ADAMTS5 Is a Biomarker for Prediction of Response to Infliximab in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To identify a biomarker for prediction of the response to infliximab (IFX) in patients with rheumatoid arthritis (RA), we focused on a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) that seems to play a key role in aggrecan degradation in cartilage.

Methods. Seventy-three randomly selected patients with active RA were treated with IFX. Peripheral blood samples were collected at baseline and ADAMTS5 messenger RNA (mRNA) was quantified using real-time polymerase chain reaction.

Results. Baseline ADAMTS5 mRNA levels in the good responder group were significantly lower (1.84 ± 1.56; p = 0.0408) than those in the moderate and nonresponder groups (2.54 ± 1.70) at 38 weeks of treatment with IFX. The 28-joint count Disease Activity Score (DAS28) at 38 weeks of treatment was significantly lower in the low ADAMTS5 group (2.30 ± 1.28; p = 0.0038) than in the high ADAMTS5 group (3.90 ± 1.61). The percentage reduction of the DAS28 was significantly higher in the low ADAMTS5 group (52.5% ± 28.8%; p = 0.0156) than in the high ADAMTS5 group (29.4% ± 27.2%). Further, the Δ Health Assessment Questionnaire (ΔHAQ) score, an estimate of the improvement in the HAQ score, at 38 weeks of treatment was significantly higher in the low ADAMTS5 group (1.18 ± 0.60; p = 0.0102) than in the high ADAMTS5 group (0.21 ± 0.78). The positive predictive value of a low baseline ADAMTS5 level for predicting good response and remission (DAS28 < 2.6 at 38 weeks) was 90.0% and 70.0%, respectively.

Conclusion. The baseline ADAMTS5 mRNA level is a candidate biomarker for prediction of the response to IFX in patients with RA. (J Rheumatol First Release June 1 2010; doi:10.3899/jrheum.091285)

Key Indexing Terms:
RHEUMATOID ARTHRITIS INFLIXIMAB ADAMTS AGGRECANASE BIOMARKERS

Rheumatoid arthritis (RA) is an autoimmune and inflammatory polyarthritis characterized by joint damage and disability. Tumor necrosis factor-α (TNF-α) plays a key role in the underlying pathological events and has been identified as a therapeutic target. Biological agents that inhibit the action of TNF-α, such as infliximab (IFX), etanercept, and adalimumab, have shown excellent clinical efficacy and can prevent structural damage in patients with RA[1-4]. Various clinical trials of anti-TNF-α biologics have shown efficacy ranging from 60% to 80% in such patients[1,5,6]. Although almost 70% of the treated patients respond to these therapies, these agents are expensive and sometimes cause side effects. For unknown reasons, 30%–40% of patients with RA do not respond well to anti-TNF biologics[1,5,6]. Indeed, at present, there are few reports about factors that might predict treatment resistance to these agents. Even the markers that have proven informative for the diagnosis or prognosis of RA, such as C-reactive protein, erythrocyte sedimentation rate, autoantibodies (e.g., rheumatoid factor and anti-cyclic citrullinated peptide antibodies), matrix metalloproteinases (MMP) including MMP-3, and bone proteins, have not been shown to have the ability to predict the response to anti-TNF biologics[7]. Therefore, biomarkers for predicting the response to anti-TNF biologics need to be identified.

Aggrecan is the major proteoglycan in cartilage, and is degraded by 1 or more aggrecanases from the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family of proteinases in arthritic cartilage. Although ADAMTS5 and ADAMTS4 have been thought to be the most important aggrecanases, studies in murine models of degenerative arthritis have indicated that ADAMTS5 may also play a key role in aggrecan degradation[8-10]. Moreover,

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studies in models of cultured bovine and porcine chondrocytes and cartilage explants have reported that ADAMTS4 is induced by stimulation with interleukin 1 (IL-1) and TNF-α, while ADAMTS5 is not[11-14]. It has also been shown that while ADAMTS4 expression in human synoviocytes is significantly inhibited by the anti-TNF biologics etanercept and an anti-IL-1ß neutralizing antibody, neither had any effect on the expression of ADAMTS5 in the same cells[15]. In our study, to identify a biomarker for prediction of the response to IFX, we focused on ADAMTS5, because it has been shown to have the ability to destroy cartilage through degradation of aggrecan, independently of TNF-α.

