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The Citrullinated and MMP-degraded Vimentin Biomarker (VICM) Predicts Early Response to Anti-TNFα Treatment in Crohn's Disease

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Background: In Crohn's disease (CD), 10% to 40% of patients do not respond to anti-tumor necrosis factor-α (TNFα) treatment. Currently, there are no biomarkers with adequate sensitivity to separate responders from nonresponders at an early stage.

Aim: The aim of this study was to investigated whether early changes in the VICM (citrullinated and matrix metalloproteinase-degraded vimentin) biomarker were associated with response to anti-TNFα treatment in patients with CD.

Methods: Serum VICM levels were measured by ELISA in 2 independent cohorts of CD patients (n = 42) treated with anti-TNFα (infliximab or adalimumab). Response was determined by achieving clinical remission (Harvey Bradshaw Index < 5).

Results: Compared with baseline, VICM serum levels were reduced by anti-TNFα in the infliximab cohort (week 6 and 14) and in the adalimumab cohort (week 8). VICM was lower in the responders compared with the nonresponders [infliximab: week 6, $P < 0.05$; area under the curve (AUC) = 0.90; adalimumab: week 1, $P < 0.01$ (AUC = 0.91), and week 8, $P < 0.05$ (AUC = 0.86)], and were able to predict response to treatment after 1 week of treatment with an odds ratio of 42.5.

Conclusions: The VICM biomarker was time dependently reduced in CD patients responding to anti-TNFα treatment. We suggest that VICM may be used as a marker for monitoring early response to anti-TNFα in patients with CD.

Key Words: serological biomarkers, anti-TNF, Crohn's disease, citrullinated vimentin, prediction of response

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Crohn's disease (CD) is a chronic inflammatory disease that can affect the entire gastrointestinal tract. Drugs targeting tumor necrosis factor α (anti-TNFα) including infliximab and adalimumab are widely used in the treatment of CD.[1] Although anti-TNFα therapy has revolutionized the management of CD, 10% to 40% of CD patients starting anti-TNFα do not achieve an adequate response to the treatment.[2–5] Consequently, there is a need for biomarkers that can be applied to monitor the outcome of anti-TNFα in the first weeks of treatment or preferably even before treatment initiation. In terms of serological biomarkers, C-reactive protein (CRP), reflecting acute inflammation, is currently the most studied serum biomarker to monitor inflammatory bowel disease (IBD) disease activity.[6,7] Increased tissue remodeling in IBD is highly affected by the chronic inflammation driven by increased protease activity, for example, matrix metalloproteinases (MMPs),[8–11] leading to excessive degradation of the intestinal tissue and release of small tissue protein fragments (neo-epitopes) into the circulation, and these neo-epitope fragments were also recently confirmed to be elevated in serum from a preclinical DSS rat model.[12] Therefore, tissue-derived biomarkers of excessive MMP activity, reflecting tissue remodeling and inflammation, may be superior to CRP in monitoring response to treatment.[13,14] Biomarkers in the form of MMPs generated extracellular matrix fragments, which specifically quantify the tissue remodeling resulting from the inflammatory process, may to a high degree reflect the anti-inflammatory and mucosal regenerative effect of anti-TNFα treatment.[15–18]

Increased MMP activity contributes to extracellular matrix remodeling of CD and is associated with accelerated disease activity and endothelial/epithelial barrier integrity.[10,11,19–21] Increased levels of the citrullinated and MMP-2 and MMP-12 generated fragment of vimentin (VICM) were previously found to be highly associated with **CD**[15] and other inflammatory-driven diseases.[15,22,23] In addition, VICM is a marker of activated macrophages[24], which play a central role in the pathogenesis of IBD. Increased expression of activated macrophages is found in the mucosa in active CD inflammation and may facilitate the development of chronic inflammation.[25,26] Mucosal migration of macrophages thereby attenuates healing of the mucosa as macrophages produce high amounts of TNF-α and interleukin (IL)-23.[25] Because anti-TNF reduces the numbers of activated

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macrophages,[27,28] we hypothesized that changes in VICM levels can be applied to monitor the outcome of early response to anti-TNF treatment in CD. We evaluated the changes in VICM serum levels as a marker for monitoring early response to anti-TNF treatment in 2 independent cohorts of patients with CD.

