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Serum interleukin-6 level is associated with response to infliximab in ulcerative colitis

Short title: IL-6 is associated with response to IFX in UC

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Abstract

Objectives:

Infliximab is effective in patients with ulcerative colitis (UC); however, one-third of patients do not respond and require additional therapies such as other biologic agents. Therefore, the aim of this study was to analyze the association between pro-inflammatory molecules and clinical efficacy to elucidate possible mechanisms for the non-response to infliximab to aid in treatment selection.

Materials and methods:

Patients with moderate-to-severe active UC receiving infliximab in our hospital between 2010 and 2016 for whom pre-treatment serum samples were available were retrospectively evaluated. We analyzed the association between serum interleukin (IL)-6, tumor necrosis factor- α (TNF- α), and soluble mucosal vascular addressin cell adhesion molecule-1 (sMAdCAM-1) and the clinical efficacy of infliximab. The primary endpoint was clinical response at the end of the induction period.

Results:

Forty-one patients were included in this study. After induction therapy, 27 patients (65.9%) showed a clinical response. Serum IL-6 levels were significantly lower in responders than in non-responders ($P = 0.012$), whereas no significant differences were noted in other factors including sMAdCAM-1 and TNF- α . Multivariate analysis identified that serum IL-6 level (odds ratio = 0.72; 95% confidence interval, 0.54–0.96; $P = 0.027$) was independently associated with response to infliximab.

Conclusions:

Serum IL-6 level is associated with response to infliximab in UC. Elevated

concentrations of IL-6 may provide insight to the mechanism of non-response to infliximab.

Key Words:

Infliximab

Tumor necrosis factor

Ulcerative colitis

Interleukin-6

sMAdCAM

Introduction

Ulcerative colitis (UC) is a chronic relapsing disorder of the gastrointestinal tract that is characterized pathologically by intestinal inflammation and epithelial injury [1].

Although treatment of UC has relied mainly on 5-amino salicylates, corticosteroids and immunomodulators [2], their efficacy has been inadequate and corticosteroid refractoriness or dependence is a clinically important problem [3]. Treatment of UC has improved by the use of anti-tumor necrosis factor (TNF)- α therapy [4], using infliximab, adalimumab, or golimumab. Treatment with infliximab constitutes an effective remission induction therapy in patients with acute, severe or moderately active UC [5], and is also effective for maintaining remission or steroid tapering [6]. Nevertheless, at least 30% of patients do not respond to anti-TNF- α agents [4]. Although several biologic agents targeting inflammatory cytokines, such as IL-12/23, IL-13, and IL-17, have been investigated over the past few decades [7, 8, 9, 10, 11], most, with the exception of anti-TNF- α agents, were not effective for UC. In contrast, natalizumab [12], a monoclonal antibody directed against α 4 integrins, and vedolizumab [13], a monoclonal antibody directed against α 4 β 7 integrin that blocks α 4 β 7 integrin interaction on lymphocytes with mucosal vascular addressin cell adhesion molecule-1 (MAdCAM-1), have been reported to be effective for UC. These biologic agents are effective not only for patients naïve to anti-TNF- α therapy but also in patients with failure to anti-TNF- α therapy, implying that other molecules and/or cell types, besides TNF- α , could be involved in the pathogenesis of UC. It is crucial to be able to predict which biologic agents would be the most effective before the initiation of treatment.

Several studies have investigated predictors of clinical efficacy of anti-TNF- α agents, such as serum albumin, C-reactive protein (CRP), hemoglobin levels, or disease activity [14, 15, 16, 17, 18, 19]. Recently some studies have reported the relationships between the efficacy of anti-TNF- α therapy and specific serum cytokines levels [20, 21, 22] or trough levels of anti-TNF- α agents [23, 24]. In particular, IL-6 levels have been reported to be associated with the efficacy of anti-TNF- α agents in rheumatoid arthritis [20], Crohn's disease [22], and psoriasis [25]. Regarding UC, Sato *et al.* reported that the IL-6 level at week 8 after administration of infliximab was predictive of response at week 26 [21]. However, the association of pre-treatment serum IL-6 values with clinical response to infliximab or infliximab trough levels has not been sufficiently evaluated.

