

# TNF- $\alpha$ and soluble forms of TNF receptors 1 and 2 in the serum of patients with Crohn's disease and ulcerative colitis

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## KEY WORDS

biomarkers, Crohn's disease, inflammatory bowel diseases, tumor necrosis factor receptors, ulcerative colitis

## ABSTRACT

**INTRODUCTION** Soluble forms of tumor necrosis factor (TNF) membrane receptors 1 and 2 (sTNFR1 and sTNFR2) are present in body fluids. Their higher concentrations are observed in a number of diseases, including inflammatory bowel diseases (IBDs). sTNFR1 and sTNFR2 are capable of binding TNF- $\alpha$ , acting as an inhibitor that competes with a membrane receptor. The results of the available studies on sTNFR1 and sTNFR2 concentrations in IBDs and their association with disease activity are ambiguous.

**OBJECTIVES** The aim of the study was to assess sTNFR1 and sTNFR2 concentrations and their correlation with disease activity in patients with IBD.

**PATIENTS AND METHODS** Plasma levels of TNF- $\alpha$ , sTNFR1, and sTNFR2 were measured in 55 consecutive patients with ulcerative colitis (UC), 50 subjects with Crohn's disease (CD), and 41 healthy controls. We assessed the associations of those markers with other inflammatory markers, disease activity and location, type of treatment, and complications.

**RESULTS** Positive correlations were observed between CD activity and sTNFR1 and sTNFR2 levels ( $r = 0.42$  for both,  $P < 0.01$ ) as well as between UC activity and sTNFR1 and sTNFR2 levels ( $r = 0.63$ ,  $P < 0.0001$ ;  $r = 0.47$ ,  $P < 0.001$ ; respectively). TNF- $\alpha$  levels correlated only with CD activity ( $r = 0.29$ ,  $P < 0.05$ ). In patients with nonactive UC, higher sTNFR2 levels were observed compared with controls. In patients with CD, higher TNF- $\alpha$  and sTNFR2 levels were demonstrated in patients who developed complications.

**CONCLUSIONS** sTNFR1 and sTNFR2 are more sensitive inflammatory markers than TNF- $\alpha$  in the assessment of disease activity in patients with CD and UC. Higher sTNFR2 levels are observed in patients with CD and complications.

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**INTRODUCTION** Inflammatory bowel diseases (IBDs) include Crohn's disease (CD) and ulcerative colitis (UC). The development of inflammatory responses in both diseases is triggered by genetic and environmental factors and disturbances of cell-mediated and humoral immunity.<sup>1-5</sup> The pathogenesis of these conditions has not been fully elucidated. A characteristic feature of CD is inflammation involving the entire intestinal wall, where various cytokines, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) play a key role.<sup>4,6,7</sup>

TNF- $\alpha$  affects the cellular signaling pathways through 2 receptors: 55 kDa TNF receptor 1 (TNFR1), also known as p55, and 75 kDa

TNF receptor 2 (TNFR2), also known as p75.<sup>6-8</sup> These are type I cysteine-rich membrane proteins, which differ in structure, ligand affinity, distribution, and function. Receptor proteins consist of 3 domains: extracellular, transmembrane, and intracellular. As a result of proteolytic detachment of the receptor extracellular domain by TNF- $\alpha$ -converting enzyme, the so called soluble forms of TNF receptors (sTNFR1 and sTNFR2) are formed.<sup>6,9</sup> Soluble receptor forms have been detected in the cerebrospinal and intraarticular fluid, peritoneal fluid, urine, and serum.<sup>9-13</sup> sTNFR1 and sTNFR2 have the ability to bind TNF- $\alpha$ , acting as an inhibitor that competes with a membrane

receptor. At low concentrations, they exhibit agonistic activity with respect to TNF- $\alpha$ , while at high concentrations, they act antagonistically, binding excessive TNF at the site of inflammation. Through their activity, sTNFR1 and sTNFR2 act as physiological inhibitors of TNF- $\alpha$ ,<sup>6,9,14</sup> and this is why they may be used in the treatment of some diseases.<sup>7,15</sup>

sTNFR1 and sTNFR2 concentrations are postulated to be strongly correlated with the clinical presentation and progression of inflammatory diseases (e.g., sepsis, human immunodeficiency virus infection).<sup>9</sup>

According to some authors, sTNFR1 and sTNFR2 are considered to be a reliable parameter in the assessment of IBD activity; some claimed that they are even better than the commonly accepted CD activity index (CDAI) score for CD. So far, studies of sTNFR1 and sTNFR2 levels and their association with IBD activity in mouse, rat, and human tissues have provided inconsistent results.<sup>4,8-10,13,14,16-19</sup>

The objective of the present study was to assess the serum levels of TNF- $\alpha$ , sTNFR1, and sTNFR2 as well as their correlations with disease activity in patients with CD and UC.

