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# Cumulative Erythropoietin negatively correlated with bone mineral density in patients on dialysis

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## Abstract

### Objectives

Numerous factors are associated with the risk of osteoporosis in CKD patients, including vitamin D deficiency, hypocalcemia, hyperphosphatemia, and secondary hyperparathyroidism. The present study aimed to assess the correlation between cumulative erythropoietin (EPO) doses and osteoporosis risk in patients on chronic dialysis. A further objective was to determine the bone mineral density (BMD) of patients undergoing dialysis and its correlation with specific clinical and biochemical factors.

### Setting

The study was undertaken at a tertiary care center within the southern region of the Taipei Metropolitan area.

### Participants

This cross-sectional study included 165 participants aged 41–90 years. Dual-energy X-ray absorptiometry was used to measure BMD. A total of 108 age- and sex-matched participants were selected for further analysis. Stepwise multiple regression analysis was used to investigate the relationship between bone measurements and bone diseases' risk factors.

### Primary and Secondary outcomes

The primary outcome of this study was to assess the T-scores of the participants who received dialysis for more than three months in our institution. The secondary outcome was using a receiver operating curve to predict osteoporosis development in

patients on dialysis who received EPO treatments.

**Results**

The mean age of the participants was 66.6 ± 11.1 years. A total of 99 (60%) participants (41 male, 58 female) with a T-score of ≤−2.5 were diagnosed as having osteoporosis. Fifty-four (32.7%) participants with T-scores >−2.5 but <−1.0 were diagnosed as having osteopenia. Osteoporotic participants received 1.61 ± 1.52 million) EPO units compared to nonosteoporotic participants, who received 1.01 ± 0.64 million units (EPO1 model), $P = 0.015$. The cumulative EPO dose negatively correlated with the T-scores of participants ($P < 0.0001$).

**Conclusion**

On the basis of the results of the study, cumulative EPO doses show a negative correlation with BMD development in patients on chronic dialysis.

**Strength of the study**

1. The present study attempts to elucidate the correlation of exogenous erythropoietin administration with the risk of reducing bone mineral density. The idea is relatively new and novel, particularly within the context of the Asian population.
2. Recent studies have shed light on the molecular mechanism involved of Erythropoietin in modulating the bone mineral microenvironment. The current study attempts to provide clinical evidence of a negative correlation of cumulative erythropoietin administration to the risk of reduction in bone mineral density among chronic dialysis patients.
3. The present study contains a complete lifetime erythropoietin dose received in

our patients. Some of the patients have received Erythropoietin for more than 25 years, and no data is missing in our medical records.

4. We also performed sex and aged-match analysis to demonstrate the negative correlation of Erythropoietin and uremic osteoporosis risk.
5. The multivariate analysis further substantiates our hypothesis of the negative correlation of Erythropoietin and uremic osteoporosis risk.

**Limitation of the study**

1. A single-center limits our study and a relatively small sample size of 165 patients recruited.
2. On average, our patients are relatively old.
3. We recruited more female patients but did not show sex as the significant factor for osteoporosis in the dialysis population.

## Introduction

Bone disease associated with chronic kidney disease (CKD) involves complicated biochemical and hormonal molecular interactions. In addition to bone abnormalities in patients with CKD–mineral bone disorders (CKD–MBDs), such as secondary hyperparathyroidism, osteomalacia, and adynamic bone disease, osteoporosis is another prevalent bone disease in patients with CKD. CKD patients with osteoporosis are at a higher risk of bone fractures[1] and have reduced quality of life[2]. Considering the increasing prevalence of CKD among aging populations, diagnosis and treatment of osteoporosis in patient with CKD deserve more attention.

In patient with CKD, biochemical alterations resulting in vitamin D deficiency, hypocalcemia, hyperphosphatemia, and secondary hyperparathyroidism can cause deterioration of the cortical bone architecture, leading to reduced cortical density and increased cortical porosity earlier in the course of CKD than previously thought[3]. Osteoporosis is a decrease in bone mineral density (BMD). Dual-energy X-ray absorptiometry (DXA) is the most common method for measuring BMD and is considered the current gold standard for osteoporosis diagnosis. According to the World Health Organization (WHO) criteria, the standard BMD value (the average in young, healthy women) is a T-score of ≥−1.0. T-score values between −1.0 and −2.5 are considered to indicate low bone density or osteopenia. A T-score of ≤−2.5 is considered to indicate osteoporosis.

More than 2 decades ago, the introduction of recombinant human Erythropoietin (EPO) in clinical practice completely altered the management of CKD. Treatment of renal anemia with EPO is now well established. The extensive use of EPO and its analogs (EPO-stimulating agents [ESAs]) for anemia correction has reduced the associated morbidity and improved functionality, exercise tolerance, cognitive function, and overall quality of life. However, over the last few years, much controversy has been raised over the possible risks of ESA therapy. Moreover, thorough investigation of the mechanism of action of EPO has revealed multiple biologic effects that extend beyond its erythropoietic effect and may have a favorable or sometimes unfavorable contribution to these outcomes.

EPO acts on erythroid progenitor cells by binding to an EPO receptor (EPOR), promoting survival, proliferation, and differentiation[4]. Functioning EPOR is present in endothelial cells[5], neurons[6], skeletal muscle progenitor cells[7], adipocytes[5], and islets[8],

suggesting that EPO signaling exerts systemic regulation and interacts with nonerythroid cells through actions beyond erythropoiesis. Growing evidence from animal studies has demonstrated the critical role of EPO in regulating skeletal homeostasis[9,10]. Moreover, recent evidence has also demonstrated that EPO resulted in reduced trabecular bone volume in a mouse model of diet-induced obesity[11]. However, for humans, insufficient evidence exists on the role of EPO in mediating the bone microenvironment.

This study aimed to assess the correlation between cumulative doses of EPO administration and the risk of osteoporosis in patients on chronic dialysis. Moreover, bone mass density in the femur and lumbar Spine of patients on dialysis was investigated, its correlation with some clinical and biochemical factors was determined.

## Materials and methods

### Patient and Public Involvement

Taipei Medical University, Wan Fang Hospital is a tertiary care hospital in Taipei. On average, there are 300 hemodialysis and 60 peritoneal dialysis patients under our maintenance renal replacement therapy program. Patients aged >20 years with end-stage renal disease and who were undergoing renal replacement therapy (hemodialysis or peritoneal dialysis) for more than one year were recruited. Patients on steroids, antiresorptive drugs (bisphosphonates), contraceptives, or calcitonin, and those who received parathyroidectomy were excluded from the study. Patients who did not

initiate dialysis in our hospital were also excluded from the study due to the limitation in calculating cumulative EPO doses. Patients who were able to complete an interview were considered eligible. Of the 170 patients who gave consent, one died, three failed to undergo a DXA scan, and one DXA scan failed due to technical problems; the remaining 165 patients (74 males [44.8%] and 91 females [55.2%]) completed the study, and their demographic data and biochemistry are summarized in Table 1. Chronic renal failure of unknown etiology in six patients (3.6%) and was due to glomerulonephritis in 37 (22.4%), adult polycystic kidney disease in 7 (4.4%), diabetic nephropathy in 90 (54.5%), hypertensive nephrosclerosis in 24 (14.5%), and chronic tubulointerstitial nephritis in 1 (0.6%) patient. The mean duration of dialysis was 6.3±5.4 years, and the number of hours of dialysis per week was 9.5–16.5 h, with a mean of 11.2 h. The dialysate calcium concentration was 2.5 meq/L in 30 patients, 3.0 meq/L in 75 patients, and 3.5 meq/L in 60 patients.

**Table 1** Basic Characteristics of the Study Subjects and Univariate Analysis between Male and Female Dialysis Patients

