Erythropoietin therapy improves endothelial function in patients with non-dialysis chronic kidney disease and anemia (EARNEST-CKD)

A clinical study

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Abstract

Background: This study investigated whether administering erythropoiesis-stimulating agents (ESAs) improves endothelial function in patients with non-dialysis chronic kidney disease (CKD) and anemia.

Methods: This single-center, prospective, single-arm comparison study enrolled patients with non-dialysis CKD (stages 4-5) and hemoglobin levels $<10g/dL$. ESA administration followed the Kidney Disease: Improving Global Outcomes guideline. The primary endpoint was the change in flow-mediated dilatation after ESA administration in individual patients. The secondary endpoints were changes in 6-minute walk test results, blood pressure, New York Heart Association class, and echocardiographic parameters. The echocardiographic parameters examined included chamber quantification, Doppler parameters, and systolic and diastolic function parameters.

Results: Initially, 13 patients were screened, but 2 discontinued due to either heart failure or voluntary withdrawal. The mean flow-mediated dilatation values significantly increased by 10.59\% (from 1.36\% ± 1.91\% to 11.95\% ± 8.11\%, $P = .001$). Echocardiographic findings showed that the left ventricular mass index decreased by 11.9 g/m\textsuperscript{2} (from 105.8 ± 16.3 to 93.9 ± 19.5 g/m\textsuperscript{2}, $P = .006$), and the left atrial volume index decreased by 10.8 mL/m\textsuperscript{2} (from 50.1 ± 11.3 to 39.3 ± 11.3 mL/m\textsuperscript{2}, $P = .004$) after 12 weeks of ESA administration. There were no significant differences between pre- and post-ESA treatment 6-minute walk test results. No significant side effects were observed during the study period.

Conclusions: This is the first clinical study to demonstrate that an ESA improves endothelial dysfunction, left ventricular hypertrophy, and left atrial volume in patients with non-dialysis CKD. Thus, ESAs may be considered as adjunctive therapy for reducing cardiovascular risk in these patients.

Abbreviations: ASE = American Society of Echocardiography, CKD = chronic kidney disease, DTI = Doppler tissue imaging, eGFR = estimated glomerular filtration rate, ESA = erythropoiesis-stimulating agents, FMD = flow-mediated dilation, Hb = hemoglobin, LA = left atrium, LAVI = left atrial volume index, LV = left ventricle, LVEDD = left ventricular end diastolic dimension, LVEF = left ventricular ejection fraction, LVESD = left ventricular end systolic dimension, LVH = left ventricular hypertrophy, LVMi = left ventricular mass index, NYHA = New York Heart Association, RVSP = right ventricular systolic pressure.

Keywords: anemia, chronic kidney disease, endothelial dysfunction, erythropoiesis-stimulating agents

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This study protocol was approved by the Gangneung Asan Hospital ethical committee of Ulsan University (GNAH IRB number 2017-05-002-007). Informed consent was obtained from all participating patients.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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1. Introduction

Cardiovascular disease is a common complication and the most common cause of death in patients with chronic kidney disease (CKD), especially those undergoing dialysis.\[^{[11]}\] After stratification by age, race, sex, and presence of diabetes, mortality due to cardiovascular disease is 10 to 20 times higher in patients undergoing dialysis than in the general population. This is difficult to explain using only cardiovascular risk factors.\[^{[2,3]}\] Therefore, a number of studies have examined the relationship between dialysis and cardiovascular disease, showing that hemodialysis impairs endothelial function.\[^{[4–6]}\] Recently, several studies have demonstrated that even patients with non-dialysis CKD show endothelial dysfunction.\[^{[5,7]}\] which may worsen arterial vessel elasticity and cause left ventricular hypertrophy (LVH).\[^{[8]}\]

Erythropoiesis-stimulating agents (ESAs) are primarily used as erythropoietic growth factors in patients with CKD and anemia. Some studies have shown that ESAs reduce plasma-oxidative stress in dialysis patients.\[^{[9–12]}\] This evidence suggests that ESAs play an important role in protecting cells by reducing oxidative stress. Other studies have demonstrated that erythropoietin directly induces heme oxygenase-1 expression, indirectly depletes iron, increases the number of circulating young red blood cells, and shows an antioxidant effect.\[^{[11,13–16]}\] In a clinical setting, ESAs have been shown to improve endothelial dysfunction in patients with coronary artery disease.\[^{[17]}\] Furthermore, many clinical trials have demonstrated the beneficial effects of ESAs, including decreasing LVH, reducing cardiac remodeling, and improving functional capacity.\[^{[18–22]}\]

