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Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at <https://dx.doi.org/10.1053/j.gastro.2022.12.042>.

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Conflicts of interest

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Anti-Integrin $\alpha v \beta 6$ Autoantibodies Are a Novel Biomarker That Antedate Ulcerative Colitis

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Abstract

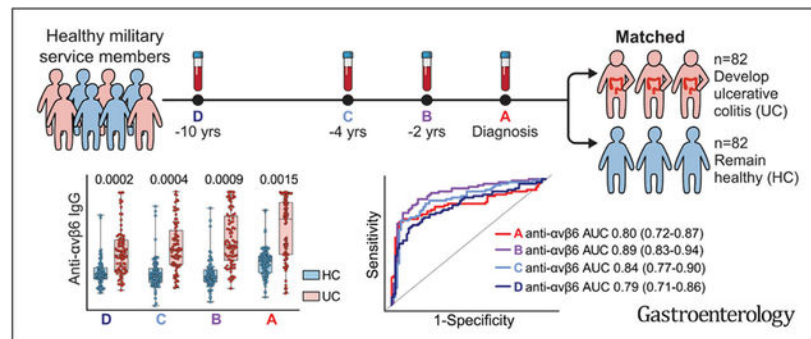
BACKGROUND & AIMS: Better biomarkers for prediction of ulcerative colitis (UC) development and prognostication are needed. Anti-integrin $\alpha v\beta 6$ (anti- $\alpha v\beta 6$) autoantibodies have been described in patients with UC. We tested for the presence of anti- $\alpha v\beta 6$ antibodies in the preclinical phase of UC and studied their association with disease-related outcomes after diagnosis.

METHODS: Anti- $\alpha v\beta 6$ autoantibodies were measured in 4 longitudinal serum samples collected from 82 subjects who later developed UC and 82 matched controls from a Department of Defense preclinical cohort (PREDICTS [Proteomic Evaluation and Discovery in an IBD Cohort of Tri-service Subjects]). In a distinct, external validation cohort (Crohn's and Colitis Canada Genetic Environmental Microbial project cohort), we tested 12 pre-UC subjects and 49 matched controls. Furthermore, anti- $\alpha v\beta 6$ autoantibodies were measured in 2 incident UC cohorts (COMPASS [Comprehensive Care for the Recently Diagnosed IBD Patients], $n = 55$ and OSCCAR [Ocean State Crohn's and Colitis Area Registry], $n = 104$) and associations between anti- $\alpha v\beta 6$ autoantibodies and UC-related outcomes were defined using Cox proportional hazards model.

RESULTS: Anti- $\alpha v\beta 6$ autoantibodies were significantly higher among individuals who developed UC compared with controls up to 10 years before diagnosis in PREDICTS. The anti- $\alpha v\beta 6$ autoantibody seropositivity was 12.2% 10 years before diagnosis and increased to 52.4% at the time of diagnosis in subjects who developed UC compared with 2.7% in controls across the 4 time points. Anti- $\alpha v\beta 6$ autoantibodies predicted UC development with an area under the curve of at least 0.8 up to 10 years before diagnosis. The presence of anti- $\alpha v\beta 6$ autoantibodies in preclinical UC samples was validated in the GEM cohort. Finally, high anti- $\alpha v\beta 6$ autoantibodies was associated with a composite of adverse UC outcomes, including hospitalization, disease extension, colectomy, systemic steroid use, and/or escalation to biologic therapy in recently diagnosed UC.

CONCLUSIONS: Anti-integrin $\alpha v\beta 6$ autoantibodies precede the clinical diagnosis of UC by up to 10 years and are associated with adverse UC-related outcomes.

Graphical Abstract



Keywords

Inflammatory Bowel Disease; Ulcerative Colitis; Anti-Integrin $\alpha v\beta 6$; Autoantibodies; Biomarkers

Inflammatory bowel diseases (IBDs), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory disorders that primarily affect the gastrointestinal tract with shared genetic and environmental risk factors.¹⁻³ Analogous to other immune-mediated diseases, there is an increasing appreciation that IBD may be preceded by subclinical immune perturbations,⁴⁻⁶ and there is a growing interest in biomarkers that can predict the occurrence of IBD.⁷ In addition, identification of biomarkers to predict disease course is a major unmet need due to the considerable variability of IBD disease progression.⁷ In this context, noninvasive blood-based biomarkers are appealing because of their ease of collection and application in clinical practice. The identification of predictive biomarkers has been more successful in CD,^{4,5,8,9} with some being used to risk stratify patients in clinical trials.¹⁰ In contrast, discovery of predictive biomarkers in UC remains elusive.

Recent studies have highlighted a novel autoantibody against integrin $\alpha v\beta 6$ in the serum of patients diagnosed with UC.¹¹⁻¹³ In a study from Japan, anti-integrin $\alpha v\beta 6$ (anti- $\alpha v\beta 6$) autoantibodies had a sensitivity of 92.0% and a specificity of 94.8% for diagnosing UC in adult patients compared with non-IBD subjects.¹¹ These results were further confirmed in a Swedish cohort and extended to a Japanese pediatric population.^{12,13} Integrin $\alpha v\beta 6$ is an epithelium-associated heterodimer that interacts with the extracellular matrix¹⁴ and enables activation of latent transforming growth factor- β , which is thought to be its primary role in vivo.^{15,16} Accordingly, critical homeostatic roles, such as maintenance of epithelial barrier integrity and suppression of epithelial inflammation, have been ascribed to integrin $\alpha v\beta 6$.¹⁷⁻¹⁹

Recognizing that loss of epithelial barrier integrity is a major and perhaps early feature of disease pathogenesis,²⁰ we hypothesized that onset of UC may be preceded by the appearance of anti- $\alpha v\beta 6$ autoantibodies and, thus, may serve as a preclinical biomarker. Furthermore, due to the important physiological role of integrin $\alpha v\beta 6$, we explored the performance of anti- $\alpha v\beta 6$ autoantibodies to predict adverse clinical outcomes in recently diagnosed UC.

