Effects of Anemia Correction by Erythropoiesis-Stimulating Agents on Cardiovascular Function in Non-Dialysis Patients With Chronic Kidney Disease

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SUMMARY

Anemia is a significant risk factor for patients with chronic kidney disease (CKD). Here, we investigated the effects of anemia correction on cardiac functions in CKD patients. Pre-dialysis CKD patients (n = 171) without known risk factors for cardiovascular disease (CVD) other than CKD with hemoglobin (Hb) concentrations < 10.0 g/dL were enrolled for evaluation of cardiac functions and biomarkers before and after the 16-week treatment of erythropoiesis-stimulating agents. The treatment significantly increased Hb concentrations in all patients who completed the study (n = 143, 8.91 ± 0.87 versus 11.27 ± 1.31 g/dL; P < 0.001) and among patients whose echocardiograms were available for evaluation (n = 77, 8.92 ± 0.94 versus 11.24 ± 1.13 g/dL; P < 0.001). The left ventricular mass index (LVMI) was decreased (121.3 ± 25.8 versus 114.7 ± 25.1 g/m²; n = 77, P = 0.012) and significant correlation between the change in the LVMI and Hb concentration was noted (P = 0.011). The levels of B-type natriuretic peptide and human atrial natriuretic peptide, and the cardio-thoracic ratio were significantly increased among subjects with Hb concentrations < 11.0 g/dL at completion of the study. The changes in these parameters were significantly correlated with the Hb concentrations (P = 0.033, P = 0.011, and P < 0.001, respectively). No significant differences were observed in the electrocardiographic parameters. Correcting Hb levels higher than those conventionally recommended reduced left ventricular hypertrophy and myocardial stress, lowering risks for CVD in pre-dialysis CKD patients. (Int Heart J 2012; 53: 238-243)

Key words: Anemia, Chronic kidney disease, Erythropoiesis-stimulating agent

Patients with chronic kidney disease (CKD) are at high risk for mortality from cardiovascular diseases (CVD) and prevention of CVD improves their prognosis. Anemia in CKD patients is a serious complication that is frequently observed in patients with stage 4 or higher CKD. While CKD itself is a significant risk factor for developing CVD, anemia is also an independent risk factor for CVD and can be treated with erythropoiesis-stimulating agents (ESAs). However, optimal target hemoglobin (Hb) levels using ESA treatment to efficiently prevent the development of CVD and to improve the prognosis of CKD patients have yet to be fully determined.

In Japan, recombinant human erythropoietin (rHuEPO) was approved in 1990 as a therapeutic reagent for anemia in patients on dialysis, and became available for non-dialysis CKD patients in Japan as of March 2010, is approved for treatment to efficiently prevent the development of CVD and to improve the prognosis of CKD patients. As of April 2010 for use in non-dialysis patients, as well.

Multiple clinical studies on treatment of anemia in CKD patients were conducted and clinical guidelines have been developed since 1997 in various countries based on these results. In 2006, the results of large-scale comparative clinical studies, such as the CHOIR study, were reported, leading to the conclusion that targeting higher Hb levels in CKD patients would not be of benefit. As a result, the KDOQI guidelines were revised, recommending that the Hb levels in CKD patients should generally be managed in the range of 11.0 to 12.0 g/dL, and should not be greater than 13.0 g/dL. The EBPG also recommends Hb levels in CKD patients more than 11.0 g/dL without setting any specific upper limits. In Japan, the Japanese Society for Dialysis Therapy issued the “Guidelines for Anemia in Chronic Kidney Disease” in 2008, and suggested a target Hb level by ESA treatments in non-dialysis patients of 11.0 g/dL or higher (recommendation) and dose reduction or interruption to be considered if the Hb levels exceed 13.0 g/dL (opinion).

However, rHuEPO, the only ESA available for pre-dialysis CKD patients in Japan as of March 2010, is approved for...
target Hb levels approximately at 10.0 g/dL, with precautions for the Hb levels not to exceed 12.0 g/dL. Therefore, little evidence regarding the possible benefits of targeting Hb levels higher than the current recommendations in non-dialysis CKD patients has been made available.

We recently conducted a dose-response study for DPO in non-dialysis CKD patients. In this study population, we evaluated the effects of Hb levels higher than those recommended in conventional rHuEPO therapy on cardiac functions in non-dialysis CKD patients.

