

# ENA-78/CXCL5 as a Predictive Factor for Baricitinib Effectiveness in Rheumatoid Arthritis

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## Research Article

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# Abstract

**Objectives:** To determine whether cytokines and chemokines are predictive factors for baricitinib effectiveness in rheumatoid arthritis (RA).

**Methods:** Eleven patients were treated with baricitinib, and the levels of fractalkine/CX3CL1, CXCL16, ENA-78/CXCL5, IL-8/CXCL8, MCP-1/CCL2 and RANTES/CCL5 in serum were measured with enzyme-linked immunosorbent assays.

**Results:** Nine of the 11 patients successfully completed the study at 12 weeks. Two patients were excluded due to noneffectiveness of baricitinib treatment. The simplified disease activity index (SDAI) at week 12 was significantly decreased in patients receiving baricitinib compared with the SDAI at week 0 (mean  $\pm$  SEM;  $5 \pm 1$  and  $15 \pm 3$ , respectively,  $p < 0.05$ ). One case of herpes zoster virus infection occurred at 12 weeks. The levels of MCP-1/CCL2, RANTES/CCL5, ENA-78/CXCL5, IL-8/CXCL8, CXCL16 and fractalkine/CX3CL1 in RA serum were not significantly different before and after baricitinib treatment. Finally, we found that the level of ENA-78/CXCL5 in patients with SDAI remission was significantly higher than that in patients without SDAI remission at 12 weeks ( $887 \pm 115$  pg/ml and  $461 \pm 102$  pg/ml, respectively).

**Conclusions:** ENA-78/CXCL5 may be a predictor of the effectiveness of baricitinib in treating RA.

## Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder affecting the cartilage of multiple joints and subsequently the cartilage of the underlying bone [1]. Cytokines and chemokines synergistically regulate joint destruction, such as bone erosion and cartilage degradation [2–7]. The therapeutic management of RA has changed dramatically in recent decades. However, despite the success of blocking various cytokines with monoclonal antibodies, not all RA patients respond to these therapies.

The Janus kinase/signal transducer and activator of transcription (JAK-STAT) signaling pathway is implicated in the pathogenesis of some autoimmune diseases, such as RA, psoriasis, and inflammatory bowel disease [8]. JAKs mediate signal transduction via cytokine receptors, playing a key role in lymphocyte activation, proliferation and function. Baricitinib is a selective small molecule inhibitor of JAK1/2 enzymes and was approved in 2018 by the European Medicines Agency (EMA) and the Pharmaceuticals and Medical Devices Agency (PMDA) in Japan for moderate to severe active RA in adult patients who have responded inadequately to DMARDs.

In this study, we investigated the clinical course and levels of cytokines in RA patients treated with baricitinib.

## Materials And Methods

## Patient

Eleven patients who fulfilled the American College of Rheumatology (ACR) 1987 revised criteria for RA between March 2018 and September 2019 were enrolled. RA disease activity was evaluated using the SDAI. Patients who at baseline had moderate or severe disease activity despite treatment with DMARDs for more than 3 months were enrolled and given baricitinib. Serum at baseline and 12 weeks was obtained from RA patients. All specimens were obtained with informed consent and collected following approval from the Showa University Institutional Review Board. All experiments were performed in accordance with relevant guidelines and regulations.

## Enzyme-linked immunosorbent assay (ELISA)

ELISAs were performed as described previously [9]. The levels of fractalkine/CX3CL1, CXCL16, ENA-78/CXCL5, IL-8/CXCL8, MCP-1/CCL2 and RANTES/CCL5 in serum were measured following the manufacturer's protocol (R&D Systems, Minneapolis, MN). Briefly, ninety-six-well plates were coated with mouse anti-human antibodies. Pretreatment and posttreatment RA serum and recombinant cytokines used as standards were added. Biotinylated mouse anti-human antibodies were used to detect cytokines using a streptavidin–peroxidase method (BD Biosciences, San Jose, CA) with a TMB substrate kit. The concentration in each sample was measured at 450 nm.