Several studies investigated improving endothelial dysfunction in patients with CKD, and some have reported success when patients receive vitamin D and omega-3 polyunsaturated fatty acids.\[^{[23–25]}\] Briet et al.\[^{[26]}\] suggested that ESA administration induces impaired endothelial function as a result of endothelin-1 secretion and oxidative stress. However, a study published in 2015 showed that endothelial dysfunction was improved when ESAs were administered to rats with CKD.\[^{[27]}\] Except for this study in rats, the beneficial effects of ESA administration to patients with CKD have not been definitively proved and it could be very controversial.

In the present study, we hypothesized that ESA administration might prevent endothelial dysfunction in patients with non-dialysis CKD and anemia. Therefore, this study evaluated the effects of ESA administrations on endothelial dysfunction and clinical outcomes in this patient population.

2. Methods

2.1. Study design and participants

This study was a single-center, prospective study involving patients attending the Gangneung Asan Hospital (South Korea). Patients with non-dialysis CKD, stages 4 to 5, having estimated glomerular filtration rate (eGFR) of <30 mL/min/1.73 m\(^2\) and with hemoglobin (Hb) levels of <10 g/dL were included; eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.

Patients were excluded from the study if they had symptoms and signs of heart failure (dyspnea more than New York Heart Association (NYHA) grade II, orthopnea, paroxysmal nocturnal dyspnea, pitting edema, pulmonary edema or pulmonary effusion), brain natriuretic peptide levels of >35 pg/mL, significant valvular heart disease (e.g., more than moderate grade severity), history of coronary artery syndrome within 60 days, significant arrhythmia (ventricular fibrillation, ventricular tachycardia, or atrioventricular block), uncontrolled hypertension, history of taking ESAs within 12 weeks before recruitment, abnormal liver function (total bilirubin >3 mg/dL or albumin <2.8 mg/dL), active bleeding requiring transfusion, pregnancy, or other causes of dyspnea.

Participating patients were scheduled to visit the hospital monthly for 12 weeks. Blood pressure measurements and laboratory tests, including Hb and creatinine levels and eGFR, were performed during each visit. Echocardiography, flow-mediated dilatation (FMD), and 6-minute walk tests were performed at the start of the study and after 12 weeks of treatment. The NYHA class, determined before and after ESA administration, was also recorded.

This study protocol was approved by the Gangneung Asan Hospital ethical committee of Ulsan University (GNAH IRB number 2017-05-002-007). The study protocol was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participating patients.

2.2. Drug administration and dosing

The ESA used in this study was methoxy polyethylene glycol-epoetin beta (Mircera, F. Hoffmann-La Roche, Basel, Switzerland). The ESA was administered by subcutaneous injection according to the following protocol. ESA administration was initiated when the patient’s Hb was ≤10 g/dL, with a starting dose of 0.6 μg/kg every 4 weeks. If a patient’s Hb level exceeded 11 g/dL, that week’s treatment was skipped; if the Hb level did not increase by >1 g/dL after 4 weeks of therapy, the dose was increased by 25%.

2.3. Study endpoints

The primary endpoint of this study was the FMD change, in each patient, following ESA administration. The secondary endpoints were the changes in each patient’s echocardiographic parameters (left ventricular mass index [LVMI], left atrial [LA] diameter, LA volume index [LAVI], right ventricular systolic pressure, E wave to e’ [E/e’] ratio, left ventricular end systolic dimension [LVESD], left ventricular end diastolic dimension [LVEDD] and left ventricular ejection fraction [LVEF]), 6-minute walk test result, systolic blood pressure, diastolic blood pressure, and NYHA class following ESA administration.