Our study leverages 2 unique preclinical cohorts of UC. In our primary preclinical cohort—PREDICTS (Proteomic Evaluation and Discovery in an IBD Cohort of Tri-service Subjects)—longitudinal serum samples were obtained up to 10 years before disease diagnosis. The Crohn's and Colitis Canada Genetic Environmental Microbial (CCC-GEM) project served as an independent external validation preclinical cohort. Finally, we also studied 2 well-characterized incident IBD cohorts to better understand the association between anti- $\alpha v\beta 6$ autoantibodies and UC-related disease outcomes.

Methods

Clinical Cohorts

Proteomic Evaluation and Discovery in an Inflammatory Bowel Disease Cohort of Tri-service Subjects cohort, primary preclinical cohort.—We studied longitudinal serum samples from 82 individuals who eventually developed UC and 82 healthy controls (HCs) matched by age, sex, and race from the PREDICTS cohort, which was created in collaboration with the Department of Defense Serum Repository.²¹ This study was approved by the Institutional Review Board of the Naval Medical Research Center, Silver Spring, MD (NMRC.2014.0019) in compliance with all federal regulations governing the protection of human volunteers, and this research was performed under a Cooperative Research and Development Agreement (NMR 17-10209). Incident cases of UC between 1998 and 2013 within the Defense Medical Surveillance System were identified by having 2 or more medical encounters with the *International Classification of Diseases, Ninth Revision, Clinical Modification* (ICD-9-CM)²² code for UC (556 [including all subgroup codes] and linked to the Department of Defense Serum Repository). For each subject, 4 serum samples were retrieved: 1 from the time of diagnosis (± 1 year) (sample A) and 3 other samples preceding diagnosis (samples B, C, and D). HCs were required to have no medical encounter with evidence of IBD, rheumatoid arthritis, celiac disease, or colon cancer (based on ICD-9-CM codes) and available serum at the time of sample A for subjects with UC (± 1 year). Controls were matched on the basis of age, sex, and race. Sample B was at a median of -2.01 years before diagnosis (sample A) for UC and -2.03 years (before sample A) for HC; sample C was at a median of -4.06 years for UC and -3.99 years for HC; and sample D, the earliest available sample, was at a median of -10.03 years for UC and -10.52 years for HC. Measurement of anti-integrin $\alpha v \beta 6$ IgG autoantibodies and total IgG was performed with blinding to diagnosis and sample time point. From sample D, 1 serum sample from an HC subject was excluded due to a discrepancy in serum identification discovered after unblinding. ICD-9-CM codes, considering the code representing the highest disease extent, were used to determine the disease extent of the patients with UC according to the Montreal classification.^{4,9,23} Specifically, the extent of disease was defined by the following 3 subgroups: E1, proctitis only (disease limited to the rectum); E2, left-sided colitis (disease distal to the splenic flexure); and E3, extensive colitis (disease beyond the splenic flexure).²³

Crohn's and Colitis Canada Genetic Environmental Microbial project cohort, validation preclinical cohort.—We evaluated 61 sera samples that were collected as part of the prospective CCC-GEM Project. As described else-where,^{5,24} this is a prospective study that recruited asymptomatic first-degree relatives of patients with CD between 2008 and 2017. Subjects were between the age of 6 and 35 years at the time of recruitment. The study was approved by the Mount Sinai Hospital Research Ethics Board (Toronto Managing Center) and local recruitment centers. All subjects were contacted every 6 months via telephone and if a subject reported being diagnosed with UC, this was confirmed by their treating physician on the basis of clinical, endoscopic, radiographic, and/or histologic reports. The present study represents a nested case-control within the CCC-GEM, including 12 individuals who developed UC (cases) matched to at least 4 controls per subject by

age, sex, geographic location (using postal and country codes), and follow-up duration. As with the PREDICTS cohort samples, all samples from the GEM cohort were blinded during sample testing.

Comprehensive Care for the Recently Diagnosed Inflammatory Bowel Disease

Patients cohort.—The Comprehensive Care for the Recently Diagnosed IBD Patients (COMPASS) cohort is a prospective cohort of recently diagnosed patients with IBD who are enrolled in a registry at The Mount Sinai Hospital (New York, NY) and approved by the Institutional Review Board (STUDY-17-01304). All patients are enrolled within 18 months of diagnosis and disease diagnosis was confirmed on the basis of standard criteria.²⁵ Baseline demographic and clinical variables were obtained via patient questionnaires and standardized data abstraction from the medical record, including age; sex; race and ethnicity; baseline medications; laboratory data, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR); and disease extent (following the Montreal classification²³). Outcomes were determined by means of chart review by a gastroenterologist to determine whether the patient had IBD-related hospitalization, disease extension (defined as E1 disease extending to either E2 or E3, or E2 disease extending to E3 disease), IBD-related surgery, systemic steroid use (defined as oral prednisone or intravenous steroid formulations), or required biologic therapy. Serum samples from the time of enrollment were available on 55 patients with UC and were used in this study. Non-IBD control subjects were defined as subjects without an IBD diagnosis who were seen at The Mount Sinai Hospital gastroenterology clinic for endoscopy and had serum or plasma samples available for testing.