| Mean ± SD | | | | |
|---|---|---|---|---|
| Variables | Values | Male ($N = 74$) | Female ($N = 91$) | $p$ value |
| Age (years) | 66.6 ± 11.1 | 66.9 ± 9.9 | 66.3 ± 12.0 | 0.519 |
| Body Mass Index (kg/m$^2$) | 23.4 ± 3.4 | 23.9 ± 3.2 | 22.8 ± 3.6 | **0.010** |
| Weight (kg) | 59.4 ± 10.6 | 66.0 ± 9.2 | 54.1 ± 8.6 | **< 0.0001** |
| Dialysis time | 6.3 ± 5.4 | 5.9 ± 5.2 | 6.1 ± 4.9 | 0.772 |
| Calcium (mg/dL) | 9.1 ± 0.8 | 9.2 ± 0.7 | 9.0 ± 0.8 | **0.036** |
| Phosphorus (mg/dL) | 5.0 ± 1.3 | 5.0 ± 1.3 | 5.1 ± 1.4 | 0.811 |
| Parathyroid hormone (pg/mL) | 362.9 ± 364.3 | 343.0 ± 345.3 | 379.1 ± 380.2 | 0.508 |
| Alkaline phosphatase (μg/L) | 97.2 ± 54.6 | 93.8 ± 53.0 | 100.0 ± 56.1 | 0.324 |
| Triglyceride (mg/dL) | 186.5 ± 131.9 | 182.5 ± 113.0 | 189.7 ± 145.9 | 0.937 |
| Total Cholesterol (mg/dL) | 153.8 ± 34.9 | 141.3 ± 30.7 | 164.0 ± 34.9 | **< 0.0001** |
| Albumin (g/dL) | 3.7 ± 0.4 | 3.8 ± 0.3 | 3.6 ± 0.4 | **0.0005** |
| Fasting glucose(mg/dL) | 146.9 ± 70.3 | 148.1 ± 73.1 | 145.9 ± 68.4 | 0.907 |
| Sodium (mmol/L) | 136.1 ± 3.5 | 136.0 ± 3.5 | 136.3 ± 3.4 | 0.474 |
| Potassium (mmol/L) | 4.4 ± 0.7 | 4.4 ± 0.8 | 4.4 ± 0.7 | 0.451 |
| Uric acid (mg/dL) | 6.9 ± 1.8 | 6.8 ± 1.8 | 7.0 ± 1.8 | 0.521 |
| Hemoglobin (g/dL) | 10.3 ± 0.9 | 10.4 ± 1.0 | 10.1 ± 0.8 | 0.093 |
| Ferritin | 531.4 ± 426.9 | 442.9 ± 307.0 | 603.4 ± 493.9 | 0.064 |
| EPO1 ($10^6$ units) | 1.38 ± 1.77 | 1.22 ± 1.38 | 1.51 ± 1.35 | 0.127 |
| EPO2 ($10^6$ units) | 1.92 ± 1.80 | 1.63 ± 1.62 | 2.15 ± 1.91 | 0.096 |
| EPO3 ($10^6$ units) | 2.45 ± 2.31 | 2.08 ± 2.01 | 2.76 ± 2.50 | 0.107 |
| T-score | -2.8 ± 2.6 | -2.5 ± 1.1 | -3.1 ± 3.3 | 0.210 |
| L-spine BMD | 1.093 ± 0.264 | 1.218 ± 0.247 | 0.991± 0.233 | **< 0.0001** |
| Right femoral BMD | 0.769 ± 0.223 | 0.820 ± 0.247 | 0.728 ± 0.194 | **0.0025** |
| Left femoral BMD | 0.757 ± 0.228 | 0.817 ± 0.240 | 0.707 ± 0.207 | **< 0.0001** |
| Hemodialysis/Peritoneal Dialysis | 125/40 | 57/17 | 68/23 | 0.186 |
| Diabetes mellitus | 97 | 48 | 49 | 0.154 |
| Hypertension | 148 | 68 | 80 | 0.406 |
| Congestive Heart Failure | 23 | 8 | 15 | 0.299 |
| Coronary artery disease | 44 | 23 | 21 | 0.249 |
| Vitamin D treatment | 35 | 14 | 21 | 0.531 |

A detailed history of related risk factors (smoking, hypertension, diabetes, steroid intake, and surgical menopause) was obtained from all patients, and medical records were checked after consent was obtained. Baseline investigations were performed, which included kidney function tests; determination of serum calcium, serum phosphorus, intact parathyroid hormone, fasting glucose, and serum alkaline phosphatase levels; liver function tests; complete blood counts; and determination of lipid profiles.

The DXA definition of osteoporosis and the bone mass criteria followed for its diagnosis were adopted from the WHO definition of osteoporosis (1994). T-scores were used for the evaluation of BMD and the definition of different stages of BMD according to the WHO definition of osteoporosis. T-scores were obtained for the femoral neck and L1 and L2.

**EPO dose conversion**

Patients receive either darbepoetin alfa (DPO) (Aranesp, Kyowa Hakko Kirin Co., Ltd.), epoetin beta (Recormon, Roche), or methoxy polyethylene glycol-epoetin beta (Mircera, Roche) at our institution. EPO doses are administered according to the patient's weekly hemoglobin levels. We maintain our patients' hemoglobin levels between 10 and 12 g/dL. For conversion from EPO alfa to darbepoetin alfa, a fixed conversion ratio of 200 IU EPO to 1 μg DPO was suggested by the manufacturer[12]. However, numerous studies have suggested that the conversion ratio should be 240–400 IU of EPO and 1 μg of DPO[13-15]. In the current study, the cumulative dose of

EPO received by the patient was calculated from the first day received EPO in our hospital until the DXA study date. We established three conversion doses of darbepoetin alpha (DPO) and methoxy polyethylene glycol-epoetin beta (Mircera) to calculate the statistical difference between patients with and without osteoporosis. EPO1 refers to the conversion of 1 μg of DPO/Mircera to 200 IU of EPO. EPO2 refers to the conversion of 1 μg of DPO/Mircera to 300 IU of EPO. EPO3 refers to the conversion of 1 μg of DPO/Mircera to 400 IU of EPO.

**Ethical approval**

The study was approved by the Taipei Medical University Institutional Review Board for Human Experimentation. The accession number: TMU-IRB N202103059.

**Statistical analysis**

Data were expressed as mean ± standard deviation unless otherwise specified. Correlations between bone measurements and cumulative EPO doses were assessed using Pearson's correlation coefficients. Stepwise multiple regression analysis was used to investigate the relationships between bone measurements and biochemical markers or risk factors for bone diseases. The backward stepwise regression method was used to select variables in the multivariate analysis. The logarithmic scale of EPOs was selected for multivariate analysis to avoid errors generated due to the collinearity of EPOs and Ln EPOs. Differences between the means of multiple subgroups were assessed using the Kruskal–Wallis test. An unpaired t-test or Mann–Whitney U test was used for continuous variables. SPSS version 25 (SPSS Inc., Chicago, IL, USA) was used for analysis. A p-value of $<0.05$ was considered statistically significant.

## Results

### Bone mineral densitometry

Bone mineral densitometry measurements of the 165 patients are shown in Table 2. A good correlation was found between BMD measurements of both the right and left femur ($r = 0.76$; $P < 0.0001$). However, lower correlation coefficients of BMD measurements were noted between lumbar spine values and right femoral neck ($r = 0.50$; $P < 0.0001$) and left femoral neck ($r = 0.54$; $P < 0.0001$) values, but they were still statistically significant. Ninety-nine patients with T-scores of ≤−2.5 were diagnosed with osteoporosis, and 54 patients with T-scores <−1.0 but > (−2.5 were diagnosed with osteopenia. Only twelve patients had T-scores of >−1.0.

**Table 2** Results of bone mineral densitometry measurements of patients on dialysis

| | BMD (g/cm²) | T-score (SD) | Osteopenia | | Osteoporosis | |
|---|---|---|---|---|---|---|
| | | | N | % | N | % |
| Lumbar spine | 1.093 ± 0.264 | −0.67 ± 1.85 | 54 | 32.7 | 27 | 16.4 |
| Right femoral neck | 0.769 ± 0.223 | −2.17 ± 1.27 | 74 | 44.8 | 51 | 30.9 |
| Left femoral neck | 0.757 ± 0.228 | −2.31 ± 1.24 | 77 | 46.7 | 53 | 32.1 |
| Total | - | −2.62 ± 1.14 | 54 | 32.7 | 99 | 60 |

Osteopenia: T-score < −1.0 but > −2.5; Osteoporosis: T-score ≤ −2.5

**Factors associated with reduced bone mineral density**

In total, 165 patients with and without osteoporosis were evaluated for differences in risk factors for and biochemical markers of bone diseases. These included all the factors in Table 3, and individual variables were evaluated using Student's t-test. Independent variables that were analyzed and reached statistical significance ($P < 0.05$) are shown in Table 3. Age, body mass index (BMI), weight, serum calcium, ferritin, and EPO doses showing statistical differences between patients with osteoporosis and patients without osteoporosis. Furthermore, 108 age- and sex-matched patients were evaluated for differences in risk factors for and biochemical markers of bone diseases. These included all the factors listed in Table 4. Cumulative EPO dosage was significantly different in age- and sex-matched patients with osteoporosis than nonosteoporotic patients on dialysis. All three EPO conversion models showed similar and significant results. To examine the association of T-scores with different models of EPO dose conversion, the process was repeated using different EPO dose conversion models; the results are shown in Figure 1. Pearson's correlation coefficient varied between −0.30 and −0.46, but *P* values were statistically significant.