MAdCAM-1 was reported to be overexpressed on vessels in active inflammatory bowel disease (IBD) [26, 27] and downregulated by anti-TNF- α therapy [28]. Soluble MAdCAM-1 (sMAdCAM-1), which can be secreted into serum under TNF- α and methylamine stimulation [29], might also be altered in active IBD and may act as a potential marker for monitoring the clinical course. However, no studies have reported the clinical utility of measuring serum sMAdCAM-1 levels in UC.

Therefore, the aim of this study was to analyze the association between serum levels of pro-inflammatory molecules including TNF- α , serum IL-6, or serum sMAdCAM-1 in patients with UC receiving infliximab and clinical efficacy of infliximab to elucidate the possible mechanism for non-response to infliximab to aid in treatment selection.

Materials and Methods

Patients

This was a retrospective study of patients with moderate-to-severe active UC (partial Mayo score ≥ 5) receiving the scheduled induction therapy of infliximab at the Department of Gastroenterology, Osaka City University Hospital during the period from June 2010 to December 2016, and whose pre-treatment serum samples were available. The diagnosis of UC was based on clinical, endoscopic, and histopathological findings according to the diagnostic criteria determined by the Japanese Ministry of Health, Labour, and Welfare. infliximab was intravenously administered at a dose of 5 mg/kg at weeks 0, 2, and 6 as remission induction therapy and then every 8 weeks as maintenance therapy. Patients who were switched from other anti-TNF- α antibody drugs to infliximab were excluded.

Assessment of serum marker profile

Serum aliquots for marker measurement were stored at -80°C until the assay was performed. Serum infliximab levels were measured using an enzyme-linked immunosorbent assay (ELISA) [30] at KAC Co., Ltd. (Kyoto, Japan). Serum TNF- α , IL-6 levels and sMAdCAM levels were determined using an ELISA according to the manufacturer's instructions. We used the following ELISA kits: Human TNF-alpha Quantikine ELISA Kit (HSTA00D, R&D, Minneapolis, US), Human IL-6 Quantikine ELISA Kit (HS600B, R&D, Minneapolis, US), and sMAdCAM-1 ELISA kit (HK337-02, Hycult Biotech, Uden, the Netherlands).

Definition

The partial Mayo score [31] was used to assess disease activity. Moderate-to-severe active disease was defined as a partial Mayo score ≥ 5 . Clinical response was defined as

a reduction in partial Mayo score of ≥ 3 points accompanied by a decrease in the rectal bleeding subscore ≥ 1 or an absolute rectal bleeding subscore of 0 or 1 [32] without proctocolectomy or secondary alternative drug use such as tacrolimus or corticosteroids. We set the baseline period as 1 week prior to the initiation of infliximab, and the induction period as 14 weeks posterior to the initiation of infliximab. Demographic, clinical, and laboratory data were obtained from medical records. The blood samples for analyses of pre-treatment markers were obtained during baseline periods. Regarding serum infliximab trough analysis, blood samples were obtained just before the infliximab infusion at the end of the induction period (at 14 or 22 weeks).

Endpoint

The primary endpoint was clinical response to infliximab at the end of the induction period (at week 14).

Statistical analysis

Continuous variables were summarized with the median and interquartile range (IQR). The differences between clinical characteristics were compared using the chi-square test or Fisher's exact test for categorical variables and Mann-Whitney U test or Wilcoxon signed-rank test for continuous variables. Independent risk factors for clinical response were evaluated using logistic regression or multivariate analysis. Correlation coefficients were calculated using the Kendall rank correlation test. A *P*-value less than 0.05 was regarded as statistically significant. Box-and-whisker plot showing median (horizontal line), interquartile range (box), maximum/minimum range (whiskers) and outliers ($> 1.5 \times$ upper quartile). All statistical analyses were performed with EZR

(Saitama Medical Center, Jichi Medical University), a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0). More precisely, it is a modified version of R commander (version 1.6–3) that includes statistical functions that are frequently used in biostatistics.

Ethical Considerations

This study was approved by the ethics committee of the Osaka City University Graduate School of Medicine (protocol number 2286). All patients had provided their informed consent to undergo the procedure.