METHODS

Study Design and Population

A total of 42 white patients with CD were included in this study. Patients were treated anti-TNFα, either infliximab or adalimumab. Thirty-two CD patients achieved clinical remission and 10 CD remained clinical active after the anti-TNFα treatment. The Harvey-Bradshaw Index (HBI) was applied to evaluate clinical disease activity. Patients achieving clinical remission (HBI < 5) were defined as responders at week 14 (infliximab cohort) and week 8 (adalimumab cohort). Patients who did not achieve clinical remission were defined as non-responders (HBI > 4).

Infliximab Cohort

Serum from 21 patients with biopsy-confirmed CD who started induction therapy with infliximab (Remicade; Janssen Biologics B.V., Leiden, Holland; intravenous infusions of 5 mg/kg body weight) was retrospectively collected from the IBD database of the University Medical Center Groningen (UMCG, IBD Center, the Netherlands) from November 2009 to March 2016. Clinical response was evaluated at week 14 after induction by applying the HBI disease activity score. Retrospectively available serum samples during induction therapy at baseline (week 0), and weeks 2, 6, and 14 were collected. Serum was always retrieved before a patient received infliximab.

Adalimumab Cohort

Serum from 21 patients with CD from Aarhus University Hospital (AUH, single center, Denmark)[29] was included retrospectively from patients with biopsy-confirmed CD who received adalimumab induction therapy between January 2009 and October 2012. The HBI disease activity score was applied to evaluate disease activity. The patients received adalimumab (n = 21) (subcutaneous injections of 160 mg on day 0, 80 mg 2 weeks later, and then 40 mg every other week) (Humira; Abbott, Chicago, IL). Retrospectively available serum samples during induction therapy at baseline (week 0), and weeks 1 and 8 were investigated. Serum was always taken before a patient received the adalimumab injection.

Exclusion Criteria

Exclusion criteria for both cohorts were as follows: clinical remission (HBI < 5) or inactive disease at baseline, age below 18 years, history of resection due to complicated disease phenotypes (intra-abdominal stenosis or fistula), no HBI data available at the end of induction, any kind of (also non-CD related) surgery or balloon dilatation within 6 months before a sample serum sample was taken or during the induction phase and solely perianal disease indication, and malignancy (except for all types of skin cancer); no patients were, however, diagnosed with any form of skin cancer at inclusion. Further exclusion criteria specific for the infliximab cohort were as follows: other fibrotic disease (eg, liver fibrosis/cirrhosis), autoimmune diseases not associated with CD, and hematologic disease. Further exclusion criteria specific for the adalimumab cohort were as follows: pregnancy, no informed consent, unable to understand/read Danish, and heart failure.

Biomarker Assay

At Nordic Bioscience (Herlev, Denmark), serum VICM (Lot. TO1505A) concentrations (a fragment of citrullinated and MMP degraded vimentin) were assessed using solid-phase competitive enzyme-linked immunosorbent assays (ELISAs).[30] In brief, precoated wells with streptavidin (cat. no. 11940279; Roche Diagnostic's Hvidovre, Denmark) were coated with a biotinylated antigen. Samples and controls were added to the wells and were incubated with horseradish peroxidase-conjugated monoclonal antibodies for 20 hours at 4°C. Subsequently, tetramethylbenzidine (cat. no. 438OH; Kem-En-Tec, Taastrup, Denmark) was added. Stop buffer (1% H_2SO_4) was added to stop the tetramethylbenzidine reaction. An ELISA reader (VersaMAX; Molecular Devices, Wokingham Berkshire, UK) was used to read optical densities at 450 and 650 nm. A standard curve was plotted using a 4-parametric mathematical fit model.

This assay has an intra-assay coefficient of variance % of <10% and interassay coefficient of variance % of <15%. Plates were rejected and reruns if they did not fulfill the intra-assay/interassay criteria. Assay controls were used to assess the intravariations and intervariations between 10 plates. The measurement range is 1.03 to 217.6 ng/mL. Samples below the lower limit of detection were therefore set to 1.03 ng/mL.

Statistical Analysis

To achieve a normal distribution, log-transformation of the data was applied before statistical analysis. Student *t* test or paired *t* test for normally distributed data was applied to analyze statistical differences between responders and non-responders for the individual timepoints and differences from baseline levels to the different timepoints, respectively. If a normal distribution was not achieved by log transformation, the Mann-Whitney *U* test or the Wilcoxon test was applied to analyze differences between responders and nonresponders for the individual timepoints and differences from baseline levels to the different timepoints for non-normal distributed data, respectively. The Bonferroni correction was applied to correct for multiple testing. Biomarker levels are presented as non-log transformed data with mean and SEM.