**Table 3** Univariate analysis of chronic dialysis patients with and without osteoporosis

| | **Mean ± SD** | | |
|---|---|---|---|
| Variables | Osteoporosis ($N = 99$) | Without Osteoporosis ($N = 66$) | $p$ value |
| Age (years) | 70.0 ± 9.9 | 61.4 ± 10.8 | **< 0.0001** |
| Men/women | 41/58 | 33/33 | 0.278 |
| Body mass index (kg/m$^2$) | 22.7 ± 3.5 | 24.1 ± 3.2 | **0.011** |
| Weight (kg) | 58.2 ± 14.6 | 62.7 ± .10.4 | **0.045** |
| Dialysis time | 6.3 ± 5.5 | 6.1 ± 5.2 | 0.762 |
| Calcium (mg/dL) | 9.0 ± 0.8 | 9.2 ± 0.7 | **0.038** |
| Phosphorus (mg/dL) | 5.0 ± 1.4 | 5.2 ± 1.4 | 0.227 |
| Parathyroid hormone (pg/mL) | 367.7 ± 398.2 | 353.4 ± 310.9 | 0.805 |
| Alkaline phosphatase(μg/L) | 99.6 ± 54.8 | 93.1 ± 54.5 | 0.456 |
| Triglyceride (mg/dL) | 187.8 ± 128.8 | 183.7 ± 137.2 | 0.843 |
| Total Cholesterol (mg/dL) | 154.2 ± 36.9 | 153.4 ± 31.6 | 0.884 |
| Albumin (g/dL) | 3.7 ± 0.4 | 3.7 ± 0.3 | 0.184 |
| Fasting Glucose mg/dL) | 147.1 ± 71.6 | 153.0 ± 80.3 | 0.618 |
| Sodium (mmol/L) | 136.1 ± 3.4 | 136.1 ± 3.7 | 0.905 |
| Potassium (mmol/L) | 4.3 ± 0.7 | 4.5 ± 0.8 | 0.201 |
| Uric acid (mg/dL) | 6.8 ± 1.8 | 7.0 ± 1.8 | 0.627 |
| Hemoglobin (g/dL) | 10.3 ± 0.8 | 10.2 ± 1.1 | 0.383 |
| Ferritin | 592.7 ± 447.8 | 439.4 ± 377.8 | **0.027** |
| EPO1 ($10^6$ units) | 1.61 ± 1.52 | 1.01 ± 0.64 | **0.015** |
| EPO2 ($10^6$ units) | 2.23 ± 1.93 | 1.42 ± 0.92 | **0.013** |
| EPO3 ($10^6$ units) | 2.82 ± 2.45 | 1.87 ± 1.22 | **0.039** |
| T-score | -3.3 ± 0.78 | -1.5 ± 0.6 | **< 0.0001** |
| L-spine BMD | 1.012 ± 0.232 | 1.214 ± 0.264 | **< 0.0001** |
| Right femoral neck BMD | 0.700 ± 0.244 | 0.871 ± 1.345 | **< 0.0001** |
| Left femoral neck BMD | 0.667 ± 0.191 | 0.893 ± 0.210 | **< 0.0001** |
| Hemodialysis/Peritoneal dialysis | 79/20 | 46/20 | 0.140 |
| Diabetes mellitus | 58 | 39 | 0.949 |
| Hypertension | 88 | 60 | 0.676 |
| Congestive heart failure | 17 | 6 | 0.148 |
| Coronary artery disease | 27 | 17 | 0.829 |

**Table 4** Age and sex-matched univariate analysis of chronic dialysis patient with and without osteoporosis

| | **Mean ± SD** | | |
|---|---|---|---|
| Variables | Osteoporosis ($N = 54$) | Without Osteoporosis ($N = 54$) | *p* value |
| Age (years) | 66.0 ± 9.0 | 62.9 ± 10.2 | 0.097 |
| Men/women | 28/26 | 28/26 | 1.0 |
| Body mass index (kg/m$^2$) | 23.0 ± 4.0 | 24.0 ± 3.0 | 0.142 |
| Weight (kg) | 59.7 ± 11.7 | 62.6 ± .10.6 | 0.176 |
| Dialysis time | 7.3 ± 5.7 | 5.7 ± 5.0 | 0.111 |
| Calcium (mg/dL) | 9.1 ± 0.8 | 9.2 ± 0.7 | 0.524 |
| Phosphorus (mg/dL) | 5.1 ± 1.4 | 5.2 ± 1.4 | 0.495 |
| Parathyroid hormone (pg/mL) | 418.0 ± 419.5 | 329.2 ± 307.0 | 0.212 |
| Alkaline phosphatase(µg/L) | 102. 8 ± 47.9 | 96.6 ± 57.6 | 0.240 |
| Triglyceride (mg/dL) | 195.9 ± 139.2 | 197.9 ± 144.6 | 0.941 |
| Total Cholesterol (mg/dL) | 148.6 ± 40.3 | 155.1 ± 30.9 | 0.355 |
| Albumin (g/dL) | 3.8 ± 0.3 | 3.8 ± 0.3 | 0.796 |
| Fasting Glucose mg/dL) | 138.8 ± 69.5 | 163.0 ± 84.4 | 0.106 |
| Sodium (mmol/L) | 136.5 ± 3.2 | 136.4 ± 3.6 | 0.844 |
| Potassium (mmol/L) | 4.4 ± 0.8 | 4.5 ± 0.8 | 0.287 |
| Uric acid (mg/dL) | 7.1 ± 1.9 | 7.2 ± 1.7 | 0.823 |
| Hemoglobin (g/dL) | 10.4 ± 0.8 | 10.3 ± 1.1 | 0.486 |
| Ferritin | 502.6 ± 365.9 | 439.3 ± 372.4 | 0.375 |
| EPO1 ($10^6$ units) | 1.54 ± 1.19 | 0.94 ± 0.69 | **0.002** |
| EPO2 ($10^6$ units) | 2.15 ± 1.56 | 1.28 ± 0.91 | **0.001** |
| EPO3 ($10^6$ units) | 2.76 ± 1.97 | 1.62 ± 1.18 | **< 0.0001** |
| T-score | -3.7 ± 4.0 | -1.6 ± 0.6 | **< 0.0001** |
| L-spine BMD | 1.028 ± 0.240 | 1.227 ± 0.271 | **< 0.0001** |
| Right femoral neck BMD | 0.711 ± 0.239 | 1.033 ± 1.092 | **0.037** |
| Left femoral neck BMD | 0.664 ± 0.147 | 0.889 ± 0.198 | **< 0.0001** |
| Hemodialysis/Peritoneal dialysis | 41/13 | 41/13 | - |
| Diabetes mellitus | 29 | 35 | 0.244 |
| Hypertension | 45 | 49 | 0.256 |
| Congestive heart failure | 10 | 6 | 0.283 |
| Coronary artery disease | 15 | 14 | 0.830 |

**Factors associated with osteoporosis in patients on dialysis**

Table 5 shows factors associated with osteoporosis in patients on dialysis after different statistical models were applied. The univariate analysis results showed a statistically significant difference in age, BMI, the logarithmic scale of ferritin, and cumulative EPO dose in patients with osteoporosis compared with those without. Backward stepwise logistic regression was used to select multiple variables. In addition to age, ferritin, and EPO, both hemoglobin and body weight were significantly different between patients with and without osteoporosis. In the age- and sex-matched multivariate analysis model, EPO3 and the logarithmic scales of EPOs are the significant factors associated with osteoporosis.

**Table 5** Factors associated with osteoporosis in dialysis patients of different statistical models

| | Univariate model | | Multivariate model | | Age-sex matched model | |
|---|---|---|---|---|---|---|
| | P values | OR (95% C.I.) | P values | OR (95% C.I.) | P values | OR (95% C.I.) |
| Age | **< 0.0001** | 1.08(1.05- 1.12) | **0.001** | 1.07(1.03 - 1.12) | - | - |
| Gender | 0.278 | 0.71(0.38- 1.32) | 0.759 | 1.21(0.37 - 3.96) | - | - |
| Weight | **0.053** | 0.97(0.95- 1.00) | **0.010** | 0.95(0.92 - 0.99) | 0.766 | 0.99(0.93 - 1.06) |
| BMI | **0.012** | 0.88(0.80- 0.97) | 0.065 | 0.95(0.74 - 1.20) | 0.461 | 0.92(0.75 - 1.14) |
| Hemoglobin | 0.508 | 1.13(0.80- 1.60) | **0.022** | 1.76(1.08 - 2.85) | 0.197 | 1.41(0.84 - 2.36) |
| Ln Ferritin | **0.003** | 1.20(1.06- 1.36) | **0.033** | 2.96(1.09 - 8.03) | 0.656 | 1.30(0.42 - 4.03) |
| EPO1 | **0.008** | 1.29(1.07- 1.56) | **0.010** | 1.07(1.02 -1.12) | 0.094 | 1.63(0.92 - 2.87) |
| EPO2 | **0.009** | 1.19(1.04- 1.35) | **0.008** | 1.05(1.01 - 1.08) | **0.055** | 1.52(0.99 - 2.32) |
| EPO3 | **0.008** | 1.15(1.04- 1.27) | **0.009** | 1.04(1.01 - 1.07) | **0.019** | 1.55(1.07 - 2.23) |
| LnEPO1 | **0.007** | 1.08(1.02- 1.13) | **0.005** | 4.25(1.56 - 11.56) | **0.002** | 9.11(2.18 - 38.01) |
| LnEPO2 | **0.007** | 1.07(1.02- 1.13) | **0.008** | 4.70(1.50 - 14.76) | **0.002** | 10.61(2.43 - 46.40) |
| LnEPO3 | **0.007** | 1.07(1.02- 1.13) | **0.007** | 4.85(1.54 - 15.29) | **0.002** | 11.32(2.52 – 50.93) |

***Abbreviation:*** BMI, body mass index; Ln Ferritin, logarithmic scale Ferritin; LnEPO1, logarithmic scale EPO1; logarithmic scale LnEPO2, LogEPO2; LnEPO3, logarithmic scale EPO3. Multivariate model represents a stepwise backward logistic regression model of the unmatched individuals. Age-sex matched model represents a stepwise backward logistic regression model of the age- and sex- matched individuals.