**METHODS**

This study was a multicenter, randomized, open-label, parallel-group study conducted from July 2004 through April 2005 at 52 medical centers and hospitals in Japan. The study was conducted in accordance with the Declaration of Helsinki and international guidelines for good clinical practice. The protocol was approved by each local Institutional Review Board prior to initiation of the study.

**Subjects:** This study enrolled adult CKD patients with anemia (serum creatinine ≥ 2 mg/dL (177 μmol/L) and Hb < 10.0 g/dL, without rHuEPO administration at least for the last 4 weeks), 20 to 80 years of age and weighing 40 to 80 kg who were not expected to start renal replacement therapy within 16 weeks. Candidates were observed during the prestudy periods for 16 weeks to exclude patients with uncontrolled hypertension, if more than one third of available diastolic blood pressure measurements up to 16 weeks prior to the initiation of the present study exceeded 100 mmHg. Patients with congestive heart failure (New York Heart Association (NYHA) class III or IV) or a known history of symptomatic myocardial, pulmonary or cerebral infarction, unstable angina or obstructive arteriosclerosis (Fontaine’s classes II through IV) were also excluded, as were patients either with malignancies, recent major surgery, major bleeding and/or transfusion, or investigational product use in the previous 16 weeks. Written informed consent was obtained from all subjects prior to study enrollment.

**Administration of the ESAs and other supplemental medications:** Eligible subjects received DPO or rHuEPO subcutaneously for 16 weeks. Subjects were allocated to receive either DPO 30, 60, or 90 μg once every two weeks, or EPO 6000 IU once every week as a dose-response study for DPO. Patients also received iron substitutes to keep the transferrin saturation (TSAT) levels above 20% or ferritin levels higher than 100 ng/ml.

**Cardiac parameters and timing of the measurement:** Echocardiograms, endocrinological tests, chest X-rays, and electrocardiograms were performed at baseline and the end of the study (week 16). Physiological tests (eg, blood pressure) and complete blood counts (CBCs and Hb concentrations) were performed once every two weeks.

Echocardiography was performed at each participating institution and left ventricular M-mode echograms, end-diastolic and systolic left ventricular long- and short axis tomograms were recorded. The recorded images were evaluated by a committee of three cardiologists. First, two cardiologists independently assessed the quality of the echocardiograms and measured the left ventricle parameters (left ventricular end-diastolic dimension [LVDd], left ventricular end-systolic dimension [LVDs], interventricular septal thickness [IVST], and posterior wall thickness [PWT]) according to the method recommended by the American Society of Echocardiography (ASE) (Figure 1). The mean values of the measurements by the two cardiologists were used as the final results. When the 2 measurements differed considerably, the third committee member was consulted for discussion with the first two to obtain agreeable measurements. To prevent any bias for the evaluation processes, subject information was kept blind to the committee members.

Devereux’s formula, \( 0.8 \times \{ 1.04 \times \left[ (LVDd + IVST + PWT) - LVDd^2 \right] \} + 0.6 \) was used to calculate the left ventricular mass (LVM) from the echocardiographic measurements, and the left ventricular mass index (LVMI) was obtained by dividing the LVM values with the body surface area (BSA). The left ventricular ejection fraction (LVEF) was calculated from the left ventricular volume determined by Teicholz’s method in order to assess left ventricular systolic functions.

Electrocardiograms obtained at each participating center were assessed by the cardiologist committee as described for echocardiogram evaluation. Parameters of ventricular load, left ventricular hypertrophy, and myocardial ischemia were obtained (heart rate, P interval, QRS interval, QRS axis, QTc, Rv5, S1, Rv5, presence of arrhythmia, ST-T changes).

**Cardio-thoracic ratio (CTR)** was calculated with chest X-ray images with a standard technique.

**Measurements of biomarkers for cardiac functions:** B-type natriuretic peptide (BNP) and human atrial natriuretic peptide (hANP) levels were measured at the baseline and at 16 weeks after completion of the ESA treatment. Blood samples were drawn with the subject in a seated position after avoiding excessive physical activities, and were sent to SRL Medisearch Inc (Tokyo) for biomarker measurements by radioimmunoassay.

**Statistical analysis:** The study included 4 different treatment groups (either with EPO or DPO at different doses) as originally designed as a dose-response study for DPO. However, subjects from any group were analyzed altogether irrespective of type or dosage of the ESAs administered based on the degree of anemia correction achieved.

