

# Relationship Between Epoetin Alfa (EPO) Treatment and Serum Hepcidin Levels in Anemic Patients with Rheumatoid Arthritis (RA)

Moyo VM,<sup>1</sup> Kersting R,<sup>1</sup> Westerman M,<sup>2</sup> Langhoff W,<sup>1</sup> Rastogi R,<sup>1</sup> Mundle SD<sup>1</sup>

<sup>1</sup>Centocor Ortho Biotech Services, LLC, Horsham, PA; <sup>2</sup>Intrinsic LifeSciences LLC, La Jolla, CA

## BACKGROUND

- Hepcidin, secreted by the liver in response to inflammation, is a key regulatory hormone involved in gastrointestinal iron absorption, iron mobilization, and distribution<sup>1,2</sup>
- Hepcidin binds and downregulates ferroportin (the principal cellular iron exporter)<sup>3</sup>
- Consequences of ferroportin downregulation include iron sequestration in duodenal enterocytes, hepatocytes, and macrophages<sup>4</sup>
- Iron sequestration results in impaired erythropoiesis (anemia of chronic disease)
- Inflammatory states including malignancy, RA, and infections are frequently associated with anemia and elevated hepcidin levels<sup>5</sup>
- Preclinical evidence suggests that EPO downregulates hepcidin levels<sup>6</sup>
- There is a lack of clinical data available to evaluate the relationship of EPO treatment and hepcidin levels in inflammatory states

## OBJECTIVE

- To assess the impact of EPO treatment on hepcidin levels in patients with RA

## METHODS

### OVERALL STUDY

- Serum hepcidin levels and iron status were analyzed from a randomized, double-blind, placebo-controlled, investigational study of 29 patients with RA treated with either EPO or placebo for 20 weeks
- Patients with a baseline hemoglobin (Hb)  $\leq 11$  g/dL were treated with EPO starting doses of 20,000 U subcutaneously (SC) once weekly (QW) that could be titrated up to 40,000 U SC QW
- Oral iron was administered to keep the serum transferrin (sTfr) index  $< 1.5$
- Iron restriction adequate to hinder erythropoiesis was defined by a transferrin saturation of  $< 16\%$ <sup>7</sup>
- Blood samples were collected at baseline and at the end of the study
- Serum hepcidin levels were analyzed using a newly developed immunodiagnostic test (ELISA)<sup>8</sup>
- Serum hepcidin levels  $\geq 300$  ng/mL were generally considered elevated<sup>8</sup>

- Serum transferrin saturation, serum ferritin, sTfr levels, c-reactive protein (CRP), and Hb concentrations were also measured

- Hematologic response to EPO was defined as achieving a Hb of  $\geq 12$  g/dL or a  $\geq 2$  g/dL increase in Hb from baseline to the end of study

### STATISTICAL ANALYSES

- Relationships between baseline values for hepcidin, serum ferritin, and serum transferrin saturation were examined graphically and by calculating Pearson's Product Moment Correlations. The Correlations were tested for significance using a *t*-test.
- Hepcidin changes from baseline for the 2 groups (EPO and placebo) were compared using an unequal variance *t*-test.
- All statistical comparisons were conducted using SAS version 9.1

## RESULTS

Table 1. Baseline Characteristics

	EPO n=15	Placebo n=14	Total n=29
Age (years)			
Mean (SD)	71.8 (9.32)	66.4 (9.39)	69.2 (9.59)
Median	71.0	69.5	71.0
Range	53–84	44–77	44–84
Sex, n(%)			
Male	2 (13.3)	2 (14.3)	4 (13.8)
Female	13 (86.7)	12 (85.7)	25 (86.2)
Race, n(%)			
White	13 (86.7)	10 (71.4)	23 (79.3)
Black or African American	1 (6.7)	4 (28.6)	5 (17.2)
Hispanic or Latino	1 (6.7)	0	1 (3.4)
Baseline Hb values (g/dL)			
Mean (SD)	10.51 (0.818)	10.00 (0.735)	10.26 (0.807)
Range	9.0–11.3	8.7–11.1	8.7–11.3

EPO, epoetin alfa; Hb, hemoglobin; SD, standard deviation.

