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Increased cell adhesion molecules, PECAM-1, ICAM-3, or VCAM-1, predict increased risk for flare in patients with quiescent inflammatory bowel disease

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Abstract

Goals and Background—Predicting the risk of flare-ups for patients with inflammatory bowel disease (IBD) is difficult. Alterations in gut endothelial regulation of mucosal immune homeostasis might be early events leading to flares in IBD. Cell adhesion molecules (CAMs), in particular, are important in maintaining endothelial integrity and regulating the migration of leukocytes into the gut.

Study—We evaluated the mRNA expression of various tight junction proteins, with an emphasis on CAMs, in 40 patients with IBD in clinical remission. Patients were retrospectively assessed at 6, 12, and 24 months after baseline colonoscopy, and at the end of all available follow-up (maximum 65 months), for flare events to determine whether baseline mRNA expression was associated with subsequent flares.

Results—At all follow-up points, the baseline expression of PECAM-1, ICAM-3, and VCAM-1 was significantly higher in patients who flared than in those who did not (2.4-fold elevation, p=0.012 for PECAM-1; 1.9-fold increased, p=0.03 for ICAM-3; and 1.4-fold increased, p=0.02 for VCAM-1). PECAM-1 and ICAM-3 expression was significantly increased in patients who flared as early as 6 months after baseline colonoscopy. In contrast, there were no significant differences between patients with and without flares in baseline expression of other CAMs (ESAM, ICAM-1, ICAM-2, E-selectin, P-selectin, and MadCAM1).

Conclusions—Increased expression of PECAM-1, ICAM-3, and VCAM-1 in colonic biopsies from patients with IBD in clinical remission is associated with subsequent flares. This suggests that increases in the expression of these proteins may be early events that lead to flares in patients with IBD.

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Keywords

inflammatory bowel disease; PECAM-1; ICAM-3; VCAM-1

INTRODUCTION

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), is immune-mediated and characterized by chronic inflammation of the intestinal mucosa. The pathogenesis of inflammation in IBD remains to be elucidated, and the disease course is often difficult to predict. However, studies have suggested multiple mechanisms that may contribute to the development of inflammation.[1] Among these proposed mechanisms, the endothelium of the gut may play an important part due to its active role in mucosal immune homeostasis.

One of the means by which the gut endothelium regulates mucosal immune homeostasis is through cell adhesion molecules (CAM). CAMs are a family of membrane molecules involved in the extravasation of leukocytes from the blood into the inflamed mucosa through a multistep process involving sequential capturing, rolling, firm adhesion, and transmigration. P-selectin, E-selectin, ICAM-1, ICAM-2, ICAM-3, VCAM-1, PECAM-1, and MadCAM1 each have a role in the process of extravasation, and all have been found to be overexpressed in IBD patients.[2–6] Moreover, clinical trials investigating various CAMs as potential targets for treatment of IBD have found some of these treatments, for example vedolizumab and natalizumab, to be effective.[7,8]

Among the different CAMs, P-selectin and E-selectin, which have an early role in the extravasation process by capturing leukocytes, are overexpressed in the colonic mucosa of UC patients compared to healthy controls; expression of P-selectin, in particular, correlates with disease activity in UC.[6] Expression of ICAM-1, which participates in leukocyte adhesion, is elevated in both CD and UC.[2,4,6] Furthermore, ICAM-1 is more elevated in active UC than in UC in remission.[4,6] The degree of ICAM-1 expression also seems to be proportional to the degree of inflammation in CD.[6,9] Additionally, expression of ICAM-3, which presumably functions in leukocyte extravasation, is significantly elevated in CD patients compared to healthy controls but is interestingly found in lower concentrations in UC than in CD.[6] CAM1, which helps hone lymphocytes to sites of inflammation in the gut, is more highly expressed in the venuoles of the colonic mucosa in CD than in UC.[5] Finally, higher concentrations of PECAM-1, which is involved in the transmigration of leukocytes into the mucosa, is associated with active inflammation in UC and CD, and the degree of expression correlates with disease activity in UC.[6] Interestingly, VCAM-1, which is involved in lymphocyte and monocyte-endothelial cell interactions, is found in higher concentrations in the serum, but not in the colonic mucosa, of patients with active UC compared to healthy controls.[4]

One of the main goals in IBD management is to prevent relapses or, at the very least, to prolong the duration of remission before the next relapse. Identifying predictive biomarkers can help guide management decisions in order to reduce the risk of relapse. While studies have evaluated the expression of various CAMs in patients with active or inactive disease,

none has evaluated the predictive value of these markers for future flares. In this study, we aimed to identify CAMs in colonic biopsies of IBD patients taken during clinical remissions that are associated with an increased risk of clinical relapse.