For the purpose of assessing the predictive power of the biomarker to differentiate between responders and non-responders to anti-TNF, receiver operating characteristic (ROC) curves were constructed. Odds ratios (ORs) were calculated using the ROC-curve defined cutoff values to carry out a contingency analysis. A *P*-value of ≤ 0.05 was considered statistically significant. Statistical analysis was carried out using Graphpad Prism 7.03 and MedCalc. Figures were created using GraphPad Prism version 7.03.

Ethical Considerations

All patients from the Aarhus cohort provided written informed consent, and the study protocol was approved by the Central Denmark Region Committees on Biomedical Research Ethics (journal no. 20080092). All patients from the Groningen cohort provided written informed consent when participating in the University Medical Centre Groningen IBD ethically approved database and for biobank (IRB no. 08/279) for the use of patient data and serum.

RESULTS

Baseline Characteristics

The 2 cohorts were similar when comparing the patient demographics (Table 1). No statistically significant differences

TABLE 1. Patient Demographics

	Infliximab Cohort (N = 21)				Adalimumab Cohort (N = 21)				
Demographics	**All**	**Nonresponders (N = 4)**	**Responders (N = 17)**	**Responders vs. Nonresponders (*P*)**	**All**	**Nonresponders (N = 6)**	**Responders (N = 15)**	**Responders vs. Nonresponders (*P*)**	**IFX vs. ADA (*P*)**
Sex (female) [n (%)]	17 (81.0)	3 (75.0)	14 (82.4)	> 0.99	10 (47.6)	3 (50.00)	7 (46.7)	> 0.99	0.052
Age at the start of treatment [mean (minimum-maximum)] (y)	37.7 (22.6-66.1)	35.7 (25.4-52.9)	38.20 (22.58-66.09)	0.752	38.5 (20.1-67.9)	32.85 (20.12-58.86)	40.7 (21.5-67.9)	0.308	0.867
Disease duration at the start of treatment [mean (minimum-maximum)] (y)	6.54 (0.3-28.4)	8.3 (0.27-28.4)	6.2 (0.5-16.4)	0.59	5.45 (0.1-12.2)	4.1 (0.5-9.4)	6.0 (0.1-12.2)	0.374	0.544
Age at diagnosis [n (%)] (y)				> 0.99				0.623	0.454
A1 (< 16)	0	0	0		0	0	0		
A2 (17-40)	18 (85.7)	4 (100)	14 (82.4)		15 (71.4)	5 (83.3)	10 (66.7)		
A3 (> 40)	3 (14.3)	0	3 (17.60)		6 (28.6)	1 (16.70)	5 (33.3)		
Disease location at the start of treatment [n (%)]				0.772				0.768	0.264
Ileal (L1)	5 (23.8)	0	5 (29.4)		2 (9.5)	0	2 (13.3)		
Colonic (L2)	4 (19.0)	1 (25.0)	3 (17.7)		8 (38.1)	3 (50.0)	5 (33.3)		
Ileocolonic (L3)	12 (57.1)	3 (75.0)	9 (53.0)		11 (52.4)	3 (49.7)	8 (53.3)		
Disease behavior at the start of IFX [n (%)]				> 0.99				0.071	> 0.999
Nonstricturing/nonpenetrating (B1)	18 (85.7)	4 (100.0)	14 (82.4)		19 (90.5)	4 (66.7)	15 (100.0)		
Stricturing (B2)	3 (14.3)	0	3 (17.60)		2 (9.5)	2 (33.3)	0		
Penetrating (B3)	0	0	0		0	0	0		
Perianal disease [n (%)]	6 (28.6)	1 (25.0)	5 (29.4)	> 0.99	4 (19.0)	1 (16.7)	3 (20.0)	> 0.99	0.719
Resection before IFX [n (%)]	4	1 (25.0)	3 (17.6)	> 0.99		0	0	NA	NA
Cause of resection before starting IFX						NA	NA	NA	NA
Persistent inflammation	2	2 (50.0)	3 (17.6)	0.228					
Stenosis	0	0	0						
Intra/abdominal fistula/abscess/ perforation (with stenosis)	0	0	0						

ADA indicates adalimumab; IFX, infliximab; NA, not applicable.

were observed between responders and nonresponders in the 2 cohorts (Table 1). Serum VICM correlated positively with CRP in the infliximab cohort ($r = 0.62$, $P < 0.001$), but not in the adalimumab cohort ($r = 0.15$, $P = 0.54$). There were no differences in baseline VICM levels between responders and nonresponders, and also among patients treated with infliximab and those treated with adalimumab.