Student’s t-test was applied to compare changes in meas-
Pressure levels were observed throughout the study period. No major changes in either diastolic or systolic blood pressure (59.4%) patients, and 12.0 g/dL or higher in 43 (30.1%) patients. The Hb level was 11.0 g/dL or higher at week 16 in 85 (53.8) patients.

The Hb concentrations increased significantly (P < 0.001), from 8.91 ± 0.87 g/dL at baseline to 11.27 ± 1.31 g/dL at week 16. The Hb levels increased significantly (P < 0.001) in patients whose Hb levels reached 11.0 g/dL or higher, with significant correlation (P = 0.011) between the Hb levels and changes in the LVMI (Figure 4A). Changes in the LVMI significantly correlated with the increase in the Hb levels (P = 0.001) as well (Figure 4B). The correlations between the Hb levels and other echocardiographic parameters were also evaluated. IVST was decreased more efficiently in those whose Hb levels were increased at week 16 with significant correlation to the Hb levels (P = 0.025), whereas no correlations to the Hb levels at week 16 were observed for other parameters (PWT; P = 0.257, LVDD; P = 0.576, LVD; P = 0.306). The LVEF, a measure of

### Results

**Patient characteristics:** After initial screening of 210 patients, 171 patients who met the study criteria were enrolled and DPO or EPO administration was started. Twenty-eight subjects withdrew from the study, meaning 143 completed the study. Table I shows the patient characteristics of these 143 patients.

**Hb concentrations and blood pressure levels:** Figures 2A and 2B show the Hb concentrations and blood pressure (diastolic/ systolic) levels. The Hb concentrations increased significantly (P < 0.001), from 8.91 ± 0.87 g/dL at baseline to 11.27 ± 1.31 g/dL at week 16. The Hb levels increased significantly (P < 0.001) at baseline (61.0%; 47 patients) compared to week 16 (45.5%; 35 patients). LVMI significantly decreased (P = 0.001) in patients whose Hb levels reached 11.0 g/dL or higher, with significant correlation (P = 0.011) between the Hb levels and changes in the LVMI (Figure 4A). Changes in the LVMI significantly correlated with the increase in the Hb levels (P = 0.001) as well (Figure 4B). The correlations between the Hb levels and other echocardiographic parameters were also evaluated. IVST was decreased more efficiently in those whose Hb levels were increased at week 16 with significant correlation to the Hb levels (P = 0.025), whereas no correlations to the Hb levels at week 16 were observed for other parameters (PWT; P = 0.257, LVDD; P = 0.576, LVD; P = 0.306). The LVEF, a measure of

### Table I. Baseline Characteristics of the Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Whole study population</th>
<th>Subjects with evaluable echocardiograms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 143</td>
<td>n = 77</td>
</tr>
<tr>
<td>Female</td>
<td>78 (54.5)</td>
<td>39 (50.6)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.7 ± 9.8</td>
<td>60.2 ± 10.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>156.9 ± 7.9</td>
<td>158.2 ± 7.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>56.4 ± 9.0</td>
<td>56.6 ± 8.5</td>
</tr>
<tr>
<td>Cause of chronic kidney disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>69 (48.3)</td>
<td>36 (46.8)</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>34 (23.8)</td>
<td>21 (27.3)</td>
</tr>
<tr>
<td>Chronic pyelonephritis</td>
<td>3 (2.1)</td>
<td>2 (2.6)</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>7 (4.9)</td>
<td>6 (7.8)</td>
</tr>
<tr>
<td>Nephrosclerosis</td>
<td>16 (11.2)</td>
<td>5 (6.5)</td>
</tr>
<tr>
<td>Other</td>
<td>14 (9.8)</td>
<td>7 (9.1)</td>
</tr>
<tr>
<td>History of rHuEpo use</td>
<td>77 (53.8)</td>
<td>41 (53.2)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>8.91 ± 0.87</td>
<td>8.92 ± 0.94</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>150.52 ± 251.58</td>
<td>148.24 ± 199.95</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>30.46 ± 13.99</td>
<td>29.91 ± 13.19</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>3.91 ± 1.43</td>
<td>3.83 ± 1.43</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>137.6 ± 18.7</td>
<td>135.4 ± 16.8</td>
</tr>
<tr>
<td>Diastolic</td>
<td>75.5 ± 10.5</td>
<td>75.6 ± 10.2</td>
</tr>
</tbody>
</table>

Values are listed as number of patients (%) or mean ± SD, unless otherwise indicated. rHuEPO indicates recombinant human erythropoietin alpha and TSAT, transferring saturation.