2.4. Echocardiographic and Doppler measurements

Each patient was assessed using 2-dimensional transthoracic echocardiography before and after ESA administration. Standard 2-dimensional pulsed-wave Doppler and pulsed-wave Doppler tissue imaging (DTI) echocardiographic parameters were collected from parasternal and apical acoustic windows based on American Society of Echocardiography (ASE) guidelines, using an IE33 (Phillips, Andover, MA) instrument.\[^{[28]}\] During the assessment, each patient was positioned in the left lateral supine position under electrocardiographic monitoring. M-mode tracings obtained just below the mitral valve leaflets were acquired using the parasternal short-axis view. We measured LVEDD, LVEDD, interventricular septal wall thickness, posterior wall thickness, and LA diameter. LVE diastolic and LVE systolic
volumes were acquired from apical 2- and 4-chamber views, using the biplane modified Simpson rule; the LVEF was calculated according to ASE recommendations.[30] The LAVI was also measured using the biplane Simpson method. Left ventricle (LV) mass was calculated using the Devereux formula and indexed to body surface area calculated using the Mosteller formula. LV filling variables were determined from pulsed-wave Doppler recordings of transmitral flow velocity. The sample volume was allocated at the tips of the mitral leaflets and the Doppler velocity recordings of 3 cardiac cycles, at a paper speed of 100 mm/s, were digitized and the variables averaged. LV diastolic function was evaluated using pulsed-wave Doppler and pulsed-wave DTI recordings, based on ASE/European Association of Cardiovascular Imaging recommendations.[30] Transmitral flow was used to gather peak early (E) and atrial (A) flow velocities. We used the mean peak early diastolic (E') velocity obtained from the septal side of the mitral annulus in the 4-chamber view with appropriate DTI settings. Systolic and late diastolic velocity and isovolumic relaxation time were computed utilizing pulsed-wave DTI at the septal insertion sites of the mitral leaflets in the apical 4-chamber view. The E/e' ratio was calculated to determine LV filling pressures. Finally, the tricuspid regurgitation pressure gradient was measured using color-flow Doppler imaging and the parasternal right ventricle inflow view.

2.5. Measurement of brachial artery diameters and flow mediated dilation

Patients were asked to rest for 10 minute in the supine position before each exam. Brachial artery images were acquired using a commercially available system (Vivid 7, GE Vingmed, Horten, Norway) equipped with a 14-MHz linear array transducer. For FMD measurements, the baseline brachial artery diameter was averaged from 6 separate images at 5-second intervals. Subsequently, a pneumatic cuff, placed on the forearm, was inflated to 230 mm Hg for 5 minute. Following cuff deflation, the brachial artery diameter was reexamined and averaged from 6 separate images at 5-second intervals. The FMD was calculated as a percentage of the maximum increase in arterial diameter. The brachial artery diameter was semiautomatically calculated from the trailing edge of the intima-blood interface to the leading edge, using a modified version of ImageJ software (National Institutes of Health, Bethesda, MD) and custom-designed software. The cutoff value for normal endothelial function assessed by FMD of the brachial artery is 7.1%.[31]

2.6. Statistical analysis

Data were stored and analyzed using SPSS version 19.0 software (IBM, Armonk, NY). Continuous variables were expressed as mean±standard deviation, and categorical variables were expressed as frequency and percentage. Continuous variables following a normal distribution were analyzed using Student t test, and non-normal data were analyzed using a Mann–Whitney U test. In all cases, P < .05 was considered statistically significant.

3. Results

3.1. Baseline characteristics

Initially, 13 patients were screened, but 2 patients were excluded from the analyses. One was excluded due to a diagnosis of heart failure, based on clinical signs, biomarker level (brain natriuretic peptide = 959.9 pg/mL), and an echocardiographic exam (LVEF = 41%); the other voluntarily withdrew from the study. Of the remaining 11 patients, the average age was 62±14 years. Six males and five females participated. Five patients had an eGFR ranging from 15 to 30 mL/min/1.73 m² and 6 had an eGFR <15 mL/min/1.73 m². The average Hb levels were 8.7 g/dL, respectively (Table 1); Hb levels were below 12 g/dL upon completion of the 12 weeks of treatment.

3.2. Effects of ESAs on endothelial function and heart functions

The mean FMD showed a significant increase of 10.59% (from 1.36%±1.91% to 11.95%±8.11%, P = .001) after 12 weeks of ESA administration as shown in Figure 1 (Table 2). Additionally, the mean LVM1 was reduced by 11.9 g/m² (from 105.8±16.3 g/m² to 93.9±19.5 g/m², P = .006), and the mean LAVI was reduced by 10.8 mL/m² (from 50.1±11.3 mL/m² to 39.3±11.3 mL/m², P = .004) after 12 weeks of ESA injection (Fig. 2, Table 3). However, the right ventricular systolic pressure, E/e', LVEDV, LVEDD, and LVEF did not show significant changes following the intervention (Table 3).

