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Biomarkers are associated with clinical and endoscopic outcomes with vedolizumab treatment in Crohn's disease

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Abstract

Background: Vedolizumab, an α4β7 integrin antagonist, is an effective therapy for Crohn's disease (CD). Biomarkers are needed to guide therapy and predict outcomes. This study evaluated biomarker concentrations and outcomes in patients with CD undergoing vedolizumab treatment.

Methods: Sera at weeks 0, 2, 6, 14, and ⩾26 were collected from vedolizumab-treated, refractory CD patients. Concentrations of soluble (s)-Vascular Cell Adhesion Molecule (VCAM)-1, s-Intercellular Cell Adhesion Molecule (ICAM)-1, s-Mucosal Addressin Cell Adhesion Molecule (MAdCAM)-1, and s-α4β7 integrin were evaluated for associations with achieving endoscopic remission.

Results: A total of 22 patients with CD were included. In all patients, s-MAdCAM-1 decreased significantly and s-α4β7 increased compared with baseline. s-VCAM-1 and s-ICAM-1 changed differentially in patients who achieved remission. At week 6, median s-VCAM-1 (859.6 ng/ml *versus* 460.3 ng/ml, $p = 0.03$) and s-ICAM-1 (545.7 ng/ml *versus* 286.2 ng/ml, $p = 0.03$) concentrations were higher in patients who achieved endoscopic remission compared with those who did not, and similar differences were observed for s-ICAM-1 concentrations in patients who achieved clinical remission, compared with those who did not (669.1 ng/ml *versus* 291.0 ng/ml, $p = 0.04$). Week 14 s-α4β7 concentrations were lower in patients who achieved endoscopic remission, compared with those who did not (7.5 ng/ml *versus* 17.6 ng/ml, $p = 0.020$).

Conclusion: In all vedolizumab-treated CD patients, s-MAdCAM-1 decreased significantly and s-α4β7 increased. However, higher concentrations of s-ICAM-1 and s-VCAM-1 at week 6 and lower concentrations of s-α4β7 at week 14 differentiated patients who achieved endoscopic remission. These findings may help identify early predictors of response to vedolizumab treatment in patients with CD. Further validation in less refractory CD patients is needed.

Keywords: biomarker, biologic, mucosal healing, predict, vedolizumab

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Introduction

Crohn's disease (CD) is a chronic inflammatory disease of the gastrointestinal tract caused by an interplay of immunologic, environmental, microbiotal, and genetic factors.[1] Vedolizumab, which inhibits the α4β7 integrin on leukocytes, and prevents trafficking and binding to Mucosal Addressin Cell Adhesion Molecule-1 (MAdCAM)-1 on endothelial cells within the gastrointestinal tract and gastrointestinal-associated lymphoid tissues, is a targeted therapy for CD.[2] Randomized clinical trials have shown that treatment with vedolizumab is associated with increased rates of corticosteroid-free remission and mucosal healing in patients

with CD.[3] In addition, due to its gut-selective mechanism, vedolizumab has a favorable safety profile.[4]

Therapeutic drug monitoring (TDM) is a widely utilized and recommended practice of measuring serum drug concentrations and presence of anti-drug antibodies in patients treated with biologic therapies.[5] Although there is robust evidence for optimal trough drug concentrations of tumor necrosis factor-alpha antagonists (TNFα antagonists) to guide management,[6] there is less consistent data on optimal target drug concentration thresholds for vedolizumab.[3,7–9] Similarly, while rising C-reactive protein (CRP) levels correlate with TNF production and TNFα antagonist response,[10] CRP is not specific for intestinal inflammation, and is not as helpful as a pharmacodynamic marker for treatment with vedolizumab.[11] For this reason, alternative tests to predict response to therapy become increasingly important to guide treatment. In a prospective cohort, non-responders to vedolizumab still displayed near maximum occupancy of peripheral and intestinal α4β7 integrin receptors, regardless of drug concentration or clinical outcomes.[12] Similarly, in phase II clinical trials, low drug concentrations of vedolizumab achieved full α4β7 receptor saturation,[13] suggesting that other mechanistic factors beyond receptor saturation may be necessary to achieve clinical outcomes. Therefore, additionally evaluating other proteins that mediate lymphocyte trafficking to the gut endothelium in CD may identify biomarkers to evaluate treatment response to vedolizumab. These include Vascular Cell Adhesion Molecule-1 (VCAM)-1 and Intracellular Adhesion Molecule-1 (ICAM)-1. These cell adhesion molecules, which are expressed in higher levels on lymphocytes of CD patients, bind lymphocytes through their integrin ligands α4β1and αLβ2, respectively. Through blockade of the shared α4 subunit,[14] dual inhibition of α4β1 and α4β7 reduces both peripheral leukocyte binding to VCAM-1 and leukocyte trafficking to the intestine. Our group reported an association between serum biomarker concentrations and outcomes in a cohort of patients with ulcerative colitis (UC) receiving vedolizumab therapy. Soluble (s)-VCAM-1 concentrations were consistently associated with differential outcomes for both clinical and endoscopic remission, and changes in this biomarker were appreciable by week 6.[15] In addition, s-MAdCAM-1, s-VCAM-1, and s-ICAM-1 decreased more rapidly in patients who achieved endoscopic remission, suggesting that further exploration of these biomarkers may be useful in predicting outcomes with vedolizumab.