#### **PATIENTS AND METHODS** Study population

The study included 105 adult patients with IBDs, 50 individuals with CD (aged 18–69 years) and 55 subjects with UC (aged 19–69 years), in whom the disease was diagnosed based on classic histological, endoscopic, and radiological criteria.<sup>1,20</sup> Patients were treated at the Department of Gastroenterology and Hepatology, University Hospital in Kraków, Poland.

The study was conducted in accordance with the Helsinki Declaration of 1975. All patients provided their written informed consent to participate in the study. The protocol was approved by the Jagiellonian University Ethical Committee.

None of the patients had been treated with TNF- $\alpha$  antibodies prior to blood sample collection. The exclusion criteria were pregnancy, diabetes, immune diseases, other serious diseases, chronic inflammatory processes, and refusal to grant informed consent. The control group consisted of 41 healthy volunteers aged from 17 to 61 years.

**Clinical assessment** The following variables were evaluated in all individuals enrolled into the study: disease duration and location, presence of complications, present therapy, past surgical procedures, cigarette smoking, presence of concomitant diseases, and relapses during follow-up. The body mass index was calculated. Complications were defined as the presence of abscesses, fistulas, obstructions, and extraintestinal diseases associated with IBDs.<sup>21</sup> Based on the medications used, the patients were divided into groups on 5-aminosalicylic acid (ASA) monotherapy, 5-ASA and glucocorticosteroids, 5-ASA and immunosuppressants, and 5-ASA, glucocorticosteroids,

and immunosuppressants. The Montreal classification was used to assess lesion location in UC and CD.<sup>22</sup>

Patients with CD were divided into 2 subgroups based on the CDAI: nonactive CD (CAI score <150) and active CD (CAI score  $\geq$ 150).<sup>23</sup>

Patients with UC were also divided into 2 subgroups based on the colitis activity index (CAI): nonactive UC (CAI score <6) and active UC (CAI score  $\geq$ 6).<sup>2,24</sup> The CAI includes the daily number of stools, visible blood in stool, presence of the colonic mucosa on endoscopy, and the physician's global assessment.<sup>2,24</sup>

In the subgroups of patients with CD and UC as well as in controls, the mean values of the assessed parameters were compared with routine inflammatory markers (white blood cell count, C-reactive protein [CRP], blood platelets, and fibrinogen).<sup>2</sup>

**Laboratory tests** Routine laboratory tests and complete blood count were performed in all patients during hospital stay. Fasting blood samples were collected from the antecubital vein in the morning. On the same day, the following laboratory parameters were determined: complete blood count, albumin, fibrinogen, and CRP. CRP and albumin were assayed using a Modular P clinical chemistry analyzer (Roche Diagnostics, Mannheim, Germany). Complete blood count was performed with a Sysmex XE-2100 hematology automated analyzer (Sysmex, Kobe, Germany). Fibrinogen was measured with a Behring Coagulation System (BCS, Dade Behring, Marburg, Germany).

Serum TNF- $\alpha$  and sTNFR1 and sTNFR2 concentrations were determined by an immunoenzymatic assay using the Quantikine Immunoassay Kit (R&D Systems, Inc., Minneapolis, United States). Following the instructions provided by the manufacturer, we used 96-well plates coated with a specific monoclonal antibody, to which standards and the tested sera were added. After 2-hour incubation at room temperature, the plate was rinsed in buffer, specific polyclonal antibodies coupled with horseradish peroxidase were added, and the plate was again incubated for 2 hours at room temperature. The plate was rinsed in buffer and incubated for 20 minutes with tetramethylbenzidine solution; subsequently, sulfuric acid solution was added to stop the reaction and the color intensity was read at 450 nm wavelength using an enzyme-linked immunosorbent assay reader (ELx808, Biokom, United States). According to the manufacturer, the sensitivity of marker determination is 0.106 pg/ml for TNF- $\alpha$ , 0.77 pg/ml for sTNFR1, and 0.60 pg/ml for sTNFR2.

**Statistical analysis** The distribution of variables in the study groups checked with the Shapiro-Wilk test showed that each of those groups was different from normal. The statistical significance between the groups was determined by the Kruskal-Wallis analysis of variance test.