Results

Patients

Forty-one patients were included in this study. The demographic characteristics of patients are summarized in Table 1. After induction therapy at week 14, 27 patients (65.9%) achieved a clinical response. Given the retrospective design of the study, nine patients did not provide serum samples at the end of the induction period; thus, only 32 serum samples (responders $n = 20$, non-responders $n = 12$) were available for trough analysis.

Relationships between serum markers and disease severity

We analyzed the association between the baseline partial Mayo score and baseline serum markers. No significant correlations were noted between the partial Mayo score and serum IL-6, TNF- α , or sMAdCAM-1 levels (Figure 1).

Factors associated with the clinical response to infliximab

We analyzed association between clinical characteristics and efficacy of infliximab. Table 1 compares the clinical characteristics and serum markers at the induction of infliximab between responders and non-responders. Pre-treatment serum IL-6 levels were significantly lower in responders in comparison to that in non-responders ($P = 0.012$) (Table 1, Figure 2), whereas no significant differences were noted in clinical characteristics and other serum markers at baseline between the two groups. In the multivariate model, we included severity of disease [14, 33], hemoglobin [14, 33], serum albumin concentration [34], CRP [33, 35], and serum IL-6 because these have previously been reported to be associated with clinical efficacy of anti-TNF- α therapy in the literature and were the focus of our study. Multivariate analysis identified that IL-6 level (odds ratio = 0.72; 95% confidence interval, 0.54–0.96; $P = 0.027$) was independently associated with response to infliximab (Table 2).

Serum infliximab trough level at the end of induction period

Higher infliximab trough levels during the induction period have been reported to be associated with a higher clinical response rate to infliximab induction therapy in UC [24, 36]. We therefore assessed the relationship of serum infliximab trough levels with pre-treatment serum markers and clinical outcome. Although no significant correlation was noted between pre-treatment serum TNF- α and infliximab trough levels (Kendall's tau = -0.075, $P = 0.548$), pre-treatment IL-6 levels negatively correlated with infliximab trough levels (Kendall's tau = -0.297, $P = 0.017$) and there was a tendency for patients with low pre-treatment sMAdCAM to have higher infliximab trough levels (Kendall's tau = -0.236, $P = 0.058$) (Figure 3). Regarding the association between response to

infliximab and infliximab trough levels, responders (median 4.32 $\mu\text{g/mL}$, IQR 2.18–7.38) had significantly higher infliximab trough levels than non-responders (median 1.24 $\mu\text{g/mL}$, IQR 0.14–2.66) ($P < 0.001$) (Figure 4).

Discussion

In this study, 27 patients achieved a clinical response among 41 patients with moderate-to-severe active UC who received infliximab, which was similar to the data from the ACT 1 and 2 trials (65.9% vs. 61–69%) [4]. Pre-treatment serum IL-6 levels were associated with response to infliximab in patients with UC.

IL-6 is a pleiotropic cytokine with a central role in a multitude of immune system reactions, including growth and terminal differentiation of activated B cells [37], T cell stimulation and proliferation [38], and differentiation of cytotoxic T cells [39]. Activated monocytes and macrophages seem to be a predominant source of IL-6 in patients with IBD [40]. Moreover, IL-6 is associated with steroid resistance in severe pediatric UC [41]. Although IL-6 is a potent cytokine involved in regulating inflammation in UC [42], how IL-6 relates to failure of anti-TNF- α therapy remains unclear. One possible mechanism is related to inflammation caused by IL-6. In the present study, there were no significant differences in pre-treatment serum TNF- α levels between responders and non-responders. From this standpoint, cytokines other than TNF- α have a crucial role in response to anti-TNF- α therapy. Furthermore, there could be an inhibitory effect of IL-6 on response to infliximab because several studies have reported a decrease in serum levels of IL-6 in response to infliximab [43, 44] and pre-treatment IL-6 levels negatively correlated with serum infliximab trough levels in our study. The degree of clinical benefit noted after anti-TNF- α therapy is probably due

to the reduction in the levels of many proinflammatory mediators apart from TNF- α , such as IL-6 [45]. High levels of IL-6 might not be able to be sufficiently suppressed by infliximab and this might lead to non-response.

