Brief communication

Hepcidin-25 gives an indication of the therapeutic effectiveness of tocilizumab in rheumatoid arthritis – Relationship between disease activity of rheumatoid arthritis and anemia

A hepcidina-25 dá uma indicação da eficácia terapêutica do tocilizumab na artrite reumatoide – Relação entre a atividade da doença na artrite reumatoide e a anemia

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Introduction

Therapy for RA has improved rapidly since the advent of biologics. However, although biologics have a greater effect compared to that with conventional disease modifying antirheumatic drugs (DMARDs), the cost of these newer therapies remains extremely high.[1] Patients in whom biologics are effective have increased employment opportunities due to reduced disease activity, meaning that the cost-effectiveness of the treatment is valid. However, in cases where the administration of biologics is not associated with a response, disease activity during the period of treatment and the progression of joint damage place an incalculable burden on both patients and the entire medical system. For this reason, the identification of factors that can predict the effectiveness of biologics in each patient, before administration, is a critical issue; tailor-made therapies using single nucleotide polymorphisms (SNPs) are currently being investigated. In Japan, biologics currently available for use in RA include those that target tumor necrosis factor (TNF)-α (infliximab, etanercept, adalimumab, golimumab, and certolizumab pegol), tocilizumab (TCZ), which targets IL-6, and abatacept, which targets CD80/86. We have previously reported on the relationship between TNF-α therapy and autoantibodies (anti-dsDNA antibodies and anti-SS-A antibodies) in patients with insufficient responses.[2] In the present study, we examined predictive factors for the therapeutic effect of biologics, while focusing

Table 1 – Patient characteristics.

Patients	Sex	Age (y.o)	Duration (years)	Hb (g/dL)	CRP (mg/dL)	MMP3 (ng/mL)	CDAI (CDAI at 24 weeks later)	MTX (mg/w)	PSL (mg/day)	Previous biologics
1	F	78	4	13.0	0.8	96	8.0 (6.2)	8	4	ETN
2	M	58	2	13.6	2.3	258	34.2 (24.9)	7.5	10	–
3	F	73	3	11.4	5.1	243	31.6 (29.7)	–	–	–
4	F	51	4	11.1	2.7	170	26 .0(1.0)	10	10	INF
5	F	61	7	12.5	0.6	284	16.3 (4.0)	8	7.5	–
6	F	46	7	13.3	1.8	176	14.3 (10.9)	6	8	INF
7	F	49	13	13.6	0.2	–	19.5 (1.1)	–	–	ETN
8	F	73	21	10.0	0.1	150	16.0 (24.0)	10	7.5	–
9	F	60	20	12.4	3.2	714	24.5 (40.0)	6	4	INF
10	F	39	6	10.1	4.2	403	29.2 (8.0)	8	4	INF

Hb, hemoglobin; CRP, C-reactive protein; MMP3, matrix metalloproteinase3; CDAI clinical disease activity index; MTX, methotrexate; PSL, prednisolone; INF, infliximab; ETN, etanercept.

on the effect of TCZ in ameliorating anemia of chronic inflammation, via direct inhibition of IL-6 receptor-mediated signaling.[3] Hepcidin-25, a major cause of anemia of chronic inflammation,[4] was measured in the serum of RA patients. We compared serum hepcidin-25 levels with clinical data and cytokine levels, to examine whether hepcidin-25 could act as a predictive factor for the effectiveness of TCZ.

Materials and methods

Patients

The 10 patients selected for inclusion in the study had previously been admitted to the Sasaki Institute Kyoundo Hospital Division of Internal Medicine and Rheumatology between September 2008 and September 2010. Patients had commenced TCZ therapy during this period, and were either naive to biologics or had changed from other biologics to TCZ. All RA patients met the American College of Rheumatology (ACR) 1987 revised criteria for the classification of rheumatoid arthritis.[5] Based on the Declaration of Helsinki and the guidelines of the institutional review board of Kyoundo Hospital, written consent was obtained from all patients prior to the start of the study. The characteristics of the 10 patients are shown in Table 1.

Methods

Hepcidin-25 and IL-6 levels were measured before introduction of TCZ and 3 months after introduction. The serum samples were stored at −80 °C. Serum hepcidin-25, which is thought to be the form of hepcidin with the greatest bioactivity, was measured using an enzyme-linked immunosorbent assay (ELISA) (Hepcidin-25 EIA Kit; BACHEM, Bubendorf, Switzerland). Serum IL-6 was also measured using ELISA (Human IL-6 Quantikine ELISA Kit; R&D Systems, Minneapolis, MN, USA). Both hepcidin-25 and IL-6 were measured using the Sandwich ELISA protocol. Test results from routine tests undertaken during general examination at Kyoundo Hospital, including serum hemoglobin, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR), were also used in our analysis. RA disease activity was assessed using clinical

disease activity index (CDAI). Student's t-test was used for statistical analysis. A p-value <0.05 was considered statistically significant.