Table 2: Differences Between EPO and Placebo Groups

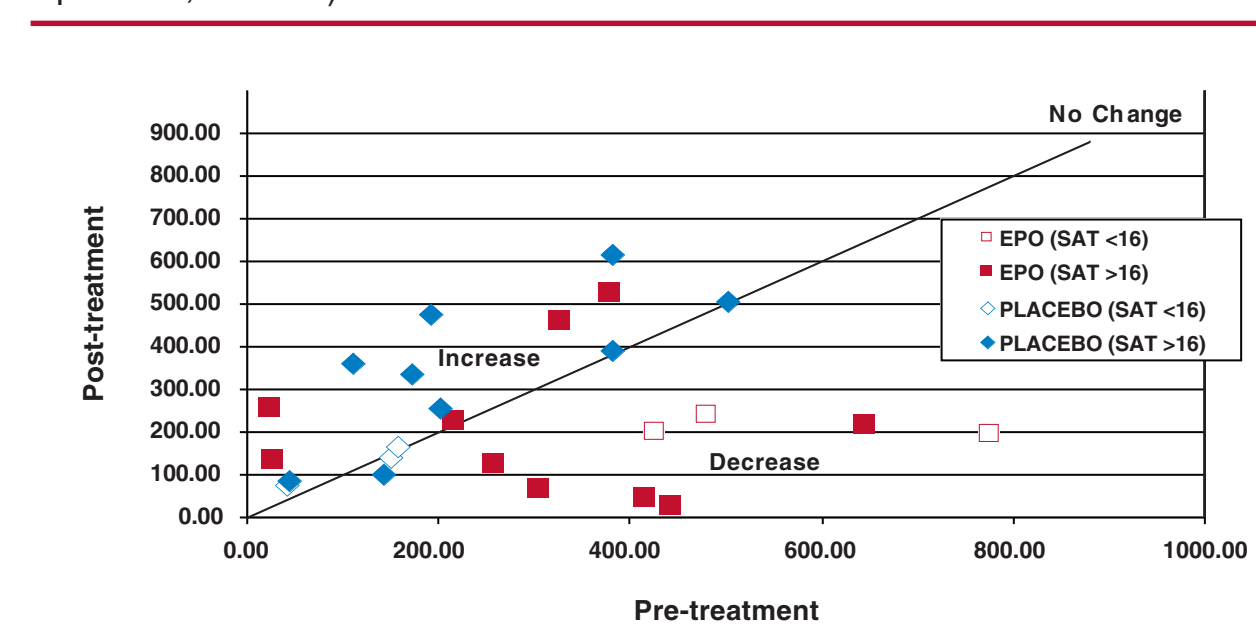
- Significant correlation between BL serum hepcidin levels and BL serum ferritin levels ( $R=0.5600$ ;  $P=0.0016$ )
- No correlation between BL hepcidin and serum transferrin saturation levels ( $R=-0.0969$ ;  $P=0.6172$ )
- No correlation between BL hepcidin and serum transferrin receptor levels ( $R=0.20527$ ;  $P=0.2854$ )

	EPO, n=15	Placebo, n=14
Baseline iron restriction, n (%)	5 (33)	2 (14)
Baseline hepcidin elevated, n (%)	10 (67)	5 (38)
Hb response, n (%)	11 (73)	1 (7)

BL, baseline; EPO, epoetin alfa; Hb, hemoglobin.

Figure 1: Serum Hepcidin Levels (ng/mL)