**Role of erythropoietin use in osteoporosis development**

A receiver operating curve was generated to assess the area under the curve (AUC) to predict the risk of osteoporosis in patients on dialysis receiving cumulative EPO doses. A logarithmic scale was used to examine all three EPO dose conversion models and the development of osteoporosis. The AUC varied between 0.698 and 0.714 and showed moderate utility in predicting osteoporosis development in patients on dialysis (Figure 2).

## Discussion

This study found a moderate reduction in the mean BMD in this unselected population of patients on chronic hemodialysis. The mean T-score of −2.17 in the DXA measurement of the femoral neck implies that these patients had moderately less favorable outcomes than age-matched controls. This is similar to the results of several other studies that used the same bone density measurement[16]. Age and weight also emerged as important determinants of BMD in our study. Age-related bone loss plays an essential role in the pathogenesis of osteoporosis, and a negative association between age and BMD in female patients with end-stage renal disease has been reported[17,18]. The mean age of patients in these two studies was 43 and 50.5 years, whereas in our study, patients were older, with a mean age of 66.6 years. With the number of older adults involved in the renal replacement program increasing and with survival rates markedly improving, age-related bone loss can be expected to become an increasingly important factor causing bone disease in these patients.

Moreover, evidence has revealed a positive correlation between weight and BMD in healthy populations[19]. This has been attributed to bone formation stimulations through weight-bearing and adipose tissues' increased peripheral conversion of adrenal androgens to estrogens. Two studies have reported a positive association between BMI and BMD measurements[20,21]. We showed a similar association in our patients. Finally, we found a significant difference in cumulative EPO use in patients with osteoporosis compared with those without osteoporosis in both univariate and multivariate analyses (Table 5).

At our institution, EPO is administered based on the patient's weekly hemoglobin levels. EPO doses received were positively correlated with patient dialysis duration. The longer the patient undergoes dialysis, the higher the dose of EPO the patient may receive. However, no statistically significant differences in dialysis duration were found between patients with osteoporosis and those without [$P = 0.762$ (unmatched), $P = 0.111$ (age- and sex-matched)]. All three models, LnEPO1, LnEPO2, and LnEPO3, showed significant differences in cumulative EPO in patients with osteoporosis compared with those without (Table 5). A negative correlation was observed between the total, lumbar, right femoral neck, and left femoral neck T-scores and EPO dose (Figure 1). Although these results showed a low and negative correlation between T-scores and EPO dose (Pearson's correlation coefficient $r$ from 0.30 to 0.46), these data reached statistical significance ($P < 0.005$ to $< 0.0001$).

Serum PTH is negatively associated with BMD measurements; cortical porosity increased in patients with hyperparathyroidism[22]. Several studies have reported a negative association between PTH levels and BMD measurements[17,23,24], whereas others were unable to show this association[25-27]. In the present study, however, we found a negative association between PTH levels and BMD measurements, suggesting that other factors affect BMD in patients on hemodialysis. Forty-three patients received active vitamin D treatment in the current study.

Aluminum overload may be responsible for adynamic bone disease and osteomalacia. At our institution, serum aluminum levels are measured annually in patients who have undergone dialysis for >5 years. Our patients had no abnormally elevated serum aluminum levels. Moreover, we did not perform a histological analysis of bone. Thus, we cannot comment on the prevalence of adynamic bone disease and osteomalacia in this population.

The relationships between calcium intake, vitamin D supplementation, and osteoporosis development remain controversial. One study has shown that oral 1$\alpha$-hydroxycalciferol treatment could prevent BMD loss in the Lumbar Spine in a study of 165 male patients[28]. All 165 patients were receiving calcium-containing phosphate binders. Only ten patients received vitamin D supplements in the nonosteoporotic group compared with 33 patients who received vitamin D supplements in the osteoporotic group.

The strengths of our study are the random sampling of the population and the high accuracy of cumulative EPO treatment history collected. Participants with and without osteoporosis were age- and sex-matched to examine the association of EPO treatment with the risk of osteoporosis development. However, the present study was limited by its cross-sectional nature. It is difficult to establish the causal relationship between EPO cumulation and the risk of osteoporosis. A further longitudinal study is required to confirm the cause and effect of EPO in reducing BMD. Moreover, the present study involving a group of elderly participants. Our subgroup analysis showed that participants aged <65 years with osteoporosis did not receive a higher EPO dose than participants aged >65 years with osteoporosis ($r = -0.21$, $P = 0.133$, data not shown).

In conclusion, we confirmed the importance of age and body weight as the risk factors affecting BMD in patients on hemodialysis. We found that the cumulative EPO dose has a negative correlation in dialysis patients' BMD.



## Declarations

### Ethics approval and consent to participate

Taipei Medical University Institutional Review Board approved the study for Human Experimentation.

### Data sharing

All data generated or analyzed during this study are included in this article; no additional data is available.

### Competing interests

The authors declare that they have no competing interests.

### Funding

The present study has no funding available.

**Authors' contributions**

YJK designed the study and helped in analyzing the data. CYC wrote the manuscript and analyzed the data.

**Acknowledgment**

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## Figure legends

**Figure 1** Descriptive plots of correlations between T-scores of L-spine, right femoral, left femoral, and cumulative Erythropoietin (EPO) dose received. EPO1, EPO2, and EPO3 represent three different dose conversion models. EPO1, 1 μg of darbepoietin/Mircera converts to 200 IU of EPO; EPO2, 1 μg of darbepoietin/Mircera converts to 300 IU of EPO; EPO3, 1 μg of darbepoietin/Mircera converts to 400 IU of EPO.

**Figure 2** Receiver operating characteristic (ROC) curves of prediction models for cumulative erythropoietin dose converted to a logarithmic scale that incorporates patients with and without osteoporosis. AUC = area under the ROC curve; LnEPO1, logarithmic scale of EPO1; LnEPO2, logarithmic scale of EPO2; logarithmic scale EPO3, LnEPO3.

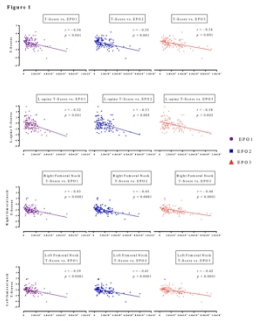

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**Figure 2**

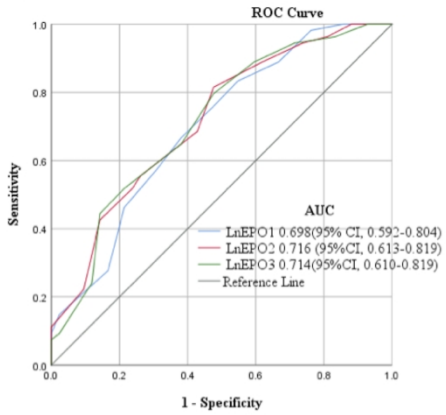


Figure 2 Receiver operating characteristic (ROC) curves of prediction models for cumulative erythropoietin dose converted to a logarithmic scale that incorporates patients with and without osteoporosis. AUC = area under the ROC curve; LnEPO1, logarithmic scale of EPO1; LnEPO2, logarithmic scale of EPO2; logarithmic scale EPO3, LnEPO3.

677x381mm (300 x 300 DPI)

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# Cumulative Erythropoietin negatively correlated with bone mineral density in patients on dialysis

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## Abstract

### Objectives

Numerous factors are associated with the risk of osteoporosis in CKD patients, including vitamin D deficiency, hypocalcemia, hyperphosphatemia, and secondary hyperparathyroidism. The present study aimed to assess the correlation between cumulative erythropoietin (EPO) doses and osteoporosis risk in patients on chronic dialysis. A further objective was to determine the bone mineral density (BMD) of patients undergoing dialysis and its correlation with specific clinical and biochemical factors.

### Setting

The study was undertaken at a tertiary care center within the southern region of the Taipei Metropolitan area.

### Participants

This cross-sectional study included 165 participants aged 41–90 years. Dual-energy X-ray absorptiometry was used to measure BMD. A total of 108 age- and sex-matched participants were selected for further analysis. Stepwise multiple regression analysis was used to investigate the relationship between bone measurements and bone diseases' risk factors.

### Primary and Secondary outcomes

The primary outcome of this study was to assess the T-scores of the participants who received dialysis for more than three months in our institution. The secondary outcome was using a receiver operating curve to predict osteoporosis development in patients on dialysis who received EPO treatments.

### Results

The mean age of the participants was 66.6 ± 11.1 years. A total of 99 (60%) participants (41 men, 58 women) were diagnosed as having osteoporosis. Fifty-four

(32.7%) participants with T-scores >−2.5 but <−1.0 were diagnosed as having osteopenia. Osteoporotic participants received 1.61 ± 1.52 million) EPO units compared to nonosteoporotic participants, who received 1.01 ± 0.64 million units (EPO1 model), $P = 0.015$. The cumulative EPO dose negatively correlated with the T-scores of participants ($P < 0.0001$).