## Statistical analysis

Data were analyzed using Student's *t*-test assuming equal variance. Data are reported as the mean  $\pm$  SEM. *P* values less than 0.05 were considered significant.

# Results

## Clinical characteristics of study subjects

The patient characteristics are shown in Table 1. Nine of the 11 patients successfully completed the experiment. Two patients were excluded due to noneffectiveness of baricitinib treatment. These 2 patients had also experienced noneffectiveness with all biologics and tofacitinib. At the start of this study, the mean age of the patients was  $59 \pm 11$  years, and the disease duration was  $10 \pm 11$  years. The baseline SDAI for the 11 patients was  $15 \pm 3$ , and the MTX dosage was  $11 \pm 3$  mg/week.

Table 1  
Summary of patient characteristics (mean  $\pm$  SE)

	<b>RA patients (n = 11)</b>
Age	59 $\pm$ 11
Female (%)	6/11 (55%)
Methotrexate dose (mg/week)	11 $\pm$ 3
Glucocorticoid dose (mg/day)	9 $\pm$ 6
SDAI	16 $\pm$ 3
Duration (years)	10 $\pm$ 11
RF positive (%)	73.3
ACPA positive (%)	66.7
Stage (I/II/III/IV)	(3/1/4/3)
Previous use of biologics in patients (%)	8/11 (73%)

### Efficacy

The SDAI at week 12 was significantly decreased in patients receiving baricitinib compared with the SDAI at week 0 (mean  $\pm$  SEM; 5  $\pm$  1 and 15  $\pm$  3, respectively,  $p < 0.05$ , Fig. 1A). Swollen joint counts, tender joint counts and the CRP at week 12 were also significantly decreased compared with the respective pretreatment values (Fig. 1B – D). The Doppler signal was improved after baricitinib treatment (Fig. 2A and B).

### Safety

One case of herpes zoster virus infection occurred at 12 weeks. This patient recovered with medication. Laboratory result changes are shown in Fig. 3A-G. High-density lipoprotein cholesterol (HDL-cho) and creatine phosphokinase (CPK) were increased at 12 weeks compared with the respective baseline values (Fig. 3A and B). On the other hand, low-density lipoprotein cholesterol (LDL-cho) was not significantly different before and after treatment. (Fig. 3C). In terms of blood cells, white blood cells (WBCs) and neutrophils were decreased with baricitinib treatment. (Fig. 3D and E). Lymphocytes and hemoglobin (Hb) were not different before and 12 weeks after treatment (Fig. 3F and G).

### Change in serum cytokines and chemokines

MCP-1/CCL2, RANTES/CCL5, ENA-78/CXCL5, IL-8/CXCL8, CXCL16 and fractalkine/CX3CL1 in RA serum were not significantly different before and after treatment with baricitinib (Fig. 4A-F).

On the other hand, we found that the level of ENA-78/CXCL5 in patients in SDAI remission was significantly higher than that in patients without SDAI remission at 12 weeks ( $887 \pm 115$  pg/ml and  $461 \pm 102$  pg/ml, respectively, Fig. 5).

## Discussion

In the present study, we have shown that treatment with baricitinib sustains improvement in the signs and symptoms of RA. We confirmed that the laboratory results of 9 patients were improved with baricitinib treatment. The ACR20 response rate for baricitinib was greater than that for placebo [10]. Two patients did not experience an effect from baricitinib treatment. Both patients had previously experienced noneffectiveness from tofacitinib and all biologics that are approved in Japan. On the other hand, 9 patients continued to receive baricitinib at 12 weeks.

In terms of safety, a 75-year-old female patient had herpes zoster virus infection at 12 weeks. RA patients have a 1.5- to 2-fold increased risk of herpes zoster virus infection compared with the similarly aged general population [11]. JAK inhibitors, including tofacitinib and baricitinib, have been reported to increase herpes zoster virus infection compared with placebo [12, 13]. Interestingly, the risk varied by geographic region, and the rates were significantly higher in Japan and Korea.