Ocean State Crohn's and Colitis Area Registry cohort.—The Ocean State Crohn's and Colitis Area Registry (OSCCAR) is a community-based prospective inception cohort established in the state of Rhode Island (Sands Med Health Rhode Island), as described previously.^{26,27} Enrollment was between January 2008 and January 2018. Patients were included if they had a new diagnosis of IBD confirmed by means of endoscopic, pathologic, or radiographic findings according to the criteria of the National Institute of Diabetes and Digestive and Kidney Diseases IBD Genetics Consortium² and were a resident of Rhode Island at the time of diagnosis. Patients were excluded if they had received a diagnosis of IBD previously or were unwilling to provide informed consent. Baseline and longitudinal data, including medication prescriptions, IBD-related hospitalizations, IBD-related surgery, and endoscopic disease extent (based on the Montreal classification²³), were collected by means of annual structured interview, self-completion of validated questionnaires, and standardized central data abstraction from the medical record. In addition, select laboratory data were available, including CRP and ESR, as well as perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) at baseline. pANCA testing was performed at Prometheus Laboratories (San Diego, CA). In this cohort, there were 151 patients with UC. Two patients were eliminated because they did not have follow-up data and 1 additional patient was removed because diagnosis was more than 6 months from enrollment. Therefore, analysis was performed on the remaining 148 samples. Of these 148 subjects, 143 had pANCA data to analyze and 104 had serum available for measurement of anti-integrin $\alpha v \beta 6$ IgG autoantibodies.

Enzyme-Linked Immunosorbent Assay for Measurement of Total IgG and Anti-Integrin $\alpha v\beta 6$ IgG Autoantibodies

Total serum IgG was measured using Mabtech human IgG enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's protocol. For detection of IgG against integrin $\alpha v\beta 6$, all serum samples were diluted to a concentration of 10 $\mu\text{g}/\text{mL}$ IgG for subsequent testing for anti-integrin $\alpha v\beta 6$ IgG, as detailed below. MaxiSorp immuno microtiter plates (Thermo Fisher Scientific) were coated with 100 μL per well of 1.5 $\mu\text{g}/\text{mL}$ human recombinant integrin $\alpha v\beta 6$ (R&D Systems) diluted in coating buffer from the ELISA Starter Accessory Kit (Bethyl Laboratories) and incubated at room temperature for 60 minutes. Next, 1 mM MgCl_2 and CaCl_2 were added to the incubation buffers to stabilize the $\alpha v\beta 6$ integrin heterodimer, as described by Kuwada et al.¹¹ The plates were subsequently washed with ELISA washing buffer and blocked with blocking buffer for 30 minutes at room temperature (all buffers were purchased from Bethyl Laboratories). The plates were then washed and incubated with 100 μL per well of samples prepared as described above and with mouse anti-human $\alpha v\beta 6$ (Millipore, MAB2077Z) from 312.5 ng/mL to 1.22 ng/mL to produce the standard curve for 60 minutes at room temperature. Plates were then washed and incubated with secondary antibodies. For the human sera samples, anti-human IgG secondary conjugated to horseradish peroxidase (Invitrogen, 31410) diluted 1:4000 was used, and for the standards, antimouse IgG secondary conjugated to horseradish peroxidase (Invitrogen, 62-6520) diluted 1:2000 was used. Plates were washed and then developed by means of incubating with 100 μL per well of 3,3',5,5'-tetramethylbenzidine for 4 minutes, at which time the reaction was stopped with 100 μL per well of 0.18 M H_2SO_4 and immediately read on the POLARstar Omega plate reader (BMG LABTECH).

In a subset of patients with established UC ($n = 6$) and HCs ($n = 2$), plasma samples were assayed for anti-integrin $\alpha v\beta 6$ IgG at a 1:200 starting dilution and 7 additional 3-fold serial dilutions from which the area under the curve (AUC) was calculated. In addition, we measured anti- $\alpha v\beta 6$ IgG as described above with samples diluted to a concentration of 10 $\mu\text{g}/\text{mL}$ IgG (IgG normalized measurement). The IgG normalized measurement strongly correlated with the AUC from the dilution curves (Pearson correlation $r = 0.97$, $P < .0001$) (Supplementary Figure 1A and B).

Statistical Analysis

Descriptive statistics were performed in GraphPad Prism, version 9.3.0. The anti- $\alpha v\beta 6$ IgG was treated as a continuous variable and binary variable. As a binary variable the cutoff for positivity was defined as greater than the mean HC plus 3 SD.¹¹ For the univariate analyses of continuous variables, we used the Mann-Whitney test (for 2 group analyses) or Kruskal-Wallis with Dunn's multiple comparisons test (for >2 group analyses). For categorical comparisons, Fisher exact test or χ^2 test were used. For comparing the anti- $\alpha v\beta 6$ IgG OD450 between pre-UC and matched HC (or asymptomatic) subjects in the PREDICTS cohort and the GEM cohort, we performed conditional logistic regressions using the R function *clogit* from the survival package.²⁸ Linear regression using *glm* function in R was used to model anti- $\alpha v\beta 6$ as a function of the disease extent in the PREDICTS cohort.²⁹ Spearman correlations were used to determine strength and direction of the associations between 2 variables.

