

Anemia correction by erythropoietin reduces BNP levels, hospitalization rate, and NYHA class in patients with cardio-renal anemia syndrome

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Abstract Little is known about the effect of anemia correction with erythropoietin (EPO) on B-type natriuretic peptide (BNP) levels, NYHA class, and hospitalization rate. The aim of the study was to investigate, in patients with cardio-renal anemia syndrome, the effects of EPO on hemochrome and renal function parameters and BNP levels. We also analyzed the effect of EPO therapy on hospitalization rate and NYHA class after 12 months in comparison with a population undergoing to standard therapy. We performed a randomized double-blind controlled study of correction of the anemia with subcutaneous α (group A $n = 13$) or β (group B $n = 14$) EPO for 12 months in addition to standard therapy with oral iron in 27 subjects. Control group ($n = 25$ patients) received only oral iron. Significant increase in hemoglobin (Hb), hematocrit (Hct), and red blood cells (RBC) were revealed in EPO groups at 12 months; Hb, group A 12.3 ± 0.6 ; group B 11.7 ± 0.8 ; control group 10.6 ± 0.5 g/dl $P < 0.0001$; Hct group A 34.2 ± 2.3 , group B 34 ± 2 , control group $32.3 \pm 1.8\%$ $P < 0.01$; RBC, group A 3.9 ± 0.2 , group B 3.8 ± 0.2 , control group 3.3 ± 0.2 , ($P < 0.0001$). Plasma BNP levels in EPO groups were significantly reduced after 12 months (group A: 335 ± 138 vs. group B: 449 ± 274 pg/ml control group 582 ± 209 pg/ml ($P < 0.01$). After 12 months of treatment, hospitalization rate and NYHA class were reduced in EPO groups with respect to control group ($P < 0.05$). Finally, an inverse correlation was observed between BNP and Hb

levels in EPO Groups ($r = -0.70$ $P < 0.001$). EPO treatment reduces BNP levels and hospitalization rate in patients with cardio-renal anemia syndrome. The correction of anemia by EPO treatment appears able to improve clinical outcome in this subset of patients with heart failure.

Keywords Anemia · Erythropoietin · Heart failure · B-type natriuretic peptide · Cardio-renal syndrome

Introduction

Anemia is a clinical manifestation commonly observed in patients with congestive heart failure (CHF) and renal disease [1–9]. Prevalence of this condition is estimated to be between 15 and 55%, depending on the clinical definition of anemia. The prevalence of anemia has been found to be greater in those with older age, more advanced New York Heart Association (NYHA) classes, diabetes, more severe systolic dysfunction, more reduced exercise tolerance, and more reduced renal function [1–9]. Anemia in patients with CHF is often associated with a more adverse outcome including increased mortality, morbidity, and hospitalization [1–9]. It is known that anemia alone, even without the presence of CHF, can increase heart rate and cardiac output, and cause increased levels of renin, angiotensin, aldosterone, antidiuretic hormone, sympathetic activity and Natriuretic Peptides, reduce renal blood flow, and glomerular filtration rate and increase plasma and interstitial volume [10]. However, it is still not certain whether the anemia in CHF is actually causing the increase in adverse effects or is merely an innocent bystander, a marker for other causes such as inflammation with increased cytokines and/or renal failure [1–10].

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Erythropoietin (EPO) is a glycoprotein growth factor produced by the kidney. In CHF, treatment with recombinant human EPO increased the hemoglobin (Hb) concentration and improved many aspects of CHF including functional status and quality of life [11–20]. However, most of interventional studies conducted in patients with CHF and anemia that showed an improvement in exercise tolerance and in functional status were uncontrolled studies [11] or controlled studies but without a placebo [12–20]. In the present study, we extend the previous studies by investigating the effects of subcutaneous (sc) EPO and oral iron versus oral iron alone on laboratory parameters, BNP levels, and on clinical outcome (hospitalization rate and NYHA classes) during one-year follow-up period.