Results

Therapeutic effectiveness of TCZ in RA patients

CDAI for patients prior to and after treatment with TCZ are shown in Fig. 1A.

Six months after initiation of treatment with TCZ, lower CDAI were noted in 8 of 10 patients.

Comparison of hepcidin-25 levels in RA patients and healthy subjects

In a comparison of serum hepcidin-25 levels in RA patients before receiving treatment with TCZ and in healthy subjects, hepcidin-25 levels were significantly elevated in RA patients (Fig. 1B).

### Changes in hepcidin-25 and IL-6 levels in RA patients

Serum hepcidin-25 and IL-6 levels in RA patients before administration of TCZ and 12 weeks (3 months) after administration are shown in Fig. 1C.

Hepcidin-25
Serum hepcidin-25 levels were high in 8 of 10 patients before administration of TCZ; in these 8 patients, levels decreased after treatment with TCZ. However, in the 2 patients that had comparatively low levels before administration, treatment with TCZ was associated with an increase in hepcidin-25. These results reflected the effectiveness of TCZ therapy. In the 8 patients who exhibited a decrease in hepcidin-25, TCZ therapy was effective, while in the latter 2 patients, TCZ therapy was determined to be ineffective.

IL-6
It is known that because TCZ is an IL-6 receptor antagonist, serum IL-6 levels temporarily increase after administration.[6] In the present study, levels increased in the majority of
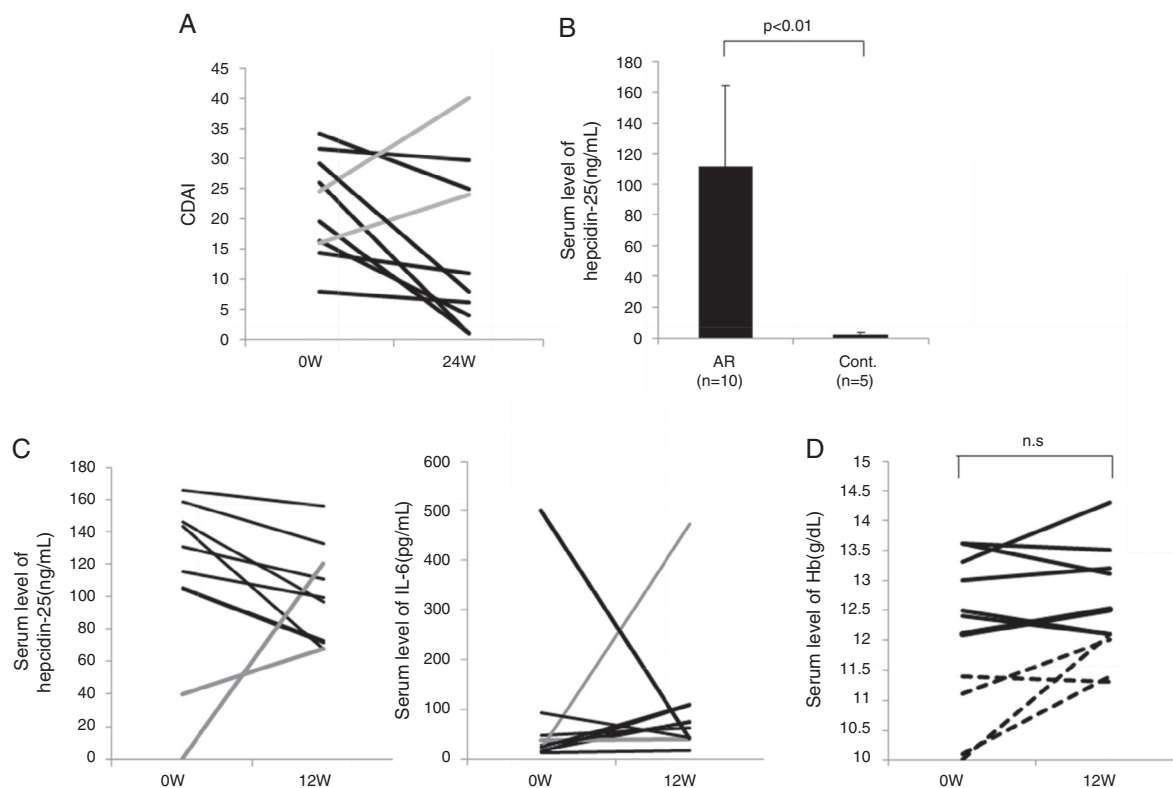
Fig. 1 – (A) Six months after initiation of treatment with TCZ, CDAI was lower in 8 of 10 patients, and was therefore considered to be effective. TCZ was found to be ineffective in 2 of 10 patients; **(B)** in a comparison of serum hepcidin-25 levels in RA patients and healthy subjects, hepcidin-25 levels were found to be significantly elevated in RA patients (*p* < 0.01); **(C)** high levels of serum hepcidin-25 were observed prior to administration of TCZ, and decreased 3 months after initiation of treatment. However, 2 of 10 patients had pre-administration levels that were comparatively low; these levels increased after treatment with TCZ. These 2 cases were consistent with the 2 patients in whom TCZ was considered to be ineffective 6 months after initiation of treatment. The gray line indicates the same patients as the gray line in (A); **(D)** we examined hemoglobin levels 3 months after administration of TCZ. Elevated hemoglobin levels were noted in 5 of 10 patients. Elevated levels were observed in 3 of 4 patients in whom anemia was present before receiving treatment (Hb <12 g/dL, dotted line).