Another possible mechanism is that IL-6 levels may be related to infliximab drug clearance. Several studies have reported an association between infliximab trough levels and clinical response to infliximab [24, 36, 46]. We therefore assessed the association between serum IL-6 levels, serum infliximab trough levels, and clinical outcome. The data showed that pre-treatment IL-6 levels negatively correlated with serum infliximab trough levels and responders had significantly lower serum IL-6 and higher infliximab trough levels than non-responders. Brandse *et al* reported that infliximab is lost through stools in patients with UC and high fecal concentrations of infliximab in the first few days after the start of therapy are associated with primary non-response to infliximab [47]. Xiao *et al* reported IL-6 was associated with intestinal permeability in dextran sulfate sodium (DSS)-induced colitis [48]. Taken together, patients with higher serum IL-6 levels might consist of a unique subset, which have lower infliximab trough levels because more infliximab is lost into feces resulting in non-response to infliximab. Therefore, patients with high IL-6 levels should possibly be treated with another type of therapy such as small molecules (e.g. Janus kinase inhibitor, tofacitinib). In fact, tofacitinib concentrations have been reported to be similar between patients who were in remission and those who were not in remission [11]. Some studies have reported a relationship between IL-6 levels and anti-TNF therapy efficacy [21, 43]. Sato *et al.* reported IL-6 levels at 8 weeks after induction therapy may be predictive of a subsequent response to infliximab for UC [21]; however, they did not identify significant differences in pre-treatment IL-6 levels between responders and

non-responders. This discrepancy might have occurred due to differences in the definition of clinical response. We assessed the clinical response at the end of the induction period (week 14), whereas they assessed it after a longer duration (at week 26). Thus, serum IL-6 levels may be associated with short-term outcomes only. Dahlen *et al.* reported no significant differences in pre-treatment serum IL-6 levels between responders and non-responders but that responders had lower mucosal mRNA expression of IL-6 than non-responders [43]. They speculated that mucosal cytokine expression is superior to serum cytokine levels for reflecting disease severity and that serum cytokine levels did not reflect mucosal cytokine expression. Serum IL-6 levels in their report were relatively low compared to those in our study or previous reports analyzing serum IL-6 levels in patients with UC. The discrepancy might have been due to differences in disease severity at the induction of infliximab. More severe active mucosal changes in our study might have influenced serum cytokine levels and led to this discrepancy. They also reported that decreased levels of serum IL-6 levels were detected at week 14 as compared with baseline in responders. Evaluating the time course of serum IL-6 levels after the induction of infliximab is quite important. As not all serum samples at the end of induction period were available in this retrospective study, we have analyzed only those serum samples that were available (15 responders and 12 non-responders). We found no significant differences in the serum IL-6 levels at the end of the induction period between responders and non-responders ($P = 0.172$) and no significant differences between pre-treatment serum IL-6 levels and those at the end of induction period among non-responders or responders (data not shown). However, in our study, as non-responders were usually treated with additional therapies such as surgery or medications such as tacrolimus or adalimumab, it would not be appropriate to

compare serum cytokines between responders and non-responders after the induction of infliximab because it is very difficult to evaluate the influence of the additional treatments on serum IL-6 levels.

Although ECCO guidelines equally recommend infliximab and vedolizumab for immunomodulatory-refractory or steroid-refractory UC as biologics [49], there are no guidelines available defining which therapy should be chosen. We focused on sMAdCAM-1 as a potentially useful biomarker for therapy selection. The overexpressed MAdCAM-1 on vessels in active IBD patients would be tightly linked to gut inflammation. The clinical efficacy of vedolizumab [13], a monoclonal antibody against $\alpha 4\beta 7$ integrin which blocks interaction with MAdCAM-1, supports the importance of the pathway and sMAdCAM-1, the soluble form of the MAdCAM-1, was reported to be secreted under TNF- α stimulation [29], therefore, we hypothesized that sMAdCAM-1 might be associated with clinical activity and responsiveness to infliximab treatment. This is the first report analyzing the serum sMAdCAM levels in patients with UC and the association between sMAdCAM and infliximab response, to the best of our knowledge. We did not identify any statistical association between serum sMAdCAM levels and disease severity or efficacy of infliximab, although patients with high pre-treatment sMAdCAM levels had tendency to have lower infliximab trough levels at the end of induction period. Further study will be needed to clarify the clinical utility of measuring serum sMAdCAM levels.