Table 1

| Baseline characteristics of study population. | Total patients n = 11 |
|-----------------------------------------------|-----------------------|
| Age (yrs), mean (SD)                          | 62±14                 |
| Male                                          | 6 (55)                |
| Diabetes mellitus                            | 6 (55)                |
| Hypertension                                 | 5 (45)                |
| BMI (kg/m²)                                  | 23.4±3.5              |
| Hb (g/dL)                                    | 8.7±0.9               |
| Cr (mg/dL)                                   | 4.16±1.27             |
| eGFR (mL/min/1.73 m²)                        | 14.45±5.71            |
| SBP (mm Hg)                                  | 141.5±19.4            |
| DBP (mm Hg), mean (SD)                       | 71.6±13.9             |
| LVEF (%), mean (SD)                          | 66.5±5.1              |

Data are presented as means±SD or n (%).

BMI = body mass index, Cr = creatinine, DBP = diastolic blood pressure, eGFR = estimated glomerular filtration rate, Hb = hemoglobin, LVEF = left ventricular ejection fraction, SBP = systolic blood pressure, SD = standard deviation.

Figure 1. FMD before and after ESA administration. (n = 11). Data are reported as means±SD or n (%). ESA = erythropoiesis-stimulating agents, FMD = flow-mediated dilation, SD = standard deviation.
Laboratory and clinical outcomes.

|                         | Baseline (n = 11) | After 12 wk (n = 11) | P value |
|-------------------------|-------------------|----------------------|---------|
| Hb (g/dL)               | 8.7 ± 0.9         | 10.7 ± 0.8           | <.001   |
| Cr (mg/dL)              | 4.16 ± 1.27       | 4.83 ± 1.71          | .003    |
| eGFR (mL/min/1.73 m²)   | 14.45 ± 5.71      | 12.27 ± 5.72         | .005    |
| SBP (mm Hg)             | 141.5 ± 19.4      | 145.7 ± 19.7         | .79     |
| DBP (mm Hg)             | 71.4 ± 13.9       | 71.1 ± 15.7          | .89     |
| FMD (%)                 | 1.36 ± 1.91       | 11.95 ± 8.11         | .001    |
| 6MWT (m)                | 378.0 ± 117       | 395.8 ± 133.4        | .437    |
| NYHA (class)            | 1.00 (1-1)        | 1.00 (1-1)           | .317    |

Data are reported as means ± SD except NYHA class data which are reported as median (IQR).

The 6-minute walk test was not performed in some participants for a variety of reasons, including arthralgia, difficulty walking, and others. However, 6 (54.5%) patients underwent the test prior to and after the study. The average results were 378 m before ESA administration, and 395 m after the intervention. Although the 6-minute walk test difference showed a tendency to increase following ESA administration, the change was not significant.

Changes in echocardiographic characteristics between baseline and 12 wk after ESA administration.

|                         | Baseline (n = 11) | After 12 wk (n = 11) | P value |
|-------------------------|-------------------|----------------------|---------|
| LVMI (g/m²)             | 105.8 ± 16.3      | 93.9 ± 19.5          | .006    |
| LAVI (mL/m²)            | 50.1 ± 11.3       | 39.3 ± 11.3          | .004    |
| RVSP (mm Hg)            | 28.8 ± 5.1        | 28.5 ± 5.2           | .983    |
| E/e’ ratio              | 13 ± 2.7          | 12 ± 3.0             | .135    |
| LVESD (mm)              | 33 ± 2.6          | 33 ± 1.3             | .999    |
| LVEDD (mm)              | 52.6 ± 1.9        | 51.7 ± 1.9           | .067    |
| LVEF (%)                | 66.5 ± 5.1        | 64.5 ± 3.9           | .295    |

Data are reported as means ± SD.

3.3. Safety profiles of ESAs

There were no significant changes in systolic or diastolic blood pressures observed during the study period. The average systolic blood pressure before ESA administration was 141.5 ± 19.4 mm Hg, compared with 145.7 ± 19.8 mm Hg after ESA administration. The average diastolic blood pressure before ESA administration was 71.4 ± 13.9 mm Hg and 71.1 ± 15.7 mm Hg after ESA administration. When reviewing the blood pressure medications administered to patients before and after the study, 2 participants added 1 blood pressure medication and the others did not change their medications (Table 2). There were no side effects associated with ESA administration, such as uncontrolled blood pressure, stroke, seizure, or injection site infection.