In order to assess the predictive performance of anti- $\alpha v\beta 6$ IgG in the PREDICTS cohort, 10-fold cross-validation was performed. For each fold, the model was trained on the remaining 9 sets of samples and the predictive performance of the estimated model was applied to predict the outcome of samples in the validation set. For this analysis, UC status was modeled via logistic regression as a function of anti- $\alpha v\beta 6$. Predictive performance was evaluated on the basis of receiver operating characteristic curve (ROC) and AUC. Logistic regressions were estimated using the R function *glm*.²⁹ The 95% CIs of AUC were evaluated on the basis of 10,000 bootstrap iterations using function *ci.auc* from the pROC package³⁰ available in R.

For the COMPASS and OSCCAR cohort analyses, multivariable regressions was performed using the R function *glm*²⁹ and ROC curves created in R using the pROC package³⁰ and graphed in GraphPad Prism. The 95% CIs of AUC were evaluated as described above, with 10,000 bootstrap iterations. Cox proportional hazards models were used to define associations between anti- $\alpha v\beta 6$ and UC-related outcomes and visualized using Kaplan-Meier curves. For these analyses, we used a composite of disease-related adverse outcomes, including the need for biologic therapy, disease extension, systemic steroid use, UC hospitalization, and/or surgery. In the COMPASS model, we used age and extensive disease (E3) at baseline as covariates. In the OSCCAR model, we included the clinical risk factors for complicated disease course, including age younger than 40 years at diagnosis, extensive disease (E3), ESR/CRP elevation, systemic steroid use, focal ulcers, and history of UC-related hospitalization.³¹

Results

Anti-Integrin $\alpha v\beta 6$ Autoantibodies Are Detected up to 10 Years Before Diagnosis of Ulcerative Colitis

We hypothesized that anti- $\alpha v\beta 6$ autoantibodies would predate UC diagnosis and analyzed longitudinal samples predating UC diagnosis by up to 10 years in 82 subjects who developed UC matched by age, sex, and race with 82 subjects who did not develop IBD (HCs) (Figure 1A and Table 1). Anti- $\alpha v\beta 6$ levels were significantly higher in sera from patients who developed UC compared with the controls at all time points tested ($P < .001$, samples A–C; $P = .0015$, sample D) (Supplementary Table 1, Figure 1B and C). During the preclinical phase (sample D–A), anti- $\alpha v\beta 6$ seropositive subjects increased from 12.2% (sample D) to 20.7% (sample C) to 30.5% (sample B) to 52.4% (sample A) of subjects who developed UC (χ^2 test for trend $P < .0001$), compared with a mean of 2.7% in the HC group across the 4 time points (Figure 1C).

Next, we examined disease-related factors that were associated with anti- $\alpha v\beta 6$ autoantibodies (in sample A, at diagnosis), and determined that anti- $\alpha v\beta 6$ autoantibodies were associated with extensive (E3) disease (odds ratio [OR], 2.76; 95% CI, 1.21–6.33; Figure 1D) and inversely associated with age of diagnosis (Spearman correlation $r = -0.34$, $P = .002$; Figure 1E).

After identifying the presence of anti- $\alpha v\beta 6$ autoantibodies in UC samples before diagnosis, we sought to determine the performance of this autoantibody as a predictive biomarker. The

predictive performance of anti- $\alpha v\beta 6$ autoantibodies was assessed via ROC curves and AUC based on 10-fold cross-validation. The AUC was 0.80 at the time of diagnosis (sample A). Notably, the AUC remained high in all 3 prediagnostic samples, with AUC of 0.89, 0.84, and 0.79 at samples B, C, and D, respectively (Figure 1F and Table 2). Furthermore, the lower bound of the 95% CI of AUCs calculated via bootstrapping was >0.71 for samples A, B, C, and D; confirming the excellent predictive performance of anti- $\alpha v\beta 6$ autoantibodies in all prediagnostic groups of samples (Figure 1F and Table 2).

To confirm our findings in a second independent cohort, we tested sera collected as part of the CCC-GEM Project. In this cohort of more than 5000 first-degree relatives of patients with CD, 12 subjects had developed UC since recruitment (median time from recruitment to diagnosis was 4.2 years [range, 0.4–8.5 years]). We tested these pre-UC samples and compared them with 49 matched subjects who remained asymptomatic (HCs) since recruitment (Table 3). The pre-UC samples had higher anti- $\alpha v\beta 6$ autoantibody levels compared with controls (conditional logistic regression $P = .05$) (Figure 1G). In the pre-UC group, 4 of the 12 subjects (33%) were positive for anti- $\alpha v\beta 6$ autoantibodies (defined as mean + 3 SD of asymptomatic subjects from the GEM cohort) and 1 of the 49 controls (2%) were anti- $\alpha v\beta 6$ autoantibody-positive (Fisher exact test, $P = .004$).

Anti- $\alpha v\beta 6$ Autoantibodies Are Associated With Adverse Disease-Related Outcomes

Due to the physiological roles ascribed to $\alpha v\beta 6$ autoantibodies in mucosal homeostasis, we hypothesized that anti- $\alpha v\beta 6$ autoantibodies would be associated with adverse clinical outcomes. To test this hypothesis, we studied anti- $\alpha v\beta 6$ autoantibodies in 2 inception cohorts of patients with UC—COMPASS ($n = 55$) and OSCCAR ($n = 104$)—and compared them with non-IBD controls (non-IBD) ($n = 54$) (Table 4). With the exception of the patients with UC being younger than controls ($P < .0001$, COMPASS; $P = .0011$, OSCCAR), cases and controls shared similar characteristics. Ninety-one percent of COMPASS patients and 96% of OSCCAR patients were naïve to any biologic therapies.