**TABLE 1** Characteristics of patients with ulcerative colitis, Crohn's disease, and controls

| Variables                             |               | UC<br>n = 55                           | CD<br>n = 50                             | Controls<br>n = 41       |
|---------------------------------------|---------------|----------------------------------------|------------------------------------------|--------------------------|
| age, y                                |               | 37.1 ± 13.2 <sup>a</sup><br>36 (21)    | 30.1 ± 10.5 <sup>b</sup><br>29.5 (12)    | 35.5 ± 11.0<br>37 (18)   |
| sex                                   | female, n (%) | 28 (50.9)                              | 23 (46)                                  | 19 (46)                  |
|                                       | male, n (%)   | 27 (49.1)                              | 27 (54)                                  | 22 (54)                  |
| BMI, kg/m <sup>2</sup>                |               | 22.6 ± 3.4                             | 21.7 ± 4.2                               | 23.8 ± 3.3               |
| WBC count, × 10 <sup>3</sup> /μl      |               | 7.9 ± 3.5<br>7.2 (3.21)                | 7.4 ± 3.2<br>6.7 (3.16)                  | 6.3 ± 1.8<br>6.0 (2.8)   |
| hematocrit, %                         |               | 40.1 ± 4.5                             | 39.6 ± 4.6                               | 42.2 ± 3.7               |
| platelet count, × 10 <sup>3</sup> /μl |               | 308.9 ± 123.2<br>293 (128)             | 329.9 ± 100.4<br>317.5 (90)              | 215.6 ± 49.7<br>198 (65) |
| CRP, mg/l                             |               | 16.5 ± 32.6 <sup>a</sup><br>5.8 (14.9) | 26.6 ± 33.0 <sup>b</sup><br>14.8 (26.71) | 0.7 ± 0.5<br>0.6 (0.65)  |
| fibrinogen, g/l                       |               | 4.4 ± 2.0 <sup>a</sup>                 | 5.2 ± 2.1 <sup>b</sup>                   | 2.8 ± 0.5                |
| albumin, g/l                          |               | 41.5 ± 6.0                             | 39.7 ± 5.7                               | 46.0 ± 2.7               |
| TNF-α, pg/ml                          |               | 3.0 ± 3.4                              | 3.7 ± 5.2 <sup>b</sup>                   | 2.3 ± 3.1                |
| sTNFR1, pg/ml                         |               | 2072.6 ± 707.8 <sup>c</sup>            | 1948.5 ± 472.8 <sup>b</sup>              | 1702.0 ± 272.8           |
| sTNFR2, pg/ml                         |               | 3226.9 ± 955.2 <sup>c</sup>            | 2964.1 ± 701.0 <sup>b</sup>              | 2344.2 ± 345.1           |

Data are presented as mean ± SD; median (interquartile range) was added for non-Gaussian distribution.

**a**  $P < 0.05$  UC vs. CD, **b**  $P < 0.05$  CD vs. controls, **c**  $P < 0.05$  UC vs. controls

Abbreviations: BMI – body mass index, CD – Crohn's disease, CRP – C-reactive protein, SD – standard deviation, sTNFR1 and sTNFR2 – soluble tumor necrosis factor membrane receptors 1 and 2, TNF-α – tumor necrosis factor-α, UC – ulcerative colitis, WBC – white blood cells

The Mann-Whitney  $U$  test was then used where applicable. Associations between the variables with normal distribution were assessed using the Pearson correlation coefficient, while those between the variables without normal distribution were assessed using the Spearman's rank correlation coefficient. All statistical analyses were conducted using the Statistica 8.0 software (StatSoft Inc., Tulsa, Oklahoma, United States). A  $P$  value less than 0.05 was considered statistically significant.

**RESULTS** The study was conducted on 105 patients with IBDs: 50 subjects with CD and 55 with UC, and in controls. The characteristics of the groups are presented in **TABLE 1**.

Patients with CD were characterized by a lower mean age compared with those with UC ( $P = 0.008$ ) and controls ( $P = 0.03$ ). No significant age difference was observed between patients with UC and controls.

In the majority of patients with CD (66%), disease-associated lesions were located both in the small intestine and in the colon. Such complications as enterocutaneous and enteroenteric fistulas and abscesses were present in 62% of patients with exacerbated CD, while subjects in remission showed no active fistulas or abscesses. The majority of patients (60%) did not undergo any CD-associated surgical procedures. In 52% of patients with UC, disease-associated lesions extended to the splenic flexure (L1), while in 35% of the patients, the lesions involved the colon,

extending proximally beyond the splenic flexure (L3) (according to the Montreal classification). None of the patients with UC were treated surgically. The CD group demonstrated higher CRP and fibrinogen levels compared with the UC group. A TNF-α level was higher in subjects with CD compared with controls. No differences in cytokine levels were observed between the CD and UC groups.

The mean sTNFR1 levels in CD and UC were higher than those in controls (1948 pg/ml,  $P = 0.004$  for CD and 2072 pg/ml,  $P = 0.02$  for UC vs. 1702 pg/ml in controls). There were no significant differences in sTNFR1 levels between CD and UC. We also observed higher sTNFR2 levels in patients with CD and UC (2964 pg/ml,  $P < 0.001$  for CD and 3226 pg/ml,  $P < 0.001$  for UC) compared with controls (2344 pg/ml); there were no differences between CD and UC. The characteristics of the active and nonactive subgroups of CD and UC are presented in **TABLE 2**. Both in CD and UC, higher levels of blood platelets, CRP, and fibrinogen were noted in the active subgroups compared with the nonactive subgroups of patients.