VICM Levels Are Reduced in Patients With Crohn's Disease Who Receive Anti-TNFα

VICM and CRP serum levels decreased during the course of anti-TNFα treatment. In infliximab-treated patients, VICM was statistically significant reduced at week 6 ($P = 0.008$) and week 14 ($P = 0.013$) compared with the baseline (Fig. 1). In the adalimumab cohort, VICM was significantly reduced at week 8 ($P = 0.009$), compared with the baseline, but not at week 1 ($P = 0.53$) (Fig. 1). CRP levels decreased in both groups from anti-TNFα treatment. In the infliximab cohort, there was a significant decrease in CRP levels at week 2 ($P = 0.023$) and week 6 ($P = 0.003$) compared with the baseline, and for the adalimumab cohort, CRP levels were significantly reduced at week 1 ($P = 0.002$) and week 8 ($P = 0.004$) compared with the baseline (Fig. 1).

VICM Levels Are Reduced in Responders to Anti-TNF Treatment in CD

In both cohorts, VICM serum levels were statistically significantly reduced at different timepoints in patients who responded to anti-TNFα treatment compared with the baseline [infliximab week 6 ($P = 0.039$), and adalimumab week 1 ($P = 0.33$), and week 8 ($P = 0.003$)] (Fig. 2). VICM was reduced in patients responding to infliximab at week 2 and week 14 compared with nonresponders; however, the reduction in VICM serum levels at these timepoints was not significant compared with the nonresponders [infliximab week 2 ($P = 0.48$) and week 14 ($P = 0.23$)] (Fig. 2).

There were no significant changes in VICM levels in nonresponding patients compared with the baseline. VICM levels were significantly lower in responders compared with nonresponders at week 6 ($P = 0.046$) in the infliximab-treated patients and at week 1 ($P = 0.007$) and week 8 ($P = 0.048$) in adalimumab-treated patients (Fig. 2).

Anti-TNFα treatment reduced CRP levels in both cohorts for responders compared with baseline [infliximab week 2 ($P = 0.094$), week 6 ($P = 0.023$) week 14 ($P = 0.69$); adalimumab week 1 ($P < 0.001$), and week 8 ($P < 0.001$)] and nonresponders [infliximab week 2 ($P = 0.25$), week 6 ($P = 0.066$)