Materials and methods

Patient population

The starting patients number consisted in 80 subjects with a history of moderate to severe CHF (NYHA Class III or IV), mild to moderate renal insufficiency with CrCl between 60 and 30 ml/min, and anemia (defined as Hb levels below 11.5 g/dl on two occasions separated by 1–2 weeks). Before the enrollment, all patients underwent optimized medical treatment for CHF, according to the last ESC guidelines [21]. The study population was homogenous for exercise tolerance, co-morbidities, laboratory investigations, and echocardiographic examination.

Exclusion criteria

Patients with isolated diastolic dysfunction, more than moderate valvular disease, recent myocardial infarction (within 12 weeks), modifiable causes of anemia, severe renal failure, and gastrointestinal bleeding were excluded. All patients undergoing fecal occult blood tests, if it was positive, were also excluded.

Study protocol: *double-blind period*

Group A, consisting of 13 patients, was treated with subcutaneous (sc) α -EPO twice weekly and daily oral iron as a ferrous gluconate 300 mg tablet.

Group B, consisting of 14 patients, was treated with subcutaneous (sc) β -EPO twice weekly and daily oral iron as a ferrous gluconate 300 mg tablet. Control group (25 patients) received the oral iron alone and sc saline injections twice weekly.

The EPO and saline injections were similar and it was impossible to know which syringes the patients received. The physicians were unaware which syringes they used.

The dose of EPO was 6,000 international units (IU). The EPO or placebo were done twice weekly for 12 months. All patients referred to our ambulatories for standard clinical checkups, as before the study, on the basis of their clinical status. If any adverse effect or treatment was manifested during follow-up, this was immediately interrupted. Both groups were re-evaluated for the study at 12 months from onset of experimental protocol.

Laboratory analysis

A complete blood count with Hb, Hct, red blood cell (RBC) count, RBC indices, serum creatinine sodium, and potassium were performed at baseline and after 12 months of treatment. Creatinine clearance was estimated from the serum creatinine values using the Cockcroft-Gault formula [22]. Plasma BNP was measured at the beginning and at the end of the study, using the quantitative immunofluorescence assay manufactured by Biosite (San Diego, CA, USA). The analytic sensitivity of the assay is <5 pg/ml and the upper normal limit is considered to be 100 pg/ml.

Primary and secondary end points

Primary end points assessed were laboratory data including Hb, hematocrit (Hct), red blood cells (RBC), and creatinine clearance; these parameters were evaluated at the start and at the end of the 12-month study period. BNP modifications were evaluated at the beginning and after 12 months. For each patient, NYHA class was obtained at baseline and at 12 months of follow-up. Secondary end points that were monitored included sudden death, hospitalizations, and myocardial infarction.

Statistical analysis

Continuous data were expressed as mean \pm SD. The data were analyzed for statistically significant differences by ANOVA/ANCOVA test for unpaired data and by linear correlation using the SPSS 17 for Windows (SPSS Inc, Chicago IL). The significant level was set at $P < 0.05$. Results were considered significant if there was, within or between group analyses, a statistical confidence level of 95%.

Results

Of the 80 consecutively recruited patients, only 56 had inclusion criteria. Of these, 2 refused to continue the study protocol and 2 needed blood transfusions to correct their anemia. Four patients died during the follow-up period (3 in control group and 1 in EPO groups). Thus, only 48

patients completed the follow-up study. During the 12 months follow-up, no adverse effects due to the treatment were reported. Thirty patients were in NYHA class III and 22 were in class IV. General and clinical characteristics of the study population are summarized in Table 1.