Figure 2. Serial changes in mean hemoglobin levels (A, mean ± SD, n = 143, P < 0.001 by one-way ANOVA) and blood pressure levels (B, mean ± SD, n = 143, not significant) during the 16-week study periods. SBP indicates systolic blood pressure and DBP, diastolic blood pressure.
contractile function, did not show significant correlation with the Hb levels.

(2) Electrocardiographic and chest X-ray evaluations. Table III shows the results of all 143 patients who completed the study. No major differences were observed between the mean values at baseline and at week 16 in any of these parameters. There was no difference in the incidence of arrhythmias or the ST-T changes (data not shown). The ESA treatment did not affect CTR, however, the changes in the CTR correlated with either the Hb levels or changes in the Hb levels ($P < 0.001$ and $P = 0.001$, respectively, Table III, Figure 5A).

(3) Endocrinological parameters. Table III shows the BNP and hANP levels (mean ± SD) at baseline and week 16 in all 143 patients who completed the study. No significant differences were observed in any of these values. On the other hand, patients whose Hb levels at week 16 were 11.0 g/dL or higher showed higher levels of BNP (Figure 5B) and hANP (Figure 5C) compared to those whose Hb were lower than 11.0 g/dL, and significant correlations were observed between the Hb levels and the changes in either BNP or hANP levels ($P = 0.033$ and 0.011, respectively). Similarly, BNP and hANP levels significantly correlated with changes in the Hb levels ($P = 0.049$ and 0.024, respectively).

(4) Safety. Incidences of adverse events in the 171 patients who participated in the study were as follows. Adverse events were observed in 155 (90.6%) of the 171 patients. The
most common event was influenza virus infection (71 patients, 41.5%). Adverse drug reactions were reported in 64 patients (37.4%). The most common adverse event was an increase in blood pressure (20 patients, 11.7%). No mortality was observed during the study. Non-fatal serious adverse events were seen in 23 (13.5%) and deteriorating renal function was the most common (5 patients, 2.9%). Non-fatal serious adverse drug reactions not evidently related to the ESA therapy were seen in 6 subjects (3.5%), and included chronic renal failure, fever, abnormal liver function, elevated blood pressure, hyponatremia, cerebral infarction, and transient cerebral ischemic attack (1 patient each, 0.6% of total study population); fever and abnormal liver function occurred in the same patient.

**DISCUSSION**

CVD is an important risk factor for determining the prognosis of patients with CKD, and left ventricular hypertrophy (LVH) is a typical risk factor for developing CVD. Foley, et al. reported that 75% of CKD patients on dialysis demonstrate LVH, and Levin, et al. showed that the prevalence of LVH increases as creatinine clearance (Ccr) decreases: 26.7, 30.8 or 45.2% in patients with Ccr > 50, = 25-49 or < 25 mL/minute, respectively. Levin, et al. further reported that the independent risk of LVH increases 32% for each decrease of 0.5 g/dL in the Hb concentration, and found that anemia is an independent predictive factor for LVH. On the other hand, Silberberg, et al. reported that patients on dialysis with LVMI ≤ 125 g/m² had significantly higher survival rates than patients whose LVMI is higher than 125 g/m². Verdecchia, et al. also showed that, in hypertensive patients, the incidence of CVD was significantly lower in patients who showed sufficient regression of LVH. Therefore, optimal management of anemia in CKD patients would be beneficial in preventing LVH progression and consequent CVD, improving their prognosis. Several prospective comparative studies have been conducted so far in order to support these hypotheses. However, there is as yet no clear evidence on whether anemia correction in CKD patients improves their cardiac function.