**Conclusion**

On the basis of the results of the study, cumulative EPO doses show a negative correlation with BMD development in patients on chronic dialysis.

**Strength of the study**

1. The present study attempts to elucidate the correlation of exogenous erythropoietin administration with the risk of reducing bone mineral density in a clinical context. The idea is novel, particularly within the chronic dialysis Asian population.
2. A sex and aged-match analysis demonstrate the negative correlation of Erythropoietin and uremic osteoporosis risk.
3. The multivariate analysis further substantiates our hypothesis of the negative correlation of Erythropoietin and uremic osteoporosis risk.

**Limitation of the study**

1. A single-center study involving a relatively old age group of patients and a relatively small sample size of 165 patients limited our study strength.
2. We recruited more female patients but did not show sex as the significant factor for osteoporosis in the dialysis population.

## Introduction

Bone disease associated with chronic kidney disease (CKD) involves complicated biochemical and hormonal molecular interactions. In addition to bone abnormalities in patients with CKD–mineral bone disorders (CKD–MBDs), such as secondary hyperparathyroidism, osteomalacia, and adynamic bone disease, osteoporosis is another prevalent bone disease in patients with CKD. CKD patients with osteoporosis are at a higher risk of bone fractures[1] and have reduced quality of life[2]. Considering the increasing prevalence of CKD among aging populations, diagnosis and treatment of osteoporosis in patient with CKD deserve more attention.

In patient with CKD, biochemical alterations resulting in vitamin D deficiency, hypocalcemia, hyperphosphatemia, and secondary hyperparathyroidism can cause deterioration of the cortical bone architecture, leading to reduced cortical density and increased cortical porosity earlier in the course of CKD than previously thought[3]. Osteoporosis is a decrease in bone mineral density (BMD). Dual-energy X-ray absorptiometry (DXA) is the most common method for measuring BMD and is considered the current gold standard for osteoporosis diagnosis. According to the World Health Organization (WHO) criteria, the standard BMD value (the average in young, healthy women) is a T-score of $\geq -1.0$. T-score values between $-1.0$ and $-2.5$ are considered to indicate low bone density or osteopenia. A T-score of $\leq -2.5$ is considered to indicate osteoporosis.

More than 2 decades ago, the introduction of recombinant human Erythropoietin (EPO) in clinical practice completely altered the management of CKD. Treatment of renal anemia with EPO is now well established. The extensive use of EPO and its

analogs (EPO-stimulating agents [ESAs]) for anemia correction has reduced the associated morbidity and improved functionality, exercise tolerance, cognitive function, and overall quality of life. However, over the last few years, much controversy has been raised over the possible risks of ESA therapy. Moreover, thorough investigation of the mechanism of action of EPO has revealed multiple biologic effects that extend beyond its erythropoietic effect and may have a favorable or sometimes unfavorable contribution to these outcomes.

EPO acts on erythroid progenitor cells by binding to an EPO receptor (EPOR), promoting survival, proliferation, and differentiation[4]. Functioning EPOR is present in endothelial cells[5], neurons[6], skeletal muscle progenitor cells[7], adipocytes[5], and islets[8], suggesting that EPO signaling exerts systemic regulation and interacts with nonerythroid cells through actions beyond erythropoiesis. Growing evidence from animal studies has demonstrated the critical role of EPO in regulating skeletal homeostasis[9,10]. Moreover, recent evidence has also demonstrated that EPO resulted in reduced trabecular bone volume in a mouse model of diet-induced obesity[11]. However, for humans, insufficient evidence exists on the role of EPO in mediating the bone microenvironment.

This study aimed to assess the correlation between cumulative doses of EPO administration and the risk of osteoporosis in patients on chronic dialysis. Moreover, bone mineral density in the femur and lumbar Spine of patients on dialysis was investigated, its correlation with some clinical and biochemical factors was determined.

## Materials and methods

### Patient and Public Involvement

Taipei Medical University, Wan Fang Hospital is a tertiary care hospital in Taipei. On average, there are 300 hemodialysis and 60 peritoneal dialysis patients under our maintenance renal replacement therapy program. Patients aged >20 years with end-stage renal disease and who were undergoing renal replacement therapy (hemodialysis or peritoneal dialysis) for more than one year were recruited. Patients on steroids, antiresorptive drugs (bisphosphonates), contraceptives, or calcitonin, and those who received parathyroidectomy were excluded from the study. Patients who did not initiate dialysis in our hospital were also excluded from the study due to the limitation in calculating cumulative EPO doses. Patients who were able to complete an interview were considered eligible. Of the 170 patients who gave consent, one died, three failed to undergo a DXA scan, and one DXA scan failed due to technical problems; the remaining 165 patients (74 males [44.8%] and 91 females [55.2%]) completed the study, and their demographic data and biochemistry are summarized in Table 1. The causes of chronic renal failure were diabetic nephropathy (DMN) (90 patients, 54.5%), chronic glomerulonephritis (37 patients, 22.4%), hypertensive nephrosclerosis (24 patients, 14.5%), adult polycystic kidney disease (7 patients, 4.4%), chronic renal failure of unknown etiology (6 patients, 3.6%), and chronic tubulointerstitial nephritis patient (1 patient, 0.6%). The mean duration of dialysis was 6.3±5.4 years, and the number of hours of dialysis per week was 9.5–16.5 h, with a mean of 11.2 h. The dialysate calcium concentration was 2.5 meq/L in 30 patients, 3.0 meq/L in 75 patients, and 3.5 meq/L in 60 patients.

**Table 1** Basic Characteristics of the Study Participants and Univariate Analysis between men and women

| Variables | Values ( N = 165) | Men (*n* = 74) | Women (*n* = 91) | *p* value |
|---|---|---|---|---|
| Age (years) | 66.6 ± 11.1 | 66.9 ± 9.9 | 66.3 ± 12.0 | 0.519 |
| BMI (kg/m$^2$) | 23.4 ± 3.4 | 23.9 ± 3.2 | 22.8 ± 3.6 | 0.010* |
| BW (kg) | 59.4 ± 10.6 | 66.0 ± 9.2 | 54.1 ± 8.6 | < 0.0001**** |
| Dialysis vintage (yrs) | 6.3 ± 5.4 | 5.9 ± 5.2 | 6.1 ± 4.9 | 0.772 |
| Ca (mg/dl) | 9.1 ± 0.8 | 9.2 ± 0.7 | 9.0 ± 0.8 | 0.036* |
| P (mg/dl) | 5.0 ± 1.3 | 5.0 ± 1.3 | 5.1 ± 1.4 | 0.811 |
| Intact PTH (pg/ml) | 362.9 ± 364.3 | 343.0 ± 345.3 | 379.1 ± 380.2 | 0.508 |
| ALP (μg/l) | 97.2 ± 54.6 | 93.8 ± 53.0 | 100.0 ± 56.1 | 0.324 |
| TG (mg/dl) | 186.5 ± 131.9 | 182.5 ± 113.0 | 189.7 ± 145.9 | 0.937 |
| T-Chol (mg/dl) | 153.8 ± 34.9 | 141.3 ± 30.7 | 164.0 ± 34.9 | < 0.0001**** |
| Alb (g/dL) | 3.7 ± 0.4 | 3.8 ± 0.3 | 3.6 ± 0.4 | 0.0005*** |
| AC glucose (mg/dl) | 146.9 ± 70.3 | 148.1 ± 73.1 | 145.9 ± 68.4 | 0.907 |
| Na (mmol/l) | 136.1 ± 3.5 | 136.0 ± 3.5 | 136.3 ± 3.4 | 0.474 |
| K (mmol/l) | 4.4 ± 0.7 | 4.4 ± 0.8 | 4.4 ± 0.7 | 0.451 |
| Uric acid (mg/dl) | 6.9 ± 1.8 | 6.8 ± 1.8 | 7.0 ± 1.8 | 0.521 |
| Hb (g/dl) | 10.3 ± 0.9 | 10.4 ± 1.0 | 10.1 ± 0.8 | 0.093 |
| Ferritin (ng/ml) | 531.4 ± 426.9 | 442.9 ± 307.0 | 603.4 ± 493.9 | 0.008* |
| EPO1 (x10$^6$ units) | 1.38 ± 1.77 | 1.22 ± 1.38 | 1.51 ± 1.35 | 0.847 |
| EPO2 (x10$^6$ units) | 1.92 ± 1.80 | 1.63 ± 1.62 | 2.15 ± 1.91 | 0.414 |
| EPO3 (x10$^6$ units) | 2.45 ± 2.31 | 2.08 ± 2.01 | 2.76 ± 2.50 | 0.295 |
| T-score | -2.8 ± 2.6 | -2.5 ± 1.1 | -3.1 ± 3.3 | 0.291 |
| L-spine BMD (g/cm$^2$) | 1.093 ± 0.264 | 1.218 ± 0.247 | 0.991± 0.233 | < 0.0001**** |
| RF-T BMD (g/cm$^2$) | 0.769 ± 0.223 | 0.820 ± 0.247 | 0.728 ± 0.194 | 0.003** |
| LF-T BMD (g/cm$^2$) | 0.757 ± 0.228 | 0.817 ± 0.240 | 0.707 ± 0.207 | < 0.0001**** |
| HD/PD | 125/40 | 57/17 | 68/23 | 0.186 |
| DM | 97 | 48 | 49 | 0.012 |
| Hypertension | 148 | 68 | 80 | 0.175 |
| CHF | 23 | 8 | 15 | 0.213 |
| CAD | 44 | 23 | 21 | 0.189 |
| Vitamin D treatment | 35 | 14 | 21 | 0.531 |