MATERIALS AND METHODS

Study Design

From 2009 through 2011, we obtained informed consent from patients at our VA Medical Center with UC and colonic CD who had minimal or no symptoms at the time they were scheduled for clinically-indicated elective colonoscopy. Disease activity was assessed prior to colonoscopy using the Colitis Activity Index (CAI) for patients with UC and the Crohn's Disease Activity Index (CDAI) for patients with Crohn's disease. Patients were eligible for participation if they had a CAI <5 or a CDAI <150. Biopsies of the right, transverse and left colon were taken during this colonoscopy and flash frozen in liquid nitrogen.

Patients' charts were retrospectively reviewed at 6, 12, 24 months after their colonoscopy, and through the end of the study period to assess whether patients had relapsed or remained in remission. The patients were then divided into two groups based on whether they relapsed or remained in remission after the index colonoscopy.

Demographic Data

Age, sex, race, current medication regimen for IBD (at time of index colonoscopy), age at diagnosis of IBD, disease duration, and any documented medication noncompliance during the study follow-up period were recorded for each patient.

Tissue sample analysis

As an exploration set, RNA was extracted from the flash frozen colonic biopsies obtained at baseline from 6 patients who subsequently flared and from 6 patients who remained in remission, and the samples were evaluated for expression of 84 candidate genes using RT2 Profiler Human Tight Junction PCR Array (Qiagen #PAHS-143ZC-12). For patients with UC, a biopsy from the left colon was used for analysis; for patients with Crohn's, the site which correlated with the area of known prior inflammation was chosen for analysis.

As a validation set, significant or close to significant candidate genes identified in the exploration set were confirmed by quantitative real-time PCR in all 40 patients, along with assessment of additional genes in the same family as those identified as significant in the exploration analysis.

Relapse of IBD

The primary outcomes of interest were IBD flares within 6 months, 12 months, and 24 months of follow up. A relapse was defined as an increase in disease activity (i.e., increased number of stools, presence of blood, and/or abdominal pain) that the treating physician deemed as a relapse and that resulted in a change in the medication regimen.

Statistical Analysis

Analyses were performed using Fisher's exact test for categorical variables, and the unpaired t-test (normally distributed) or Mann-Whitney Test (not normally distributed) for continuous variables. Numbers are expressed as mean ± standard deviation.

ETHICAL CONSIDERATIONS

The Dallas VA Medical Center's institutional review board approved this study.

All authors have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting of the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

RESULTS

A total of 40 patients underwent a colonoscopy while in clinical remission and had tissue sampling performed for analysis. Among these patients, 15 (37.5%) eventually relapsed and 25 remained in remission.

Baseline Characteristics

Of the 40 patients included in the study, 35 were male and 5 were female (Table 1). Age at index colonoscopy ranged from 37 to 85 years with a mean of 59.2 years. After the index colonoscopy, relapses occurred in 8 patients by 6 months (20%), 9 patients by 12 months (23%), 11 patients by 24 months (28%), and 15 patients by then end of all available follow-up (37.5%).

The two study cohorts when divided based on documentation of relapse during the follow up period were similar in regard to IBD type (for UC, 10 vs. 13 patients, relapse vs. remission, p=0.36; for CD, 6 vs. 12 patients, relapse vs. remission, p= 0.18) and disease duration (15.9 vs. 19.0 years, relapse vs. remission, p=0.01). The two groups differed significantly in regard to mean age at index colonoscopy (50.5 vs. 64.4 years old, relapse vs. remission, p<0.01), BMI (34.8 vs. 29, relapse vs. remission, p=0.002) and documented non-compliance (9 vs. 1 patients, relapse vs. remission, p= 0.002).

Analysis of Mucosal Biopsies

In the exploration set, 6 patients who subsequently flared and 6 patients who remained in remission were evaluated for RNA expression of 84 candidate genes known to modulate tight junctions. From this analysis, only PECAM-1 mRNA expression differed significantly between the groups (see Figure 1). Other genes in the cell adhesion molecule (CAM) family included in the array (ESAM, ICAM1, ICAM2) were not significantly altered. For validation, qRT-PCR was performed in all 40 patients to confirm altered PECAM-1 expression and candidate genes that were close to significantly altered ($p \geq 0.05$–0.10) shown in Figure 1. Among these genes, PECAM-1 remained significantly elevated in patients who subsequently flared compared to those who did not (2.4-fold elevation, p=0.012, Figure 2).

In addition to PECAM-1, we assessed 5 additional CAMs not included in the initial array including ICAM-3, VCAM-1, E-selectin, P-selectin, and MadCAM1 with qRT-PCR in the complete cohort of 40 patients. Of these, ICAM-3 and VCAM-1 were also significantly elevated in the patients who flared compared to those who remained in remission (ICAM-3, 1.9-fold increased, p=0.03; VCAM-1, 1.4-fold increased, p=0.02; Figure 3) while E-selectin (0.96-fold changed), P-selectin (0.95-fold changed), and MadCAM1 (1.06-fold changed) were not significantly changed.