A flow-cytometry based study of peripheral blood memory T cells in vedolizumab-treated patients with inflammatory bowel disease (IBD) showed near-complete α4β7 integrin occupancy on peripheral blood mononuclear cells (PBMCs) during induction and maintenance therapy, with no associations between receptor occupancy and vedolizumab drug concentrations.[12] Another flow cytometry based study of vedolizumab-treated patients with IBD showed that pre-treatment α4β7 integrin expression on PBMCs was higher in responders, and during therapy, responders maintained higher α4β7 receptor saturation on effector memory T cells as compared with non-responders.[16] Although PBMC analyses have elucidated important data about integrin expression and receptor saturation, a knowledge gap persists on the relationship between free circulating protein serum biomarkers in vedolizumab-treated CD patients and patient outcomes.

While the relationship between these serum biomarkers and treatment response to vedolizumab has been reported in UC, data are lacking in CD. For this reason, this study aimed to address this gap, by prospectively analyzing the relationship between serum biomarkers, drug concentrations and outcomes in vedolizumab-treated patients with CD.

Materials and methods

Patients and study design

A convenience sample of adult patients (⩾18 years) at the University of California, San Diego (UCSD) were included in the analysis who met the following criteria: (1) a diagnosis of CD confirmed by clinical symptoms, endoscopic and histologic data; (2) active treatment with vedolizumab. Patients received intravenous vedolizumab 300 mg during induction at week 0, 2, and 6, followed by maintenance infusions every 8 weeks. The requirement for dose escalation to every 4 weeks was determined by the treating physician during maintenance therapy. Patients had prospective serum collection, in addition to clinical and endoscopic evaluations. Serum

samples were prospectively obtained from patients at trough, prior to vedolizumab infusions, during induction at week 0 (baseline), 2, 6 and during maintenance at week 14 and week ⩾26 between January 2014 and October 2016. Biomarker analysis included one patient sample per time point.

Endpoints and definitions

The primary aim of this study was to identify whether s-VCAM-1 concentrations at week 6 were associated with achieving endoscopic remission during the maintenance phase of vedolizumab therapy in patients with CD. As secondary exploratory outcomes, we analyzed whether serum biomarkers were associated with endoscopic remission and symptom-based clinical remission, and aimed to analyze whether absolute values or changes in biomarker concentrations from baseline were associated with these outcomes.

Clinical and endoscopic scoring was performed prospectively by physicians subspecialized in IBD (SS, WJS, BSB). Endoscopic disease activity was prospectively assessed by using the simple endoscopic score for Crohn's disease (SES-CD). Endoscopic remission was defined as SES-CD of ⩽2 with no intestinal segment scoring greater than 1.

A physician's global assessment (PGA) or a physician's overall impression of a patient's symptoms was used to define clinical remission. Complete clinical remission was defined by complete relief of CD-related symptoms defined by a PGA of 0.[17] In addition, clinical remission required neither treatment discontinuation, nor IBD-related surgery. Biomarker concentrations from week 0 (baseline), 2, 6, 14, and ⩾26 were compared between patients who had achieved endoscopic and clinical remission during maintenance therapy with those not achieving remission. Correlations between vedolizumab concentrations and s-VCAM-1, s-ICAM-1 and s-α4β7 were explored separately during induction and maintenance phases. Effects of corticosteroid use on biomarker concentrations was also explored at baseline and at maintenance week ⩾26.

Biomarker assays

Vedolizumab and antibodies to vedolizumab (ATV) measurements used a homogenous mobility shift assay (HMSA), Anser® VDZ (Prometheus Biosciences; San Diego, CA, USA). Serum TNFα measurements used the Erenna® SMC™ Human TNFα Immunoassay Kit (EMD Millipore, St. Charles, MO, USA). CRP, s-Amyloid A (s-AA), s-ICAM-1, and s-VCAM-1 measurements used V-Plex Vascular Injury Panel-2 (human) Kits (Meso Scale Discovery, Rockville, MD, USA). s-MAdCAM-1 and s-α4β7 measurements used enzyme-linked immunosorbent assays (ELISA, Prometheus Biosciences; San Diego, CA, USA).