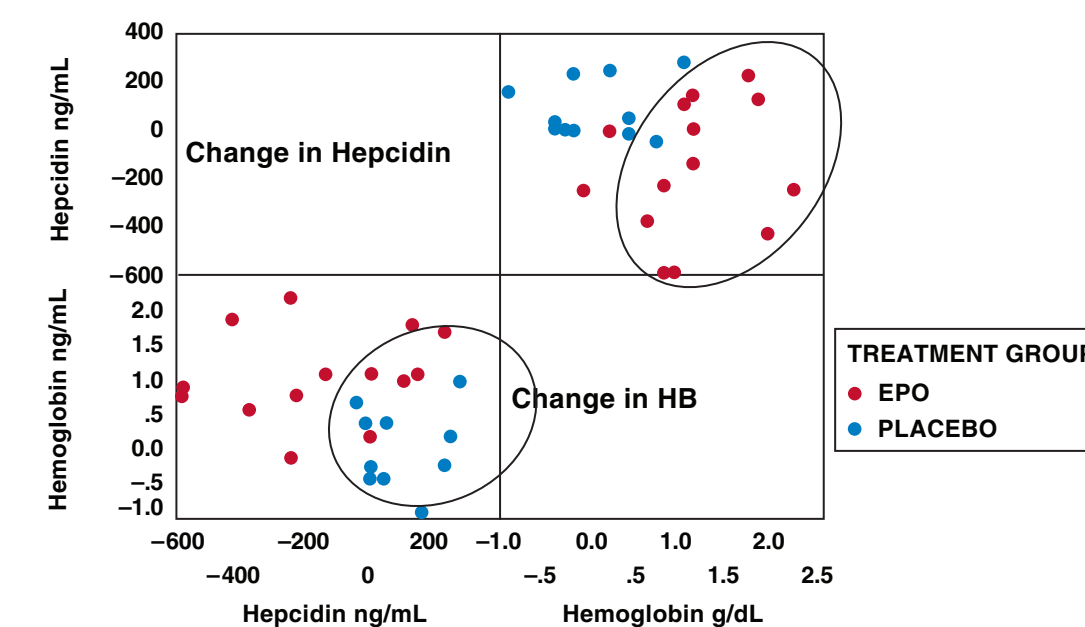
- Serum hepcidin levels appeared to decrease in EPO-treated patients, but not in those receiving placebo ( $-155.6 \pm 186.6$ , EPO;  $85.51 \pm 81.2$ , placebo;  $P<0.01$ )



EPO, epoetin alfa; Hb, hemoglobin.

Figure 2: Change From Baseline to End of Study

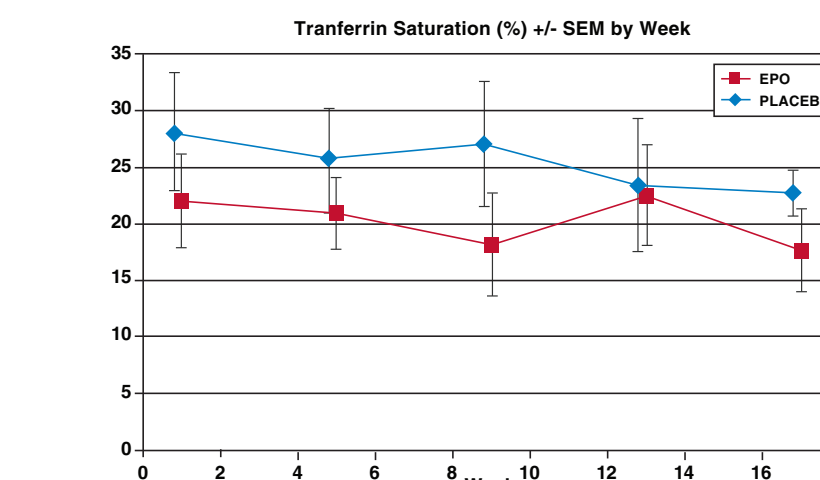
- Hb levels increased in EPO-treated patients but not in the patients receiving placebo



EPO, epoetin alfa; Hb, hemoglobin.