Abbreviations: A.C. glucose, fasting glucose; Alb, albumin; ALP, alkaline phosphatase; BMI, body mass index; B.W., body weight; CAD, coronary artery disease; Ca, calcium; CHF, congestive heart failure; D.M., diabetes mellitus; EPO, erythropoietin; Hb,

hemoglobin; H.D., hemodialysis; K, potassium; LF-T BMD, total left femur bone mineral density; L-spine BMD, lumbar-spine bone mineral density; Na, sodium; P, phosphorus; intact PTH, intact parathyroid hormone; P.D., peritoneal dialysis; RF-T BMD, total right femur bone mineral density; T.G., triglyceride; T-Chol, total cholesterol; yrs, years.

A detailed history of related risk factors (smoking, hypertension, diabetes, steroid intake, and surgical menopause) was obtained from all patients, and medical records were checked after consent was obtained. Before initiating the dialysis session, baseline investigations were performed at the patient's regular blood test session. Blood tests included kidney function tests, serum calcium, serum phosphorus, intact parathyroid hormone, fasting glucose, serum alkaline phosphatase levels, liver function tests, complete blood counts, ferritin, and determination of lipid profiles.

The DXA definition of osteoporosis and the bone mass criteria followed for its diagnosis were adopted from the WHO definition of osteoporosis (1994). T-scores were used for the evaluation of BMD and the definition of different stages of BMD according to the WHO definition of osteoporosis. T-scores were obtained for the femoral necks and lumbar spines (L1-L4). The average of lumbar Spine BMD was to evaluate the lumbar Spine T-score, use of three vertebrae if four cannot be used, and two if three cannot be used for the diagnosis according to the (The International Society for Clinical Densitometry, ISCD) guideline.[12] The lowest T-score among femoral necks and lumbar spines was accounted for established osteoporosis. The T-score Normative Database is calculated by using USA (Combined NHANES (ages 20-30)/Lunar (ages 20-40) A.P. spine and Femur Reference Population).

**EPO dose conversion**

Patients receive either darbepoetin alfa (DPO) (Aranesp, Kyowa Hakko Kirin Co.,

Ltd.), epoetin beta (Recormon, Roche), or methoxy polyethylene glycol-epoetin beta (Mircera, Roche) at our institution. EPO doses are administered according to the patient's weekly hemoglobin levels. We maintain our patients' hemoglobin levels between 10 and 12 g/dl. For conversion from EPO alfa to darbepoetin alfa, a fixed conversion ratio of 200 IU EPO to 1 μg DPO was suggested by the manufacturer[13]. However, numerous studies have suggested that the conversion ratio should be 240–400 IU of EPO and 1 μg of DPO[14-16]. In the current study, the cumulative dose of EPO received by the patient was calculated from the first day received EPO in our hospital until the DXA study date. The patient might receive various combination of EPOs during their dialysis treatment in our institution. We established three conversion doses of darbepoetin alpha (DPO) and methoxy polyethylene glycol-epoetin beta (Mircera) to calculate the statistical difference between patients with and without osteoporosis. EPO1 refers to the conversion of 1 μg of DPO/Mircera to 200 IU of EPO. EPO2 refers to the conversion of 1 μg of DPO/Mircera to 300 IU of EPO. EPO3 refers to the conversion of 1 μg of DPO/Mircera to 400 IU of EPO.

**Ethical approval**

The study was approved by the Taipei Medical University Institutional Review Board for Human Experimentation. The accession number: TMU-IRB N202103059.

**Statistical analysis**

Data were expressed as mean ± standard deviation unless otherwise specified. Pearson's correlation coefficients assessed correlations between bone measurements and cumulative EPO doses. Stepwise multiple regression analysis was used to investigate the relationships between bone measurements and biochemical markers or risk factors for bone diseases. The backward stepwise regression method was used to

select variables in the multivariate analysis. Only a single logarithmic scale of EPOs was selected at every entry for multivariate analysis to avoid errors generated due to the collinearity of EPOs and Log EPOs. Differences between the means of multiple subgroups were assessed using the Kruskal–Wallis test. An unpaired t-test or Mann–Whitney U test was used for continuous variables. The chi-square test was used to compare frequencies between categorical variables. SPSS version 25 (SPSS Inc., Chicago, IL, USA) was used for analysis. A p-value of <0.05 was considered statistically significant.

## Results

### Bone mineral densitometry

Bone mineral densitometry measurements of the 165 patients are shown in Table 2. A good correlation was found between BMD measurements of both the right and left femur ($r = 0.76$; $P < 0.0001$). However, lower correlation coefficients of BMD measurements were noted between lumbar spine values and right femoral neck ($r = 0.50$; $P < 0.0001$) and left femoral neck ($r = 0.54$; $P < 0.0001$) values, but they were still statistically significant. Ninety-nine patients with T-scores of ≤−2.5 were diagnosed with osteoporosis, and 54 patients with T-scores <−1.0 but > (−2.5 were diagnosed with osteopenia. Only twelve patients had T-scores of >−1.0.

**Table 2** Results of bone mineral densitometry measurements of patients on dialysis

| | BMD (g/cm$^2$) | T-score (SD) | Osteopenia | | Osteoporosis | |
|---|---|---|---|---|---|---|
| | | | N | % | N | % |
| L-spine | 1.093 ± 0.264 | -0.67 ± 1.85 | 54 | 32.7 | 27 | 16.4 |
| RF Neck | 0.769 ± 0.223 | -2.17 ± 1.27 | 74 | 44.8 | 51 | 30.9 |
| RF Total | 0.842 ± 0.225 | -1.72 ± 1.31 | 68 | 41.2 | 48 | 29.1 |
| LF Neck | 0.757 ± 0.228 | -2.31 ± 1.24 | 77 | 46.7 | 53 | 32.1 |
| LF Total | 0.839 ± 0.231 | -1.78 ± 1.29 | 72 | 43.6 | 54 | 32.7 |
| Total | - | -2.62 ± 1.14 | 54 | 32.7 | 99 | 60 |

Osteopenia: T-score < −1.0 but > −2.5; Osteoporosis: T-score ≤ −2.5
Total: the lowest T-score found among femoral necks and lumbar spines.
Abbreviations: BMD, bone mineral density; L.F. Neck, left femoral neck; L-spine, lumbar-spine; R.F. Neck, right femoral neck.

**Factors associated with reduced bone mineral density**

In total, 165 patients with and without osteoporosis were evaluated for differences in risk factors for and biochemical markers of bone diseases. These included all the factors in Table 3, and individual variables were evaluated using Student's t-test. Independent variables that were analyzed and reached statistical significance ($P < 0.05$) are shown in Table 3. Age, body mass index (BMI), body weight (BW), serum calcium, ferritin, and EPO doses show statistical differences between patients with osteoporosis and patients without osteoporosis. Furthermore, 108 age- and sex-matched patients were evaluated for differences in risk factors for and biochemical markers of bone diseases. These included all the factors listed in Table 4. Cumulative EPO dosage was significantly different in age- and sex-matched patients with osteoporosis than nonosteoporotic patients on dialysis. All three EPO conversion models showed similar and significant results. Three models of EPO dose conversion were used to examine the association between EPO and T-scores of participants. The statistical calculation process was repeated using different EPO dose models to avoid collinearity. The results are shown in Figure 1. Pearson's correlation coefficient varied between −0.30 and −0.46, but *p* values were statistically significant.