Lastly, we evaluated the difference in expression of the significant CAMs (PECAM-1, ICAM-3, and VCAM-1) between the subgroups of patients with either Crohn's or ulcerative colitis. For patients with Crohn's, PECAM-1 was still found to be significantly elevated in those who flared as compared to those who did not (2.5-fold increased, p=0.03) while ICAM-3 and VCAM-1 were was no longer significantly altered. For patients with ulcerative colitis, VCAM-1 was still found to be significantly altered in those who flared (1.4-fold increase, p=0.01) while ICAM-3 only trended toward significant (1.2-fold increase, p=0.07) and PECAM-1 was no longer significant. These data, however, should be interpreted with caution as the sample sizes became very small upon subgroup analysis.

Rates of flare over time

We then analyzed rates of flare over the months following the index colonoscopy, specifically by 6 months, 12 months, and 24 months, for the significantly altered CAMs: PECAM-1, ICAM-3, and VCAM-1. PECAM-1 mRNA expression was significantly higher in patients who flared than in those who remained in remission at each time point (Figure 4A). ICAM-3 mRNA expression was increased in patients who flared compared to those who did not flare at all time points, with the 6 month and 24 month time points being significant (p=0.029 and p=0.034, respectively) and the 12 month time point being close to significance (p=0.056). For VCAM-1, when analyzed over a time course, the values were higher for those who flared than for those who did not, but the differences did not achieve statistical significance.

DISCUSSION

Characterizing the role of CAMs in IBD might contribute to the understanding of the pathogenesis of inflammation in IBD and could offer new potential therapeutic targets to induce and maintain disease remission. Previous studies have examined the levels of various CAMs in the colonic mucosa and serum in active and inactive IBD. However, we are the first to characterize which CAMs are associated with a higher risk of relapse for patients in remission. In our study, using a group of patients in clinical remission, we found that ICAM-3, PECAM-1, and VCAM-1 expression were significantly higher in the colonic mucosa of the patients who relapsed than in those who remained in remission, while these groups did not differ in their expression of E-selectin, P-selectin, ICAM1, and MadCAM1.

ICAM-3 is expressed permanently on leukocytes and participates in leukocyte adhesion.[10] Currently, the role of ICAM-3 in IBD is poorly characterized. Vainer, et al. demonstrated that ICAM-3 concentrations in the colonic mucosa were significantly elevated in active CD compared to UC and healthy controls.[6] In contrast, Bloom, et al. found that there was no

detectable difference in ICAM-3 mRNA expression in inflamed IBD mucosa compared to non-inflamed mucosa and healthy controls.[11] In the current study, increased ICAM-3 expression was associated with a higher risk of relapse in IBD patients in clinical remission, suggesting that increased ICAM-3 expression is likely an early event leading to inflammation. Further studies are required to characterize the role of ICAM-3 in IBD and to establish that its increased expression is indeed a cause of subsequent inflammation.

PECAM-1, which is localized to the intercellular junctions of endothelial cells and the surface of human platelets, supports the transmigration of leukocytes to the site of inflammation in the colonic mucosa.[12,13] Recent reports describe upregulation of PECAM-1 in the colonic mucosa of active IBD, and show that this upregulation is associated with disease activity.[6] PECAM-1 expression also is increased in the non-inflamed tissue adjacent to inflamed tissue, further suggesting that PECAM-1 is involved in the early phases of inflammation.[14] Furthermore, immunoblockade of PECAM-1 results in significantly diminished disease activity in experimental colitis.[13] In the present study, we found that increased PECAM-1 expression is associated with a higher risk of relapse in IBD patients in clinical remission. Collectively, the data suggest that PECAM-1 upregulation may be a precondition for the development and maintenance of inflammation in IBD, and that its upregulation is predictive of future flares.

VCAM-1 is expressed uniquely on activated endothelium and plays a critical role in regulating lymphocyte and monocyte adhesion, particularly T-cell extravasation at sites of inflammation.[15] Studies have demonstrated significantly increased serum concentrations in active disease, but no significant difference in expression in the colonic tissue of patients with active IBD compared to healthy controls.[3,4,9] It has been suggested that VCAM-1 is involved in the homing of lymphocytes to the colonic mucosa.[16] Interestingly, in experimental colitis, chronic therapy with anti-VCAM-1 leads to a significant attenuation of disease.[17] Our current study found that increased expression of VCAM-1 in the colonic mucosa is associated with a higher risk of relapse. Thus, given the known function and the expression patterns described, VCAM-1 is likely more actively involved in chronic inflammation rather than in the acute inflammatory response in IBD. This may help to explain why natalizumab has greater efficacy in maintenance therapy than in induction therapy.[7]