Anti- $\alpha v\beta 6$ autoantibody levels were significantly higher in both the COMPASS and OSCCAR patients with UC compared with non-IBD controls ($P < .0001$) (Figure 2A), which was consistent with published data.^{11,13} ROC analysis revealed an AUC of 0.99 (95% CI, 0.97–1.00) for COMPASS and AUC of 0.96 (95% CI, 0.93–0.98) for OSCCAR (Figure 2B). With a cutoff of 0.99 (mean non-IBD + 3 SD), the sensitivity of anti- $\alpha v\beta 6$ autoantibodies for distinguishing UC from non-IBD controls was 85.5% and 70.2% for the COMPASS cohort and for the OSCCAR cohort, respectively, with specificity of 98.1%. Anti- $\alpha v\beta 6$ remained significantly associated with UC diagnosis in a multivariable model, including age, sex, and race (OR, 64.05; 95% CI, 7.41–553.74; $P = .0002$ for COMPASS and OR, 156.29; 95% CI, 12.53–1949.04; $P = .0001$ for OSCCAR) (Supplementary Tables 2 and 3).

Next, we examined whether anti- $\alpha v\beta 6$ autoantibodies were associated with any UC-related adverse outcomes representing more complicated disease, defined as a composite of need for biologic therapy, disease extension, systemic steroid use, IBD-related hospitalization, and/or surgery. In the COMPASS cohort, we found that anti- $\alpha v\beta 6$ autoantibodies were significantly associated with the composite of the above disease-related outcomes (hazard ratio [HR],

1.39; 95% CI, 1.03–1.89, with inclusion of disease extent–adjusted HR, 1.35; 95% CI, 0.99–1.85) (Figure 2C, Supplementary Table 4). In the OSCCAR cohort, we confirmed these findings and again found that subjects with higher autoantibody levels were more likely to have a more complicated course, as defined by the same composite outcome even when correcting for baseline clinical risk factors for complications, including age younger than 40 years at diagnosis, extensive disease (E3), elevated CRP or ESR, use of systemic steroids or hospitalization at diagnosis, and ulcers on baseline endoscopy³¹ (adjusted HR, 1.24; 95% CI, 1.01–1.53) (Figure 2D, Supplementary Table 5). In contrast, pANCA, an established UC-associated biomarker,^{32,33} was not associated with disease outcomes (HR, 0.75; 95% CI, 0.36–1.54) (Figure 2E, Supplementary Table 6); however, we did note a moderate positive correlation between anti- $\alpha v\beta 6$ and pANCA ($r = 0.43$, $P < .0001$) (Figure 2F).

Discussion

We identified anti- $\alpha v\beta 6$ autoantibodies as a new preclinical biomarker that precedes the diagnosis of UC by up to 10 years. Furthermore, we provide evidence in support of anti- $\alpha v\beta 6$ autoantibodies as a prognostic biomarker associated with adverse UC-related outcomes in 2 well-characterized incident cohorts of patients recently diagnosed with IBD.

Prior work had shown that anti- $\alpha v\beta 6$ autoantibodies were present in 92% of patients with established UC and had a high sensitivity and specificity for the diagnosis of UC.¹¹ The present study reported that anti- $\alpha v\beta 6$ autoantibodies are associated with the preclinical phase of UC. Importantly, these data were replicated in an independent external validation cohort.

The predictive performance of anti- $\alpha v\beta 6$ autoantibodies as assessed via AUROC with 10-fold cross-validation was at least 0.8 at all time points and remained predictive even up to 10 years before diagnosis. Furthermore, the number of seropositive patients significantly increased before the development of clinically overt disease. The increasing prevalence of anti- $\alpha v\beta 6$ autoantibodies with time is in contrast to other autoantibodies, such as anti-*Saccharomyces cerevisiae* antibodies and pANCA, which remained relatively stable before diagnosis.⁴ The predictive performance of anti- $\alpha v\beta 6$ autoantibodies is superior to that of pANCA, an established UC-associated diagnostic biomarker.⁴ Specifically, pANCA was found to be positive in a small subset of preclinical subjects with UC (2 of 8 [25%] in Israeli et al),³⁴ but had poor predictive performance, with AUCs in the range of 0.59–0.61.⁴ The combination of antimicrobial antibodies and pANCA also had a low predictive performance for UC, with AUCs ranging from 0.57 at 5 years to 0.61 at 1 year before diagnosis in a prior study using samples derived from the PREDICTS cohort. In the same study, 1129 proteins were measured using the SomaLogic platform and predictive performances using select disease-associated markers also were suboptimal, with AUCs ranging from 0.49 at 5 years before diagnosis to 0.68 at diagnosis.⁴ In the EPIC (European Prospective Investigation into Cancer and Nutrition) study, which included a single prediagnostic sample from 54 subjects who developed UC a mean of 4.4 years after collection, a combination of antimicrobial antibodies (including pANCA, anti-CBir1, anti-OmpC, and as *anti-Saccharomyces cerevisiae* antibody IgA) had an AUC <0.70.³⁵ In

cytotoxicity, an important mechanism of action of autoantibodies in other autoimmune conditions, such as vitiligo.⁴⁵

Our study has several strengths, including the detection of anti- $\alpha v \beta 6$ in 2 independent preclinical UC cohorts. In our primary preclinical cohort, PREDICTS, multiple longitudinal prediagnostic samples allowed us to study the evolution of anti- $\alpha v \beta 6$ autoantibodies over time. In addition, we have provided evidence of anti- $\alpha v \beta 6$ autoantibodies as a potential prognostic biomarker using 2 independent incident cohorts. It is possible that subjects had unrecognized symptoms of UC before the clinical diagnosis in the PREDICTS cohort. However, we believe this is limited, given the routine medical evaluations and physical rigor required of active component military populations. Furthermore, this potential limitation does not apply to samples collected up to 10 years before diagnosis and subjects in our preclinical validation cohort (GEM), who were required to be asymptomatic at enrollment. Finally, we were unable to perform functional studies due to limited sample availability.