Indeed, the hypothesis has been challenged by multiple studies in the past. Levin, et al. reported in a Canadian randomized trial that no suppression of LVH was observed in a 2 year monitoring of 152 pre-dialysis CKD patients treated with rHuEPO, either in a patient group whose Hb levels were corrected to 12.0 to 14.0 g/dL or in a group whose Hb levels were between 9.0 and 10.5 g/dL. Roger, et al. also found that, in 155 Stage 3-4 CKD patients, changes in the LVMI were not significantly different in either a group maintained at Hb = 12.0-13.0 g/dL or in a group maintained at Hb = 9.0-10.0 g/dL by rHuEPO regimen for 2 years. Furthermore, Ritz, et al. demonstrated in the ACORD study, in which 172 Stage 1-3 CKD patients with diabetes mellitus were randomized to a group maintained at Hb = 13.0-15.0 g/dL or those targeted at Hb = 10.5-11.5 g/dL and observed for 15 months, that no significant differences in the decrease of the LVMI were seen between the two groups. In 2006, the results of two new large-scale clinical studies were reported. In the CHOIR study, 1,432 CKD patients were randomized to a high Hb group targeted for Hb levels of 13.5 g/dL and another group targeted for Hb levels of 11.3 g/dL, and the patients were monitored for 3 years to determine the incidence of CVD. The high Hb group had a significantly higher incidence of CVD. In addition, the CREATE study with 603 Stage 4 CKD patients compared a high Hb group (maintained at Hb = 13-15 g/dL) and a low Hb group (maintained at 10.5-11.5 g/dL) and found no reduction in the risk of CVD or LVH progression even in the high Hb group. The results of the CHOIR study were further analyzed in 2008 to evaluate the correlations between the Hb levels achieved and dose of rHuEPO required. In this report, it was found that, in the high Hb-targeted group, subjects who actually achieved the target Hb levels had a lower incidence of CVD events than those whose Hb levels did not reach the targeted levels, while the risk of CVD events was significantly higher in the high-dose rHuEPO group compared to the low-dose rHuEPO group. Furthermore, a COX hazard model analysis corrected for these factors found no significant association between the randomized group and the incidence of CVD events. In 2009, the TREAT study with 4,038 predialysis CKD patients with type 2 diabetes also reported that subjects who received DPO with a target Hb level of 13.0 g/dL were at a similar risk for either mortality or CVD events compared to those who received a placebo. These reports suggest that intensive anemia correction by ESA administration might not improve cardiac function or prevent CVD events in patients with CKD.

The present study is distinct from previous studies such as CHOIR, CREATE, and TREAT in that CKD patients at higher risk for developing CVD, such as patients with a history of severe congestive cardiac failure or uncontrolled hyperten-

**Figure 5.** Relationship between the changes in values of chest X-ray measurements or biomarkers and Hb concentrations. A, cardiothoracic ratio (CTR). B, B-type natriuretic peptides (BNP), C, human atrial natriuretic peptides (hANP). The adjusted mean values were calculated using the baseline measurements as covariant. Adjusted mean ± 95% CI. P < 0.001 (A), = 0.033 (B), = 0.011 (C) by ANCOVA, n = 143.
sion, were excluded. Furthermore, the study is unique as we were able, by utilizing subjects enrolled for a DPO dose re- sponse study comparing with EPO, to investigate the effects of Hb concentrations higher than those generally recommended by various current guidelines. Treatment with DPO or EPO for 16 weeks significantly increased Hb levels, and decreased the LVMI as the left ventricular wall thickness was decreased. Significant correlation between the Hb levels at week 16 and the changes in the LVMI was observed, suggesting that achieving Hb levels higher than those conventionally recommended is beneficial for regression of LVH in the CKD patients. The BNP, hANP, and CTR values showed significant negative correlations with the Hb levels. The values of these cardiac function biomarkers did not increase in patients with Hb ≥ 11.0 g/dL, while they were elevated in those with Hb < 11.0 g/dL. BNP and hANP levels reflect left ventricular diastolic pressure. The improvement of these neurohormonal factors represents a decrease in left ventricular overload. The achievement of near-normal Hb levels helps to ameliorate the hyperdynamic cardiac state caused by anemia, as well as decrease left ventricular overload, leading to regression of LVH.

Conclusions: The findings of the present study suggest that Hb levels higher than 11.0 g/dL are desirable in predialysis CKD patients to suppress LVH and improve cardiac function. In particular, CKD patients at relatively low risk for CVD would benefit from maintaining their Hb levels higher than 11.0 g/dL to suppress the risk of CVD via the regression of LVH and reduction of myocardial stress. Further large-scale and long-term comparative studies are necessary to support these conclusions.

Acknowledgments

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Appendix

The Darbepoetin Alpha Study Advisers: Fumitake Gejyo (Niigata University), Shinichi Nishi (Kobe University Graduate School of Medicine), Masashi Suzuki (Shirakuen Hospital), Takashi Akiba (Tokyo Women’s Medical University), Yasuhiro Iino (Nippon Medical University Hospital), Akira Saito (Yokohama Dai-ichi Hospital), Yuzou Watanabe (Kasugai Municipal Hospital), Hideaki Hirakata (Fukuoka Red Cross Hospital), and the KRN321 STUDY group

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