Our study has some limitations. First, we did not measure the levels of cytokines other than IL-6 and TNF- α that may be upregulated in patients with IBD (e.g., IL-12, IL-1 β , IFN- γ , and IL-17). The expression of other inflammatory cytokines is also upregulated in patients with IBD [50]. Measuring and analyzing the levels of other

cytokines may identify factors related to response to anti-TNF therapy and may help to select appropriate therapeutic regimens for patients with UC. Another limitation of this study is its retrospective nature and the relatively small cohort. Therefore, a further large prospective study will help to evaluate predictors for the patients with UC receiving anti-TNF therapy.

In conclusion, our study findings suggest that pre-treatment serum IL-6 level is associated with response to infliximab and infliximab trough level in patients with UC. Elevated concentrations of IL-6 may provide insight to the mechanism of non-response to infliximab.

Abbreviations

CRP: C-reactive protein

IBD: inflammatory bowel disease

IL: interleukin

IQR: interquartile range

MAdCAM-1: mucosal vascular addressin cell adhesion molecule-1

sMAdCAM-1: soluble mucosal vascular addressin cell adhesion molecule-1

TNF: tumor necrosis factor

UC: ulcerative colitis

Declaration of conflict of interest

We declare that we have no conflict of interest.

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Table 1. Baseline characteristics of the study population and comparison of clinical factors between responders and non-responders

Characteristic	All patients	Responders	Non-responders	<i>P</i> value
Number of patients, n	41	27	14	
Sex: male/female, n	16 / 25	9 / 18	7 / 7	0.332
Age at diagnosis, years, median (IQR)	34.9 (23.0–44.3)	34.9 (24.7–40.7)	36.2 (23.4–44.5)	0.869
Age at initiation of infliximab, years, median (IQR)	41.5 (30.8–51.0)	41.5 (31.1–52.2)	42.6 (32.0–50.5)	0.956
Disease duration, years, median (IQR)	5.1 (2.7–8.5)	5.13 (2.62–9.51)	5.22 (3.62–5.71)	0.956
Location: left-sided colitis/pancolitis	13 / 28	7 / 20	6 / 8	0.307
Response to corticosteroids				
Dependent, n (%)	22 (53.7%)	15 (55.6%)	7 (50.0%)	0.754
Resistant, n (%)	13 (31.7%)	9 (33.3%)	4 (28.6%)	1
Concomitant therapy at initiation of infliximab, n (%)				
Mesalamine	38 (92.7%)	24 (88.9%)	14 (100%)	0.539
Corticosteroids	15 (36.6%)	11 (40.7%)	4 (28.6%)	1
Immunomodulators (azathioprine or 6-mercaptopurine)	17 (41.5%)	13 (48.1%)	4 (28.6%)	0.512
Tacrolimus	5 (12.2%)	3 (11.1%)	2 (14.3%)	1
Cytapheresis	16 (39.0%)	10 (37.0%)	6 (42.9%)	0.747
Partial Mayo score, median (IQR)	6 (5–7)	6 (5–8)	6 (5–7)	0.337
WBC (/μL), median (IQR)	6500 (5000–8600)	6200 (5000–8500)	8000 (6600–8900)	0.221
Hemoglobin (g/dL), median (IQR)	11.7 (10.9–12.5)	11.4 (10.9–12.1)	12.3 (11.5–13.2)	0.091
Albumin (g/dL, median (IQR)	3.60 (3.40–4.10)	3.80 (3.50–4.10)	3.55 (3.40–4.02)	0.362
CRP (mg/dL), median (IQR)	0.35 (0.04–1.27)	0.27 (0.03–1.16)	0.37 (0.26–2.06)	0.277
Serum interleukin-6 (pg/mL), median (IQR)	4.15 (1.49–7.09)	2.01 (1.14–6.26)	6.67 (4.07–12.7)	0.012