MATERIALS AND METHODS

Patient selection. A total of 73 randomly selected patients (62 women and 11 men) were enrolled in our study. They fulfilled the American College of Rheumatology (ACR) criteria for RA[16] and were followed in Saitama Medical Center of Saitama Medical University. The characteristics of the patients are shown in Table 1. At least 1 disease-modifying antirheumatic drug (DMARD), including methotrexate (MTX), had failed to control the disease activity before treatment with IFX was started. Of the total, 84.9% of the patients were women; the mean age ± SD was 55.5 ± 12.6 years, and the mean disease duration was 94 ± 105 months. Most patients had active disease, as indicated by a mean 28-joint count Disease Activity Score (DAS28) of 5.56. Every patient was continued on MTX and prednisolone at the same doses as before and was, in addition, started on treatment with IFX as recommended by the manufacturer and the Japanese Ministry of Health, Labor and Welfare (3 mg/kg IFX by intravenous injection at Weeks 0, 2, 6, and every eighth week). Whole blood specimens were collected before the start of the IFX treatment (baseline). Patients were assessed before IFX treatment and after 38 weeks for overall disease activity using the DAS28[17] and for physical function using the Health Assessment Questionnaire (HAQ) for RA[18]. Patients were categorized according to the EULAR response criteria and EULAR remission criteria using the DAS28[19]. All the patients and the controls gave their written informed consent for the present research. The protocols of the study were approved by the ethics committee of our institution, and are in compliance with the Declaration of Helsinki.

Sample preparation. Whole blood specimens were collected in PAXgene® Blood RNA tubes (PreAnalytiX GmbH, Hombrechtikon, Switzerland). Total RNA was isolated from the whole blood specimens using the PAXgene blood RNA isolation kit and converted to whole cDNA using reverse transcriptase[20]. The whole cDNA was quantified and used as the template DNA standard for real-time polymerase chain reaction (RT-PCR).

Quantitative RT-PCR. Expression levels of a subset of genes were measured by quantitative RT-PCR performed with TaqMan assay reagents, in accord with the manufacturer's instructions on an ABI Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA, USA), using predesigned primers and probes (ADAMTS5, Hs00199841_m1; ß-actin, human ACTB endogenous control). To prepare template DNA standards, a target DNA fragment was amplified by PCR and fused into a PCR vector with the TA Cloning Kit (Invitrogen, Carlsbad, CA, USA), amplified, and refined. The amount of standard DNA construct per well was adjusted to 10 pg, followed by serial dilution to yield samples containing 10, 1, 10^{-1}, 10^{-2}, and 10^{-3} pg, which were then used to construct standard plots.

Isolation of peripheral blood mononuclear cells. Peripheral blood (20 ml) was collected in a preheparinized tube, and peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density-gradient centrifugation (GE Healthcare, Waukesha, WI, USA).

Western blot analysis. PBMC were lysed with 1 ml of lysis buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP-40, 10 mM EDTA, 1 mM sodium orthovanadate, 1 mM PMSF, 10 μg/ml aprotinin, and 10 μg/ml leupeptin) at 4˚C for 15 min and disrupted by sonication. After centrifuging at 10,000 g for 5 min, 15 μg of the supernatant was loaded on a 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were electrophoretically blotted onto PVDF membranes (Millipore, Bedford, MA, USA), and the membranes were soaked at 37˚C for 1 h in blocking agents (Blockace, Dainippon Pharmaceuticals, Tokyo, Japan). The blots were then probed with a rabbit anti-ADAMTS5 antibody (AP7447c; Abgen) or a mouse anti-ß-actin (A5441; Sigma-Aldrich, St. Louis, MO, USA) at 16˚C for 1 hour. AP7447c and A5441 were visualized using horseradish peroxidase (HRP)-conjugated antirabbit IgG (GE Healthcare) and HRP-conjugated anti-mouse IgG (GE Healthcare), respectively. Biotinylated proteins were detected using streptavidin-peroxidase (Southern Biotechnology Associates, Birmingham, AL, USA). After washing 3 times, the signals were detected by chemiluminescence-enhancing reagents (GE Healthcare). The treated membranes were visualized on electrochemiluminescent radiographic film (GE Healthcare). The density of the specific bands was quantified by scanning with a Scan Jet II (Hewlett-Packard, Palo Alto, CA, USA) and Image J (version 1.38) as an index.