Regarding cytokines and chemokines, we could not find differences in MCP-1/CCL2, RANTES/CCL5, ENA-78/CXCL5, IL-8/CXCL8, CXCL16 and fractalkine/CX3CL1 levels in RA serum with baricitinib treatment. Migita K et al. demonstrated that tofacitinib was also associated with reduced serum IL-6 but had no effect on serum levels of soluble IL-6 receptor [14]. Chen Y. et al reported that tofacitinib suppressed expression of the chemokine ENA-78/CXCL5 and PMN infiltration in the infected tissues of mice [15]. However, the relationship between baricitinib and ENA-78/CXCL5 was not clarified. We found that the level of ENA-78/CXCL5 in patients in SDAI remission was significantly higher than that in patients without SDAI remission. These results indicate that ENA-78/CXCL5 is involved in RA inflammation, especially neutrophil migration. The levels of ENA-78/CXCL5 at baseline in baricitinib responders were higher than those in baricitinib nonresponders. These results suggest that the level of ENA-78/CXCL5 at baseline may be a predictor of the effectiveness of baricitinib treatment in RA.

## Conclusions

In summary, we showed that RA disease activity was significantly decreased in patients receiving baricitinib at 12 weeks. Two of 12 patients did not receive an effect from baricitinib treatment. One of 11 patients had herpes zoster virus infection. In addition, ENA-78/CXCL5 levels at baseline in those patients who responded to baricitinib were elevated compared with the baseline levels in those patients who did not respond to baricitinib.

## Declarations

## Authors' contributions

T.I., K.K., N.K. and T.K. conceived of the study, and participated in its design and coordination. S.F. performed all assays with assistance from T.I. T.I. also assisted with the acquisition of data. T.I., R.G. and K.S. performed the statistical analysis. All authors read and approved the final manuscript.