Statistical analysis

Continuous variables were reported as mean ± standard deviation for normally distributed data and median with interquartile ranges (IQR) for non-normally distributed data. Categorical variables were reported as numbers (*n*) and percentages (%). Continuous variables were compared using the Mann–Whitney *U* test and categorical variables were compared using the Fishers exact test or chi square test, as appropriate. The Wilcoxon signed-rank test was used for paired continuous data. A univariate logistic regression was applied to assess the relationship between biomarkers on clinical and endoscopic outcomes by performing univariate analyses on biomarkers at baseline, week 2, week 6, week 14, and week ⩾26, separately as co-variates for outcomes during maintenance therapy at week ⩾26. These analyses were repeated for changes in biomarker concentrations with vedolizumab treatment for all patients from baseline to each subsequent time point 0, 2, 6, 14, and ⩾26. In addition, biomarker concentrations were compared at individual timepoints of week 0, 2, 6, 14, and ⩾26 between clinical or endoscopic remitters to non-remitters. As s-VCAM-1 and s-ICAM-1 are both induced by TNF,[18–20] and previous studies have implicated various effects of corticosteroid use on CAM's,[18,19] Pearson's correlations between s-VCAM-1 and s-ICAM-1 with s-TNFα, and relationships between s-VCAM-1 and s-ICAM-1 and corticosteroid use were explored. Pearson's correlation coefficients were calculated between s-TNFα and s-VCAM-1 and s-ICAM-1 during maintenance therapy using the latest available sample collection.

An outcome was considered significant with a p-value ⩽ 0.05. Analyses were performed STATA SE version 15.1.

Table 1. Baseline patient characteristics.

Baseline characteristics	Total patients *n* = 22	Endoscopic remission *n* = 8	No endoscopic remission *n* = 14
Age, mean (SD), years	41.5 (17.7)	50.4 (17.4)	37.9 (15.8)
Female sex, *n* (%)	13 (59.1%)	6 (75.0%)	7 (50.0%)
Age at diagnosis, *n* (%)			
<16 years	3 (13.6%)	2 (25.0%)	1 (7.1%)
16–40 years	17 (77.3%)	6 (75.0%)	11 (78.6%)
>40 years	2 (9.1%)	0	2 (14.3%)
Disease duration, mean (SD), years	19.6 (16.9)	29.5 (19.4)	13.9 (12.7)
Disease extent, *n* (%)			
Ileal, pouch	6 (27.3%)	2 (25.0%)	4 (28.6%)
Colonic	10 (45.5%)	2 (25.0%)	8 (57.1%)
Ileocolonic	6 (27.3%)	4 (50.0%)	2 (14.3%)
Current smoker, *n* (%)	1 (4.5%)	0	1 (7.1%)
Body mass index, mean (SD), kg/m^2	24.0 (5.3)	24.0 (6.8)	24.1 (4.6)
Prior TNF-inhibitor use, *n* (%)	20 (90.9%)	7 (87.5%)	13 (92.9%)
Baseline albumin, mean (SD), g/dl	3.8 (0.6)	3.8 (0.5)	3.8 (0.7)
Mean baseline SES-CD score (SD)	8.5 (6.2)	5.7 (4.1)	9.9 (6.7)
Baseline corticosteroid requirement, *n* (%)	15 (68.2%)	4 (50.0%)	11 (78.6%)
Concomitant immunomodulator use, *n* (%)	18 (81.8%)	6 (75.0%)	12 (85.7%)
Dose escalation, *n* (%)	15 (68.2%)	2 (25.0%)	13 (92.9%)

SD, standard deviation; SES-CD, simple endoscopic score for Crohn's disease; TNF, tumor necrosis factor.

Ethical considerations

The protocol was approved by the Human Research Protections Program at UCSD (IRB number: 160411) and patients provided written informed consent prior to enrollment. All authors had access to study data, reviewed and approved the final manuscript.

Results

Patients

In total, 22 patients with CD were included (Table 1). Serum samples were collected at baseline ($n = 8$), week 2 ($n = 8$), week 6 ($n = 12$), week 14 ($n = 14$), and week ⩾26 ($n = 17$). At baseline, 59.1% of patients were female, 27.3% had ileocolonic involvement, 90.9% received prior TNFα-inhibitor therapy, and 68.2% were receiving corticosteroids. The mean baseline SES-CD for all patients with available scores (18/22) was 8.5. The mean baseline SES-CD for patients who achieved endoscopic remission and for those who did not achieve endoscopic remission was 5.7 and 9.9, respectively. Four patients without baseline SES-CD scores had other assessments of baseline mucosal inflammation. Two patients had moderately active disease on endoscopy, although SES-CD was unable to be calculated. One patient underwent magnetic resonance enterography demonstrating stenosis of the terminal ileum and developed worsening clinical

Table 2. Vedolizumab concentrations in remitters compared with non-remitters.

	Median	IQR 25–75	*n*	Median	IQR 25–75	*n*	*p* value
	Endoscopic remission			No endoscopic remission			
Week 2	27.4	22.6–32.1	3	27.0	23.7–27.6	4	0.9
Week 6	26.9	15.5–38.1	4	19.9	14.0–27.5	7	0.6
Week 14	12.6	7.1–17.7	6	7.2	5.0–10.2	7	0.4
Week ⩾26	9.6	7.9–18.3	5	8.8	6.2–16.0	11	0.5

IQR, interquartile range.

symptoms, and one patient developed clinical symptoms characterized by bloody, frequent stools and was corticosteroid dependent. The majority of patients received concomitant immunosuppression, with 81.8% of patients taking either azathioprine, mercaptopurine, or methotrexate. The median (IQR) time to endoscopic assessment was 34.9 weeks (27.9–41.3).