TNF-α levels were higher in patients with active CD ( $P = 0.02$ ) compared with the nonactive group, while no differences were observed between the active and nonactive subgroups of patients with UC.

We observed higher levels of sTNFR1 and sTNFR2 in active UC (2389.31 ± 763.18 pg/ml vs. 1663.51 ± 326.83 pg/ml, respectively,  $P < 0.001$ ) and CD (2146.91 ± 470.39 pg/ml vs. 1674.55

**TABLE 2** Characteristics of patients with active and nonactive ulcerative colitis and Crohn's disease

| Variables                             | Nonactive UC<br>n = 23       | Active UC<br>n = 32          | Nonactive CD<br>n = 20    | Active CD<br>n = 30         |
|---------------------------------------|------------------------------|------------------------------|---------------------------|-----------------------------|
| age, y                                | 37.3 ± 14.6<br>33.5 (25; 50) | 37 ± 12.3<br>38 (26; 45)     | 30.4 ± 9.6<br>30 (24; 34) | 29.8 ± 11.3<br>28 (21; 35)  |
| sex                                   | female, n (%)                | 17 (55)                      | 9 (39)                    | 14 (52)                     |
|                                       | male, n (%)                  | 13 (54)                      | 14 (61)                   | 13 (48)                     |
| BMI, kg/m <sup>2</sup>                | 23.0 ± 3.5                   | 22.6 ± 3.4                   | 22.8 ± 4.0                | 20.8 ± 4.3 <sup>b</sup>     |
| WBC count, × 10 <sup>3</sup> /μl      | 6.6 ± 1.8                    | 8.9 ± 4.1 <sup>a</sup>       | 7.6 ± 4.0                 | 7.2 ± 2.5                   |
|                                       | 6.8 (5.2; 7.8)               | 8.2 (5.6; 10.0)              | 6.8 (5.6; 8.5)            | 6.7 (5.0; 9.1)              |
| hematocrit, %                         | 41.8 ± 4.5                   | 38.7 ± 4.0                   | 42.8 ± 3.5                | 36.9 ± 3.5 <sup>b</sup>     |
| platelet count, × 10 <sup>3</sup> /μl | 254.5 ± 66.0                 | 351.0 ± 140.6 <sup>a</sup>   | 277 ± 69.4                | 375.0 ± 101.8 <sup>b</sup>  |
|                                       | 249 (212.5; 305.5)           | 326 (252; 399)               | 280 (210; 338)            | 341 (296; 464)              |
| CRP, mg/l                             | 3.0 ± 4.9                    | 27.0 ± 40.3 <sup>a</sup>     | 9.7 ± 18.8                | 41.0 ± 35.8 <sup>b</sup>    |
|                                       | 1.4 (0.8; 2.5)               | 11.2 (7.2; 34.8)             | 3.6 (1.2; 8.7)            | 28.5 (16.4; 61)             |
| fibrinogen, g/l                       | 3.1 ± 1.3                    | 5.3 ± 1.9 <sup>a</sup>       | 4.1 ± 1.6                 | 6.1 ± 2.1 <sup>b</sup>      |
| albumin, g/l                          | 43.9 ± 5.6                   | 39.6 ± 4.9 <sup>a</sup>      | 42.9 ± 3.7                | 37.1 ± 5.8 <sup>b</sup>     |
| TNF-α, pg/ml                          | 2.2 ± 2.5                    | 3.6 ± 3.8                    | 2.4 ± 4.0                 | 4.6 ± 5.8 <sup>b</sup>      |
| sTNFR1, pg/ml                         | 1663.5 ± 326.8               | 2389.3 ± 763.2 <sup>a</sup>  | 1674.6 ± 319.4            | 2146.9 ± 470.4 <sup>b</sup> |
| sTNFR2, pg/ml                         | 2736.5 ± 524.8               | 3606.6 ± 1043.9 <sup>a</sup> | 2579.7 ± 564.4            | 3242.4 ± 664.4 <sup>b</sup> |

Data are presented as mean ± SD; median (lower and upper quartile) was added for asymmetrical distribution.

**a**  $P < 0.05$  active UC vs. nonactive UC, **b**  $P < 0.05$  active CD vs. nonactive CD

Abbreviations: see [TABLE 1](#)

±319.35 pg/ml, respectively,  $P < 0.001$ ) compared with the subgroups in remission.