Significant increase in hemoglobin (Hb), hematocrit (Hct), and red blood cells (RBC) was revealed in EPO groups at 12 months (Hb, group A: 10.4 ± 0.6 g/dl baseline and 12.3 ± 0.6 g/dl 12 months, $P < 0.01$; group B: 9 ± 3.4 g/dl baseline, 11.7 ± 0.8 g/dl 12 months $P < 0.01$; control group: 10.6 ± 0.4 g/dl baseline 11.9 ± 0.8 g/dl 12 months P ns; Hct, group A: $30 \pm 1.2\%$ baseline, $34.2 \pm 2.3\%$ 12 months, $P < 0.001$; group B: $30.8 \pm 1.3\%$ baseline, $34 \pm 2\%$ 12 months, $P < 0.00001$; control group $32 \pm 1.7\%$ baseline and $34 \pm 2\%$ at 12 months, P ns; RBC, group A 3.6 ± 0.7 mil/mm³ baseline, 3.90 ± 2 mil/mm³ 12 months $P < 0.00001$; group B: 3.2 ± 0.2 mil/mm³ baseline, 3.8 ± 0.2 mil/mm³ 12 months, $P < 0.00001$; control group: 3.2 ± 0.2 mil/mm³ baseline vs. 3.8 ± 0.2 mil/mm³ at 12 months, P ns). Significant increases in CrCl were revealed in the study population neither after 12 months of treatment nor between EPO groups with respect to control group. Plasma BNP levels in EPO groups were significantly reduced after 12 months (group A: 512 ± 127 pg/ml baseline vs. 335 ± 138 pg/ml 12 months $P < 0.01$; group B: 659 ± 321 pg/ml baseline vs. 449 ± 274 pg/ml 12 months $P < 0.01$). No significant difference was found for BNP plasma levels after 12 months in control group (610 ± 231 baseline vs. 582 ± 209 pg/ml, P ns) with a significant difference between EPO groups and control groups after 12 months of treatment (405 ± 235 pg/ml vs. 582 ± 209 pg/ml $P < 0.01$). (Table 2). NYHA class was significantly reduced in EPO groups after 12 months of follow-up (group A: 3.38 ± 0.5 baseline vs. 2.7 ± 0.7 12 months, $P < 0.05$; group B: 3.5 ± 0.5 baseline vs. 2.78 ± 0.8 12 months, $P < 0.01$; control group: 3.32 ± 0.47 baseline vs. 3.2 ± 0.6 , P ns; P intergroups < 0.01) and showed a significant inverse correlation with Hb levels ($r = -0.45$; $P < 0.05$, Fig. 1). Finally, an inverse correlation was observed between BNP and Hb levels in EPO groups ($r = -0.70$, $P < 0.001$; Fig. 2a, b).

Secondary end points

Four patients died during the year study: 3 in control group (1 for sudden death, 2 for refractory CHF) and 1 in EPO groups (from sudden death). After 12 months of treatment, hospitalization rate was significantly reduced in EPO groups with respect to control group (25% vs. 56% $P < 0.05$, Fig. 3). No significant change in blood pressure, body weight, and myocardial events were seen.