In conclusion, anti- $\alpha v \beta 6$ autoantibodies are a novel biomarker associated with the preclinical phase of UC and are a prognostic biomarker associated with development of complicated disease in patients recently diagnosed with UC. These findings further highlight potential key early events in the development of UC, providing opportunities for diagnostic and therapeutic interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability

All requests for raw and analyzed data and materials will be promptly reviewed by the corresponding authors and study team. The authors will provide source data files for all of the figures. Additional raw data are provided in the Supplementary Material.

Code Availability

The R code developed for analyses in this study will be made available.

Abbreviations used in this paper:

anti-$\alpha\text{v}\beta\text{6}$	anti-integrin $\alpha\text{v}\beta\text{6}$
AUC	area under the curve
CCC-GEM	Crohn's and Colitis Canada Genetic Environmental Microbial project cohort
CD	Crohn's disease
COMPASS	Comprehensive Care for the Recently Diagnosed IBD Patients
CRP	C-reactive protein
ELISA	enzyme-linked immunosorbent assay
ESR	erythrocyte sedimentation rate
HC	healthy control
HR	hazard ratio
IBD	inflammatory bowel disease
ICD-9-CM	International Classification of Diseases, Ninth Revision, Clinical Modification
OR	odds ratio
OSCCAR	Ocean State Crohn's and Colitis Area Registry
pANCA	perinuclear anti-neutrophil cytoplasmic antibody
PREDICTS	Proteomic Evaluation and Discovery in an IBD Cohort of Tri-service Subjects
ROC	receiver operating characteristic curve
UC	ulcerative colitis

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WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Biomarkers for predicting the development of ulcerative colitis (UC) and disease course are currently limited.

NEW FINDINGS

In samples derived from 2 independent cohorts, we found autoantibodies directed against integrin $\alpha v\beta 6$ among individuals who go on to develop UC, which were not present in healthy controls. In addition, in 2 independent incident cohorts, we found an association between anti-integrin $\alpha v\beta 6$ autoantibodies and disease-related adverse outcomes.

LIMITATIONS

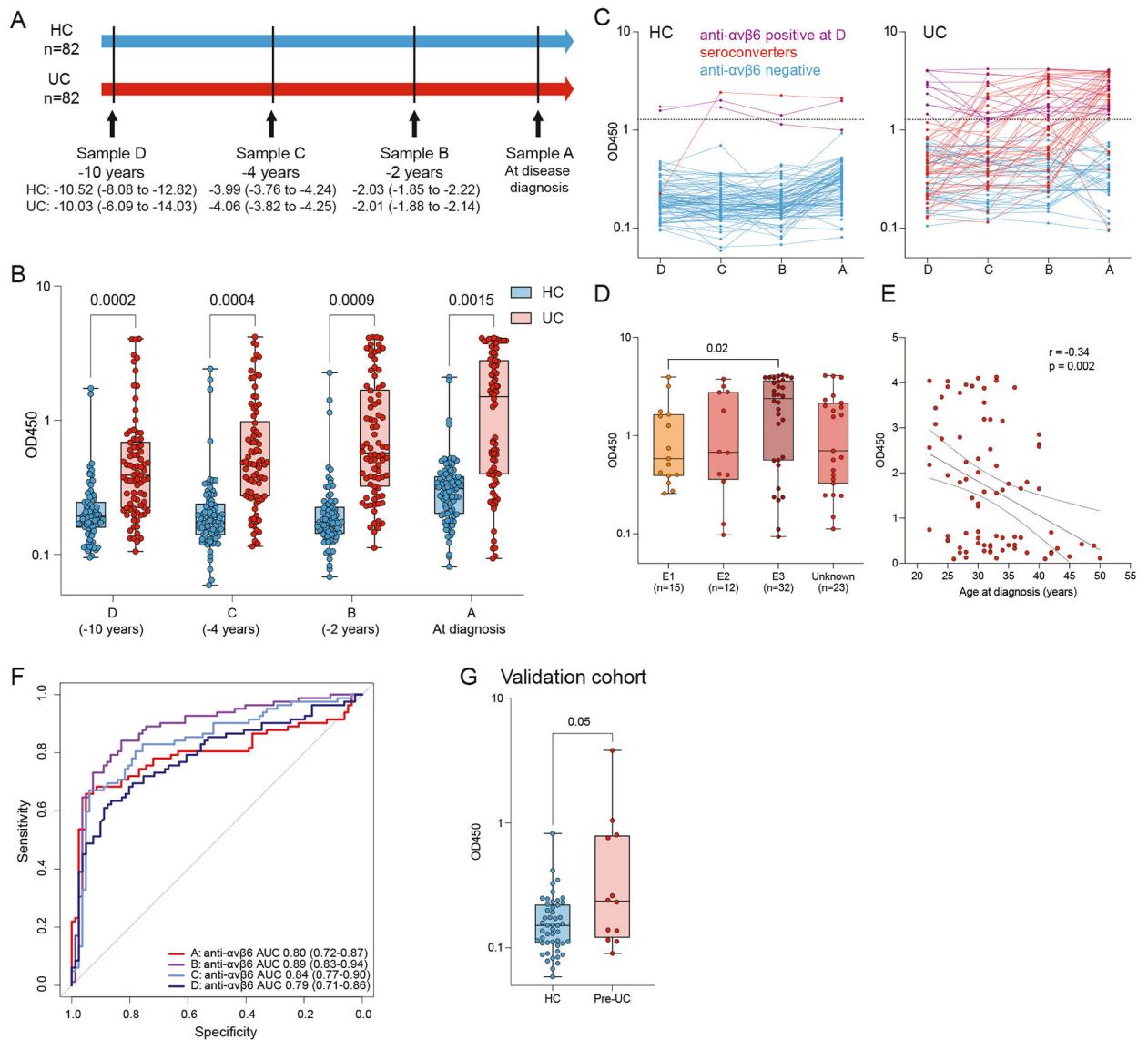
Subjects enrolled in the preclinical PREDICTS cohort may have had symptoms before the clinical diagnosis of UC. However, we believe this is limited, given the routine medical evaluations and physical rigor required of active military personnel and the disturbing character of UC manifestations. Furthermore, this limitation would not apply to samples collected up to 10 years before diagnosis and in the preclinical validation cohort (GEM), as subjects were required to be asymptomatic at enrollment.