**Competing interests:** The authors declare no competing interests.

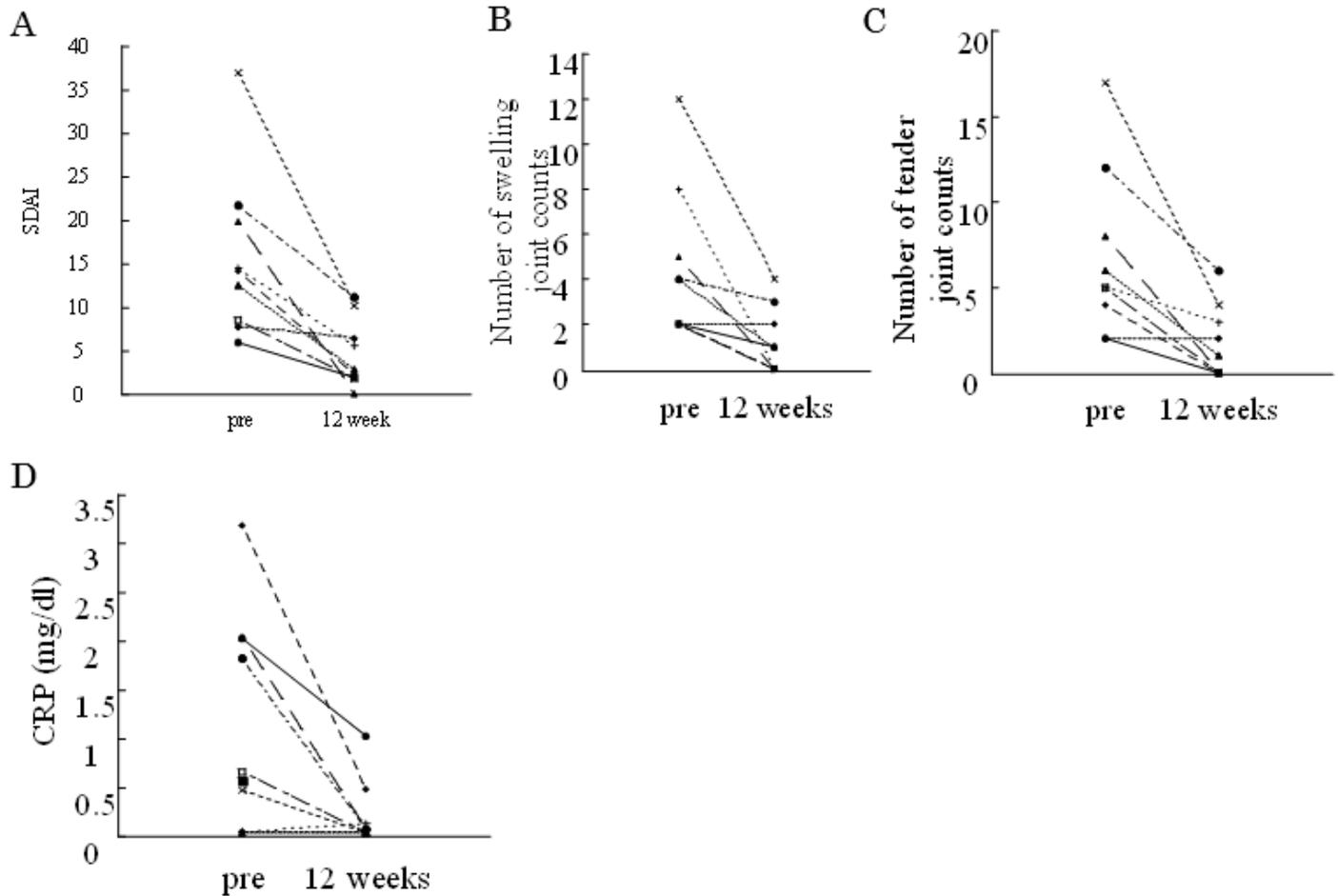
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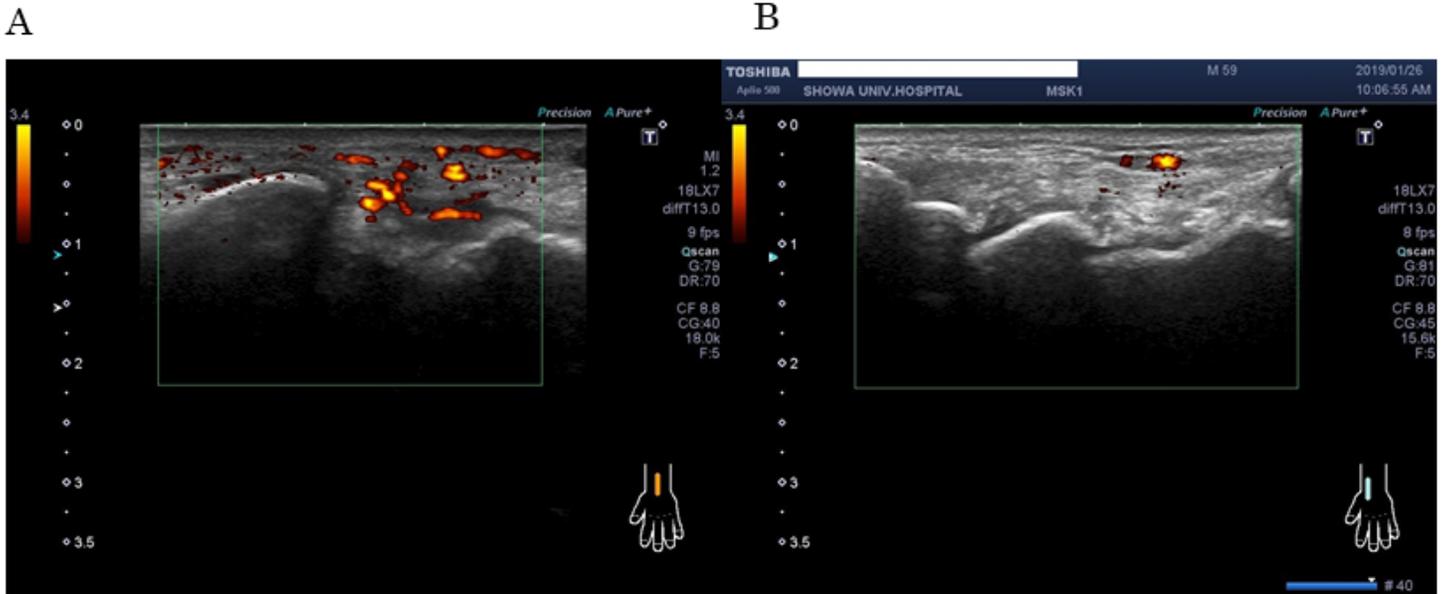
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## Figures