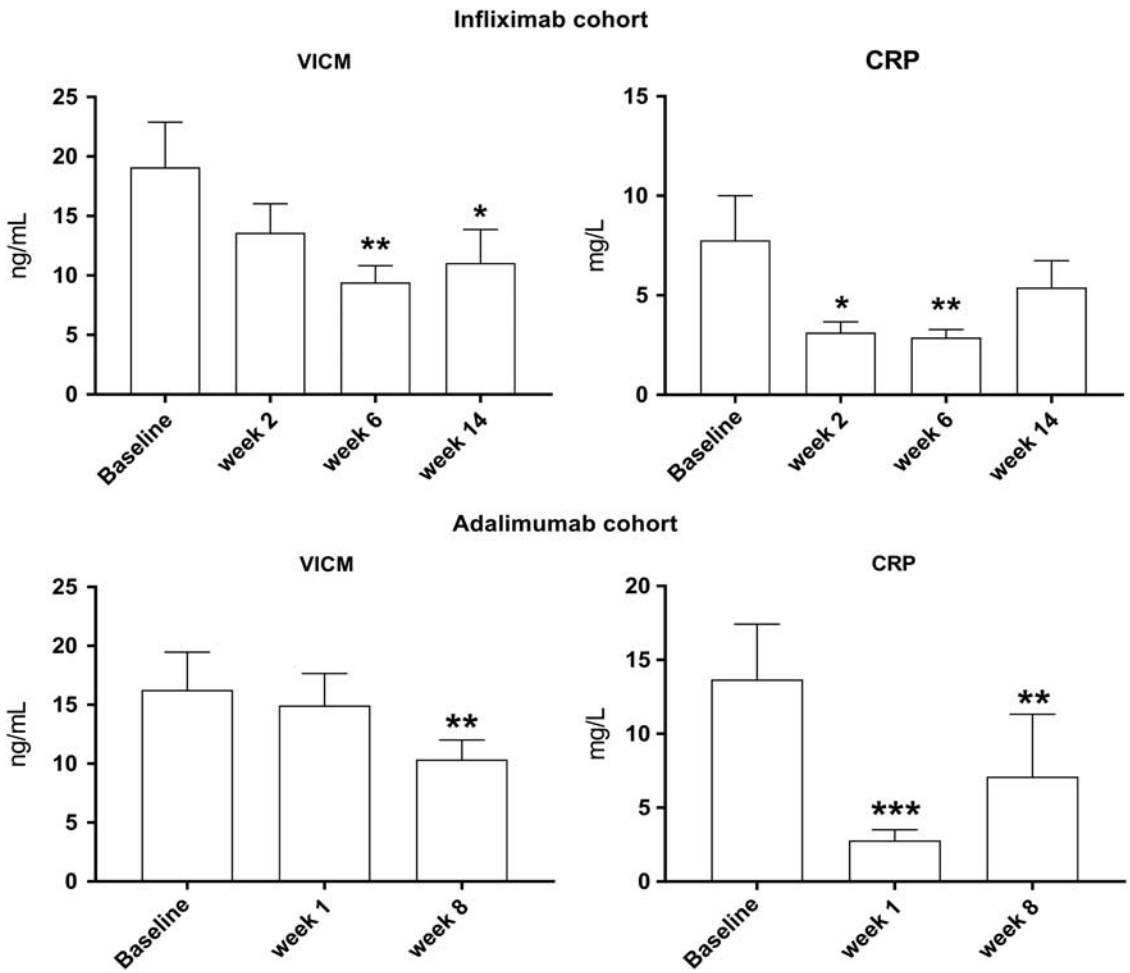

FIGURE 1. Representation of the VICM biomarker serum level during the course of anti-TNFα treatment in CD, including anti-TNF responders and anti-TNFα nonresponders for both cohorts. Asterisks indicate the level of significance, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Error bars indicate SEM. CD indicates Crohn disease; CRP, C-reactive protein; TNF, tumor necrosis factor.