Serum tumor necrosis factor- α (pg/mL), median (IQR)	2.96 (1.56–6.81)	2.08 (1.19–6.38)	4.15 (2.48–6.56)	0.153
Serum sMAdCAM (ng/mL), median (IQR)	34.0 (24.7–43.0)	31.6 (24.7–42.8)	36.8 (27.7–48.6)	0.509

IQR, interquartile range;

WBC: white blood cell;

CRP: C-reactive protein;

sMAdCAM-1: soluble mucosal vascular addressin cell adhesion molecule-1

1

Table 2. Multivariate analysis of variables associated with clinical response to infliximab

	Odds ratio (95% CI)	Multivariate <i>P</i> -value
Interleukin-6	0.72 (0.54–0.96)	0.027
Hemoglobin	0.42 (0.16–1.10)	0.077
Albumin	3.97 (0.32–49.4)	0.284
CRP	1.63 (0.80–3.30)	0.180
Disease severity (partial Mayo score)	0.45 (0.16–1.22)	0.116

CI: Confidence interval

CRP: C-reactive protein

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5 **Legends to Figures**

6

7 **Figure 1. Association between the partial Mayo score and serum markers.**

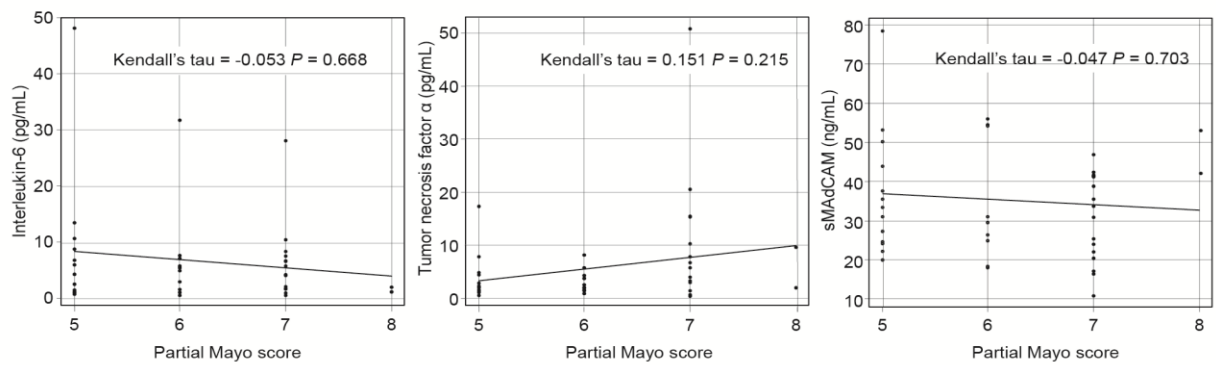
8 No significant correlations were identified between the partial Mayo score and serum

9 interleukin-6 (Kendall's tau = -0.053, $P = 0.668$), serum tumor necrosis factor- α

10 (Kendall's tau = 0.151, $P = 0.215$) or serum soluble mucosal vascular addressin cell

11 adhesion molecule-1 (sMAdCAM-1) levels (Kendall's tau = -0.047, $P = 0.703$, Kendall

12 rank correlation test).

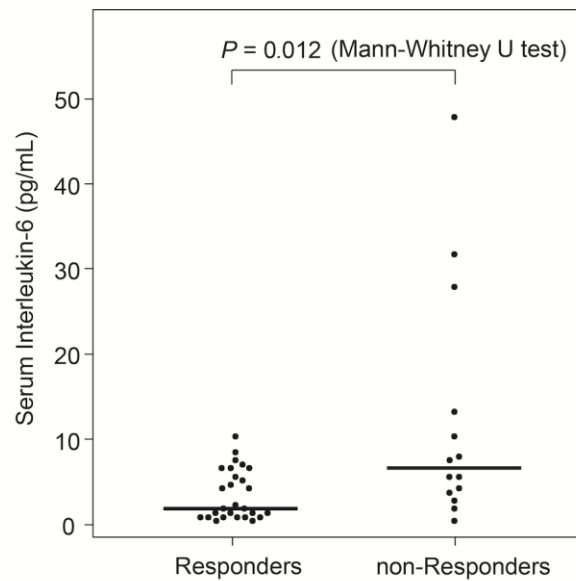


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15 **Figure 2. Comparison of pre-treatment serum interleukin-6 levels between**
16 **responders and non-responders to infliximab.**

17 Pre-treatment serum IL-6 levels were significantly lower in responders than in
18 non-responders ($P = 0.012$; Mann-Whitney U test). Horizontal bars indicate median
19 values.