Statistical analysis. Statistical significance was calculated using the Student t-test for unpaired data and Fisher's exact test for the distribution on Statview software (version 4.5; Abacus, Berkeley, CA, USA). Receiver-operating characteristics (ROC) curves were analyzed using Jmp software (version 8.0; SAS Institute Inc., Cary, NC, USA).

RESULTS

Patients with RA and response to infliximab. Before the start of the IFX treatment, all patients with RA had high disease

Table 1. Demographic and clinical data of patients with rheumatoid arthritis at entry into this study.

Characteristic	All Patients, n = 73	GR, n = 33	NGR, n = 40	p*
Women, no. (%)	62 (84.9)	28 (84.9)	34 (85.0)	0.986
Age, yrs	55.5 ± 12.6	52.9 ± 11.4	57.6 ± 13.4	0.113
Disease duration, mo	94 ± 105	85 ± 92	101 ± 116	0.526
Rheumatoid factor, IU/ml	117.6 ± 218.4	79.0 ± 39.0	148.3 ± 34.8	0.190
MMP-3	206.7 ± 203.3	176.8 ± 205.3	232.6 ± 200.8	0.258
Methotrexate, mg/wk	8.05 ± 2.16	8.38 ± 1.88	7.79 ± 2.35	0.247
Prednisone, mg/day	3.25 ± 3.73	2.45 ± 2.89	4.90 ± 4.23	0.152
DAS28 at baseline	5.56 ± 1.20	5.40 ± 1.21	5.68 ± 1.19	0.323
HAQ at baseline	1.31 ± 0.79	1.19 ± 0.73	1.40 ± 0.83	0.271

* Each measure was compared between the GR and NGR groups by 2-sample t-test. GR: good responder; NGR: moderate + nonresponder; MMP: matrix metalloproteinase; DAS28: 28-joint count Disease Activity Score; HAQ: Health Assessment Questionnaire.

activity, as reflected by a DAS28 (mean ± SD) of 5.56 ± 1.20 (Table 1). At the end of 38 weeks of IFX therapy, a significant decrease in the DAS (3.68 ± 1.65) was observed in the entire group of treated patients. At 38 weeks after the first infusion of IFX, good and moderate responders and nonresponders were noted in 33 (45.2%), 20 (27.4%), and 20 (27.4%) of the 73 patients, respectively. Overall, most patients were classified into the R response category (moderate + good response; 72.6%) after 38 weeks of treatment. Further, 24 (32.9%) patients had entered remission by 38 weeks of treatment. We categorized patients into 2 groups, GR (good response) and NGR (moderate + no response), after 38 weeks of treatment. Table 1 also provides demographic and clinical information for these 2 groups at entry. None of the clinical characteristics at baseline was significantly associated with the clinical outcome.

Quantification of baseline ADAMTS5 messenger RNA. One microgram of total RNA was isolated from whole blood specimens of the patients before the first infusion of IFX and converted to whole cDNA using reverse transcriptase. A 5-μl sample of the whole cDNA was used as the template, and ß-actin and ADAMTS5 cDNA were quantified by RT-PCR. To validate RT-PCR, the standard curves for ADAMTS5 and ß-actin cDNA were constructed from the PCR vector fused with the ADAMTS5 cDNA (430 bp) and ß-actin cDNA (467 bp), respectively. The critical threshold cycle (Ct) for each cDNA was inversely proportional to the logarithm of the initial amount of the standard template DNA (correlation coefficients 0.911 for ß-actin and 0.999 for ADAMTS5). Then the Ct of each of the cDNA was measured by RT-PCR, and the relative expression level of each gene was evaluated as a ratio to the level of ß-actin cDNA. The results revealed an average baseline ADAMTS5 mRNA expression, relative to ß-actin mRNA before the start of treatment with IFX, of $(2.22 \pm 1.66) \times 10^{-4}$.

Reduced expression of baseline ADAMTS5 mRNA in responders to IFX. Baseline ADAMTS5 mRNA expression was compared between the GR and NGR groups. ADAMTS5 mRNA levels before the treatment with IFX in the GR group [$(1.84 \pm 1.56) \times 10^{-4}$] were significantly lower than those in the NGR group [$(2.54 \pm 1.70) \times 10^{-4}$; $p = 0.0408$; Figure 1], suggesting the association of low expression of baseline ADAMTS5 mRNA with the responders to IFX treatment.