Patient outcomes

During maintenance, 8 out of 22 patients (36.4%) achieved endoscopic remission by 26 weeks, 5 out of 20 patients with available scores (25.0%) achieved clinical remission, and 13 out of 22 patients (59.1%) were corticosteroid-free at the end. In total, 15 out of 22 patients (68.2%) underwent dose escalation as part of routine clinical care [median time (IQR) to escalation: 36.5 (24.5–56) weeks].

Vedolizumab and anti-vedolizumab antibody concentrations

Median vedolizumab concentrations at weeks 2, 6, 14 and ⩾26 were 27.4 mcg/ml (IQR: 22.2–27.7), 19.9 mcg/ml (IQR: 14.0–32.6), 9.6 mcg/ml (IQR: 5.8–15.0) and 9.2 mcg/ml (IQR: 7.6–18.6), respectively. Antibodies to vedolizumab (ATV) were detected in one patient at week 6 while on vedolizumab monotherapy. Although ATV resolved with dose escalation, this patient was ultimately switched to an alternate therapy due to persistence of active perianal disease. While vedolizumab concentrations at all time points were not significantly different between endoscopic remitters and non-remitters, concentrations were numerically higher in endoscopic remitters at all time points (Table 2).

Biomarkers

Baseline biomarkers and changes in biomarkers during therapy for all patients

There were no differences between baseline biomarker concentrations (s-TNFα, s-α4β7, s-MAdCAM-1, CRP, s-AA, s-ICAM-1, and s-VCAM-1) between groups for any measured outcome (Table 3).

In the entire cohort of vedolizumab-treated patients with available baseline serum biomarker measurements, s-α4β7 increased over time to week ⩾26 (concentration compared with baseline: week 2: $p=0.003$, week 6: $p=0.001$, week 14: $p=0.0008$, week ⩾26: $p=0.001$), and s-MAdCAM-1 decreased over time to week ⩾26 (concentration compared with baseline: week 2: $p=0.005$, week 6: $p=0.001$, week 14: $p=0.0008$, week ⩾26: $p=0.001$) (Figure 1). Neither s-ICAM-1 nor s-VCAM-1 concentrations significantly changed at week ⩾26 as compared with baseline (concentration compared with baseline, $p=$NS for all time points, Supplemental Figure S1). In addition, s-TNFα, CRP, and s-SAA concentrations did not significantly change at ⩾week 26 as compared with baseline (concentration compared with baseline, $p=$NS for all time points).

s-VCAM-1 concentrations

Median concentrations of s-VCAM-1 at week 6 were higher in patients who subsequently achieved endoscopic remission compared with those who did not achieve endoscopic remission (859.6 ng/ml *versus* 460.3 ng/ml, $p=0.03$) (Table 4). In addition, patients who achieved endoscopic remission had numerically greater median increases in s-VCAM-1 concentrations from baseline to week

Table 3. Baseline concentrations of all biomarkers stratified by endoscopic and clinical remission.

Biomarker (ng/ml)	Median	IQR 25–75	*n*	Median	IQR 25–75	*n*	*p* value
Baseline	Endoscopic remission			No endoscopic remission			
s-TNFα	0.01	0.01–0.01	1	0.004	0.003–0.006	6	0.3
s-MAdCAM-1	65650.0	47725.0–83575.0	2	21415.0	16501.3–32823.8	6	0.3
s-CRP	7540.5	7254.1–7826.9	2	1923.5	927.0–3745.3	6	0.3
s-SAA	6794.1	4852.7–8735.5	2	4567.9	1947.8–47826.2	6	0.9
s-ICAM-1	367.4	318.9–415.8	2	265.0	244.6–297.0	6	0.6
s-VCAM-1	502.1	446.1–558.1	2	475.0	390.6–520.0	6	0.9
	Clinical remission			No clinical remission			
s-TNFα	NA	NA	0	0.004	0.004–0.007	7	NA
s-MAdCAM-1	NA	NA	0	25775.0	19553.8–36525.0	8	NA
s-CRP	NA	NA	0	3466.5	934.3–7254.1	8	NA
s-SAA	NA	NA	0	4666.0	2459.3–23414.7	8	NA
s-ICAM-1	NA	NA	0	277.7	244.9–341.8	8	NA
s-VCAM-1	NA	NA	0	475.0	386.7–544.3	8	NA

CRP, C-reactive protein; ICAM-1, intercellular cell adhesion molecule-1; IQR, interquartile range; MAdCAM-1, mucosal addressin cell adhesion molecule-1; s-, soluble; SAA, serum amyloid A; TNFα, tumor necrosis factor alpha; VCAM, vascular cell adhesion molecule-1.