Finally, we found that E-selectin, P-selectin, ICAM-1, and MadCAM1 expression was not significantly greater in patients who relapsed than in those who did not. Studies have demonstrated increased expression of all four molecules in active IBD.[4–6,9,18,19] Interestingly, E-selectin expression is absent in the colonic mucosa of quiescent disease.[3] Additionally, P-selectin and ICAM-1 expression are similar in non-inflamed tissue in IBD patients compared to healthy controls.[14,18] The constellation of data suggests that E-selectin, P-selectin, ICAM-1, and MadCAM1 have active roles in the maintenance of acute inflammation in IBD, but they may not play an important role in inducing it.

Limitations of the present study include its retrospective nature in assessing the presence of flares. Moreover, given that the study included only patients with colitis (either UC or Crohn’s colitis), the results may not be applicable to patients with ileal Crohn’s. Moreover,

an additional limitation is that our analysis only used mRNA and no other localizing techniques, like immunohistochemistry. Future studies will utilize immunohistochemistry or immunofluorescent staining on mucosal biopsies to localize the cell type(s) in which ICAM-3, VCAM-1, and PECAM-1expression changes occur. On the other hand, strengths include a large group of patient samples for tissue analysis, with a focused approach evaluating endothelial junctional function. Our findings can form the basis for a prospective study to establish the predictive value of ICAM-3, VCAM-1, and PECAM-1 expression for patients with IBD in clinical remission.

In conclusion, we have demonstrated that increased expression of ICAM-3, VCAM-1, and PECAM-1 in the colonic mucosa of IBD patients in remission is associated with a higher risk of relapse. These findings suggest that increased expression of these CAMs might be an early event leading to inflammation in IBD. If verified by further studies, clinicians might use biopsy specimens taken during colonoscopy of IBD patients in remission for prognostic information. Furthermore, our data suggest that CAMs might be fruitful target molecules in the rapidly growing field of targeted therapy. Further studies are needed to characterize the role of these CAMs in the pathogenesis of inflammation in order to develop better therapies to induce and maintain remission in IBD.

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References

1. Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. Gastroenterology. 1998; 115:182–205. [PubMed: 9649475]
2. Malizia G, Calabrese A, Cottone M, et al. Expression of leukocyte adhesion molecules by mucosal mononuclear phagocytes in inflammatory bowel disease. Gastroenterology. 1991; 100:150–159. [PubMed: 1670578]
3. Koizumi M, King N, Lobb R, Benjamin C, Podolsky DK. Expression of vascular adhesion molecules in inflammatory bowel disease. Gastroenterology. 1992; 103:840–847. [PubMed: 1379955]
4. Jones SC, Banks RE, Haidar A, et al. Adhesion molecules in inflammatory bowel disease. Gut. 1995; 36:724–730. [PubMed: 7541009]
5. Arihiro S, Ohtani H, Suzuki M, et al. Differential expression of mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in ulcerative colitis and Crohn's disease. Pathol Int. 2002; 52:367–374. [PubMed: 12100519]
6. Vainer B, Nielsen OH. Changed colonic profile of P-selectin, platelet-endothelial cell adhesion molecule-1 (PECAM-1), intercellular adhesion molecule-1 (ICAM-1), ICAM-2, and ICAM-3 in inflammatory bowel disease. Clin Exp Immunol. 2000; 121:242–247. [PubMed: 10931137]
7. Sandborn WJ, Colombel JF, Enns R, et al. Natalizumab induction and maintenance therapy for Crohn's disease. N Engl J Med. 2005; 353:1912–1925. [PubMed: 16267322]
8. McLean LP, Shea-Donohue T, Cross RK. Vedolizumab for the treatment of ulcerative colitis and Crohn's disease. Immunotherapy. 2012; 4:883–898. [PubMed: 23046232]
9. Nakamura S, Ohtani H, Watanabe Y, et al. In situ expression of the cell adhesion molecules in inflammatory bowel disease. Evidence of immunologic activation of vascular endothelial cells. Lab Invest. 1993; 69:77–85. [PubMed: 7687311]

