

Case Report

Anemia of Inflammation in the Elderly Patients. The Significance of Measuring Serum Level of Hcpidin25- for Diagnosis, and the Therapy with Peroral and Intravenous Iron Administration

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Abstract

The illustrative cases of patients with Anemia of inflammation (AI) encountered in the outpatient clinic in the rural area in Japan during a year were retrospectively studied. AI was diagnosed based on normocytic and normochromic anemia with low serum iron (Fe) and elevated ferritin levels, with or without elevated inflammatory markers such as C-reactive protein (CRP) and interleukin-6 (IL-6). Six patients were diagnosed with AI. Serum levels of hepcidin-25 were markedly elevated in 5 patients and slightly elevated in 1 patient. The administration of peroral (PO) iron was performed in 4 patients: it was ineffective in 2 patients whose serum hepcidin levels were extremely high, and effective in 1 patient whose hepcidin level was slightly elevated at diagnosis, and effective in 1 patient whose hepcidin level decreased before administration. Intravenous(IV) administration of iron was performed in 2 patients, being effective and ineffective in 1 patient in which CRP level decreased before administration, and ineffective in 1. The results indicate that the measurement of serum levels of hepcidin-25 is a reliable diagnostic tool for AI, and is useful for assessing the effectiveness of iron administration. The administration of IV iron only is controversial, and further study is necessary.

Keywords: Anemia of Inflammation (AI); Anemia of chronic disease (ACD); Hcpidin-25; PO and IV Iron Administration

Introduction

Anemia is common in elderly persons increasing their morbidity and mortality rates. The cause of anemia is multifactorial in elderly persons. Causes include nutritional deficiency such as iron deficiency, vitamin B12 and folic acid deficiency,

and anemia of inflammation (AI), which has been classically referred as anemia of chronic disorder (ACD), associated with inflammation, heart and renal failure, malignancy and autoimmune disorder such as rheumatoid arthritis, and unexplained anemia (UA)[1-4].

Iron deficiency anemia (IDA) is characterized by microcytic and hypochromic anemia [low values of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)], and low serum iron (Fe) and ferritin levels, while AI is characterized with normocytic and normochromic anemia (normal values of MCV, MCH and MCHC), and low serum Fe and elevated ferritin levels [3,4].

The pathogenesis of anemia of AI were considered as defects of iron availability and utilization, impaired erythropoiesis due to decreased erythropoietin (EPO) production, and an impaired response to EPO of erythroid progenitors [5-10].

Hepcidin is a key regulator of iron homeostasis [6,7,11-13]. It is produced in the liver, induced by inflammatory cytokines such as interleukin-6 (IL-6). Hepcidin blocks the intestinal absorption of iron, and also inhibits the release of iron from macrophage stores by acting on ferroportin [11-13]. The restriction of iron delivery to erythrocyte precursors can limit erythropoiesis, iron-restricted hematopoiesis [6,7].

The diagnostic criteria for AI, and also therapeutic strategies for these patients have yet to be established [1-4,8-10]. Such patients were previously untreated in most cases.

Here, we retrospectively studied several cases of AI in a rural area of Japan where the proportion of elderly persons is increasing, measured the serum level of hepcidin-25 for diagnosis, employed the therapy with iron administration by PO and/or IV, and reviewed the literature concerning the disease mechanism, diagnosis, and therapeutic aspects.

Methods

Complete blood cell counts (CBC), blood chemistry, serum levels of Fe and ferritin, and CRP were measured using the standard methods. The serum levels of EPO and IL-6 were measured in some patients. Serum levels of hepcidin-25, the main element of hepcidin, were measured in all patients by liquid chromatography and mass spectrometry using the 4000QTRAP LC-MS/MS assay system (Applied Biosystems, Foster City, CA, USA) by Medical Care Proteomics Biotechnology Co., Ltd. (Ishikawa, Japan). The approval of institutional review board (IRB) for this clinical study was obtained. Informed consent was obtained from the patients for IV iron administration.

Patients

Six illustrative elderly patients with AI treated in my outpatient clinic at a hospital in the rural area of Japan where the population was approximately 6,000, and the percentage of elderly persons over 65 years-old was approximately 50%, over the last 1 year are presented. The patients were diagnosed with AI based on normocytic and normochromic anemia and low serum Fe and high ferritin levels, with or without positive

inflammatory markers such as CRP and IL-6. The diagnosis of myelodysplastic syndrome (MDS) was ruled out by the absence of bicytopenia or pancytopenia of the peripheral blood cells.