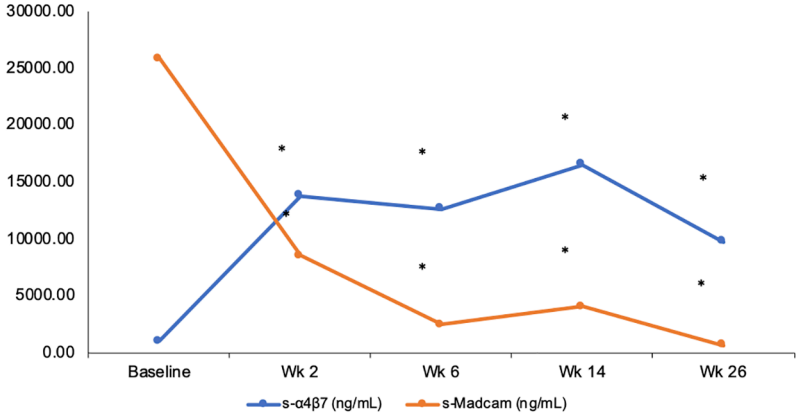

Figure 1. Concentrations of s-α4β7 and s-MAdCAM-1 longitudinally in all patients concentrations of s-α4β7 increased overtime in patients with baseline available measurements, and sMAdCAM-1 decreased overtime.
*Significantly lower concentrations of biomarkers at individual timepoints compared with baseline.
s-MAdCAM-1, soluble mucosal addressin cell adhesion molecule-1.

≥26, compared with those who did not achieve endoscopic remission ($p=0.05$). However, concentrations were similar between remitters and non-remitters at other time points.

s-ICAM-1

Median concentrations of s-ICAM-1 at week 6 were significantly higher in patients who achieved endoscopic remission during maintenance therapy compared with those who did not achieve endoscopic remission: 545.7 ng/ml *versus* 286.2 ng/ml ($p=0.03$), and in patients who achieved clinical remission compared with those who did not achieve clinical remission: 669.1 ng/ml *versus* 291.0 ng/ml ($p=0.04$). In addition, patients who achieved endoscopic remission had numerically greater median increases in s-ICAM-1 concentrations from baseline to week ≥26 compared with those who did not achieve endoscopic remission ($p=0.05$). However, concentrations were similar between remitters and non-remitters at other time points.

s-α4β7

Median concentrations of s-α4β7 at week 14 were lower in patients who achieved endoscopic remission compared with those who did not (7.5 ng/ml *versus* 17.6 ng/ml, $p=0.02$). There was no difference in median changes of s-α4β7 concentrations for patients who achieved endoscopic remission

Table 4. Concentrations of s-α4β7, s-ICAM-1, s-VCAM-1 stratified by endoscopic and clinical remission.

Biomarker (ng/ml)	Median	IQR 25–75	*n*	Median	IQR 25–75	*n*	*p* value
Week 2		**Endoscopic remission**			**No endoscopic remission**		
s-VCAM-1	897.0	621.1–906.5	3	449.0	394.5–494.7	5	1.0
s-ICAM-1	650.9	423.4–675.1	3	287.0	253.1–333.1	5	0.6
s-α4β7	9.9	6.3–10.1	3	14.6	13.0–21.7	5	0.1
		Clinical remission			**No clinical remission**		
s-VCAM-1	906.5	901.6–911.3	2	421.7	364.1–483.3	6	0.3
s-ICAM-1	675.1	663.0–687.2	2	270.1	242.5–321.6	6	0.07
s-α4β7	10.1	10.0–10.2	2	13.75	10.6–19.9	6	0.6
Week 6		**Endoscopic remission**			**No endoscopic remission**		
s-VCAM-1	859.6	730.8–961.5	4	460.3	403.3–525.8	8	0.03*
s-ICAM-1	545.7	401.2–667.7	4	286.2	259.3–311.3	8	0.03*
s-α4β7	12.2	11.3–13.6	4	12.6	11.2–15.5	8	0.9
		Clinical remission			**No clinical remission**		
s-VCAM-1	859.6	838.7–880.5	2	460.6	411.0–520.0	9	0.2
s-ICAM-1	669.1	667.7–670.5	2	291.0	270.0–330.0	9	0.04*
s-α4β7	14.4	13.6–15.1	2	12.5	11.4–13.6	9	0.4
Week 14		**Endoscopic remission**			**No endoscopic remission**		
s-VCAM-1	639.1	492.6–720.4	6	615.7	475.7–895.5	8	0.7
s-ICAM-1	414.9	321.4–459.8	6	465.8	306.8–655.5	8	0.7
s-α4β7	7.5	4.4–11.6	6	17.6	12.8–20.9	8	0.02*
		Clinical remission			**No clinical remission**		
s-VCAM-1	710.5	563.3–717.1	3	526.8	473.0–823.1	10	0.8
s-ICAM-1	471.0	372.0–516.3	3	395.7	300.0–514.6	10	0.9
s-α4β7	8.7	5.9–10.6	3	16.5	10.6–18.7	10	0.1
Week ⩾26		**Endoscopic remission**			**No endoscopic remission**		
s-VCAM-1	470.0	439.3–1012.2	5	491.2	407.9–675.7	12	0.7
s-ICAM-1	361.3	333.0–442.9	5	328.7	281.0–528.1	12	0.5
s-α4β7	7.7	7.3–11.8	5	9.2	7.4–11.4	12	0.8
		Clinical remission			**No clinical remission**		
s-VCAM-1	788.0	554.5–1021.5	2	467.0	434.5–675.7	12	1.0
s-ICAM-1	629.8	481.4–778.2	2	328.7	291.3–461.5	12	0.4
s-α4β7	9.7	8.7–10.7	2	7.8	7.2–9.8	12	0.5