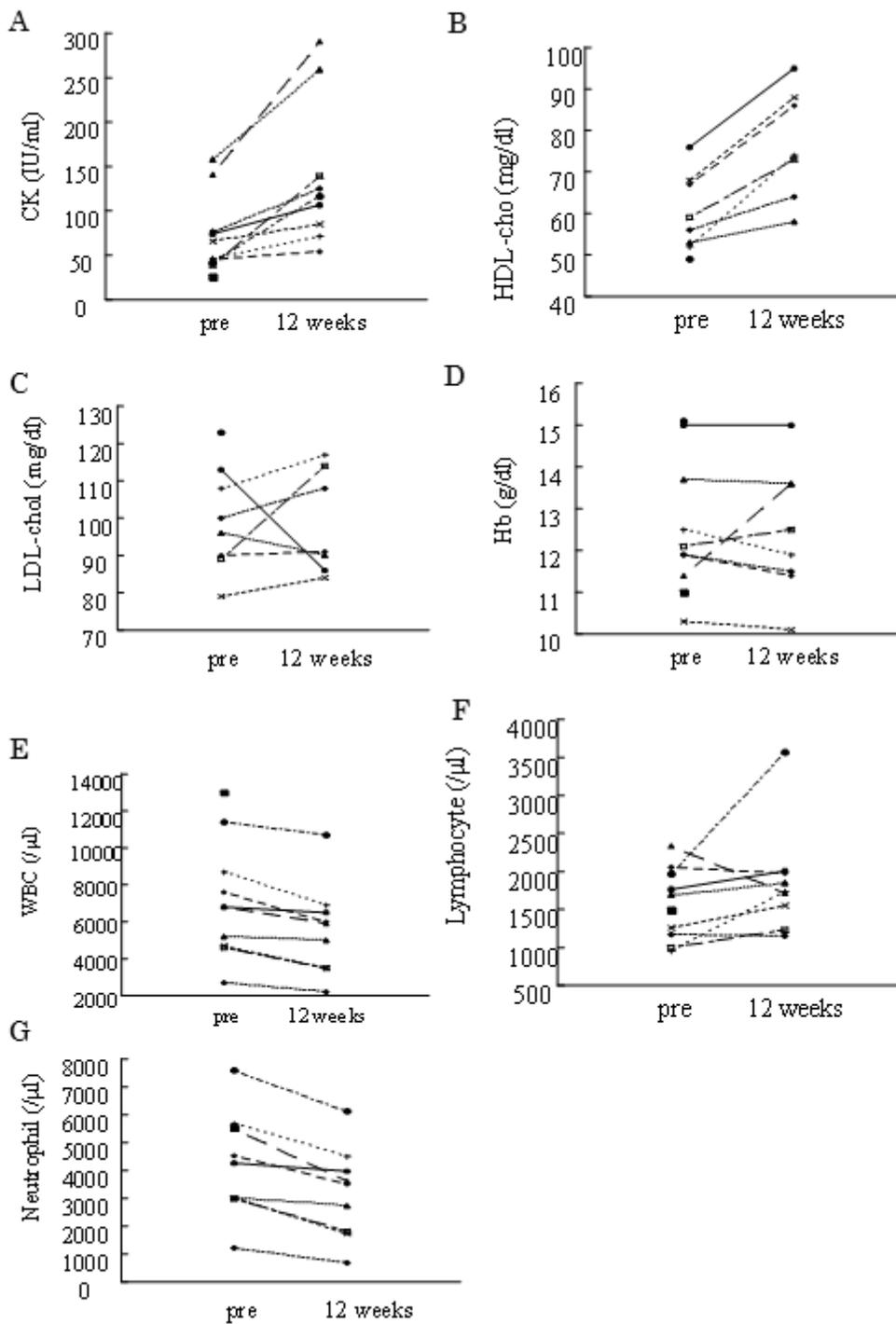
**Figure 1**

Effect of baricitinib in patients with RA. A) The SDAI in RA patients was assessed from baseline to 12 weeks after adding baricitinib to the MTX administration. Significant improvements in the SDAI were seen at 12 weeks ( $*p < 0.05$ ) compared with baseline. B, C and D) Swelling joint counts, tender joint counts and serum CRP levels were assessed from baseline to 12 weeks after adding baricitinib to the MTX administration.