The patient's laboratory data on admission and the therapeutic outcomes with iron administration are presented in Table 1. The present study includes the patients with heterogeneous characteristics of causes of inflammation. The ages of the patients ranged from 79 to 89. Four were female and two were male. The values of Hb ranged from 8.3 to 10.1 g/dL. Serum levels of Fe were reduced, and those of ferritin increased in all patients. The unsaturated iron binding capacity (UIBC) was normal, and the total iron-binding capacity (TIBC) was decreased in almost all patients. The serum levels of vitamin B12 and folic acid were normal in most of the patients. The values of CRP were elevated in 5 patients (patients 1,3-6), but almost normal in one patients (patient 2). A decrease of the elevated CRP values was observed in the patients 1, 4 and 5 during the clinical course. The levels of IL-6 at the diagnosis of AI were markedly elevated in 2 patients (patients 1 and 6), and slightly elevated in 2 patients (patients 4 and 5). The serum levels of hepcidin-25 at the diagnosis were markedly elevated in 5 patients (patients 1-4, 6), and slightly elevated in 1 patient (patient 5), and decreased in two patient during clinical course before treatment, (patients 1 and 4). The serum levels of EPO were normal in all of the 5 patients measured. The doses of IV administration were calculated according to the recommended formula [14].

Clinical Courses

Patients were treated by the IV(saccharated ferric oxide) and/or PO(sodium ferrous citrate) administration of iron. The outcome of the treatment is shown in Table 1. The illustrative clinical course of the treated patients is shown in Figures 1-3. PO administration was performed before IV administration in patients 1 and 2. In patient 1 (Figure 1), PO administration was ineffective, whereas IV administration, with a total amount of 960mg, was effective for the amelioration of anemia. The CRP level decreased before IV administration without specific treatment. The serum levels of Fe, ferritin and hepcidin-25 remained high. Over 1 year after IV iron, the increased Hb level was maintained. In patient 2 (Figure 2), PO administration was ineffective as well as IV administration, with a total amount of 880mg. The levels of Fe and ferritin remained high after a few months of IV iron in these patients. However, adverse effects including organ damage after PO and IV iron administration were not observed in these patients. Patient 4 suffered from fracture of the femoral bone soon after diagnosis, consequently underwent orthopedic surgery. PO administration was effective after the operation and administration of antibiotics, and the levels hepcidin and CRP had decreased before administration (Figure 3). Patient 5 was treated with PO administration, and it was effective for the amelioration

of anemia and CRP dropped without administration of antibiotics. The other two patients were untreated (patients a and 6). Patient 3 died of pneumonia soon after the diagnosis of AI. Patient 6 suffered from swallowing difficulty due to cerebral infarction and aspiration pneumonia.

Discussion

According to the report by the National Health and Nutrition Examination Survey III (NHANES III) of the distribution of types of anemia in persons 65 years and older in the United States, IDA was 16.6%, AI was 19.3%, and UA was 33.6%[1].

Patient	1	2	3	4	5	6
Sex	F	F	M	F	F	M
Age	81	83	89	89	79	83
Cause	unknown	renal failure	pneumonia	fracture	dementia	pneumonia
Antibiotics	NA	NA	NA	+	NA	+
RBC x10 ¹² /L	3.07	2.62	3.30	3.12	3.34	3.10
Hb g/dL	8.5	8.3	9.7	9.5	10.0	8.9
Ht %	27.9	26.4	28.6	29.8	31.3	30.6
MCV fl (86-100)	91	101	86	96	94	99
MCH pg (29-35)	28	32	29	30	30	29
MCHC % (32-36)	31	31	34	32	32	29
Plt x10 ⁹ /L	312	176	305	261	250	160
WBC x10 ⁹ /L	6.2	5.2	4.4	6.1	7.7	5.9
Fe µg/dL (M 80-199,F 70-179)	16	34	34	54	24	35
UIBC µg/dl (M 81-351,F 126-388)	201	171	ND	181	158	117
TIBC µg/dl (M 237-458,F 253-509)	177	224	ND	214	216	160
Ferritin ng/mL (M 30-310,F 6-138)	496	564	417	387	486	496
CRP mg/dL (<0.3)	8.7	0.5	18.7	6.5	7.7	14.3
IL-6 pg/mL (<4.0)	26.4	3.8	ND	5.6	6.4	23.0
Hepcidin-25 ng/mL (7.8±7.0)	80.8	88.7	88.7	67.6	17.6	88.5
				*46.8		
Vit B12 pg/mL (233-914)	358	1590	521	505	663	718
Folic Acid ng/mL (3.6-12.9)	7.6	4.2	3.4	7.1	4.4	2.9
Epo mU/mL (9.1-32.8)	27.7	27.7	ND	20.3	22.1	52.5
Uric Acid mg/dL	3.4	8.8	4.9	6.5	3.8	6.9
Creatinine mg/dL	0.7	1.5	0.6	0.9	0.6	1.5
BUN mg/dL	7.7	34.0	39.8	26.1	16.3	24.2
Iron Administration						
PO	I	I	NA	E	E	NA
IV	E	I	NA	NA	NA	NA