Determination of the cutoff value of baseline ADAMTS5 mRNA. From these observations, it might be supposed that baseline ADAMTS5 mRNA expression is a biomarker for prediction of response to IFX in patients with RA. To confirm this hypothesis, the optimal cutoff of the ADAMTS5 mRNA level for categorization of patients into low and high ADAMTS5 groups at baseline was investigated. ROC analysis was performed using samples from the 33 patients of the GR group and 40 patients of the NGR group. The ROC analysis revealed an area under the curve of 0.7271, with a specificity of 97.5% at the cutoff level of 0.75×10^{-4} ($p < 0.0001$; Figure 2). Therefore, patients with baseline ADAMTS5 mRNA levels $< 0.75 \times 10^{-4}$ and $\geq 0.75 \times 10^{-4}$ were classified into the low and high ADAMTS5 groups, respectively. Table 2 gives the baseline demographic and clinical information for these 2 groups at study entry. None of the clinical characteristics at baseline was significantly associated with the baseline ADAMTS5 mRNA levels.

ADAMTS5 mRNA level and response to IFX. At the end of

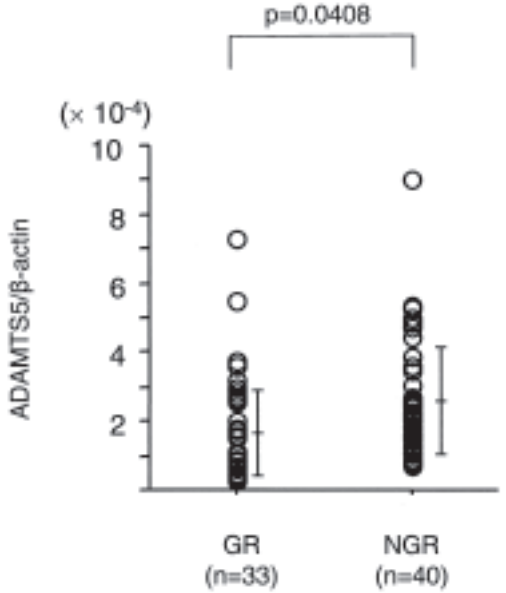

Figure 1. Reduced expression of the baseline ADAMTS5 mRNA in the responders to infliximab treatment. Baseline ADAMTS5 mRNA expression was compared between the GR (good response) and NGR (moderate + no response) groups.

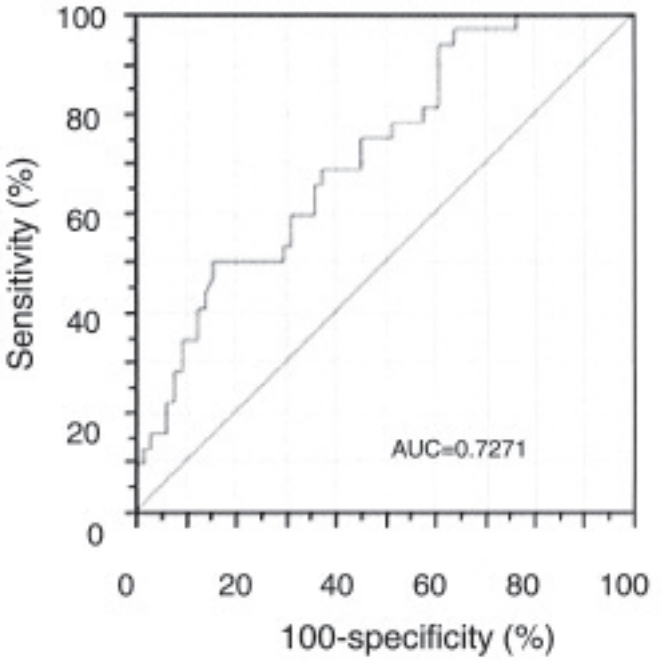

Figure 2. Receiver operating characteristic (ROC) curves for the baseline ADAMTS5 messenger RNA levels [area under the ROC curves (AUC), 0.7271] to detect good responders to infliximab.

Table 2. Baseline characteristics of patients in low and high ADAMTS5 groups before the first injection of infliximab.