4. Discussion

This study is the first to suggest that ESA administration improves endothelial dysfunction, LVMI and LAVI in patients with non-dialysis CKD. The FMD was significantly increased after 12 weeks of ESA administration, suggesting that ESAs can be considered as adjunctive therapies for improving cardiovascular risk without introducing serious side effects.

Since endothelial dysfunction is known to be a primary mechanism of cardiovascular outcome deterioration, a major problem in patients with CKD, numerous trials involving vitamin C, growth hormone, and L-arginine have been conducted to improve endothelial dysfunction in these patients. Some studies have suggested that oxidative stress markers are consistent with endothelial dysfunction in patients with CKD; as reactive oxygen species production increases, nitric oxide bioavailability decreases and vasodilation becomes ineffective. Several studies have explored the effects of ESAs on endothelial function, with inconclusive results. One study demonstrated that ESAs aggravate endothelial function through endothelin-1 secretion and oxidative stress. That study had the advantage of directly measuring endothelium-dependent relaxation using acetylcholine, but was limited by being conducted ex vivo, without measuring clinical parameters. Additionally, a significant number of patients included in the study had histories
of cardiovascular disease and severe arterial stiffness, which likely impacted some study parameters. Conversely, Bartnicki et al.\textsuperscript{[37]} showed that ESA administration may decrease oxidative stress in patients with non-dialysis CKD by improving the antioxidant defense system and reducing ROS production. In a 2015 study on rats, ESA administration corrected anemia and prevented endothelial dysfunction in a rat model of CKD; the results were explained by reduced local oxidative stress and enhanced endothelial nitric oxide synthase phosphorylation.\textsuperscript{[27]} Our present study showed that ESA administration resulted in clinically improved endothelial dysfunction in patients with CKD, correlating with the previous animal study results. As previously mentioned, endothelial dysfunction has been considered to be a prognostic indicator of cardiovascular and metabolic diseases, such as CKD. Currently, there are no perfect therapeutics to prevent endothelial dysfunction. However, according to our data, ESA therapy might be a promising therapeutic option for CKD with endothelial dysfunction.

Since Furchgott and Zawadzki, among others, discovered endothelium dependent relaxing factors, rapid progress has been made in evaluating endothelial function.\textsuperscript{[38]} Presently, numerous methods exist to measure endothelial dysfunction, including intravascular coronary FMD, ultrasound brachial artery FMD, flow-mediated magnetic resonance imaging, and pulsed-wave analysis. Among those methods, brachial artery FMD is widely used because it provides fast, inexpensive, and noninvasive measurements that have proven reproducible and useful in many clinical studies.\textsuperscript{[39–41]} Therefore, to evaluate endothelial dysfunction in this study, we used brachial FMD to measure endothelium-dependent vasodilation.

The Kidney Disease: Improving Global Outcomes (KDIGO) guideline recommends initiating ESA therapy in consideration of prior responses to iron therapy, rate of Hb concentration decline, transfusion risk, and symptoms attributable to anemia when patient Hb concentrations are $<$10 g/dL. Several studies have reported benefits associated with ESA administration, such as reduced mortality, reduced hospitalization risk, shortened hospitalization, fewer transfusion-related complications, and improved quality of life.\textsuperscript{[42]} However, concerns exist about the safety and long-term effects of ESA administration, including increased hypertension, seizures, and stroke risk, in patients with CKD.\textsuperscript{[43,44]} Thus, KDIGO and the US Food and Drug Administration recommend that Hb levels should not exceed 11.5 g/dL or 11 g/dL, respectively.

In previous studies, ESA therapy reduced the LVMI in patients with non-dialysis CKD.\textsuperscript{[45,46]} Specifically, several studies have suggested that the effect of ESA administration on LVH is only evident in patients with low Hb levels ($<$10 g/dL) and underlying LVH.\textsuperscript{[47,48]} Our findings showed that the LVMI was significantly reduced after ESA administration, consistent with previous studies, although only 36% of the patients in our study population demonstrated LVH. This might be explained by an increase in Hb leading to a decreased LV load and reduced LVH. Additionally, ESA therapy had an impact on the decrease in LA volume. This suggested that ESA administration had a positive effect on atrial remodeling. LA structural remodeling is a complicated phenotypic expression following changes in LA size,\textsuperscript{[49]} shape, and architecture and alterations in the cardiomyocyte, fibroblast, and non-collagenous infiltrative compartments of the atrium.\textsuperscript{[50]} LA enlargement, which is simple to measure, is the default clinical indication of structural remodeling that develops most frequently in response to LA pressure and volume overload. In the absence of mitral valvular disease, atrial fibrillation, and high cardiac output states such as thyrotoxicosis, it is an exemplary biomarker for the presence and severity of LV diastolic dysfunction.\textsuperscript{[51]} Further, there were no significant changes in the functional capacities of the patients who underwent the 6-minute walk test.