Table 1. The cause of AI and laboratory data on admission and treatment outcome of iron therapy in patients with AI. (): normal range. ND: not determined. * Decreased before PO administration. IV: intravenous, PO: peroral. E: effective, I: ineffective, NA: not administered.

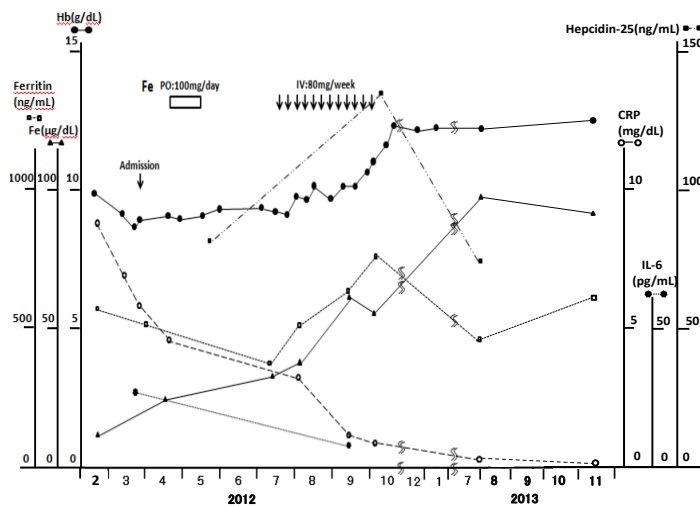


Figure 1. Clinical course of patient 1. PO iron administration was ineffective, while IV was effective for amelioration of anemia. The level of CRP decreased spontaneously before IV administration without administration of antibiotics. The level of Hb was maintained normal after IV administration and those levels of hepcidin-25, serum Fe and ferritin remained high without long-term adverse effects.

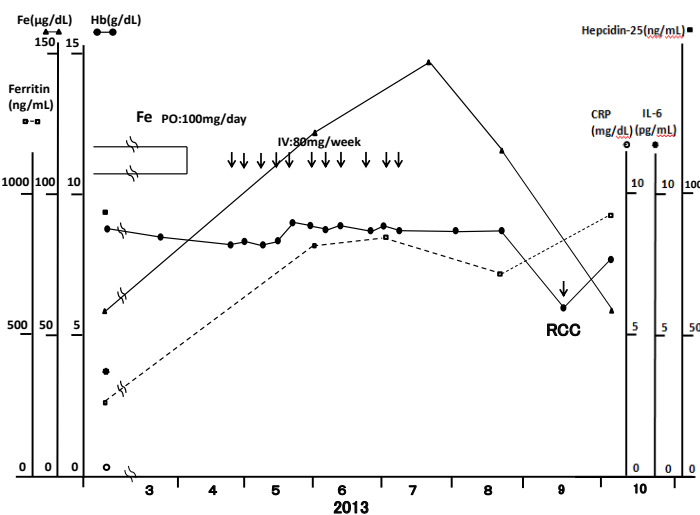


Figure 2. Clinical course of patient 2. PO and IV iron administration was ineffective. The levels of CRP and ferritin remained high after IV administration. RCC: red cell concentrate.

AI initially presents as mild normocytic and normochromic anemia, but it becomes microcytic and hypochromic as it progresses, as in IDA [3,4], since serum iron is unavailable due to an elevated serum hepcidin level, resulting in functional iron deficiency (FID) or iron-restricted erythropoiesis [6-10].

The serum level of hepcidin-25, the main element of hepcidin, is currently measured using mass spectrometry or an immunoassay, such as the enzyme-linked immunosorbent assay (ELISA) [15].