**Table 3** The t-test analysis of chronic dialysis patients with and without osteoporosis

| Variables | OS ($n$ = 99) | Without OS ($n$ = 66) | $p$ value |
|---|---|---|---|
| Age (years) | 70.0 ± 9.9 | 61.4 ± 10.8 | < 0.0001**** |
| Men/women | 41/58 | 33/33 | 0.278 |
| BMI (kg/m$^2$) | 22.7 ± 3.5 | 24.1 ± 3.2 | 0.009** |
| BW (kg) | 58.2 ± 14.6 | 62.7 ± .10.4 | 0.040* |
| Dialysis vintage (years) | 6.3 ± 5.5 | 6.1 ± 5.2 | 0.762 |
| Ca (mg/dL) | 9.0 ± 0.8 | 9.2 ± 0.7 | 0.028* |
| P (mg/dL) | 5.0 ± 1.4 | 5.2 ± 1.4 | 0.227 |
| Intact PTH (pg/mL) | 367.7 ± 398.2 | 353.4 ± 310.9 | 0.805 |
| ALP (μg/L) | 99.6 ± 54.8 | 93.1 ± 54.5 | 0.456 |
| TG (mg/dL) | 187.8 ± 128.8 | 183.7 ± 137.2 | 0.843 |
| T-Chol (mg/dL) | 154.2 ± 36.9 | 153.4 ± 31.6 | 0.884 |
| Alb (g/dL) | 3.7 ± 0.4 | 3.7 ± 0.3 | 0.184 |
| AC Glucose mg/dL) | 147.1 ± 71.6 | 153.0 ± 80.3 | 0.618 |
| Na (mmol/L) | 136.1 ± 3.4 | 136.1 ± 3.7 | 0.905 |
| K (mmol/L) | 4.3 ± 0.7 | 4.5 ± 0.8 | 0.201 |
| Uric acid (mg/dL) | 6.8 ± 1.8 | 7.0 ± 1.8 | 0.627 |
| Hb (g/dL) | 10.3 ± 0.8 | 10.2 ± 1.1 | 0.383 |
| WBC (/μl) | 7090 ± 636.7 | 6366 ± 199.5 | 0.365 |
| Platelet ( x10$^3$/μl) | 182.50 ± 6.30 | 179.20 ± 7.08 | 0.732 |
| Ferritin (ng/ml) | 592.7 ± 45.03 | 439.4 ± 36.51 | 0.023* |
| EPO1 (10$^6$ units) | 1.61 ± 1.52 | 1.01 ± 0.64 | 0.015* |
| EPO2 (10$^6$ units) | 2.23 ± 1.93 | 1.42 ± 0.92 | 0.013* |
| EPO3 (10$^6$ units) | 2.82 ± 2.45 | 1.87 ± 1.22 | 0.039* |
| T-score | -3.3 ± 0.78 | -1.5 ± 0.6 | < 0.0001**** |
| L-spine BMD | 1.012 ± 0.232 | 1.214 ± 0.264 | < 0.0001**** |
| RF-T BMD | 0.770 ± 0.025 | 0.952 ± 0.015 | < 0.0001**** |
| LT-T BMD | 0.749 ± 0.021 | 0.979 ± 0.024 | < 0.0001**** |
| HD/PD | 79/20 | 46/20 | 0.140 |
| DM | 58 | 39 | 0.949 |
| Hypertension | 88 | 60 | 0.676 |
| CHF | 17 | 6 | 0.148 |
| CAD | 27 | 17 | 0.829 |

T-scores represents the lowest value among the three areas of BMD measurements. Abbreviations: A.C. glucose, fasting glucose; Alb, albumin; ALP, alkaline phosphatase; BMI, body mass index; B.W., body weight; CAD, coronary artery disease; Ca, calcium; CHF, congestive heart failure; D.M., diabetes mellitus; EPO, erythropoietin; Hb, hemoglobin; H.D., hemodialysis; K, potassium; LF-T BMD, total left femur bone mineral density; L-spine BMD, lumbar-spine bone mineral density; Na, sodium; O.S., osteoporosis; P, phosphorus; intact PTH, intact parathyroid hormone; P.D., peritoneal dialysis; RF-T BMD, total right femur bone mineral density; T.G., triglyceride; T-Chol, total cholesterol; WBC, white cell count.

**Erythropoietin dosage associated with osteoporosis among three different sites of bone mineral density measurement**

Significantly higher EPO dosages were found among osteoporotic participants using BMD measured from lumbar spines, right total, and left total femur (Table 4a-4c). However, no statistical difference was found on the cumulative EPO doses (all three models) using different sites to diagnose osteoporosis (Figure 2).

**Table 4a** Association of cumulative dose of erythropoietin with L-spine BMD

| L-Spine | OS ($n$ = 27) | Without OS ( $n$ = 138) | p Value |
|---|---|---|---|
| M/F | 6/21 | 68/70 | |
| BMD | 0.95±0.20 | 1.14±0.26 | 0.001*** |
| EPO1 | 1.82±1.57 | 1.22±1.13 | 0.020* |
| EPO2 | 2.59±2.35 | 1.71±1.41 | 0.010** |
| EPO3 | 3.34±3.21 | 2.19±1.76 | 0.009** |

**Table 4b** Association of cumulative dose of erythropoietin with the total right femur BMD

| Right femur total | OS ( $n$ = 48 ) | Without OS ( $n$ = 117 ) | p Value |
|---|---|---|---|
| M/F | 15/33 | 59/58 | |
| BMD | 0.71±0.17 | 0.90±0.22 | <0.0001**** |
| EPO1 | 1.71±1.29 | 1.15±1.17 | 0.008** |
| EPO2 | 2.46±1.92 | 1.61±1.43 | 0.002** |
| EPO3 | 3.21±2.61 | 2.04±1.75 | 0.001*** |

**Table 4c** Association of cumulative dose of erythropoietin with the total left femur BMD

| Left femur total | OS ( *n* = 54 ) | Without OS ( *n* = 111 ) | p Value |
|---|---|---|---|
| M/F | 18/36 | 56/55 | |
| BMD | 0.71±0.18 | 0.90±0.23 | <0.0001**** |
| EPO1 | 1.61±1.30 | 1.17±1.17 | 0.028* |
| EPO2 | 2.34±1.91 | 1.62±1.42 | 0.007** |
| EPO3 | 3.05±2.57 | 2.05±1.75 | 0.004** |

Abbreviations: BMD, bone mineral density; EPO, erythropoietin; L-spine, lumbar-spine; OS, osteoporosis.



**Factors associated with osteoporosis in patients on dialysis**

Table 5 shows clinical factors assicated with osteoporosis in age- and sex-matched chronic dialysis patients. All three EPO conversion models show significant cumulative EPO use among osteoporotic dialysis patients than non-osteoporotic dialysis patients. Table 6 shows factors associated with osteoporosis in patients on dialysis after different statistical models were applied. The univariate analysis results showed a statistically significant difference in age, BMI, the logarithmic scale of ferritin, and cumulative EPO dose in patients with osteoporosis compared with those without osteoporosis. Backward stepwise logistic regression was used to select multiple variables. Age, sex, BW, BMI, hemoglobin, the logarithmic scale of ferritin, and a single entry of logarithmic scale of EPO were selected as variables to enter the logistic regression model. In addition to age, ferritin, and EPO, both hemoglobin and BW were significantly different between patients with and without osteoporosis. In the age- and sex-matched multivariate analysis model, EPO3 and the logarithmic scales of EPOs are the significant factors associated with osteoporosis.

**Table 5** Age- and sex-matched t-test analysis of chronic dialysis patient with and without osteoporosis

| Variables | OS ($n$ = 54) | Without OS (n = 54) | $p$ value |
|---|---|---|---|
| Age (years) | 66.0 ± 9.0 | 62.9 ± 10.2 | 0.097 |
| Men/women | 28/26 | 28/26 | 1.0 |
| BMI (kg/m$^2$) | 23.0 ± 4.0 | 24.0 ± 3.0 | 0.142 |
| BW (kg) | 59.7 ± 11.7 | 62.6 ± .10.6 | 0.176 |
| Dialysis vintage (yrs) | 7.3 ± 5.7 | 5.7 ± 5.0 | 0.111 |
| Ca (mg/dL) | 9.1 ± 0.8 | 9.2 ± 0.7 | 0.524 |
| P (mg/dL) | 5.1 ± 1.4 | 5.2 ± 1.4 | 0.495 |
| Intact PTH (pg/mL) | 418.0 ± 419.5 | 329.2 ± 307.0 | 0.212 |
| ALP (μg/L) | 102. 8 ± 47.9 | 96.6 ± 57.6 | 0.240 |
| TG (mg/dL) | 195.9 ± 139.2 | 197.9 ± 144.6 | 0.941 |
| T-Chol (mg/dL) | 148.6 ± 40.3 | 155.1 ± 30.9 | 0.355 |
| Alb (g/dL) | 3.8 ± 0.3 | 3.8 ± 0.3 | 0.796 |
| AC Glucose mg/dL) | 138.8 ± 69.5 | 163.0 ± 84.4 | 0.106 |
| Na (mmol/L) | 136.5 ± 3.2 | 136.4 ± 3.6 | 0.844 |
| K (mmol/L) | 4.4 ± 0.8 | 4.5 ± 0.8 | 0.287 |
| Uric acid (mg/dL) | 7.1 ± 1.9 | 7.2 ± 1.7 | 0.823 |
| Hb (g/dL) | 10.4 ± 0.8 | 10.3 ± 1.1 | 0.486 |
| WBC (/μl) | 7595 ± 1142 | 6518 ± 231.3 | 0.357 |
| Platelet ( x10$^3$/μl) | 178.89 ± 7.79 | 183.37 ± 9.76 | 0.721 |
| Ferritin | 502.6 ± 365.9 | 439.3 ± 372.4 | 0.375 |
| EPO1 (x10$^6$ units) | 1.54 ± 1.19 | 0.94 ± 0.69 | 0.002*** |
| EPO2 (x10$^6$ units) | 2.15 ± 1.56 | 1.28 ± 0.91 | 0.001** |
| EPO3 (x10$^6$ units) | 2.76 ± 1.97 | 1.62 ± 1.18 | < 0.0001**** |
| T-score | -3.7 ± 4.0 | -1.6 ± 0.6 | < 0.0001**** |
| L-spine BMD | 1.029 ± 0.033 | 1.227 ± 0.037 | < 0.0001**** |
| RF-T BMD | 0.775 ± 0.033 | 0.962 ± 0.020 | < 0.0001**** |
| LF-T BMD | 0.737 ± 0.022 | 0.974 ± 0.026 | < 0.0001**** |
| HD/PD | 41/13 | 41/13 | - |
| DM | 29 | 35 | 0.244 |
| Hypertension | 45 | 49 | 0.256 |
| CHF | 10 | 6 | 0.283 |
| CAD | 15 | 14 | 0.830 |