Characteristic	All Patients, n = 73	Low Group, n = 10	High Group, n = 63	p*
Women, no. (%)	62 (84.9)	9 (90.0)	53 (84.1)	0.614
Age, yrs	55.5 ± 12.6	51.7 ± 13.7	56.1 ± 12.5	0.311
Disease duration, mo	94 ± 105	115 ± 63	91 ± 111	0.498
DAS28 at baseline	5.56 ± 1.20	5.27 ± 1.64	5.60 ± 1.13	0.421
HAQ at baseline	1.31 ± 0.79	1.14 ± 0.83	1.33 ± 0.78	0.471
Rheumatoid factor, IU/ml	117.6 ± 218.4	48.1 ± 79.6	127.9 ± 230.6	0.311
MMP-3	206.7 ± 203.3	148.6 ± 167.5	216.5 ± 208.4	0.332
Methotrexate, mg/wk	8.05 ± 2.16	8.25 ± 1.64	8.02 ± 2.24	0.761
Prednisone, mg/day	3.25 ± 3.73	1.95 ± 2.83	3.45 ± 3.84	0.241

* Each measure was compared between the low and high ADAMTS5 groups by 2-sample t-test. DAS28: 28-joint count Disease Activity Score; HAQ: Health Assessment Questionnaire; MMP: matrix metalloproteinase.

38 weeks of treatment (Table 3), the percentage of patients in the GR category from the low ADAMTS5 group (90.0%) was significantly higher than that from the high ADAMTS5 group (38.1%; p = 0.0013 by chi-squared test). In addition, the percentage of patients entering remission from the low ADAMTS5 group (70.0%) was significantly higher than that from the high ADAMTS5 group (27.0%; p = 0.0092 by chi-squared test).

The average DAS28 at 38 weeks after the start of treatment was significantly lower in the low ADAMTS5 group (2.30 ± 1.28; p = 0.0038) than in the high ADAMTS5 group (3.90 ± 1.61; Figure 3A). Improvement of the DAS28 as estimated by ΔDAS28 at the end of 38 weeks of treatment was significantly higher in the low ADAMTS5 group (2.97 ± 2.31; p = 0.0331) than in the high ADAMTS5 group (1.70 ± 1.60). We defined the percentage of DAS28 reduction, which could be another marker of improvement of the DAS28, as ΔDAS28 divided by the baseline DAS28. The percentage of DAS28 reduction at the end of 14 weeks of treatment was significantly higher in the low ADAMTS5 group (52.5 ± 28.8; p = 0.0156) than in the high ADAMTS5 group (29.4 ± 27.2; Figure 3B). Further, ΔHAQ, an estimate of the improvement of the HAQ score, at the end of 38 weeks of treatment was also significantly higher in the low ADAMTS5 group (1.18 ± 0.60; p = 0.0102) than in the high ADAMTS5 group (0.21 ± 0.78; Figure 4).

These results suggest that patients with RA who have a low baseline ADAMTS5 mRNA level may show significant improvement with IFX treatment.

The positive predictive value (PPV) of a low baseline ADAMTS5 level for categorization as GR at 38 weeks after the first infusion of IFX was 90.0%, while the negative predictive value (NPV) was 61.9% (Table 4). Further, focusing on remission, the PPV of a low baseline ADAMTS5 level for remission at 38 weeks after the first infusion of IFX was 70.0% and the NPV was 73.0%.

ADAMTS5 protein expression in PBMC from 9 patients with RA was quantified using Western blot and compared with ADAMTS5 mRNA expression (Figure 5A). The expression level of ADAMTS5 proteins correlated well with that of mRNA levels ($p < 0.0001$, $r^2 = 0.962$; Figure 5B), demonstrating the expression of the ADAMTS5 as a protein level as well in PBMC.

DISCUSSION

Biomarkers usually used for the diagnosis of RA or predic-

Table 3. Difference in response to infliximab between the low and high ADAMTS5 groups at 38 weeks.