10. Campanero MR, del Pozo MA, Arroyo AG, et al. ICAM-3 interacts with LFA-1 and regulates the LFA-1/ICAM-1 cell adhesion pathway. J Cell Biol. 1993; 123:1007–1016. [PubMed: 7901223]
11. Bloom S, Simmons D, Jewell DP. Adhesion molecules intercellular adhesion molecule-1 (ICAM-1), ICAM-3 and B7 are not expressed by epithelium in normal or inflamed colon. Clin Exp Immunol. 1995; 101:157–163. [PubMed: 7542573]
12. Newman PJ, Hillery CA, Albrecht R, et al. Activation-dependent changes in human platelet PECAM-1: phosphorylation, cytoskeletal association, and surface membrane redistribution. J Cell Biol. 1992; 119:239–246. [PubMed: 1527170]
13. Rijcken E, Mennigen RB, Schaefer SD, et al. PECAM-1 (CD 31) mediates transendothelial leukocyte migration in experimental colitis. Am J Physiol Gastrointest Liver Physiol. 2007; 293:G446–G452. [PubMed: 17510197]
14. Schuermann GM, Aber-Bishop AE, Facer P, et al. Altered expression of cell adhesion molecules in uninvolved gut in inflammatory bowel disease. Clin Exp Immunol. 1993; 94:341–347. [PubMed: 7900941]
15. Briscoe DM, Cotran RS, Pober JS. Effects of tumor necrosis factor, lipopolysaccharide, and IL-4 on the expression of vascular cell adhesion molecule-1 in vivo. Correlation with CD3+ T cell infiltration. J Immunol. 1992; 149:2954–2960. [PubMed: 1383333]
16. Vainer B. Role of cell adhesion molecules in inflammatory bowel diseases. Scand J Gastroenterol. 1997; 32:401–410. [PubMed: 9175198]
17. Soriano A, Salas A, Sans M, et al. VCAM-1, but not ICAM-1 or MAdCAM-1, immunoblockade ameliorates DSS-induced colitis in mice. Lab Invest. 2000; 80:1541–1551. [PubMed: 11045571]
18. Schurmann GM, Bishop AE, Facer P, et al. Increased expression of cell adhesion molecule P-selectin in active inflammatory bowel disease. Gut. 1995; 36:411–418. [PubMed: 7535284]
19. Oshitani N, Campbell A, Bloom S, Kitano A, Kobayashi K, Jewell DP. Adhesion molecule expression on vascular endothelium and nitroblue tetrazolium reducing activity in human colonic mucosa. Scand J Gastroenterol. 1995; 30:915–920. [PubMed: 8578193]

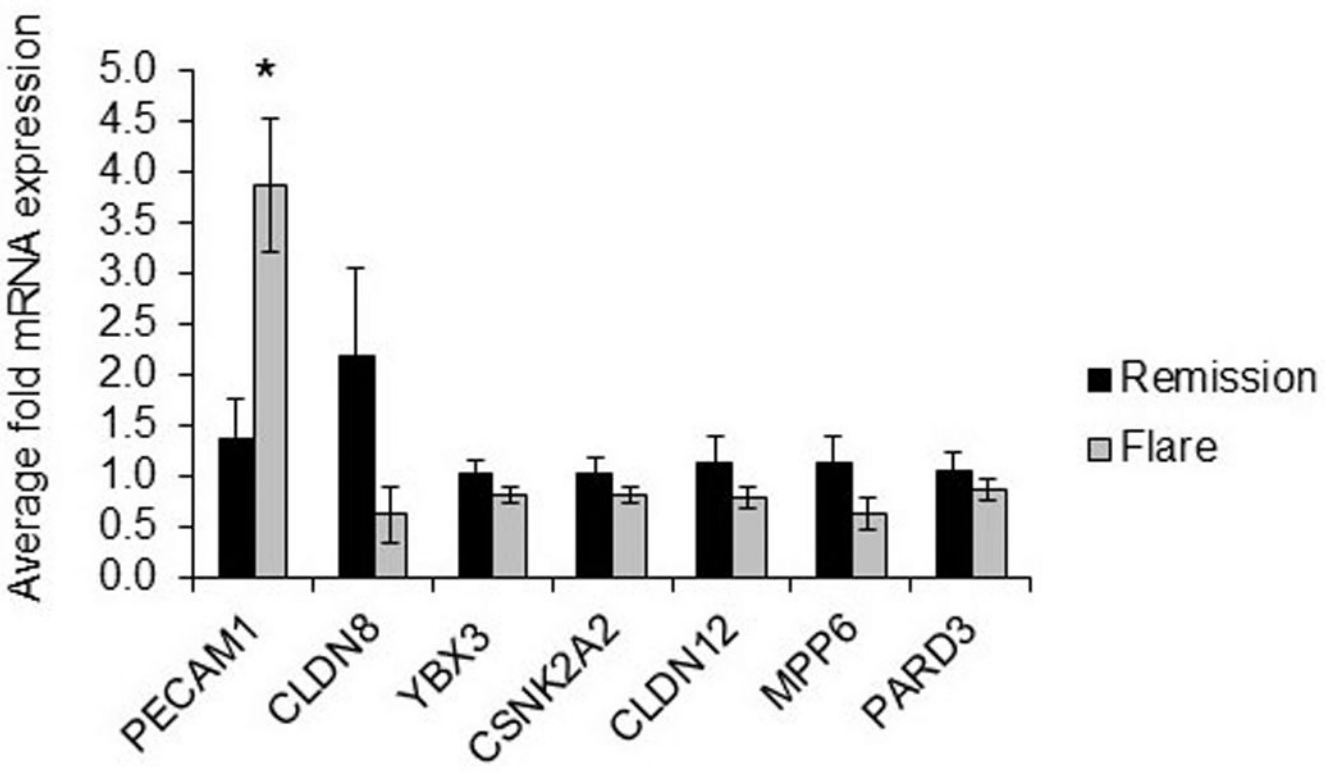

Figure 1.