CLINICAL RESEARCH RELEVANCE

In patients with UC, anti-integrin $\alpha v\beta 6$ autoantibodies are a novel biomarker associated with disease development and disease-related adverse outcomes.

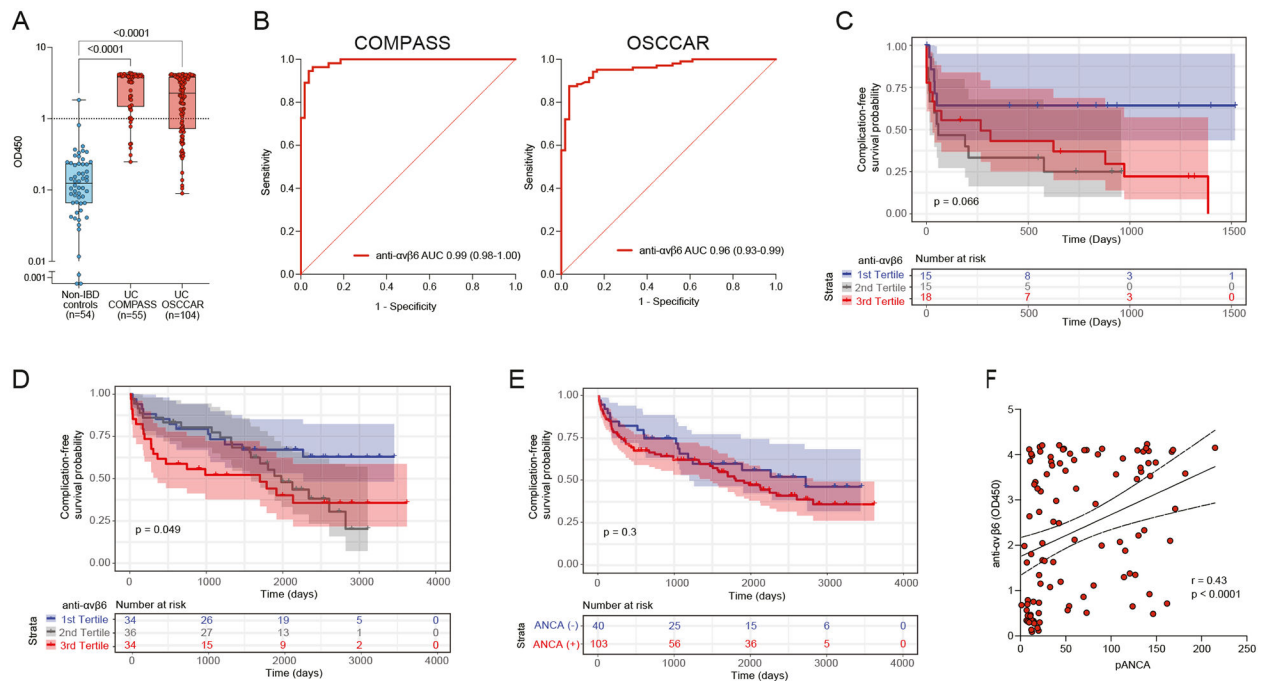
BASIC RESEARCH RELEVANCE

Given the presence of anti-integrin $\alpha v\beta 6$ autoantibodies before diagnosis and their physiologic role in intestinal epithelial homeostasis, these autoantibodies may play a role in disease pathogenesis, which will require more detailed functional studies to further elaborate.

**Figure 1.**

Anti-*avβ6* autoantibodies in preclinical subjects with UC. (A) Timeline of sample collection in the PREDICTS cohort from 82 subjects who developed UC and 82 matched controls (HC). Sample A was collected at the time of diagnosis for subjects with UC and samples B, C, and D were collected before diagnosis. Median (interquartile range [IQR]) time in years for samples A, B, C, and D are detailed for both UC and HC. (B) Anti-*avβ6* autoantibody absorbance values (OD450) determined by means of ELISA in UC (n = 82, shown in red) and HC (n = 82, shown in blue) sera obtained before diagnosis (samples B, C, and D) and at the time of diagnosis (sample A) from the PREDICTS cohort. In sample D, 1 HC sample was excluded due to an identification issue. For all boxplots, the box represents the IQR, the center line represents the median, and the whiskers indicate the minimum to maximum value. Statistical significance determined by means of conditional logistic regressions at each time point comparing subjects who developed UC with matched controls. (C) Dynamics of anti-*avβ6* in each HC or UC subject across the 4 longitudinal samples