	All , n = 73	Low Group, n = 10	High Group, n = 63	p*
GR vs NGR				
GR, no. (%)	33 (45.2)	9 (90.0)	24 (38.1)	0.0013[a]
Remission vs nonremission				
Remission, no. (%)	24 (32.9)	7 (70.0)	17 (27.0)	0.0092[a]
R' vs NR'				
R', no. (%)	47 (64.4)	9 (90.0)	38 (60.3)	0.0473[a]
DAS28 at 38 weeks	3.68 ± 1.65	2.30 ± 1.28	3.90 ± 1.61	0.0038[b]
Δ DAS28	1.87 ± 1.75	2.97 ± 2.31	1.70 ± 1.60	0.0331[b]
% DAS28 reduction	32.5 ± 28.3	52.5 ± 28.8	29.4 ± 27.2	0.0156[b]

* Statistical tests ([a] Fisher exact test, [b] 2-sample t-test) were applied to examine if any of the parameters were associated with baseline ADAMTS5 mRNA level. GR: good responder; NGR: moderate + nonresponder. R': responder as ΔDAS28 ≥ 1.2; NR': nonresponder as ΔDAS28 < 1.2; DAS28: 28-joint count Disease Activity Score.

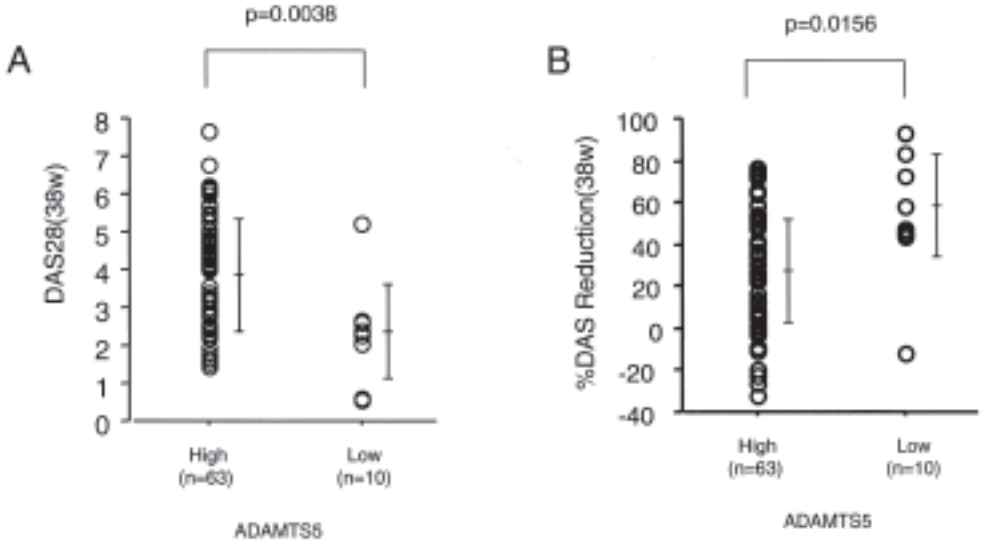

Figure 3. A. Decrease in the 28-joint count Disease Activity Score (DAS28) at 38 weeks of treatment with infliximab in the low ADAMTS5 group. The DAS28 at 38 weeks of treatment was significantly lower in the low ADAMTS5 group (2.30 ± 1.28; p = 0.0038) than in the high ADAMTS5 group (3.90 ± 1.61). B. Increase in percentage of DAS28 reduction at 38 weeks of treatment with IFX in the low ADAMTS5 group. The percentage of DAS28 reduction was significantly higher in the low ADAMTS5 group (52.5 ± 28.8; p = 0.0156) than in the high ADAMTS5 group (29.4 ± 27.2).

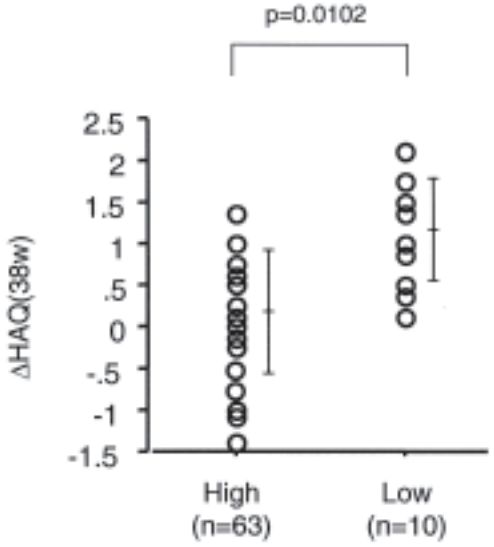

Figure 4. Health Assessment Questionnaire (HAQ) score improvement at 38 weeks of treatment with infliximab (IFX) in the low ADAMTS5 group. ΔHAQ at 38 weeks of treatment was significantly higher in the low ADAMTS5 group (1.18 ± 0.60; p = 0.0102) than in the high ADAMTS5 group (0.21 ± 0.78).