Raising Hb levels through ESA administration has been performed cautiously because of the potential side effects, including exacerbation of hypertension and an increased risk of seizures.\textsuperscript{[52]} Thus, we also examined the effect of ESA administration on blood pressure, as blood pressure rises can be managed if the Hb correction target is not excessively high. In the present study, significant changes in systolic and diastolic blood pressures were not observed during the study period; only 2 participants added additional blood pressure medications (1 each) during the study. Although this was a short-term study, uncontrolled hypertension, injection site infections, strokes, and seizures were not observed.

Our study has some limitations. First, this was a single-center, prospective, single-arm comparison study without a parallel control group involving a small number of patients over a midterm study period. Due to the narrow and strict eligibility criteria that allowed for only patients with non-dialysis CKD (stages 4 to 5) and anemia to be enrolled and various tests to be performed, registering a larger cohort of patients from a single institution was difficult. Second, we could not analyze the effect of medication, sex, or lifestyle patterns due to the lack of a parallel control group. Although this study did not demonstrate correction of the LVH effect caused by anemia, a multi-center study would be expected to better evaluate whether the Hb level is a confounding factor of the LVMI. Third, although the mechanism of action of all ESAs is the same, the half-life, receptor binding affinity, and in vivo bioactivity of each ESA is different. Each ESA may show variable clinical effects due to these differences in pharmacokinetics and pharmacodynamics. In our study, only continuous erythropoietin receptor activator (Mircera) was used as an ESA, and it is necessary to proceed with research using other ESAs. Despite these limitations, this study showed that patients with non-dialysis CKD and anemia showed improved endothelial dysfunction, LVH and LA volume following ESA administration. As the number of these patients is gradually increasing and the potential impact of ESA administration was taken into consideration, we believe that large-scale, multi-center, prospective studies are needed to further investigate the effects of ESA administrations on endothelial dysfunction in patients with CKD. Finally, although it is not a long duration, our study showed the safety and efficacy of ESA for 12 weeks. We believe that it helps to demonstrate the feasibility of a large-scale long-term study in the future.

5. Conclusion

This is the first clinical study to demonstrate that an ESA can improve endothelial dysfunction, left ventricular hypertrophy and left atrial volume in patients with non-dialysis CKD and anemia. The present results suggest that ESA administration may be considered as adjunctive therapy for reducing cardiovascular risk in these patients.

Author contributions

LJA collected, analyzed, and interpreted the data and drafted the manuscript. YCJ collected, analyzed, and interpreted the data.
YH and HSJ conceptualized and conceived the study design; collected, analyzed, and interpreted the data; and edited and revised the manuscript. All authors read and approved the final manuscript.

Data curation: Jina Lim, Chung Jo Yu, Sang Jin Ha, Hoon Yu. Supervision: Sang Jin Ha, Hoon Yu.

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Writing – review & editing: Sang Jin Ha, Hoon Yu.

References
[1] Foley RN, Parfrey PS, Harnett JD, et al. Clinical and echocardiographic disease in patients starting end-stage renal disease therapy. Kidney Int 1995; 47:186–92.

[2] Foley RN, Parfrey PS, Sarnak MJ. Epidemiology of cardiovascular disease in chronic renal disease. J Am Soc Nephrol 1998; 9:12 Suppl: S16–23.

[3] Cases A, Vera M, López Gómez JM. Cardiovascular risk in patients with chronic renal failure. Patients in renal replacement therapy. Nefrologia 2002; 22(Suppl 1):68–74.

[4] van Guldener C, Lambert J, Janssen MJ, Donker AJ, Stehouwer CD. Endothelium-dependent vasodilatation and distensibility of large arteries in chronic haemodialysis patients. Nephrol Dial Transplant 1997; 12 Suppl 2):14–8.

[5] Rescio-Mayoral A, Banerjee D, Streather C, Kaski JC. Endothelial dysfunction, inflammation and atherosclerosis in chronic kidney disease—a cross-sectional study of predialysis, dialysis and kidney-transplantation patients. Atherosclerosis 2011; 216:464–51.