*Statistically significant, $p \leqslant 0.05$.
Week 6 concentrations of s-VCAM-1 and s-ICAM-1 were significantly higher in endoscopic remitters *versus* non-remitters ($p = 0.03$), week 6 concentrations of s-ICAM-1 were significantly higher in clinical remitters *versus* non-remitters ($p = 0.04$), week 14 α4β7 concentrations were lower in endoscopic remitters *versus* non-remitters ($p = 0.02$).
ICAM-1, intercellular cell adhesion molecule-1; IQR, interquartile range; s-, soluble; VCAM, vascular cell adhesion molecule-1.

compared with those who did not achieve endoscopic remission. At other time points, concentrations were similar between remitters and non-remitters.

s-TNF-α, s-MAdCAM-1, CRP, and s-SAA

Median concentrations of s-TNFα, s-MAdCAM-1, CRP, and s-SAA were not significantly different between remitters and non-remitters for both clinical and endoscopic endpoints (p = NS for all time points, Table 5). In addition, there was no difference in median changes in these biomarker concentrations over time for patients who achieved endoscopic remission compared with those who did not achieve endoscopic remission (p = NS for all time points).

Correlations between biomarkers and vedolizumab concentrations

Although correlations between maintenance vedolizumab and s-α4β7 concentrations were observed, significant correlations between individual biomarker and vedolizumab concentrations were not consistently observed (Table 6). s-TNFα concentrations correlated with both s-ICAM-1 (r = 0.6, p = 0.01) and s-VCAM-1 (r = 0.5, p = 0.04).

Relationship between corticosteroid use and biomarkers concentrations

Cell adhesion molecule concentrations were not significantly different in those requiring corticosteroids at week ⩾26 as compared with those not requiring corticosteroids (s-MAdCAM-1: p = 0.7, s-ICAM-1: p = 0.3, s-VCAM-1: p = 0.9).

Discussion

A significant proportion of patients with CD develop a complicated and progressive disease course requiring biologic therapy. While vedolizumab is an effective therapy in CD, many patients will not respond. Serum biomarkers are attractive and needed clinical tools to guide management decisions such as medication selection, dose escalation and switching to alternate drug classes. This study prospectively collected serum biomarkers and evaluated endoscopic and clinical outcomes in patients with CD initiating vedolizumab therapy. Specifically, we measured serum concentrations of s-VCAM-1, s-ICAM-1, s-MAdCAM-1, and s-α4β7 during vedolizumab therapy at different time intervals, and compared concentrations between patients who achieved endoscopic and clinical remission with those who did not. Given previous data in UC, we analyzed primarily s-VCAM-1 at week 6 and found higher concentrations were associated with subsequent endoscopic remission in maintenance therapy. Exploratory analyses of other biomarkers also demonstrated similar findings for s-ICAM-1. Furthermore, greater increases during maintenance as compared with baseline were observed for s-VCAM-1 and s-ICAM-1 in patients who achieved endoscopic remission compared with those who had ongoing endoscopic activity. Across the entire cohort of vedolizumab-treated patients, concentrations of s-α4β7 increased over time to week ⩾26 and s-MAdCAM-1 decreased over time to week ⩾26.

In the setting of inflammation, α4 integrins recognize and bind to vascular addresins on the endothelium, which facilitates lymphocyte migration to the intestine.[21] Vedolizumab selectively prevents adhesion of α4β7 on lymphocytes to MAdCAM-1 on the gut endothelium, without interfering with α4β1 binding to VCAM-1. ICAM-1 is associated with neutrophil adhesion through β2 integrins, and is similarly involved in cellular trafficking during inflammation. The findings of this study are, in part, contrary to our previous observations for biomarker trends in vedolizumab-treated UC patients.[15] In the UC cohort, s-VCAM-1 concentrations were significantly lower in patients who achieved endoscopic remission and s-VCAM-1 significantly decreased from baseline to week 6 in all patients. In this cohort of CD patients, findings were consistent in the time point at which differential s-VCAM-1 concentrations were observed in remitters. However, as opposed to previous findings, higher s-VCAM-1 concentrations demonstrated in the present study suggest that there may be an alternative pathway to circumvent inhibition of leukocyte migration, which may be specific and unique to CD. Zundler *et al.*[22] described differences in cellular homing in CD patients treated with vedolizumab, which were not apparent in UC. Inhibition of α4β7 by vedolizumab was associated with a compensatory increase in cellular homing through α4β1, which was expressed in greater levels on T effector cells. Similar to these findings, Harvey Bradshaw index (HBI) scores were inversely correlated with α4β1 expression in

Table 5. Concentrations of s-TNFα, s-MAdCAM-1, CRP, s-SAA stratified by endoscopic and clinical remission.