tion of prognosis in RA do not appear to be useful to predict the individual responsiveness to anti-TNF biologics[7]. We demonstrated that the baseline ADAMTS5 mRNA level prior to the start of treatment was much lower in the IFX responder group than in the nonresponder group, and that it can be a biomarker for prediction of the response to IFX. As recently recommended by international experts[21], we chose 38 weeks of treatment as the duration for our study, because the objective of efficient RA treatment is a rapid response. As estimated by

Table 4. Prediction of response to infliximab based on a low baseline ADAMTS5 level.

	Prediction of GR	Prediction of Remission
Sensitivity, %	27.3	29.2
Specificity, %	97.5	93.9
PPV, %	90.0	70.0
NPV, %	61.9	73.0

GR: good responder; PPV: positive predictive value; NPV: negative predictive value.

the PPV, the baseline ADAMTS5 mRNA level can efficiently predict not only the GR (PPV 75.0%) but also remission (PPV 68.8%), following IFX treatment for 38 weeks. The finding of a significant difference in the ΔHAQ at the end of 14 weeks of treatment between the low ADAMTS5 and the high ADAMTS5 groups suggests that the low baseline ADAMTS5 level is the first biomarker that can predict a patient's improvement in ADL after treatment with IFX.

There have been several reports of biomarkers, including genes, for prediction of the response to IFX. Mugnier, *et al*[22] reported that the –308G/G genotype of the TNF-α gene promoter was associated with a better response to IFX [PPV for response (ΔDAS28 ≥ 1.2), 80.5%] than the –308A/G or A/A genotype[11]. Lequerré, *et al*[23] identified 41 mRNA using microarray analysis as a function of the response to IFX (ΔDAS28 ≥ 1.2) and demonstrated that analysis of 8 of the 41 transcripts could yield a 100% PPV for response (ΔDAS28 ≥ 1.2). On the other hand, Trocmé, *et al*[24] showed that a combination of several protein biomarkers could predict the response to IFX with a specificity of 97.5% and sen-