[6] Miyazaki H, Matsuoka H, Itabe H, et al. Hemiendothelium impairs endothelial function via oxidative stress: effects of vitamin E-coated dialyzer. Circulation 2000; 101:1002–6.

[7] Thambirajah A, Landray MJ, McGlenn FJ, Jones HJ, Wheeler DC, Townend JN. Abnormalities of endothelial function in patients with predialysis renal failure. Heart 2000; 83:205–9.

[8] Luksha N, Luksha L, Carrero JJ, Hammarsqvist F, Stenwinkel P, Kublikiene K. Impaired resistance artery function in patients with end-stage renal disease. Clin Sci (Lond) 2011; 120:525–36.

[9] Inal M, Kanhak G, Sen S, Akyuz F, Sunal E. Antioxidant status and lipid peroxidation in hemodialysis patients undergoing erythropoietin and erythropoietin-vitamin E combined therapy. Free Rad Res 1999; 31: 211–6.

[10] Uberti M, Gerardi G, Bufano G, et al. Effects of erythropoietin and vitamin E-modified membrane on plasma oxidative stress markers and the number of haemolysed patients. Am J Kidney Dis 2002; 40:590–9.

[11] Mimic-Oka J, Smic M, Djukanovic L. Epoetin treatment improves red blood cell and plasma antioxidant capacity in hemodialysis patients. Ren Fail 2002; 24:77–87.

[12] Calò LA, Stanic L, Davis PA, et al. Effect of epoetin on HO-1 mRNA level in chronic kidney disease patients treated with cyclosporin A and azathioprine. J Am Soc Nephrol 2002; 13:694–701.

[13] Abraham NG, Nelson JC, Ahmed T, Konwalinka G, Spaak J. Treating endothelial dysfunction with vitamin D in chronic kidney disease: a meta-analysis. BMC Nephrology 2018; 19:247.

[14] Zanetti M, Gorton Cappelli G, Barbetta D, Semolic A, Barazzoni R. Omega 3 polyunsaturated fatty acids improve endothelial dysfunction in chronic renal failure: role of eNOS activation and of oxidative stress. Nutrients 2017; 9:895.

[15] Jalal DI, Decker E, Perrenoud L, et al. Vascular function and uric acid lowering in stage 3 CKD. J Am Soc Nephrol 2017; 28:943–52.

[16] Briend M, Barhoumi T, Miao MO, et al. Effects of recombinant human erythropoietin on resistance artery endothelial function in stage 4 chronic kidney disease. J Am Heart Assoc 2013; 2:e000128.

[17] Serizawa K, Yogo K, Tashiro Y, et al. Epoetin beta pegol prevents endothelial dysfunction as evaluated by flow-mediated dilation in chronic kidney disease rats. Eur J Pharmacol 2015; 767:10–6.

[18] Zogbi WH, Chambers JB, Dumesnil JG, et al. Recommendations for evaluation of prosthesis with echocardiography and doppler ultrasound: a report From the American Society of Echocardiography’s Guidelines and Standards Committee and the Task Force on Prosthetic Valves, developed in conjunction with the American College of Cardiology Cardiovascular Imaging Committee, Cardiac Imaging Committee of the American Heart Association, the European Association of Echocardiography, a registered branch of the European Society of Cardiology, the Japanese Society of Echocardiography and the Canadian Society of Echocardiography, endorsed by the American College of Cardiology Foundation, American Heart Association, European Association of Echocardiography, a registered branch of the European Society of Cardiology, the Japanese Society of Echocardiography, and Canadian Society of Echocardiography. J Am Soc Echocardiogr 2009; 22:975–1014. quiz 1082–1014.

[19] Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging, endorsed by the American College of Cardiology Foundation, American Heart Association, European Association of Echocardiography, a registered branch of the European Society of Cardiology, the Japanese Society of Echocardiography, and Canadian Society of Echocardiography. J Am Soc Echocardiogr 2016; 29:377–416.

[20] Li AM, Celermajer DS, Chan MH, Sung RY, Woo KS. Reference range for brachial artery flow-mediated dilation in healthy Chinese children and adolescents. Hong Kong Med J 2018; 24(Suppl 3):36–8.

[21] Nagweh SF, Sneatha OA, Appleton CP, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2016; 29:377–314.

[22] Ruiz J, Gómez-Cuesta S, Ponce D, et al. Oxidative stress and cardiac function in rats with chronic kidney disease. J Am Soc Nephrol 2014; 25:2135–43.