in the PREDICTS cohort. *Purple lines* represent samples that are positive for anti- $\alpha v \beta 6$ in sample D, *red lines* indicate samples that seroconvert between sample D and A, and *blue lines* indicate subjects that are negative at all sample time points. (D) Anti- $\alpha v \beta 6$ autoantibody levels as a function of UC disease extent as defined by Montreal classification (E1: proctitis only, n = 15; E2: left-sided disease, n = 12; E3: extensive disease, n = 32; unknown, n = 23) and (E) by age at diagnosis, shown as *scatterplot* in the PREDICTS cohort. Statistical significance determined by means of linear regression and Spearman correlation, respectively. (F) Predictive performance of anti- $\alpha v \beta 6$ autoantibodies based on 10-fold cross-validation and the 95% CI of the AUC based on 10,000 bootstrap iterations in the PREDICTS cohort. Sample A (time of diagnosis) is in *red*, sample B (-2 years) is in *purple*, sample C (-4 years) is in *light blue*, and sample D (-10 years) is in *dark blue*. (G) Anti- $\alpha v \beta 6$ autoantibody absorbance values (OD450) determined by means of ELISA in 12 pre-UC subjects and 49 matched control subjects from the GEM cohort. Statistical significance determined by conditional logistic regression comparing pre-UC subjects with matched control subjects.

**Figure 2.**

Anti- $\alpha v\beta 6$ autoantibodies in patients with newly diagnosed UC and their association with adverse disease-related outcomes. (A) Anti- $\alpha v\beta 6$ autoantibody absorbance values (OD450) determined by means of ELISA in non-IBD controls (n = 54, shown in blue), in COMPASS patients with UC (n = 55, shown in red), and in OSCCAR patients with UC (n = 104, shown in red). (B) ROC analysis of anti- $\alpha v\beta 6$ autoantibodies in both COMPASS patients with UC (left) and OSCCAR patients with UC (right) compared with the non-IBD controls. (C, D) Kaplan-Meier curve for composite outcome of IBD hospitalization, proximal disease extension, need for surgery, systemic steroid use, and/or requiring new biologic therapy in the COMPASS cohort (C) and OSCCAR cohort (D) stratified by anti- $\alpha v\beta 6$ titer tertiles. The blue line represents the first tertile (lowest), the gray line represents the second tertile, and the red line the third (highest) tertile. (E) Kaplan-Meier curve for the same composite outcomes in the OSCCAR cohort stratified according to the presence or absence of pANCA. (F) Spearman correlation between pANCA and anti- $\alpha v\beta 6$ titers.

Table 1.

Demographic and Clinical Characteristics of PREDICTS Cohort

Characteristic	HCs	UC
No. of individuals	82	82
No. of samples	327 ^a	328
Age at diagnosis or sample A, y, mean \pm SD	32.6 \pm 5.6	32.0 \pm 6.4
Male/female, n	79/3	79/3
Race, n		
White	62	62
Black	18	18
Other	2	2
Disease extent at diagnosis, n		
E1: proctitis	NA	15
E2: left-sided colitis	NA	12
E3: extensive	NA	32
Unknown	NA	23

NA, not applicable.

^aSample D from 1 HC subject excluded due to an identification discrepancy.

Table 2.

Predictive Model for UC Development in PREDICTS Samples

Sample	AUC ^a	95% CI ^a	
		Lower	Upper
A: anti- $\alpha v\beta 6$	0.80	0.72	0.87
B: anti- $\alpha v\beta 6$	0.89	0.83	0.94
C: anti- $\alpha v\beta 6$	0.84	0.77	0.90
D: anti- $\alpha v\beta 6$	0.79	0.71	0.86

^aBased on 10-fold cross-validation.

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Table 3.

Demographic and Clinical Characteristics of the CCC-GEM Cohort

Characteristic	Subjects that remained asymptomatic (HC)	Subjects that developed UC (pre-UC)
No. of individuals	49	12
Age at recruitment, <i>y</i> , mean ± SD	17.7 ± 7.1	17.3 ± 7.5
Male/female, n	24/25	6/6
Country recruitment, n		
Canada	40	10
Israel	5	1
United States	4	1
Relation to proband, n		
Offspring	23	1
Sibling	26	11
Time from recruitment to diagnosis, <i>y</i> , mean (range)	NA	4.2 (0.4–8.5)

NA, not applicable.

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Table 4.

Demographic Characteristics of 2 UC Inception Cohorts and Non-IBD Controls

Characteristic	Non-IBD controls	COMPASS UC	OSCCAR UC
No. of individuals	54	55	104
No. of samples	54	55	104
Age at diagnosis, <i>y</i> , mean ± SD	46.3 ± 15.9	31.3 ± 12.4	36.8 ± 20.1
Male/female, n	31/23	28/27	42/62
Race, n			
White	19	35	92
Black	14	4	4
Asian	3	5	0
Other	18	11	8
Disease extent at diagnosis, n			
E1: proctitis	NA	7	27
E2: left-sided colitis	NA	24	66
E3: extensive	NA	24	44
Mayo Endoscopic score, n			
0	NA	5	NA
1	NA	15	NA
2	NA	16	NA
3	NA	14	NA
Unknown	NA	5	104
Biologic-naïve (at enrollment/blood draw), n (%)	54 (100)	50 (90.9)	100 (96.2)

NA, not applicable or not available.