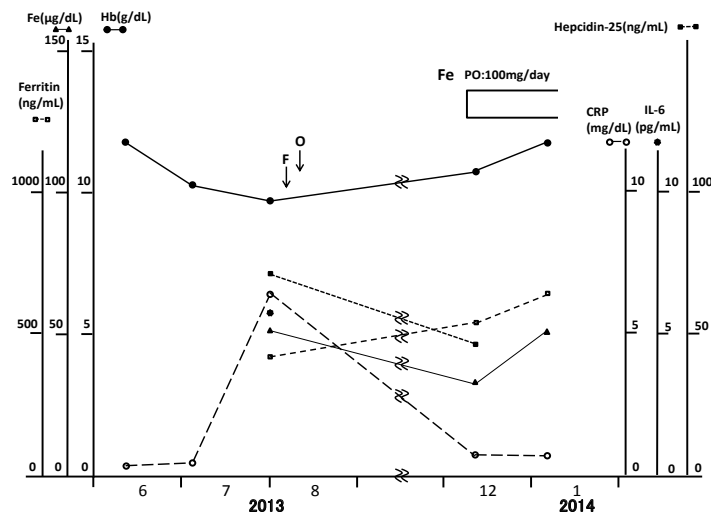


Figure 3. Clinical course of patient 4. PO iron administration was effective. The levels of CRP and hepcidin-25 decreased after surgery with administration of antibiotics before PO administration. F: fracture, O: operation.

Serum levels of hepcidin are elevated in acute and chronic inflammatory states, such as inflammation, rheumatoid arthritis, inflammatory bowel disease (IBD), and heart and renal failure [4,8-10].

The measurement of serum ferritin is frequently of less value for distinguishing inflammation and iron overload, since ferritin is an acute-phase protein, which is elevated in inflammation and malignancy, as well as an indicator of iron stores [8,9]. The level of serum ferritin is high in AI, whereas it is low in IDA, and normal in AI complicated with IDA (AI/IDA) [4,6,8]. Elevated hepcidin blunts the response of erythroid cells to EPO in AI [4-10], and patients with AI usually have normal serum EPO levels, except for patients with renal failure, who usually show decreased EPO production. Patients with AI have relatively low serum levels of EPO than that observed in equally anemic patients with IDA [10], although the present patients had normal or elevated serum levels of EPO. In addition, a significant proportion of patients with AI have concurrent IDA (AI/IDA) due to blood loss or nutritional deficiency [6-9].

The levels of serum hepcidin-25 were markedly elevated in 5 patients and slightly elevated in 1 patient (patient 5) in the present study. Inflammatory markers such as CRP and IL-6 were not elevated significantly in all of the cases. Inflammatory conditions and increased levels of CRP and IL-6 were also observed in patients with heart failure [5]. Besides hepcidin, other inflammatory cytokines such as interleukin-1 (IL-1), interferon- γ (IFN- γ), and tumor necrosis factor- α (TNF- α) might also have a detrimental role in heart and renal failure, and may have effects on the inhibition of hematopoiesis [3-5,9,10]. These might contribute for the pathogenesis of AI/ACD. Patient 5 in the present study might have had AI/IDA since the

hepcidin level was only slightly elevated and PO administration was effective for the amelioration of anemia. These results indicate that measuring the serum hepcidin-25 level is a significant diagnostic tool for AI, and is also useful for distinguishing AI, IDA, and AI/IDA [4,6,8]. However, the assay of hepcidin-25 for the diagnosis of AI still remains experimental, and has yet to be approved for routine practice by the Food and Drug Administration (FDA) in USA and also in Japan. There is also a lack of standardization of hepcidin measurement, and there are variations of measured values among laboratories [8,9]. These remain to be elucidated.

The treatment strategies for the patients with AI have yet to be established [4,8-10].

Treatment of the underlying diseases associated with AI is the mainstay; however, they are not always easy to diagnose the associated diseases and also to treat, especially in elderly patients.

Currently available agents for the treatment of anemia in AI are erythropoiesis stimulating agents, EPO, and iron preparations, administered by PO and IV [4,8].

The rationale for the use of EPO is based on the blunted EPO response seen in patients such as with cancer and renal failure of AI due to the impaired response of erythropoiesis to EPO in the presence of an excess of hepcidin, or decreased EPO production [4,5,8,9]. The administration of a sufficient amount of EPO ameliorated anemia in these patients [4,5,8,9]. However, the use of EPO has not been approved by medical insurance for AI patients in Japan.