Fold change of significantly ($p<0.05$) and close to significantly altered ($p \geq 0.05–0.10$) candidate genes from the exploratory analysis using the RT^2 Profiler Array comparing gene expression in 6 patients who flared compared to 6 who remained in remission. *$p<0.05$

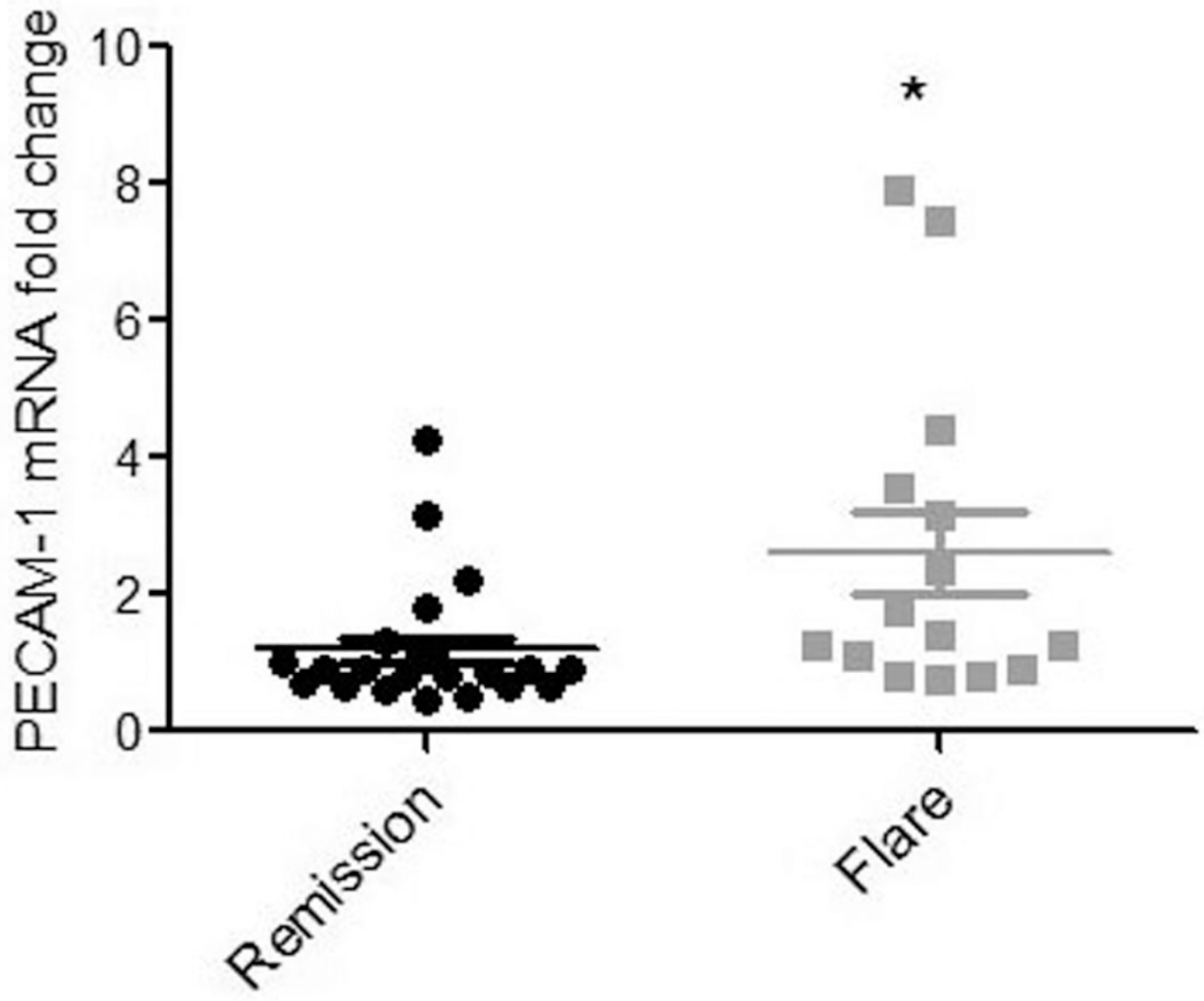

Figure 2.