Discussion

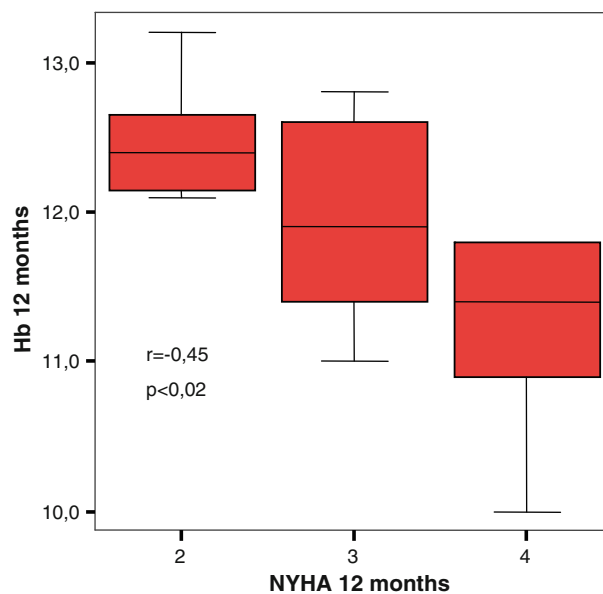
Small clinical trials have shown that the correction of anemia with erythropoietin or its derivatives with oral or intravenous iron treatment has been associated with lower hospitalization rates, better quality of life, improved exercise tolerance, and reduced neuro-hormonal activation and pro-inflammatory cytokine expression [11–19]. Despite these reports, the only large multicenter randomized placebo controlled interventional trial was not able to demonstrate significant clinical benefits with darbepoetin alfa therapy, a long-acting erythropoiesis stimulating agent (ESA) [20]. In the current study, we found the increase in Hb, RBC, and HCT values lead to NYHA class reduction and BNP level decreases. In this paper, we extend previous published data showing that EPO treatment in patients with CRS anemia leads to reverse ventricular remodeling together with systolic function improvement [18]. All these changes were supported by the fall in BNP that we found in the treated patients: since BNP is one of the most powerful markers of adverse outcomes in CHF, its plasma lowering might indirectly signal an improvement in cardiac function and outcome in patients with CRS in whom the anemia is corrected [23]. Secondary end points also demonstrated significantly reduced hospitalization without any modification in body weight, blood pressure, or other potential adverse effects. Our results appear in contrast with respect to recent clinical trials showing negative or neutral effects of EPO administration in patients with chronic kidney diseases (CKD). However, we need to keep in mind that patients enrolled in the cited studies are quite different: only 23 and 31% had a

Table 1 Clinical and laboratory characteristics in the treated group (A and B) and control group

NYHA class	Group A 13 pz 8 III/5 IV	Group B 14 pz 7 III/7 IV	Control group 25 pz 15 III/10 IV
Coronary artery disease	6	8	12
Hypertension	7	11	15
Body mass index (g/cm ²)	27.9 ± 6	28.1 ± 1	26.5 ± 3
LVEF (%)	28.3 ± 4.3	31.2 ± 8.4	30.9 ± 5.9
Hb concentration (g/dl)	10.4 ± 0.6	9 ± 3.4	9.3 ± 3.4
Creatinine clearance (ml/min)	45.8 ± 5.9	44.2 ± 6.2	44.8 ± 5.2

Table 2 NYHA class, B-type natriuretic peptide levels, and laboratory parameters during the time periods in each group

	Group A		Group B		P intra-group		Control group		P intra-group		P intergroup	
	0	12	0	12	0	12	0	12	0	12	0	12
NYHA class	3.38 ± 0.5	2.7 ± 0.7	3.5 ± 0.5	2.78 ± 0.8	0.009	0.009	3.32 ± 0.47	3.2 ± 0.6	ns	0.01		
BNP (pg/ml)	512 ± 127	335 ± 138	659 ± 321	449 ± 274	0.004	0.004	610 ± 232	582 ± 209	ns	0.007		
Hb (g/dl)	10.4 ± 0.6	12.3 ± 0.6	9 ± 3.4	11.7 ± 0.8	0.0001	0.006	9.3 ± 3.4	10.6 ± 0.5	ns	0.00001		
Red blood cell (mil/mm ³)	3.6 ± 0.7	3.9 ± 0.2	3.2 ± 0.2	3.8 ± 0.2	0.00001	0.00001	3.2 ± 0.2	3.3 ± 0.2	ns	0.00001		
Hct (%)	30.6 ± 1.2	34.2 ± 2.3	30.8 ± 1.3	34 ± 2	0.0004	0.00001	31.6 ± 1.8	32.3 ± 1.8	ns	0.002		
Creatinine (mg/dl)	2.28 ± 0.3	2 ± 0.3	2.3 ± 0.4	2.2 ± 0.4	ns	ns	2.3 ± 0.4	2.3 ± 0.4	ns	ns		
Creatinine Cl (ml/m ²)	45.8 ± 5.9	47.3 ± 5.1	44.2 ± 6.2	44.7 ± 5.3	ns	ns	44.8 ± 5.2	44.5 ± 5	ns	ns		

**Fig. 1** Correlation between Hb levels and NYHA class in the EPO groups at the end of the follow-up period

CKD history. Therefore, Hb target was higher with respect to our study [24–26]. Patients with anemic HF are often affected by primary iron deficiency, for this reasons, all the group included in our study were submitted to oral iron therapy. Recently, Anand et al. demonstrated the pivotal role of iron administration in this context [27].