[23] Li AM, Celemajer DS, Chan MH, Sung RY, Woo KS. Reference range for brachial artery flow-mediated dilation in healthy Chinese children and adolescents. Hong Kong Med J 2018; 24(Suppl 3):36–8.

[24] Cross JM, Donald AE, Nutall SL, Deanfield JE, Woolfon RG, MacAllister RJ. Vitamin C improves resistance but not conduit artery endothelial function in patients with chronic renal failure. Kidney Int 2003; 63:143–42.

[25] Liken MR, Schröder CH, Levtchenko EN, Koomans HA. Growth hormone therapy influence endothelial function in children with renal failure. Pediatr Nephrol 2004; 19:785–9.

[26] Cross JM, Donald AE, Kharbanda R, Deanfield JE, Woolson RG, MacAllister RJ. Acute administration of L-arginine does not improve arterial endothelial function in chronic renal failure. Kidney Int 2001; 60:2135–23.

[27] Annuk M, Zilmer M, Lind L, Linde T, Fellström B. Oxidative stress and endothelial function in chronic renal failure. J Am Soc Nephrol 2001; 12:2747–52.

[28] D’Apolito M, Du X, Pisaneli D, et al. Urea-induced ROS cause endothelial dysfunction in chronic renal failure. Atherosclerosis 2015; 239:393–400.

[29] Bivarnick P, Fijalkowski P, Męczczyk M, Błaszczzyk J, Banach M, Rysz J. Effect of methoxy polyethylene glycol-epoetin beta on oxidative stress in predialysis patients with chronic kidney disease. Med Sci Monit 2013; 19:954–9.

[30] Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 288:373–6.

[31] Al-Qaisi M, Kharbanda RK, Mittal TK, Donald AE. Measurement of endothelial function and its clinical utility for cardiovascular risk. Vasc Health Risk Manag 2008; 4:647–52.
Yeboah J, Folsom AR, Burke GL, et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the multi-ethnic study of atherosclerosis. Circulation 2009;120:502–9.

Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39:257–65.

Strippoli GF, Craig JC, Manno C, Schena FP. Hemoglobin targets for the anemia of chronic kidney disease: a meta-analysis of randomized, controlled trials. J Am Soc Nephrol 2004;15:3154–65.

Agarwal R. Mechanisms and mediators of hypertension induced by erythropoietin and related molecules. Nephrol Dial Transplant 2018;33:1690–8.

Noshad H. Blood pressure increase after erythropoietin injection in hemodialysis and predialysis patients. Iran J Kidney Dis 2013;7:220–5.

Portolés J, Torralbo A, Martin P, Rodrigo J, Herrero JA, Barrientos A. Cardiovascular effects of recombinant human erythropoietin in predialysis patients. Am J Kidney Dis 1997;29:541–8.

Hayashi T, Suzuki A, Shoji T, et al. Cardiovascular effect of normalizing the hematocrit level during erythropoietin therapy in predialysis patients with chronic renal failure. Am J Kidney Dis 2000;35:250–6.

Roger SD, McMahon LP, Clarkson A, et al. Effects of early and late intervention with epoetin alpha on left ventricular mass among patients with chronic kidney disease (stage 3 or 4): results of a randomized clinical trial. J Am Soc Nephrol 2004;15:148–56.

Ayus JC, Go AS, Valderrabano F, et al. Effects of erythropoietin on left ventricular hypertrophy in adults with severe chronic renal failure and hemoglobin <10g/dL. Kidney Int 2005;68:788–95.

Biebal F, Gómez-Pulido F, Cabanas-Grandio P, et al. Left atrial geometry improves risk prediction of thromboembolic events in patients with atrial fibrillation. J Cardiovasc Electrophysiol 2016;27:804–10.

Goette A, Kalman JM, Agunaga L, et al. EHRA/HRS/APHRS/SOLAECE expert consensus on atrial cardiomyopathies: definition, characterization, and clinical implication. Heart Rhythm 2017;14:e3–40.

Tsang TS, Barnes ME, Gersh BJ, Bailey KR, Seward JB. Left atrial volume as a morphophysiologic expression of left ventricular diastolic dysfunction and relation to cardiovascular risk burden. Am J Cardiol 2002;90:1284–9.

Eschbach JW, Egrie JC, Downing MR, Browne JK, Adamson JW. Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. Results of a combined phase I and II clinical trial. N Engl J Med 1987;316:73–8.