Platelet endothelial cell adhesion molecule-1 (PECAM-1) mRNA expression was significantly increased in patients who flared versus those that remained in remission on confirmation by qRT-PCR of all 40 patients.

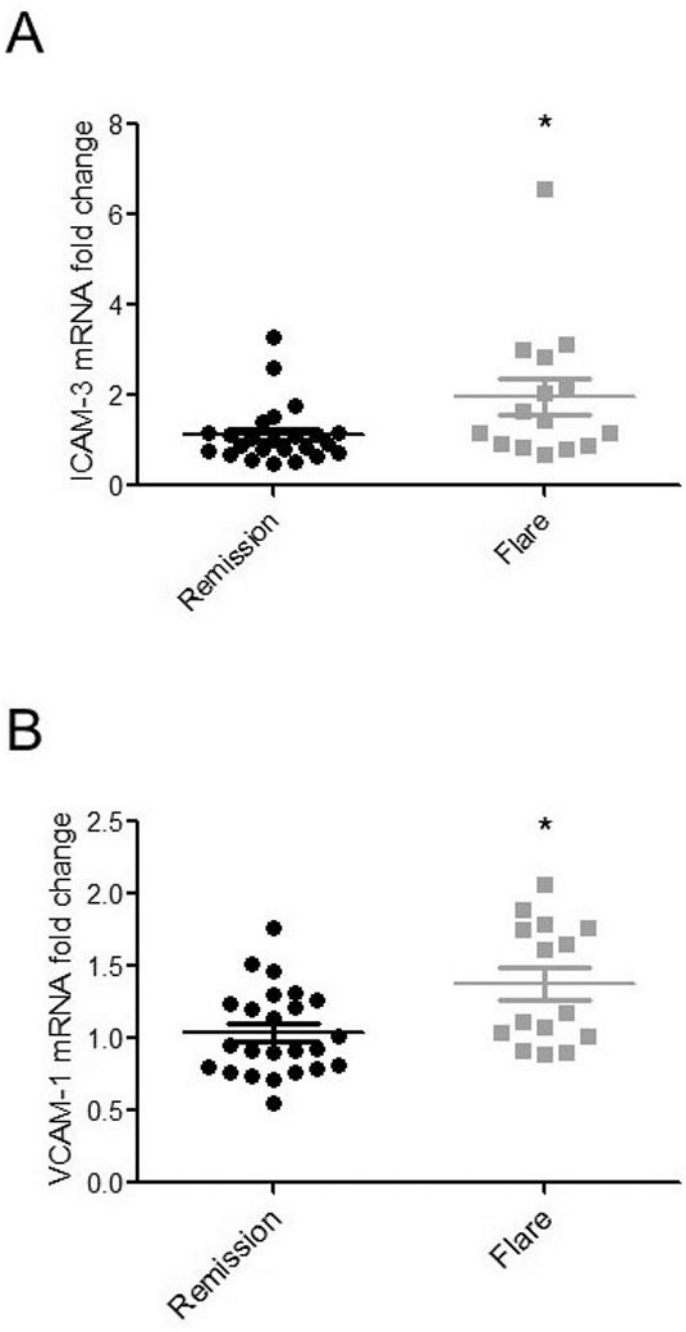

Figure 3.

ICAM-3 (panel A) and VCAM-1 (panel B) mRNA expression was significantly increased in patients who flared versus those that remained in remission. Analysis via qRT-PCR of all 40 patients.