Biomarker (ng/mL)	Median	IQR 25–75	*n*	Median	IQR 25–75	*n*	*p* value
WEEK 2		**Endoscopic remission**			**No endoscopic remission**		
s-TNFα	0.004	0.004–0.005	2	0.004	0.003–0.004	5	0.9
s-MAdCAM-1	9294.0	5479.5–12512.0	3	7357.0	5397.0–9582.0	5	1.0
CRP	4783.0	2484.1–10624.0	3	4386.1	3078.0–11103.0	5	1.0
s-SAA	10291.0	5889.6–18694.0	3	17339.0	5352.4–17389.0	5	0.8
		Clinical remission			**No clinical remission**		
s-TNFα	0.003	0.003–0.003	1	0.004	0.003–0.004	6	0.6
s-MAdCAM-1	5479.5	3572.3–7386.8	2	8469.5	5887.0–12209.3	6	0.4
CRP	10624.0	7703.5–13544.5	2	3732.0	2886.7–9423.8	6	0.3
s-SAA	18694.0	14492.5–22895.5	2	11345.7	3847.7–17376.5	6	0.6
WEEK 6		**Endoscopic remission**			**No endoscopic remission**		
s-TNFα	0.007	0.007–0.007	2	0.004	0.004–0.006	8	0.2
s-MAdCAM-1	2165.8	1604.1–2791.4	4	2332.5	2045.6–2903.4	8	0.9
CRP	2937.7	1226.9–5248.0	4	2829.0	871.4–10176.5	8	0.8
s-SAA	5515.4	3739.0–7079.9	4	9891.0	1601.9–28300.3	8	0.8
		Clinical remission			**No clinical remission**		
s-TNFα	NA	NA	0	0.004	0.004–0.007	9	NA
s-MAdCAM-1	2536.5	2131.0–2942.0	2	2410.0	2229.5–2606.0	9	1.0
CRP	4518.1	2967.8–6068.3	2	1445.0	671.6–4457.9	9	0.7
s-SAA	5515.4	4950.8–6080.0	2	5883.0	1635.0–13899.0	9	1.0
WEEK 14		**Endoscopic remission**			**No endoscopic remission**		
s-TNFα	0.003	0.003–0.009	4	.004	0.004–0.006	7	0.2
s-MAdCAM-1	4145.5	2115.8–5047.3	6	3141.5	2624.4–5005.1	8	0.8
CRP	2554.1	1003.4–3469.3	6	5679.0	3051.5–8776.6	8	0.2
s-SAA	4665.0	2668.9–7959.6	6	3574.4	2005.6–14223.9	8	1.0
		Clinical remission			**No clinical remission**		
s-TNFα	0.003	0.003–0.003	1	0.004	0.004–0.006	10	0.5
s-MAdCAM-1	1686.0	1609.3–3286.0	3	4099.5	3123.8–5504.1	10	0.3
CRP	3482.5	2580.6–11053.8	3	3450.3	1321.1–6264.5	10	0.6
s-SAA	2676.4	2286.6–18320.2	3	3574.4	2209.2–7959.6	10	0.9

(Continued)

Table 5. (Continued)

Biomarker (ng/mL)	Median	IQR 25–75	*n*	Median	IQR 25–75	*n*	*p* value
WEEK ⩾26	**Endoscopic remission**			**No endoscopic remission**			
s-TNFα	0.004	0.003–0.007	5	0.004	0.004–0.007	9	0.8
s-MAdCAM-1	3094.5	1402.5–3756.0	5	998.3	1.4–2747.3	12	0.1
CRP	4501.0	1232.6–4812.9	5	4147.8	2708.5–9904.6	12	0.8
s-SAA	5402.7	4836.4–12938.0	5	7156.3	4191.5–18240.8	12	1.0
	Clinical remission			**No clinical remission**			
s-TNFα	0.02	0.01–0.03	2	0.004	0.003–0.005	10	0.1
s-MAdCAM-1	1153.5	1029.0–1278.0	2	2059.4	460.4–3603.4	12	0.8
CRP	22927.8	13714.4–32141.2	2	3493.2	1189.2–5569.2	12	0.2
s-SAA	152799.8	82868.9–222730.8	2	6127.7	4191.5–15197.8	12	0.2

CRP, C-reactive protein; IQR, interquartile range; MAdCAM-1, mucosal addressin cell adhesion molecule-1; s-, soluble; SAA, serum amyloid A; TNFα, tumor necrosis factor alpha.

Table 6. Correlations between individual biomarkers and vedolizumab concentrations.

	r value	*p* value
Induction		
s-TNFα	14.0	0.7
s-α4β7	−0.4	0.3
s-ICAM-1	0.2	0.5
s-VCAM-1	−0.04	0.9
Maintenance		
s-TNFα	−0.2	0.6
s-α4β7	0.7	0.002*
s-ICAM-1	−0.1	0.7
s-VCAM-1	−0.06	0.8

*Statistically significant, $p \leqslant 0.05$.
Pearson's correlation coefficients were calculated between vedolizumab and individual biomarker concentrations. This was performed separately during induction and maintenance using the latest available sample collection in each phase.
ICAM-1, intercellular cell adhesion molecule-1; s-, soluble; TNFα, tumor necrosis factor alpha; VCAM, vascular cell adhesion molecule-1.