Figure 3: Mean Transferrin Saturation by Week of Treatment

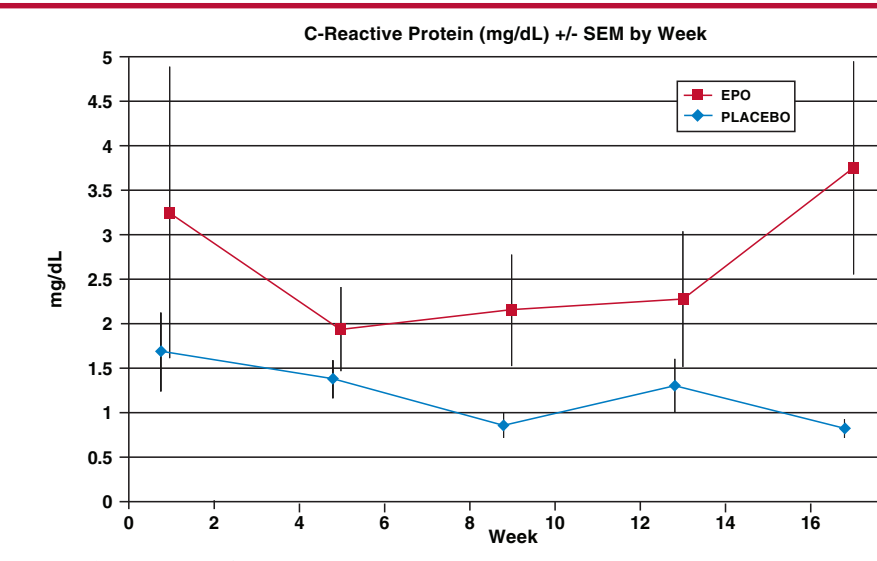
- Although the mean transferrin saturations tended to be lower in EPO treated patients they were generally maintained above the erythropoietic threshold of 16%<sup>7</sup>



EPO, epoetin alfa; SEM, standard error of the mean.

Figure 4: Mean CRP Levels by Week of Treatment

- Mean CRP levels were generally higher in EPO-treated subjects



CRP, c-reactive protein; EPO, epoetin alfa; SEM, standard error of the mean.

## LIMITATIONS

- Small data set only allows for a limited analysis
- Serum interleukin (IL)-6 levels were not measured
  - Hence EPO effect on hepcidin levels cannot be inferred to be a direct IL-6-mediated EPO effect
- Other confounders, such as RA-specific treatment and worsening inflammatory status, were not addressed by current analysis

## CONCLUSIONS

- EPO treatment may inhibit hepcidin in RA thereby preventing iron sequestration and improving erythropoiesis
- The specific EPO-hepcidin regulatory mechanisms require further investigation
  - Since CRP is a surrogate for IL-6<sup>9,10</sup> the persistence of elevated CRP levels, despite falling serum hepcidin concentrations, in EPO-treated patients suggests that EPO's effect on hepcidin may be direct and independent of IL-6<sup>6</sup>
- Further study is warranted in other inflammatory states, eg, cancer and chronic renal failure, in which EPO is used to treat anemia

### References:

- Ganz T. *Blood*. 2003;102(3):783.
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### PUBLISHED ABSTRACT

**Introduction:** Some patients treated with EPO and oral iron supplementation respond poorly to EPO, possibly due to inflammation-induced iron sequestration. In response to inflammation, hepcidin, a hormone secreted by the liver, binds to and downregulates ferroportin, the principal cellular iron exporter. In turn, ferroportin downregulation leads to sequestration of iron in absorption sites within duodenal enterocytes, hepatocytes, and in macrophages that break down senescent red blood cells and normally recycle iron back into circulation. This sequestration results in reduced amounts of plasma iron that is bio-available for erythropoiesis. Patients with RA, malignancies, or infections frequently manifest inflammatory-associated anemia and elevated hepcidin levels.

**Methods:** Serum hepcidin levels and iron status were analyzed from a randomized, double-blind, placebo-controlled, investigational study of 29 patients with RA treated with either EPO or placebo for 20 weeks. Serum hepcidin levels were measured at baseline and at the end of study. Serum transferrin saturation, serum ferritin, serum transferrin receptor (sTfr) levels and hemoglobin (Hb) concentrations were also measured. Patients with baseline Hb  $\leq 11$  g/dL were treated with EPO starting doses of 20,000 U subcutaneously (SC) once weekly (QW) that could be titrated up to 40,000 U SC QW. Oral iron was administered to keep the sTfr index  $< 1.5$ . Iron restriction adequate to hinder erythropoiesis was defined by a transferrin saturation of  $< 16\%$ . Serum hepcidin levels of  $\geq 300$  ng/mL were considered elevated. Hematologic response to EPO was defined by achieving a Hb of  $\geq 12$  g/dL or a  $\geq 2$  g/dL increase in Hb from baseline to the end of study.