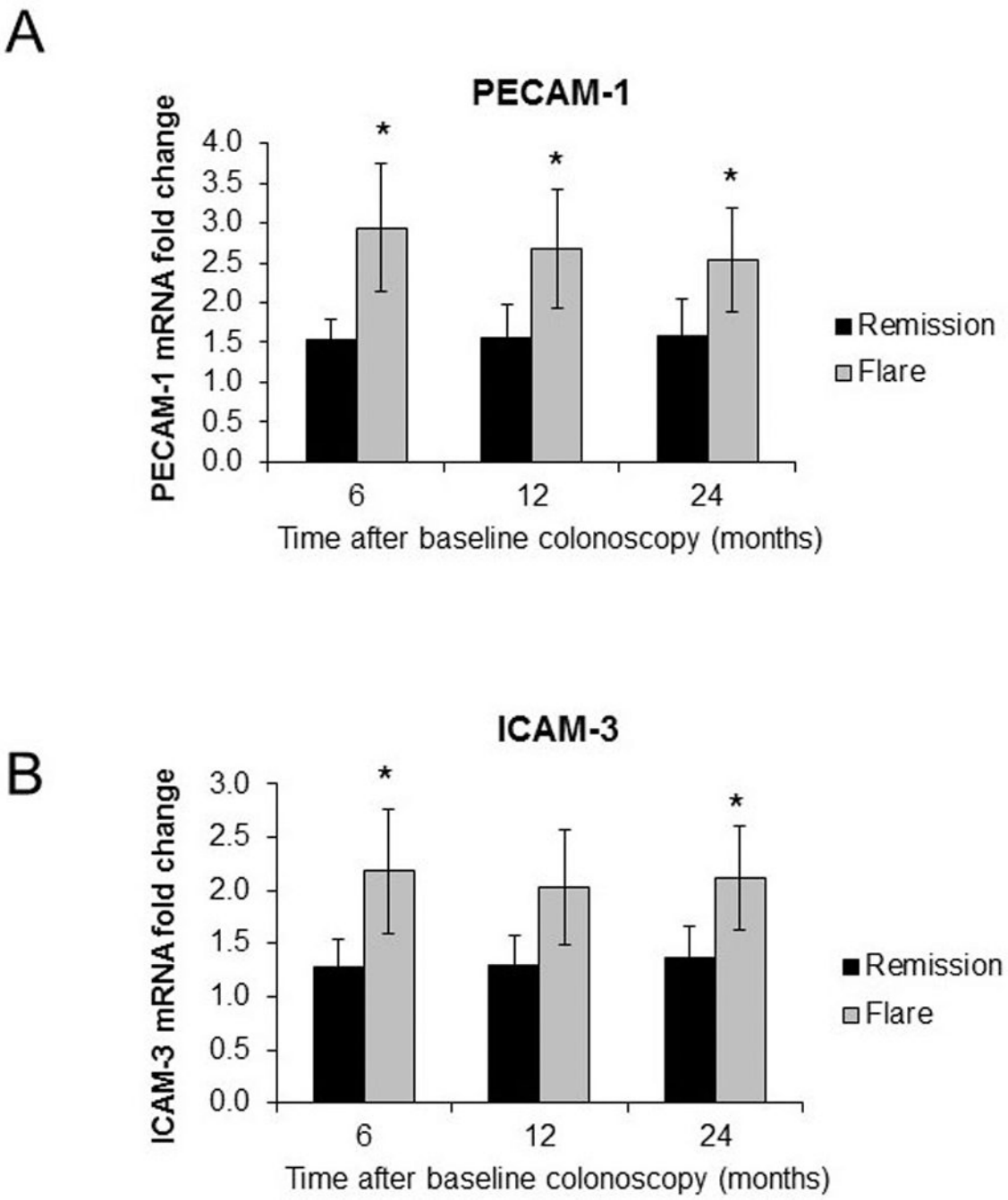

Figure 4.

Comparison of the expression of mRNA at baseline colonoscopy between patients who flared and did not flare at 6, 12 and 24 months after the baseline colonoscopy. mRNA fold change is normalized to patients who remained in remission at the respective time point.

Table 1

Baseline characteristics of study cohort and comparison between patients who experienced flares versus those that remained in remission.

	All Patients	Flare	Remission	*p-value* *
Number	40	15	25	
Average Age at Index Colonoscopy	59.2	50.5	64.4	***<0.01***
Sex (%male)	87.5%	80.0%	92.0%	0.27
Race (%)				
White	87.5%	86.7%	88.0%	0.90
Black	7.5%	13.3%	4.0%	0.28
Hispanic	5.0%	0%	8.0%	0.26
Other	0%	0%	0%	-
Tobacco Use (%current users)	12.9%	6.7%	20%	0.38
Body Mass Index (kg/m^2)	31.1	34.8	29.0	***0.002***
Disease (%)				
UC	57.5%	66.7%	52.0%	0.36
Extensive	37.5%	40.0%	36.0%	0.80
Left-sided	17.5%	20.0%	16.0%	0.74
Proctitis	0%	0%	0%	-
Unclear extent	2.5%	6.7%	0%	0.19
CD	40.0%	26.7%	48.0%	0.18
Colonic	22.5%	20.0%	20.0%	1.00
Ileocolonic	20.0%	6.7%	28.0%	0.10
Ileal	0%	0%	0%	-
Indeterminate Colitis	2.5%	6.7%	0%	0.19
Medication use at baseline				
Oral 5-ASA	80%	86.7%	76%	0.69
Rectal 5-ASA	12.5%	6.7%	16%	0.63
Thiopurine	32.5%	33.3%	32%	1.0
Biologic (infliximab or adalimumab)	22.5%	33.3%	16%	0.26
Steroid	2.5%	6.7%	0%	0.38
Disease Activity at baseline				
Colitis activity index (UC)	0.73	0.83	0.69	0.90
Crohn's disease activity index	31.5	85	19.7	***0.011***
Average disease duration (Months)	17.9	15.9	19.0	0.19
Average months of follow-up	52.7	51.7	53.3	0.73

* comparison between flare and remission groups