There were no differences in TNF-α, sTNFR1, and sTNFR2 levels depending on disease location and duration, smoking status, development of exacerbated or recurrent disease in the follow-up period, and the type of therapy either in CD or UC.

Positive correlations were demonstrated between disease activity, expressed by the CDAI and CAI scores, and sTNFR1 and sTNFR2. The correlation coefficients for both receptors were higher in UC compared with CD. For TNF-α, a positive correlation with disease activity was noted only in CD ([TABLES 3-5](#), [FIGURE](#)).

We also assessed correlations between routine inflammatory markers and disease activity. In the CD group, we found statistically significant correlations with platelet count ( $r = 0.45$ ), CRP ( $r = 0.69$ ) and fibrinogen ( $r = 0.44$ ). In the UC group, we found correlations with platelet count ( $r = 0.47$ ), CRP ( $r = 0.69$ ), and fibrinogen ( $r = 0.64$ ).

The correlations between TNF-α, sTNFR1, and sTNFR2 and routine laboratory parameters in patients with CD and UC are presented in [TABLES 3-5](#).

We also analyzed the correlations between TNF-α, sTNFR1, and sTNFR2. Both in UC and CD, there were positive correlations between the levels of TNF-α and sTNFR1 ( $r = 0.41$  for UC and  $r = 0.51$  for CD) and sTNFR2 ( $r = 0.58$  for UC and  $r = 0.5$  for CD), as well as between sTNFR1 and sTNFR2 ( $r = 0.83$  for UC and  $r = 0.8$  for CD).

**DISCUSSION** To our knowledge, there is a limited number of studies on the correlations of TNF-α with sTNFR1 and sTNFR2. A positive correlation between serum concentrations of those markers was reported in patients with impaired glucose tolerance and diabetes,<sup>25,26</sup> but we have not found any data concerning correlations between those markers in IBDs.

In the present study, sTNFR1 and sTNFR2 levels were higher in patients with CD and UC compared with controls. TNF-α levels were also higher in patients with CD and UC, but a significant difference was observed only between patients with CD and controls.

Other investigators also demonstrated the higher values of sTNFR1 and sTNFR2 in patients with CD and UC compared with controls.<sup>9,17</sup> Hadziselimovic et al.<sup>10</sup> showed a correlation between urinary sTNFR1 and sTNFR2 concentrations and the activity of CD and UC as well as therapeutic effects in these diseases.<sup>10</sup> Higher urinary sTNFR1/2 levels were observed in patients with active CD and UC compared with subjects in remission, which was correlated with the CDAI and CAI.<sup>10</sup>

**Crohn's disease** In a study by Gustot et al.,<sup>19</sup> the levels of sTNFR1 and sTNFR2 were higher in patients with active CD compared with those with nonactive disease and controls. However, in patients in remission, sTNFR1 and sTNFR2 levels were comparable to those in controls. Similar results were reported by Spoettl et al.<sup>9</sup> and Hudson et al.<sup>10</sup> In contrast, Noguchi et al.<sup>27</sup> performed

**TABLE 3** Correlations of TNF- $\alpha$  in patients with ulcerative colitis, Crohn's disease, and controls

| Variables                      | UC                 |          | CD                 |          | Controls |          |
|--------------------------------|--------------------|----------|--------------------|----------|----------|----------|
|                                | <i>r</i>           | <i>P</i> | <i>r</i>           | <i>P</i> | <i>r</i> | <i>P</i> |
| WBC                            | 0.03               | 0.84     | 0.25               | 0.08     | 0.25     | 0.1      |
| hematocrit                     | -0.04              | 0.78     | -0.11              | 0.45     | 0.08     | 0.61     |
| platelets                      | 0.36 <sup>a</sup>  | 0.008    | 0.34 <sup>a</sup>  | 0.017    | -0.12    | 0.47     |
| CRP                            | 0.3 <sup>a</sup>   | 0.027    | 0.3 <sup>a</sup>   | 0.027    | -0.12    | 0.47     |
| fibrinogen                     | 0.3 <sup>a</sup>   | 0.027    | 0.34 <sup>a</sup>  | 0.015    | -0.05    | 0.74     |
| albumin                        | -0.33 <sup>a</sup> | 0.015    | -0.33 <sup>a</sup> | 0.021    | 0.13     | 0.43     |
| disease activity (CDAI or CAI) | 0.21               | 0.12     | 0.29 <sup>a</sup>  | 0.04     | –        | –        |

**a** *P* < 0.05

Abbreviations: CAI – ulcerative colitis activity index, CDAI – Crohn's disease activity index, others – see **TABLE 1**