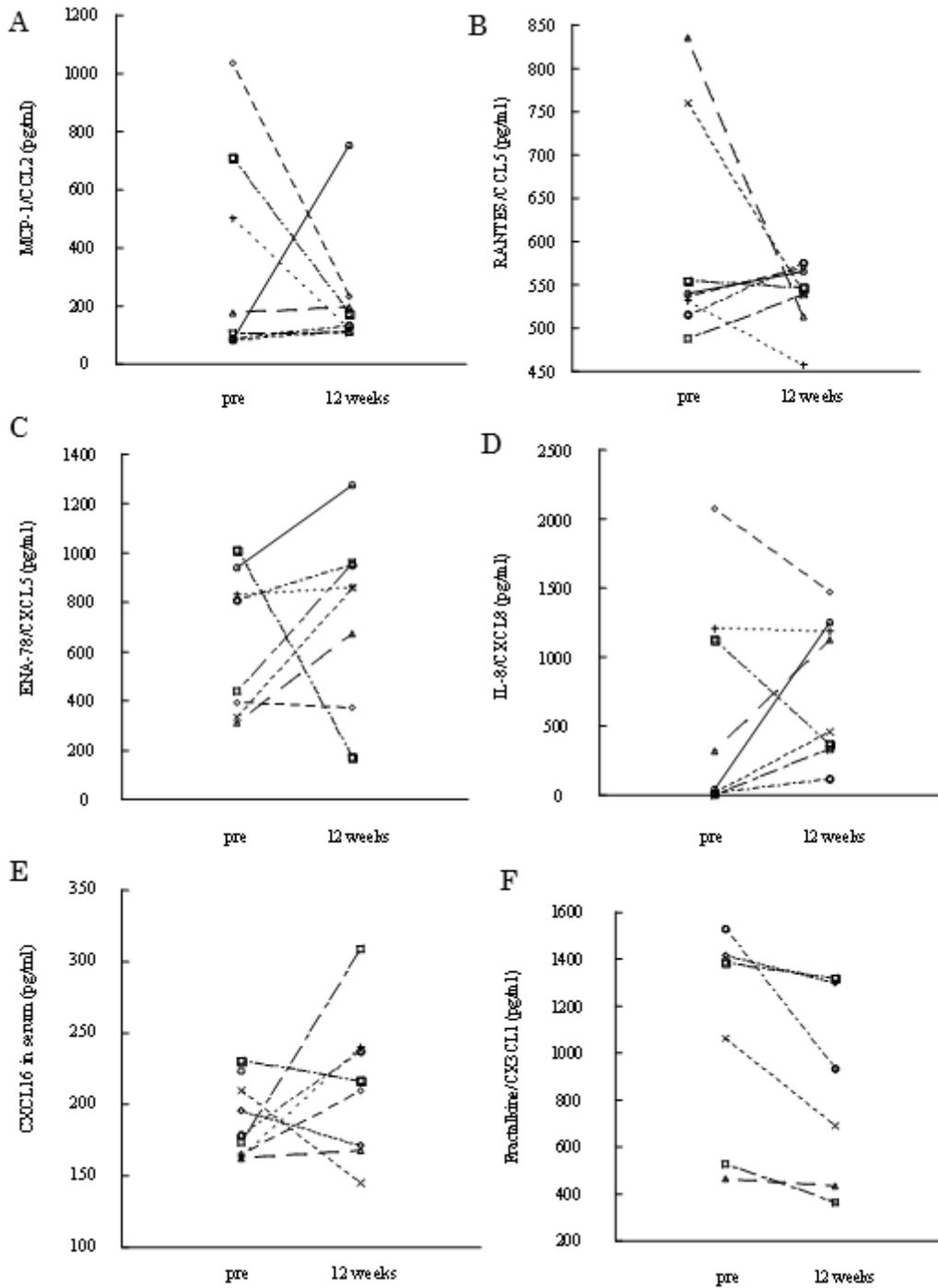
**Figure 2**

Ultrasound findings in a patient's hand (A: pretreatment, B: posttreatment). A) High-intensity Doppler signals in the wrist joint.



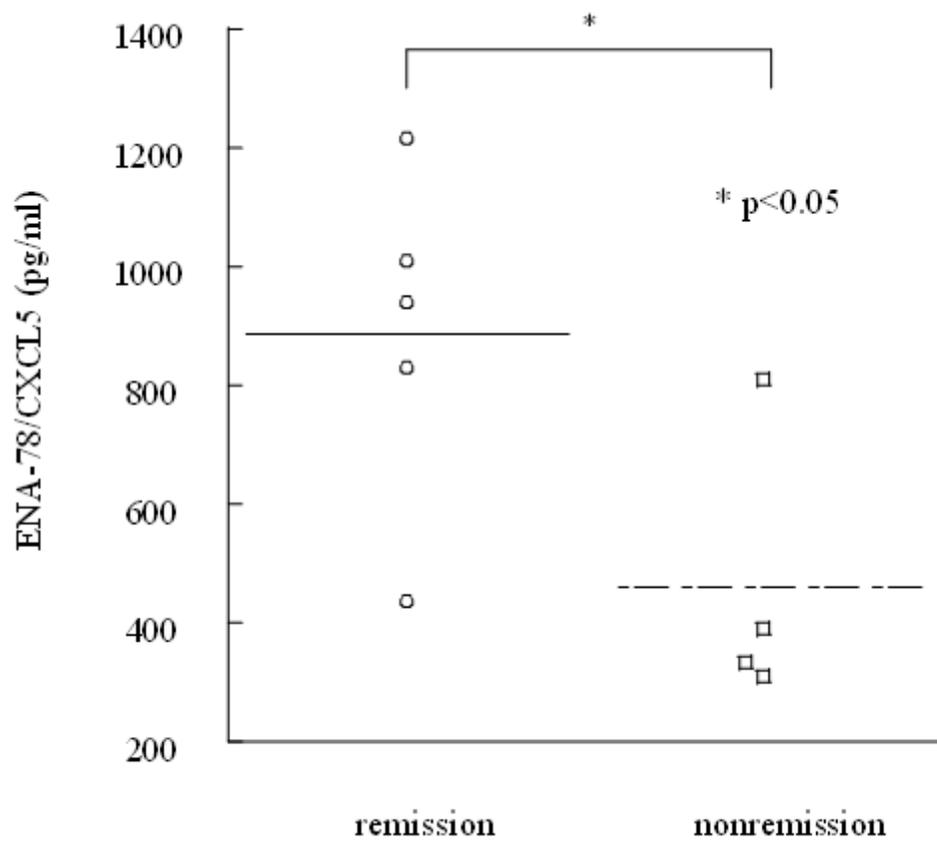
**Figure 3**

Laboratory data in patients. A and B) HDL-cho and CPK were increased at 12 weeks. C) LDL-cho was not significantly different before and after treatment. D and E) WBC and neutrophil counts were decreased. F and G) Lymphocytes and Hb were not different before and 12 weeks after treatment. (n=number of patients)



**Figure 4**

Chemokine data in patients. A-F) The MCP-1/CCL2, RANTES/CCL5, ENA-78/CXCL5, IL-8/CXCL8, CXCL16 and fractalkine/CX3CL1 levels in RA serum were not significantly different before and after treatment with baricitinib. (n=number of patients)



**Figure 5**

The ENA-78/CXCL5 level in patients with SDAI remission was significantly higher than that in patients without SDAI remission at 12 weeks. (n=number of patients)