The effects we saw may be related not only to the EPO-induced correction of anemia and the decrease in volume overloading but also to EPO's cytoprotective properties that lead to a significant increase in cardiac contractile reserve. Recently, an experimental study showed prevention of cardiac remodeling by low doses of EPO treatment independent of hematocrit increase [28]. This is in keeping with other animal studies demonstrating that EPO appears able to directly improve LV function even without increasing the hemoglobin [29, 30]. EPO can induce myocardial neovascularization and prevent myocardial cell apoptosis, myocardial fibrosis, and oxidative stress in animal models secondary to myocardial infarction, CHF or ischemic injury.

Study limitations

This single-center study was limited by its small sample size. The investigators were blinded to the EPO medication. Many of the parameters evaluated during follow-up that improved with treatment were objective and could not be easily biased by the investigator. It is likely that the baseline weight of some subjects was above their ideal weight due to fluid retention upon hospital admission. We did not measure all iron parameters and particularly

Fig. 2 Inverse correlation in controls and in treated patients between hemoglobin and B-type natriuretic peptide (BNP)

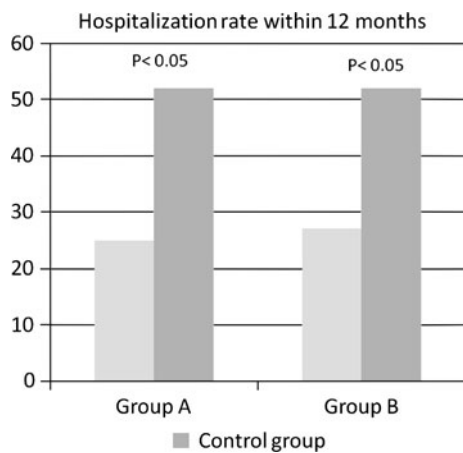
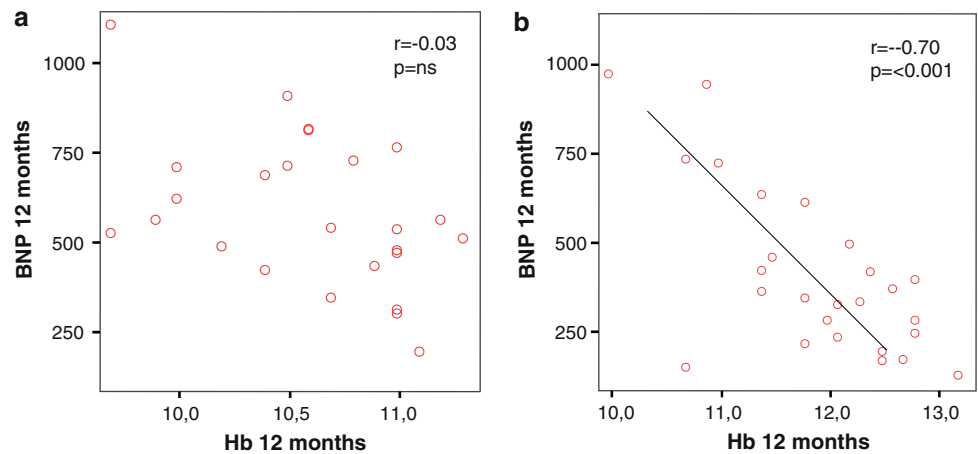


Fig. 3 Hospitalization rate in group A and B with respect to controls during one-year follow-up period

transferrin saturation, even if all patients took oral ferrous gluconate.

Conclusions

Correction of anemia in patients with CRS leads to an improvement in NYHA class with hospitalization rate reduction. All these positive changes occur together with BNP decrease and appear related to anemic status correction.

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Conflict of interest None.

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