hypothesize that anti-integrin αvβ6 autoantibodies may emerge not only as clinically relevant diagnostic biomarkers, but also as components of future biomarker panels for preclinical UC detection, with the ultimate goal of identifying and treating high-risk individuals early to prevent intestinal damage [60]. Preliminary data indicate that anti-integrin αvβ6 autoantibodies have the potential to predict the clinical diagnosis of UC up to 10 years before disease manifestation [40].

In summary, anti-integrin αvβ6 autoantibodies represent a novel biomarker with commercially available testing which has superior accuracy for distinguishing UC from CD compared with conventional serological assays such as ANCA and ASCA tests.

Author Contributions

Joel Dütschler: writing – review and editing, investigation. **Jakob Heimer:** software, data curation, methodology, formal analysis, validation, visualization, writing – review and editing. **Regine Garcia Boy:** investigation, validation, methodology, writing – review and editing, data curation. **Christina Lins:** writing – review and editing, investigation. **Marius König:** investigation, writing – review and editing, validation. **Claudia Krieger:** investigation, writing – review and editing. **Peter Schönenberger:** writing – review and editing, software, data curation, methodology, formal analysis. **David Waber:** investigation, methodology, validation, writing – review and editing, data curation, conceptualization. **Seraina Koller:** investigation, project administration, writing – review and editing. **Nicola Fabian Frei:** writing – review and editing, investigation. **Matthias Friedrich:** writing – review and editing, resources, funding acquisition, supervision, methodology, validation. **Stephan Brand:** conceptualization, methodology, writing – original draft, writing – review and editing, funding

acquisition, validation, supervision, resources, project administration, investigation. **Alfred Mahr:** conceptualization, writing – review and editing, methodology, resources, supervision, funding acquisition. **Simon Woelfel:** writing – review and editing, resources, supervision, funding acquisition, methodology, validation. **Samuel Truniger:** conceptualization, investigation, validation, supervision, formal analysis, writing – original draft, writing – review and editing, methodology, data curation, project administration, visualization, funding acquisition. **Isabelle Kamm:** writing – review and editing, investigation, methodology, validation, data curation.

Funding

This work was supported by Wellcome Trust (225928/Z/22/Z, 222426/Z/21/Z), Crohn's and Colitis UK (M2021/2), Lee Placito Medical Fund, Medical Research Council (MR/W025981/1, MR/T030410/1), Oxford Colon Cancer Trust (Octopus) and HOCH Health Ostschweiz, Cantonal Hospital St. Gallen. The funders had no role in the study's design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

Conflicts of Interest

S.T. received funding from the Cantonal Hospital St. Gallen and participated on advisory boards of Takeda, BMS, Lilly, Johnson & Johnson, Pfizer, and Medinova. S.B. received speaker's honoraria from Abbvie, Falk Foundation, Ferring, Johnson & Johnson, Lilly, MSD, Takeda, UCB, and Vifor and participated on advisory boards of Abbvie, BMS, Celgene, Janssen, Johnson & Johnson, MSD, Pfizer, Pierre Fabre, Roche, Takeda, and UCB. S.B. has received an educational grant from Takeda. J.D. received funding from the Cantonal Hospital St. Gallen. M.F. received support by the Wellcome Trust (225928/Z/22/Z and 222426/Z/21/Z), Crohn's and Colitis UK (M2021/2), the Lee Placito Medical Fund, the Medical Research Council (MR/W025981/1 and MR/T030410/1), and the Oxford Colon Cancer Trust (Octopus). He received speaker and/or consultancy fees from Lilly, UCB Pharma, Quell Therapeutics, Ono Pharma, Polaroid Therapeutics and received research funding from Lilly and UCB. The other authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Correlation of αvβ6 status (> 2.44 U/mL) with phenotype and disease activity markers for UC patients. EIM extraintestinal manifestations. **Table S2:** Correlation of αvβ6 status (> 2.44 U/mL) with phenotype and disease activity markers for CD patients. EIM extraintestinal manifestations. **Table S3:** Comparison of αvβ6 titres across CD Montreal locations and UC. Kruskal–Wallis omnibus test followed by pairwise Wilcoxon rank-sum (Mann–Whitney *U*) tests with Holm correction for multiple comparisons. **Table S4:** Comparison of αvβ6 titres across PRO-2 severity categories. αvβ6 titres were compared with the Kruskal–Wallis omnibus test followed by pairwise Wilcoxon rank-sum (Mann–Whitney *U*) tests with Holm correction for multiple comparisons. **Table S5:** Comparison of αvβ6 titres across PRO-2 severity categories in CD. Kruskal–Wallis omnibus test followed by pairwise Wilcoxon rank-sum (Mann–Whitney *U*) tests with Holm correction for multiple comparisons. **Table S6:** Comparison of αvβ6 titres across PRO-2 severity categories in UC. Kruskal–Wallis omnibus test followed by pairwise Wilcoxon rank-sum (Mann–Whitney *U*) tests with Holm correction for multiple comparisons.