**Results:** Iron restriction was noted in 5/15 (33%) EPO-treated patients and in 2/14 (14%) receiving placebo. Hepcidin levels were elevated in 10/15 (67%) EPO-treated patients and in 5/13 (38%) receiving placebo. There was a strong correlation between baseline serum hepcidin and serum ferritin levels ( $R=0.5598$ ;  $P=0.0019$ ), but not serum transferrin saturation. Eleven of 15 (73%) EPO-treated patients had a Hb response compared to 1 of 14 (7%) receiving placebo. Mean hepcidin levels decreased significantly from baseline in the EPO-treated patients ( $-155.6 \pm 186.6$ , EPO;  $90.28 \pm 83.1$ , placebo;  $P<0.01$ ). Serum hepcidin levels appeared to decrease in EPO-treated patients, but not in those receiving placebo, while Hb levels increased in EPO-treated patients but not in the patients receiving placebo.

**Conclusion:** EPO treatment may effectively elicit Hb response in patients with RA and may be associated with suppression of inflammation-induced serum hepcidin levels. Further study is warranted in conditions associated with inflammation, such as cancer and chronic renal failure, in which EPO is used to treat anemia.

Poster presented at the 50th American Society of Hematology Annual Meeting and Exposition;  
December 6-9, 2008; San Francisco, CA.

This study was supported by Centocor Ortho Biotech Services, LLC, Horsham, PA, USA.

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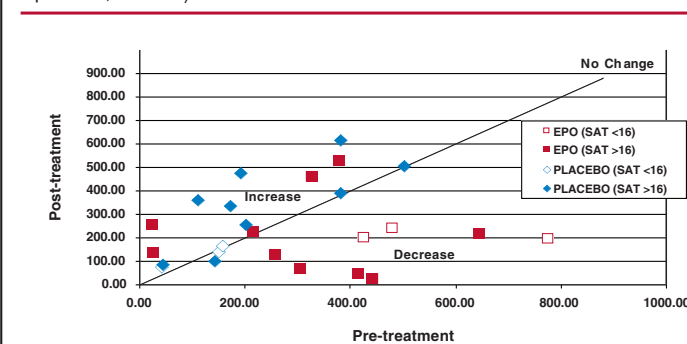
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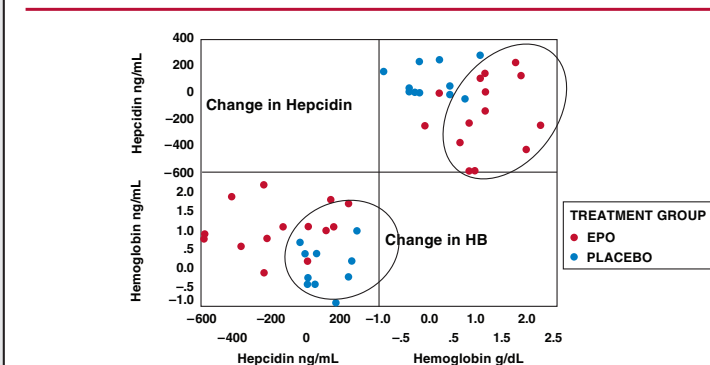
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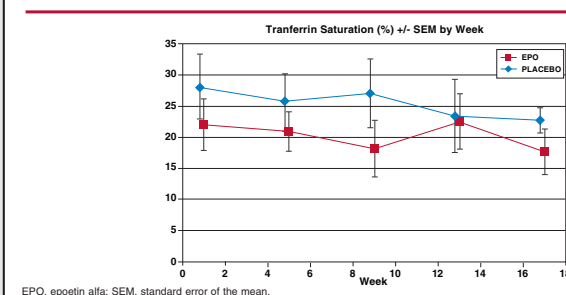
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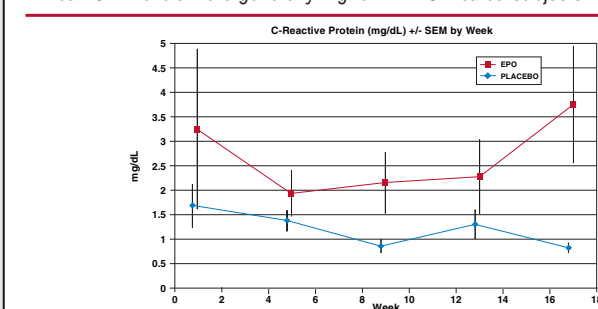
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