PO administration of iron has been the mainstay of treatment for patients with IDA. However, iron is not available for erythropoiesis in almost all patients with AI when serum hepcidin is increased. In contrast, in patients 4 and 5 of the present study, PO administration was effective for the amelioration of anemia. This might be due to only a slightly elevated hepcidin level in patient 5 at diagnosis, improvement of inflammation before PO iron administration in patient 4 as evidenced by the decrease of CRP levels, or concurrent IDA, and orally administered iron might be absorbed from the intestine and used for erythropoiesis. The results also indicate the usefulness of measuring the hepcidin level to judge the effectiveness of PO iron administration for AI.

There is controversy regarding the administration of IV iron only in the treatment of AI [4,8]. The infused iron is primarily trapped by cells of the reticuloendothelial system (RES); however RES blockade of infused iron might not be complete [8,16-18]. Hence, a very small amount of the infused IV iron might directly bind to plasma transferrin, bypassing macrophages, be gradually released into the circulation, used for erythropoiesis, and help to relieve iron-restricted hematopoiesis [8,16-18]. Thus, anemia in AI is probably overwhelmed by adminis-

tered more IV iron [8,16-18].

Several forms of safer parenteral iron with less adverse effects have become available in recent years in Europe and the USA: low-molecular-weight iron dextran, iron sucrose, ferric carboxymaltose, and sodium ferric gluconate [14,16-19], and these are widely used in conjunction with EPO in the management of patients with renal anemia and cancer-associated anemia [14,16-19]. IV iron has been reported to be safe and effective for patients with AI, and is superior to PO iron when used in combination with EPO [4,8]. Also, there is evidence that IV iron in addition to EPO may enhance response to the latter in patients with cancer-associated anemia [19].

In dialysis patients' response to IV iron with elevated ferritin (DRIVE) trial, infusion of iron in conjunction with EPO was effective in patients who had serum ferritin above 500 ng/mL, and as high as 1,200 mg/mL, and transferrin saturation (TSAT) < 25% [16]. The administration of IV iron 500mg was insufficient to increase the level of Hb; however, 1,000 mg increased the Hb level [16].

In Japan, only two formulations of IV iron are available: cideferron and saccharated ferric oxide. Limited studies have shown that IV iron given alone without EPO may improve Hb levels in patients with AI [4], and further study is necessary.

In the present study, IV iron was effective for the amelioration of anemia in patient 1, but was ineffective in patient 2. In patient 1, the serum levels of CRP, IL-6, and hepcidin were elevated at the diagnosis of AI. In patient 1, the level of CRP, and in patient 4, the levels of CRP and hepcidin, gradually decreased during the clinical course. The amelioration of anemia after the drop of CRP or hepcidin levels might suggest that the iron was utilized effectively for erythropoiesis after the resolution of inflammation, by IV in patient 1, or by PO in patient 4. In patient 2, the levels CRP and IL-6 were not elevated at the diagnosis of AI, and the patient had mild renal dysfunction. There may be a difference in the blunted response to endogenous EPO for erythropoiesis in patients with renal dysfunction, since the serum levels of EPO were normal or elevated in all patients in the present study. In patient 2, the concomitant administration of IV iron with EPO might be an acceptable treatment option. Indeed, this patient died of renal failure 6 months later.

In patients whose anemia was ameliorated, they felt an improvement of the quality of life (QOL).

Of course, iron overload and organ damage caused by generated free radicals of oxygen should be avoided when IV iron is administered [4,8]. The long-term effects of supraphysiologic IV iron therapy in AI patients have not been investigated [4,5]. Adverse reactions and organ damage were not observed in 2 patients, (patients 1 and 2), during the observation periods. In patient 1, serum levels of Fe and ferritin remained high after over 1 year of IV iron, although the level of CRP decreased.

In patient 2, these remained high after IV iron administration. These may be due to iron overload, since the levels of CRP were normal in both patients after IV iron.

A future directions for the treatment of AI is targeting the hepcidin-ferroportin axis [8,19,20]. Several agents that inhibit hepcidin function (direct hepcidin antagonists), prevent the transcription of hepcidin (hepcidin production inhibitors), or promote the resistance of ferroportin to hepcidin's action (ferroportin agonists/stabilizers) are currently under investigation [8,19]. They include anti-hepcidin antibody [8,19], and antiIL-6 receptor antibody[8,20], which also down-regulated hepcidin expression and improved AI in patients with multicentric Castleman's disease [20].

The patients of AI will increase in the rural area where elderly persons increase.

The developments of laboratory tools, including the incorporation of hepcidin measurement into the standard diagnostic criteria, and the appropriate therapeutic strategies for AI, are necessary.

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