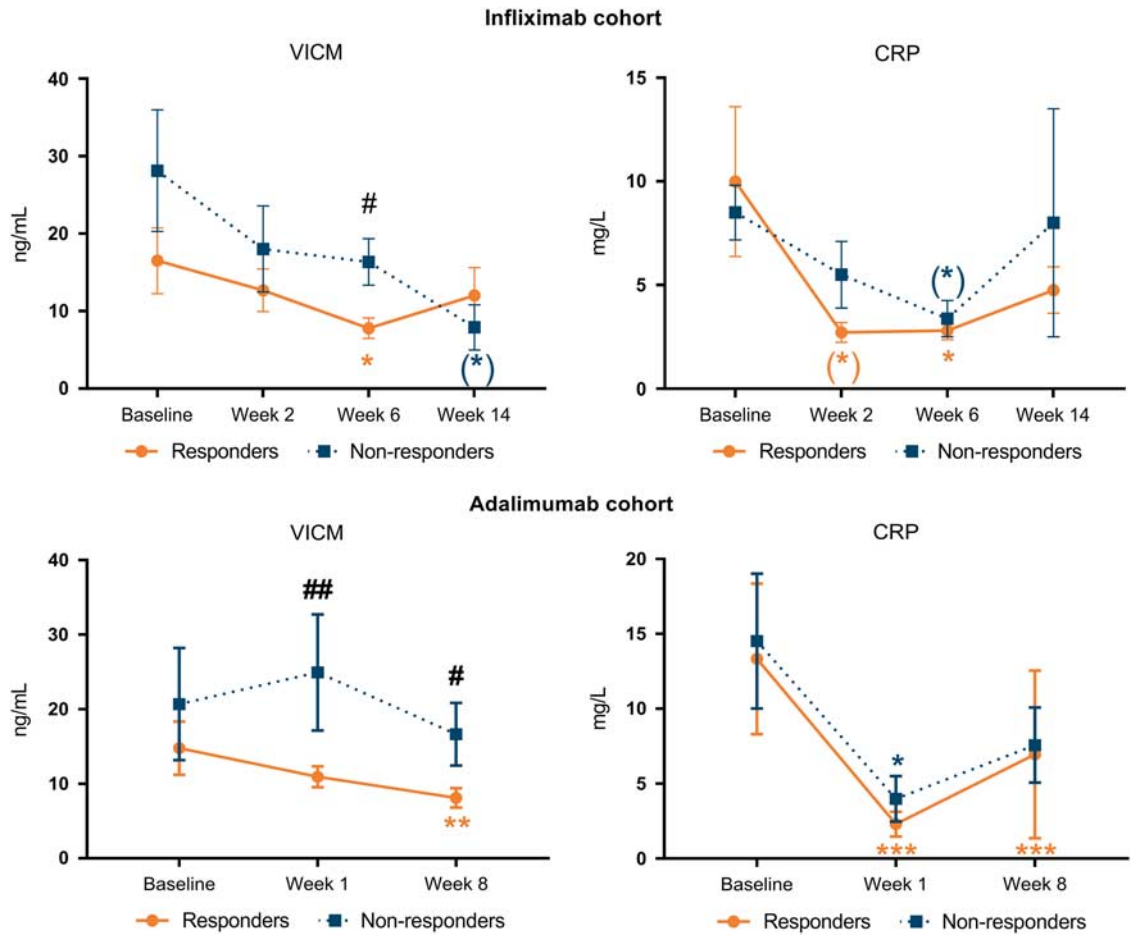

FIGURE 2. Representation of the VICM and CRP biomarkers serum levels as a response to anti-TNFα. Responders (filled orange line) and nonresponse (dashed blue line) to anti-TNFα treatment in CD. Asterisks* indicate the level of significance, *$P<0.05$, **$P<0.01$, ***$P<0.001$ at different timepoints compared with the baseline. Hashtags # indicate the level of significance #$P<0.05$, ##$P<0.01$ between responders and nonresponders at a given timepoint. Parentheses with an asterisk (*) indicate borderline significant biomarker reduction compared with the baseline. Error bars indicate SEM. CD indicates Crohn disease; CRP, C-reactive protein; TNF, tumor necrosis factor. full color online

week 14 ($P=0.53$); and adalimumab week 1 ($P=0.016$), and week 8 ($P=0.38$)]. However, no significant differences in the changes in CRP levels were observed when comparing responders with nonresponding patients (Fig. 2).

Reduced VICM Serum Levels are Associated With Response to Anti-TNFα Treatment in CD

A clinically relevant cut-off value of 12.5 ng/mL for VICM was identified by ROC-curve analysis for the infliximab and adalimumab cohort (Fig. 3, Table 2). Using this cut-off, VICM levels were able to differentiate responders from nonresponders at week 6 [area under the curve (AUC): 0.89, specificity: 75%, sensitivity: 88%, $P<0.01$] for the infliximab cohort and week 1 (AUC: 0.91, specificity: 87%, sensitivity: 100%, $P<0.01$) and week 8 (AUC: 0.86, specificity: 86%, sensitivity: 40%, $P<0.05$) for the adalimumab cohort (Fig. 3, Table 2).

A cut-off value for VICM serum levels <12.5 ng/mL for both cohorts was applied. Patients with VICM levels <12.5 ng/mL were 22.5 to 42.0 times more likely to respond to anti-TNFα compared with those with a VICM level >12.5 ng/mL (infliximab cohort week 6: OR = 22.5; adalimumab cohort week 1: OR = 42.0) (Fig. 4). Comparable analysis using CRP levels to predict the treatment outcome could not predict response to treatment for either cohort (Fig. 4).

DISCUSSION

In this study, we investigated whether reduced serum levels of the biomarker VICM are associated with response to anti-TNFα treatment. To summarize, our main finding was that VICM may be used as a pharmacodynamic biomarker to monitor early response to anti-TNFα treatment in patients with CD.

An unmet clinical need in the treatment of CD is noninvasive biomarkers that can accurately predict the therapeutic efficacy of a given treatment modality[5] and such biomarkers could aid clinicians in selecting optimal treatment options. Changes in VICM serum levels in the induction phase of anti-TNFα treatment may provide an early identification of responders and nonresponders to treatment, and thereby assist clinicians in making decisions to switch drugs in the 30% to 40% of CD patients who will not respond to anti-TNFα in the early treatment phase.[2,3]