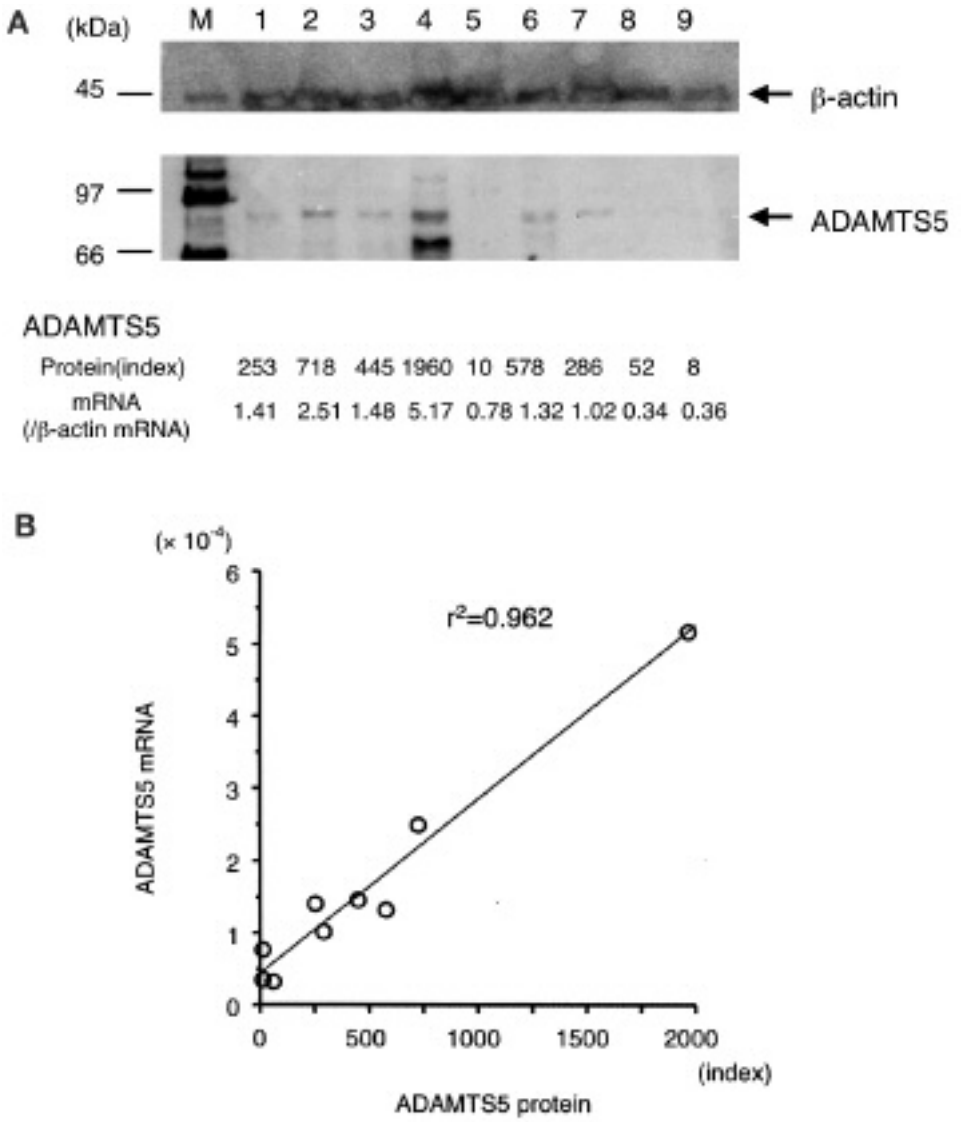

Figure 5. A. Western blot analysis of ADAMTS5 protein in peripheral blood mononuclear cells (PBMC) from patients with RA. PBMC lysate from 9 patients was electrophoresed in 10% SDS-PAGE and blotted onto PVDF membrane. Membranes were incubated with rabbit anti-ADAMTS5 or mouse anti-ß-actin antibody and then with HRP-conjugated anti-rabbit IgG or anti-mouse IgG. After treatment with chemiluminescence-enhancing reagents, membranes were visualized on electrochemiluminescent radiographic films, and density of ADAMTS5 protein bands (arrows) was quantified as index. B. Expression of ADAMTS5 protein and mRNA in peripheral blood was correlated. ADAMTS5 protein expression in PBMC from 9 patients with RA was compared with ADAMTS5 mRNA expression in whole blood from each patient.

sitivity of 97.1%, when response was defined as a 70% improvement in ACR criteria measures and nonresponse was defined as a 20% decline in ACR criteria measures. In our study, the PPV of a low baseline ADAMTS5 mRNA level for categorization as GR was 90.0% (Table 3), which was almost the same as in the other reports. Ours is the first cohort study to demonstrate significant improvement of not only ΔDAS28, but also of the DAS28 itself and ΔHAQ at the end of 38 weeks of treatment in the low ADAMTS5 group. Although we used ß-actin mRNA for normalizing molecular expression, the other RT-PCR systems using housekeeping genes other than ß-actin should be certified because of recent reports of fluctuating expression of ß-actin mRNA. Not only ADAMTS5 mRNA but also ADAMTS5 protein could be increased in the patients who did not respond to IFX.

ADAMTS5-deficient mice (but not ADAMTS4-deficient mice) were found to be protected from cartilage erosion in models of experimental arthritis, and recombinant human ADAMTS5 was found to be substantially more active than ADAMTS4[8-10]. In human synovium, especially in patients with osteoarthritis, the upregulation of ADAMTS4 was

dependent on TNF-α and IL-1 produced by synovial macrophages, while the ADAMTS5 level was not altered by these cytokines[15]. It has been reported that upregulation of ADAMTS4 was nuclear factor-κB-dependent, while that of ADAMTS5 was not, since ADAMTS4, but not ADAMTS5, has several nuclear factor-κB-binding sites on its 5' flanking region that are conserved between species[25]. The less pronounced improvement following IFX treatment in patients with RA who have high baseline blood levels of ADAMTS5 mRNA could be because in these patients, IFX cannot inhibit the degradation of aggrecan by ADAMTS5. Therefore, agents that inhibit the expression of ADAMTS5 might be effective for improving the results of IFX treatment.

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