**TABLE 4** Correlations of sTNFR1 in patients with ulcerative colitis (UC), Crohn's disease (CD), and controls

| Variables                      | UC                 |          | CD                 |          | Controls          |          |
|--------------------------------|--------------------|----------|--------------------|----------|-------------------|----------|
|                                | <i>r</i>           | <i>P</i> | <i>r</i>           | <i>P</i> | <i>r</i>          | <i>P</i> |
| WBC count                      | 0.24               | 0.07     | 0.08               | 0.58     | 0.37 <sup>a</sup> | 0.02     |
| hematocrit                     | -0.3 <sup>a</sup>  | 0.02     | -0.26              | 0.07     | 0.17              | 0.3      |
| platelet count                 | 0.49 <sup>a</sup>  | 0.0002   | 0.37 <sup>a</sup>  | 0.008    | 0.07              | 0.67     |
| CRP                            | 0.69 <sup>a</sup>  | <0.0001  | 0.47 <sup>a</sup>  | 0.0006   | 0.46 <sup>a</sup> | 0.002    |
| fibrinogen                     | 0.61 <sup>a</sup>  | <0.0001  | 0.49 <sup>a</sup>  | 0.0003   | 0.3               | 0.06     |
| albumin                        | -0.66 <sup>a</sup> | <0.0001  | -0.54 <sup>a</sup> | <0.0001  | -0.22             | 0.17     |
| disease activity (CDAI or CAI) | 0.63 <sup>a</sup>  | <0.0001  | 0.42 <sup>a</sup>  | 0.002    | –                 | –        |

**a** *P* < 0.05

Abbreviations: see **TABLES 1 and 3**

**TABLE 5** Correlations of sTNFR2 in patients with ulcerative colitis (UC), Crohn's disease (CD), and controls

| Variables                      | UC                 |          | CD                 |          | Controls |          |
|--------------------------------|--------------------|----------|--------------------|----------|----------|----------|
|                                | <i>r</i>           | <i>P</i> | <i>r</i>           | <i>P</i> | <i>r</i> | <i>P</i> |
| WBC count                      | 0.19               | 0.16     | 0.1                | 0.47     | 0.24     | 0.12     |
| hematocrit                     | -0.23              | 0.1      | -0.25              | 0.08     | 0.04     | 0.82     |
| platelet count                 | 0.37 <sup>a</sup>  | 0.006    | 0.26               | 0.07     | -0.11    | 0.51     |
| CRP                            | 0.64 <sup>a</sup>  | <0.0001  | 0.39 <sup>a</sup>  | 0.006    | 0.25     | 0.12     |
| fibrinogen                     | 0.45 <sup>a</sup>  | 0.0006   | 0.32 <sup>a</sup>  | 0.02     | 0.29     | 0.07     |
| albumin                        | -0.61 <sup>a</sup> | <0.0001  | -0.54 <sup>a</sup> | <0.0001  | -0.06    | 0.72     |
| disease activity (CDAI or CAI) | 0.47 <sup>a</sup>  | 0.0003   | 0.42 <sup>a</sup>  | 0.002    | –        | –        |