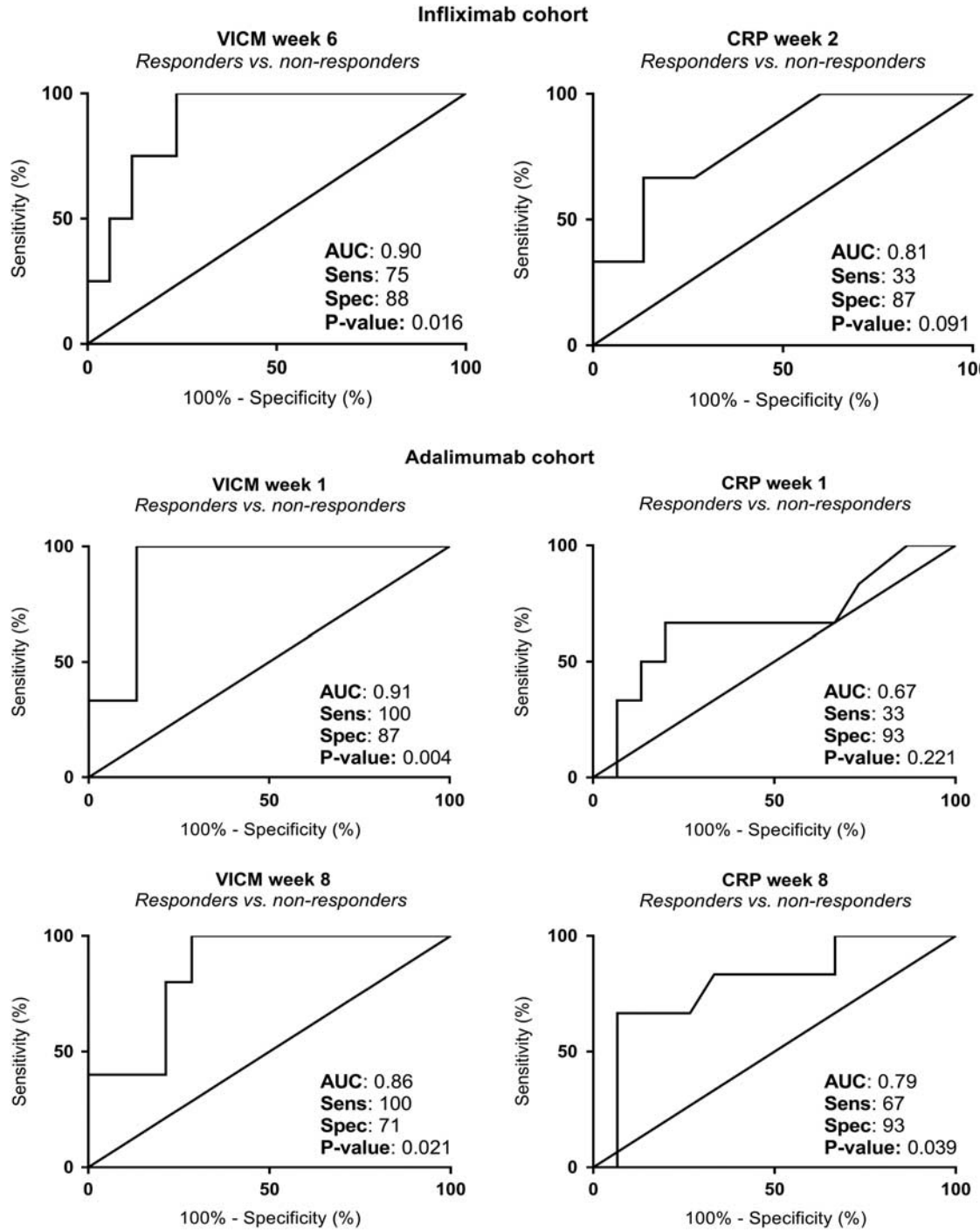

FIGURE 3. Representation of the diagnostic accuracy of biomarkers VICM and CRP to predict response to anti-TNFα by ROC-curve analysis. AUC indicates area under the curve; CRP, C-reactive protein; ROC, receiver operating characteristic; Sens, sensitivity; Spec, specificity; TNF, tumor necrosis factor.

VICM was previously demonstrated to be a biomarker of activated macrophages *in vitro* and serum VICM levels are reduced in rheumatoid arthritis patients treated with anti-granulocye macrophage-colony stimulating factor,[24] leading to diminished activation of macrophages.[31,32] Activated macrophages contribute to the chronic inflammation in CD by producing a plethora of proinflammatory cytokines, including granulocye macrophage-colony stimulating factor, TNFα, and IL-23, converging into sustained macrophage activation and recruitment of other inflammatory cells.[25,27,28,31,33] Anti-TNFα have also been demonstrated to attenuate macrophage activity but also MMP activity.[34] In addition, MMP-12, which is partly responsible for the generation of the VICM fragment, has been

TABLE 2. ROC-curve Analysis of VICM and CRP to Differentiate Between Responders and Non-responders

Cohorts	Cutoff	AUC (CI)	Sensitivity (%)	Specificity (%)	*P*
Infliximab cohort					
Responders vs. nonresponders					
VICM (wk 6)	< 12.5 ng/mL	0.90 (0.76-1.00)	75	88	0.016*
CRP (wk 2)	< 6 mg/L	0.81 (0.47-1.00)	33	87	0.091
Adalimumab cohort					
Responders vs. nonresponders					
VICM (wk 1)	< 12.5 ng/mL	0.91 (0.78-1.00)	100	87	0.004**
CRP (wk 1)	< 6 mg/L	0.67 (0.31-86)	33	93	0.221
VICM (wk 8)	< 12.5 ng/mL	0.86 (0.68-1.00)	100	71	0.021*
CRP (wk 8)	< 6 mg/L	0.79 (0.50-1.00)	67	93	0.039*