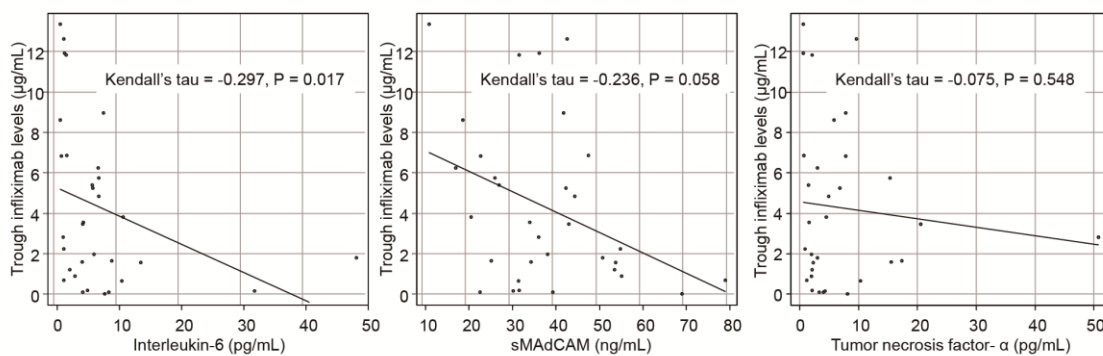


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22 **Figure 3. Correlation between the pre-treatment serum markers and serum**
23 **infliximab trough levels.**

24 Although no significant correlation was noted between pre-treatment serum TNF- α and
25 infliximab trough levels (Kendall's tau = -0.075, $P = 0.548$; Kendall rank correlation
26 test), pre-treatment IL-6 levels negatively correlated with infliximab trough levels
27 (Kendall's tau = -0.297, $P = 0.017$; Kendall rank correlation test), and there was a
28 tendency for patients with low pre-treatment sMAdCAM levels to have higher
29 infliximab trough levels (Kendall's tau = -0.236, $P = 0.058$; Kendall rank correlation
30 test). Blood samples for infliximab trough levels were obtained just before the
31 infliximab infusion at the end of the induction period (at 14 or 22 weeks).



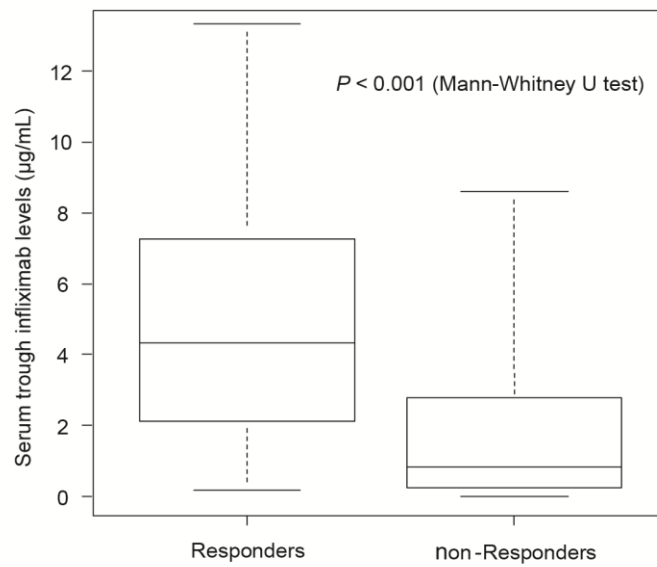
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34 **Figure 4. Comparison of serum infliximab trough levels between responders and**
35 **non-responders to infliximab.**

36 Responders (median, 4.32 $\mu\text{g/mL}$; IQR, 2.18–7.38) had significantly higher infliximab
37 trough levels than non-responders (median, 1.24 $\mu\text{g/mL}$; IQR, 0.14–2.66) ($P < 0.001$;
38 Mann-Whitney U test).

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