CD patients, with lower α4β1 levels associated with higher HBI scores.[23] However, clinical symptoms alone are neither sensitive nor specific for the assessment of intestinal inflammation in CD. While it may be counterintuitive that concentrations of s-VCAM-1 and s-ICAM-1 were higher in remitters, this may be explained by compensatory upregulation and shedding of alternate integrin endothelial ligands to circulation in individuals with effective blockade of α4β7-MAdCAM-1 interactions. An alternative explanation for higher s-VCAM-1 concentrations may be related to conformational state changes in α4β7 integrin affecting ligand-binding specificity.[24] Interestingly, s-TNFα and both s-VCAM-1 and s-ICAM-1 were positively correlated in this study, consistent with prior studies showing that TNF induces these CAMs. Analogous to our findings, pro-inflammatory cytokines such as TNF have also been shown to be present at higher concentrations in CD patients responding to induction therapy with TNF antagonists.[25] As these CAMs correlate with, and are induced by, TNF, the observations in this study of higher CAM concentrations in remitters is consistent with existing data.

Overall, s-α4β7 concentrations increased from baseline to week 26 in the entire cohort, independent of remission status. Total s-α4β7 levels were measured using a sandwich ELISA with the ability to detect drug-bound and free α4β7 concentrations, which may represent a surrogate for

α4β7 on lymphocytes during vedolizumab therapy. As vedolizumab binds to circulating α4β7 integrin, adhesion to gut endothelium is expected to decrease, and, therefore, the total concentration of s-α4β7 may rise. This finding is in line with our previous results from patients with UC. While s-α4β7 concentrations at week 14 were lower in patients who achieved endoscopic remission compared with those who did not, this was not consistently seen at other time points, and median increases of s-α4β7 from baseline to maintenance time points were similar between remitters and non-remitters. Consistent with our findings, a prior study of peripheral blood mononuclear cells from vedolizumab-treated patients demonstrated increased expression of α4β7 on CD4+T cells in patients with UC, but not patients with CD, and these increase inversely correlated with clinical symptoms in UC.[23] Thus, the current and prior findings suggest significant differences in the pharmacodynamic and clinical responses to treatment with vedolizumab between CD and UC.

Concentrations of s-MAdCAM-1 decreased in the entire cohort from baseline to week 26, irrespective of remission status. While tissue expression of MAdCAM-1 is upregulated during periods of gastrointestinal inflammation, a study using flow cytometry has shown that administration of vedolizumab completely inhibits MAdCAM-1 adhesion to α4β7.[26] This was also seen in our prior study with vedolizumab-treated UC patients. In addition, MAdCAM-1 concentrations decreased over time in a subset of IBD patients, and undetectable concentrations of MAdCAM-1 in maintenance were associated with clinical remission.[27] In our present study, there was no association between remission status and s-MAdCAM-1 levels; however, our sample size was limited.

The most notable study limitation includes our small sample size with a limited number of patients and samples in subgroups at different time intervals. The results from the smaller analyses are largely intended to be hypothesis-generating. In addition, this cohort represents a largely refractory group of CD patients, and these findings require validation in less refractory and biologic naïve patients. Also, while the PGA was used to define clinical remission, it is a subjective interpretation and may not correlate with objective endpoints. For this reason, we identified endoscopic remission as our primary endpoint. Although prospective endoscopic scoring was performed, our endoscopic scores were not read centrally, which may introduce some bias. Moreover, although all patients had baseline active disease, a numerically higher baseline SES-CD existed in non-remitters. While this difference was not statistically significant, this may have impacted outcomes. Lastly, while serum biomarkers are easily measurable surrogates, further targeted studies evaluating the cellular expression on PBMCs, tissue expression of these markers, and mechanistic validation with functional assays are needed to expand upon these findings. Furthermore, tissue concentrations of drug and biomarkers may provide additional mechanistic insight.

Conclusion

This study describes the association between early serum biomarker concentrations in vedolizumab-treated CD patients with endoscopic and clinical outcomes. Overall, s-α4β7 concentrations increased in all patients during treatment through week 26 while s-MAdCAM-1 concentrations decreased. While there was no association between serum vedolizumab concentrations and clinical or endoscopic outcomes in this cohort, higher concentrations of s-VCAM-1 and s-ICAM-1 early in treatment were associated with subsequent endoscopic remission, and these biomarkers increased more from baseline to maintenance in remitters compared with non-remitters. These findings may suggest that serum biomarker concentrations such as s-VCAM-1 or s-ICAM-1 during induction may be useful predictors of response to therapy. Further targeted studies are required to confirm these findings in less refractory patient populations and validate them in large cohorts for potential use in clinical practice.

Author contributions

Planning and conducting the study (AKH, RB, AJ, JN, JRN, WJS, BSB), data collection (AKH, RB, PSD, JN, SS, WJS, BSB), test interpretation (AKH, RB, AJ, WJS, BSB) interpreting data (AKH, RB, AJ, JRN, WJS, BSB), drafting the manuscript (all authors).

Conflict of interest statement

AKH reports no conflicting interests. RB reports no conflicting interests. PSD reports research

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Supplemental material

Supplemental material for this article is available online.

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