patients after administration of TCZ; however, a decrease was noted in 3 patients.

Relationship between hepcidin-25 and anemia

We observed changes in serum hemoglobin levels 3 months after the start of TCZ treatment, compared with the pre-TCZ hemoglobin level (Fig. 1D). Hemoglobin levels, a general indicator of anemia, increased in 5 of 10 patients after administration of TCZ. When analysis was limited to those patients in whom anemia was present prior to receiving treatment (Hb <12 g/dL), most patients showed an increase in hemoglobin levels.

Discussion

Hepcidin is a peptide hormone that is produced in the liver. There are several known isoforms of hepcidin, including hepcidin-20 and hepcidin-22; hepcidin-25 has the highest bioactivity and is considered to have a strong relationship with iron metabolism.[7] Iron taken orally is absorbed by intestinal epithelial cells. In the presence of iron deficiency, iron is

released to the peripheral blood. When no deficiency of iron is present, iron is excreted fecally as the epithelial cells are sloughed off into the intestinal lumen. Most iron absorbed in the intestinal tract is stored in the liver. A suitable amount of iron is supplied from the liver to the peripheral blood, for use in erythrocyte production in the bone marrow. Old erythrocytes are trapped and destroyed in the reticuloendothelial system, and the iron is taken up by the system, and released again into the peripheral blood. In the case of chronic inflammatory diseases such as RA, there is an overproduction of IL-6. When IL-6 binds to its receptors in the liver, hepcidin-25 is produced. Hepcidin-25 binds to ferroportin, expressed in hepatocytes and reticuloendothelial macrophages, leading to inhibition of intestinal absorption of iron, and inhibition of iron release from the reticuloendothelial system and hepatocytes; together these factors result in the development of anemia.[8,9] Hepcidin expression is up-regulated by iron and inflammation and down-regulated by anemia and hypoxia.[10]

On the basis of this knowledge, it is considered that RA patients with high disease activity and high hepcidin-25 levels are especially influenced by IL-6[11]; TCZ treatment is

anticipated to be particularly effective in these patients. However, in the present study TCZ was not effective in 2 cases, despite the fact that serum IL-6 levels before treatment were abnormally high; in 1 of these cases serum hepcidin-25 levels were low (Fig. 1C). It has previously been reported that although anemia of chronic inflammation may be present in RA patients, a low level of hepcidin that is strongly indicative of iron deficiency, was also noted.[12] In the present study, marked iron deficiency was noted in patients who had abnormally high serum IL-6 levels with concomitant low hepcidin-25 levels.

We concluded that because hepcidin-25 levels decreased after TCZ administration and improvement in the associated anemia was noted, TCZ can ameliorate anemia of chronic inflammation.

Patients that had high hepcidin-25 levels before administration of TCZ and lower levels 3 months after treatment, were still evaluated as having had a good response 6 months after treatment. Based on this, we consider that hepcidin-25 can act as a predictive factor for the effectiveness of TCZ treatment. However, caution is needed in determining cases of iron deficiency anemia. Measurement of hepcidin can be easily performed, and the application of hepcidin as a clinical indicator in RA patients can be expected in the future.

Conflicts of interest

The authors declare no conflicts of interest.

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