**a** *P* < 0.05

Abbreviations: see **TABLES 1 and 3**

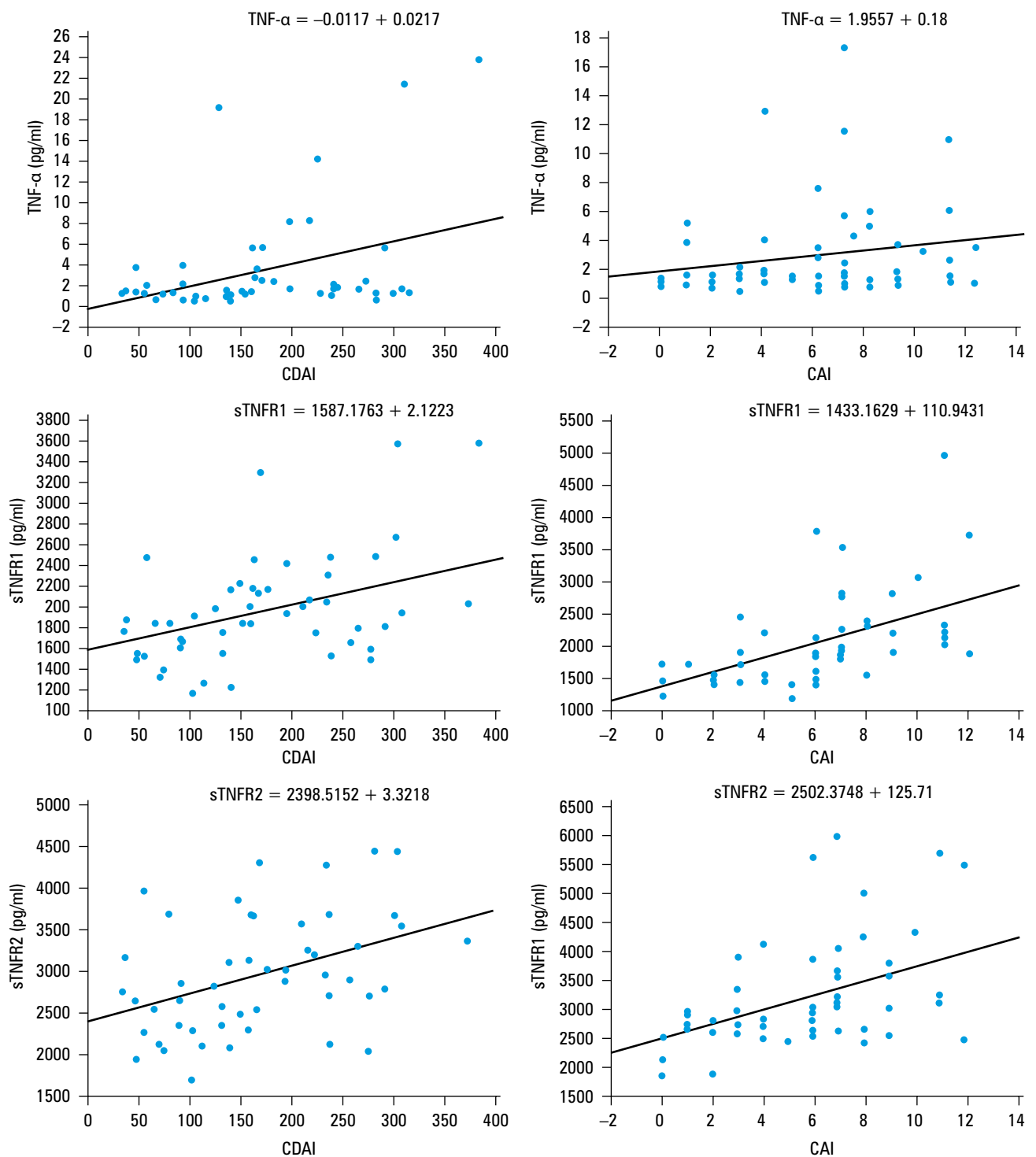
a histopathological evaluation and showed higher sTNFR1 and sTNFR2 levels in the intestinal tissue in active CD compared with nonactive CD and controls. The investigators failed to demonstrate differences between patients in remission and controls.<sup>27</sup>

In our study, we observed that sTNFR1 and sTNFR2 levels in patients with CD were higher not only during exacerbation but also during remission compared with controls.

We did not observe any differences in TNF- $\alpha$ , sTNFR1, and sTNFR2 levels depending on disease location and duration, cigarette status, development of exacerbated or recurrent symptoms during follow-up, and the type of therapy

used either in CD or UC. This finding might suggest that these biomarkers reflect only the current inflammatory status.

It should be emphasized that the immune response in CD is predominantly associated with a proinflammatory T helper 1 lymphocyte-dependent reaction, in which TNF- $\alpha$  activates proinflammatory transcription factors and the NF- $\kappa$ B initiates the production of interleukin (IL) 1 and IL-6 as well as induces chemokines and their receptors, thus leading to neutrophil aggregation and increased inflammatory response. The activation of intestinal mucosa apoptosis by TNFR1 and TNFR2, followed by the loss



**FIGURE** Correlations between serum concentrations of TNF-α, sTNFR1, sTNFR2 and Crohn's disease activity index (CDAI) and colitis activity index (CAI)  
Abbreviations: see TABLE 1

of connections between these cells results in the dysfunction of the endothelial barrier.<sup>4</sup>

In our analysis of the CDAI score, both sTNFR1 and sTNFR2 demonstrated similar correlations with disease activity. These correlations are more potent than those observed for TNF-α but weaker than the associations of sTNFR1 and sTNFR2 with other routine inflammatory markers. However, Kohut et al.<sup>18</sup> postulated that the TNFR2 level was a better indicator of CD activity than the commonly accepted CDAI.

In our study, we additionally observed an association between the presence of complications and the levels of TNF-α and TNFR2 in CD. No such associations were demonstrated for sTNFR1.

Higher TNF-α and sTNFR2 levels may indicate a more severe course of CD, including a higher risk of complications. To our knowledge, there have been no other reports on this issue. For this reason, the hypothesis would have to be validated in further studies involving larger groups of patients with CD.

**Ulcerative colitis** In patients with active UC, we also observed higher sTNFR1 and sTNFR2 levels compared with the nonactive subgroup. Somewhat different results were reported by Gustot et al.<sup>18</sup> who showed similar sTNFR1 levels both in patients with active and nonactive UC. Hanai et al.<sup>14</sup> demonstrated increased serum levels of

TNFR1 and TNFR2 and their strong correlations with disease activity in patients with UC. However, in patients in remission, the levels were comparable to those observed in controls and markedly lower than those reported in the active phase.<sup>14</sup>

In our study, in the UC group, sTNFR1 and sTNFR2 levels showed a significant correlation with the CAI. Considering its higher level even in patients in remission compared with controls, sTNFR2 seems to be a more sensitive indicator of UC, comparable with CRP and fibrinogen.