AUC indicates area under the curve; CI, confidence interval; CRP, C-reactive protein; ROC, receiver operating characteristic; TNF, tumor necrosis factor.
*$P < 0.05$.
**$P < 0.01$.

demonstrated to contribute to loss of response to anti-TNFα treatment through proteolytic degradation of the antibodies (including adalimumab, infliximab, and etanercept),[35] which may indicate that loss of response to anti-TNFα is associated with elevated MMP-12 activity and thus also VICM serum levels. Therefore, the results obtained from this study are in agreement with previous data demonstrating that VICM may be attenuated by therapies that diminish inflammation and macrophage activity.[24,31,32,34,35]

Our study demonstrates that VICM may be a valuable biomarker for monitoring and predicting the early outcome of anti-TNFα treatment in CD. Changes in VICM levels performed better than CRP in predicting the early outcome of anti-TNFα treatment. Thus, VICM may offer additional value to monitoring response to treatment and to predicting early response, within the first weeks, in CD patients and may potentially benefit decision-making on dose escalation or a different treatment. These data may also illustrate that high serum levels of VICM are indicative of flare recurrence or nonresponse, and therefore, could be utilized as a tool for decision-making for when endoscopic assessment should be prioritized or initiated to confirm a flare. We can only speculate to what extent changes in VICM levels can also be used monitor the outcome of other treatment modalities, for example, anti-α4β7 integrin and anti-IL-23 therapies. However, as the VICM biomarker is not specific for anti-TNFα treatment, it is highly likely that VICM would perform equally well as a marker for monitoring of early response in for other treatments for CD. Our findings of high VICM levels in nonresponding patients may indicate that a lack of response to anti-TNFα in CD patients corresponds with insufficient suppression of macrophage activity from this treatment.

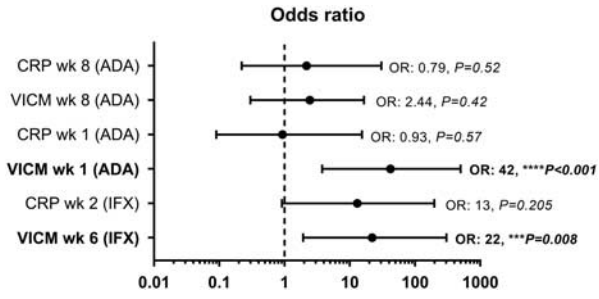

FIGURE 4. OR for predicting response to anti-TNFα with a 95% confidence interval. ADA indicates adalimumab; CRP, C-reactive protein; IFX, infliximab; OR, odds ratio; TNF, tumor necrosis factor.

A major strength of this study is the inclusion of 2 independent well-characterized CD patient cohorts treated with anti-TNFα, and VICM performed equally well as a biomarker of early response in both cohorts. However, there are several limitations of the study that need to be addressed in future studies on the role of the VICM biomarker as a treatment response biomarker. The sample size of both cohorts is small, despite significant findings, which should be validated in larger prospective cohorts, preferably > 100 patients to increase the statistical power and will also allow to take into the account the heterogeneity of CD, including patient demographics, disease behavior (luminal, fibrostenosis, and fistula), disease location, ethnicity, and environmental factors. In addition, the ability of VICM to predict a prolonged anti-TNFα response should be investigated in a CD cohort treated for > 14 weeks. Another limitation of the study is that the timepoints for end of treatment and evaluation of response in the 2 cohorts were very different. Comparing response at week 14 for infliximab cohort and at week 8 for adalimumab cohort makes the groups heterogenous in this respect. ECCO recommends primary response to anti-TNF to be assessed at week 12, and there is some evidence that for adalimumab in CD week 14 should be more suitable. Future studies should also focus on evaluating VICM and its association with endoscopic and histologic outcomes, and correlation to fecal calprotectin to address whether VICM can predict mucosal healing at an early timepoint in CD patients treated with biologics including anti-TNFα, anti-α4β7 integrin, and anti-IL-23 therapies or immunosuppressants.

In conclusion, the reduction in VICM serum levels, but not reduction in CRP levels, was associated with early response to anti-TNFα treatment in patients with CD. Thus, VICM may help to facilitate early decision-making of the best possible treatment option for CD patients.

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