We did not observe any associations between TNF- $\alpha$ , sTNFR1, and sTNFR2 levels and the presence of complications in patients with UC.

**Conclusions** The results obtained to date indicate that sTNFR1 and sTNFR2 may play an important role in modulating the immune response via binding and inactivation of TNF- $\alpha$ . High concentrations of soluble receptor forms were found not only in the active forms of IBD but also in patients with rheumatoid arthritis, osteomyelitis, and endometriosis.<sup>13,17</sup> For this reason, they are increasingly more commonly used in these diseases (for example in CD or rheumatoid arthritis) as a therapeutic strategy based on blockage of the TNF- $\alpha$ /TNFR1/2 signal induction.<sup>8</sup>

We demonstrated a correlation of sTNFR1 and sTNFR2 with inflammatory markers and disease activity in patients with CD and UC. Moreover, we confirmed that sTNFR1 and sTNFR2 levels are higher in the active subgroups of CD and UC compared with nonactive subgroups. These findings allow us to recognize sTNFR1 and sTNFR2 as more sensitive inflammatory markers in IBDs than TNF- $\alpha$ . One might additionally hypothesize that sTNFR2 is a more accurate and more sensitive marker useful in monitoring the inflammatory activity of UC compared with CD. On the other hand, sTNFR2 may be correlated with a more severe disease course associated with the risk of complications in CD.

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# TNF- $\alpha$ oraz rozpuszczalne formy receptorów TNF 1 i 2 w surowicy krwi u pacjentów z chorobą Crohna i wrzodziejącym zapaleniem jelita grubego

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## SŁOWA KLUCZOWE

choroba  
Leśniowskiego  
i Crohna, markery  
biologiczne,  
nieswoiste zapalenia  
jelit, receptory  
czynnika martwicy  
guza, wrzodziejące  
zapalenie jelita  
grubego

## STRESZCZENIE

**WPROWADZENIE** Rozpuszczalne formy receptorów błonowych czynnika martwicy guza (*tumor necrosis factor* – TNF) 1 i 2 (sTNFR1 i sTNFR2) występują w płynach ustrojowych. Ich zwiększone stężenie stwierdza się w niektórych schorzeniach, m.in. w nieswoistych stanach zapalnych jelit (NZJ). sTNFR1 i sTNFR2 mają zdolność do wiązania TNF- $\alpha$ , działając jako inhibitor współzawodniczący z receptorem błonowym. Wyniki dotychczasowych badania stężeń sTNFR1 i sTNFR2 w NZJ oraz ich zależności od aktywności choroby nie są jednoznaczne.

**CELE** Celem badania była ocena stężeń sTNFR1 i sTNFR2 oraz ich korelacji z aktywnością choroby u pacjentów z NZJ.

**PACJENCI I METODY** Osoczowe stężenia TNF- $\alpha$ , sTNFR1 i sTNFR2 oznaczano u 55 kolejnych pacjentów z wrzodziejącym zapaleniem jelita grubego (WZJG), 50 pacjentów z chorobą Leśniowskiego i Crohna (ChLC) i 41 zdrowych ochotników. Oceniano korelację pomiędzy powyższymi parametrami a innymi wskaźnikami zapalnymi, aktywnością i lokalizacją choroby, rodzajem leczenia i powikłaniami.

**WYNIKI** Zaobserwowano dodatnią korelację pomiędzy aktywnością ChLC a sTNFR1 i sTNFR2 ( $r = 0,42$  dla obu,  $p < 0,01$ ) i aktywnością WZJG a sTNFR1 i sTNFR2 (odpowiednio,  $r = 0,63$ ;  $p < 0,0001$  i  $r = 0,47$ ;  $p < 0,001$ ). Poziom TNF- $\alpha$  korelował tylko z aktywnością ChLC ( $r = 0,29$ ;  $p < 0,05$ ). W grupie z nieaktywnym WZJG stwierdzono większe stężenia sTNFR2 niż w grupie kontrolnej. W grupie z ChLC wykazano większe stężenia TNF- $\alpha$  i sTNFR2 u pacjentów, u których wystąpiły powikłania.

**WNIOSKI** sTNFR1 i sTNFR2 są markerami stanu zapalnego czulszymi niż TNF- $\alpha$  w monitorowaniu aktywności zapalnej u chorych z WZJG i ChLC. Zwiększone stężenia sTNFR2 obserwuje się u chorych z ChLC i powikłaniami.

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