Abbreviations: A.C. glucose, fasting glucose; Alb, albumin; ALP, alkaline phosphatase; BMI, body mass index; B.W., body weight; CAD, coronary artery disease; Ca, calcium; CHF, congestive heart failure; D.M., diabetes mellitus; EPO, erythropoietin; Hb, hemoglobin; H.D., hemodialysis; K, potassium; LF-T BMD, total left femur bone mineral density; L-spine BMD, lumbar-spine bone mineral density; Na, sodium; P, phosphorus; intact PTH, intact parathyroid hormone; P.D., peritoneal dialysis; RF-T BMD, total right femur bone mineral density; T.G., triglyceride; T-Chol, total cholesterol; WBC, white cell count.



**Table 6** Factors associated with osteoporosis in dialysis patients of different statistical models

| | Univariate model | | Multivariate model | | Age-sex matched model | |
|---|---|---|---|---|---|---|
| | p Values | OR (95% C.I.) | p values | OR (95% C.I.) | p values | OR (95% C.I.) |
| Age (yrs) | < 0.0001**** | 1.08(1.05- 1.12) | 0.001*** | 1.07(1.03 - 1.12) | - | - |
| Sex | 0.278 | 0.71(0.38- 1.32) | 0.759 | 1.21(0.37 - 3.96) | - | - |
| BW (kg) | 0.053 | 0.97(0.95- 1.00) | 0.010* | 0.95(0.92 - 0.99) | 0.766 | 0.99(0.93 - 1.06) |
| BMI(kg/m$^2$) | 0.012* | 0.88(0.80- 0.97) | 0.065 | 0.95(0.74 - 1.20) | 0.461 | 0.92(0.75 - 1.14) |
| Hb (g/dl) | 0.508 | 1.13(0.80- 1.60) | 0.022* | 1.76(1.08 - 2.85) | 0.197 | 1.41(0.84 - 2.36) |
| LogFerritin | 0.003*** | 1.20(1.06- 1.36) | 0.033* | 2.96(1.09 - 8.03) | 0.656 | 1.30(0.42 - 4.03) |
| LogEPO1 | 0.007** | 1.08(1.02- 1.13) | 0.005** | 4.25(1.56 - 11.56) | 0.002*** | 9.11(2.18 - 38.0) |
| LogEPO2 | 0.007** | 1.07(1.02- 1.13) | 0.008** | 4.70(1.50 - 14.76) | 0.002*** | 10.61(2.43 - 46.4) |
| LogEPO3 | 0.007** | 1.07(1.02- 1.13) | 0.007** | 4.85(1.54 - 15.29) | 0.002*** | 11.32(2.52 - 50.9) |

Abbreviations: BMI, body mass index; LogFerritin, logarithmic scale Ferritin; LogEPO1, logarithmic scale EPO1; LogEPO2, logarithmic scale, LogEPO2; LogPO3, logarithmic scale EPO3. Multivariate model represents a stepwise backward logistic regression model of the unmatched individuals. The age-sex matched model represents a stepwise backward logistic regression model of the age- and sex-matched individuals.

**Role of erythropoietin use in osteoporosis development**

A receiver operating curve was generated to assess the area under the curve (AUC) to predict the risk of osteoporosis in patients on dialysis receiving cumulative EPO doses. A logarithmic scale was used to examine all three EPO dose conversion models and the development of osteoporosis. The AUC varied between 0.698 and 0.714 and showed moderate utility in predicting osteoporosis development in patients on dialysis (Figure 3).

## Discussion

This study found a moderate reduction in the mean BMD in this unselected population of patients on chronic hemodialysis. The mean T-score of -2.17 in the DXA measurement of the femoral neck implies that these patients had moderately less favorable outcomes than age-matched controls. This is similar to the results of several other studies that used the same bone mineral density measurement[17]. Age and weight also emerged as important determinants of BMD in our study. Age-related bone loss plays an essential role in the pathogenesis of osteoporosis, and a negative association between age and BMD in female patients with end-stage renal disease has been reported[18,19]. The mean age of patients in these two studies was 43 and 50.5 years, whereas in our study, patients were older, with a mean age of 66.6 years. With the number of older adults involved in the renal replacement program increasing and with survival rates markedly improving, age-related bone loss can be expected to become an increasingly important factor causing bone disease in these patients.

Moreover, evidence has revealed a positive correlation between weight and BMD in healthy populations[20]. This has been attributed to bone formation stimulations through weight-bearing and adipose tissues' increased peripheral conversion of adrenal androgens to estrogens. Two studies have reported a positive association between BMI and BMD measurements[21,22]. We showed a similar association in our patients. Finally, we found a significant difference in cumulative EPO use in patients with osteoporosis compared with those without osteoporosis in both univariate and multivariate analyses (Table 5).

EPO is administered based on the patient's weekly hemoglobin levels at our institution. EPO doses received were positively correlated with patient dialysis duration. The longer the patient undergoes dialysis, the higher the dose of EPO the patient may receive. However, no statistically significant differences in dialysis vintage were found between patients with osteoporosis and those without [$P = 0.762$ (unmatched), $P = 0.111$ (age- and sex-matched)]. All three models, LogEPO1, LogEPO2, and LogEPO3, showed significant differences in cumulative EPO in patients with osteoporosis compared with those without (Table 5). A negative correlation was observed between the total, lumbar, right femoral neck, and left femoral neck T-scores and EPO dose (Figure 1). Although these results showed a low and negative correlation between T-scores and EPO dose (Pearson's correlation coefficient $r$ from 0.30 to 0.46), these data reached statistical significance ($P < 0.005$ to $< 0.0001$).

Higher EPO dosages were administered in patients with lumbar spine osteoporosis than patients with cortical bone osteoporosis (right or left femur). However, no statistical significance was reached in the current study (Figure 1). Effects of erythropoietin-induced bone loss had been demonstrated in experimental mice.[10,11]However, clinical evidence concerning EPO with bone mineral density is lacking. Whether EPO exerts more trabecular bone loss or cortical bone loss remains to be elucidated.

Serum PTH is negatively associated with BMD measurements; cortical porosity increased in patients with hyperparathyroidism[23]. Several studies have reported a negative association between PTH levels and BMD measurements[18,24,25], whereas others were unable to show this association[26-28]. In the present study, however, we found a negative association between PTH levels and BMD measurements, suggesting that other factors affect BMD in patients on hemodialysis. Forty-three patients received active vitamin D treatment in the current study.

Aluminum overload may be responsible for adynamic bone disease and osteomalacia. At our institution, serum aluminum levels are measured annually in patients who have undergone dialysis for >5 years. Our patients had no abnormally elevated serum aluminum levels. Moreover, we did not perform a histological analysis of bone. Thus,

we cannot comment on the prevalence of adynamic bone disease and osteomalacia in this population.

The relationships between calcium intake, vitamin D supplementation, and osteoporosis development remain controversial. One study has shown that oral 1$\alpha$-hydroxycalciferol treatment could prevent BMD loss in the Lumbar Spine in a study of 165 male patients[29]. All 165 patients were receiving calcium-containing phosphate binders. Only ten patients received vitamin D supplements in the nonosteoporotic group compared with 33 patients who received vitamin D supplements in the osteoporotic group.

Clinical and molecular evidence suggests that chronic inflammation significantly influences bone turnover.[30,31]Uremic syndrome, hemodialysis, use of a catheter, and persistent infection may contribute to the development of the inflammatory state in CKD. In hemodialysis patients, inflammation has been associated with EPO resistance mainly because the inflammatory state decreases the bone marrow response to ESA, changing iron regulation through hepcidin upregulation and/or causing red blood cell/erythrocyte hemolysis.[32]In the current study, we had not studied the inflammatory status among patients with/without osteoporosis. However, some markers of inflammatory reaction had included in our laboratory study, including WBC, platelets, ferritin, and albumin. Both platelet and white cell counts have been implicated in playing an essential role in inflammatory reaction.[33,34]Similarly, both ferritin and albumin have also known as acute-phase proteins. WBC, platelets, ferritin, and albumin have not shown statistical differences between osteoporotic and non-osteoporotic patients in the age- and sex-matched model.

The strengths of our study are the random sampling of the population and the high accuracy of cumulative EPO treatment history collected. Participants with and without osteoporosis were age- and sex-matched to examine the association of EPO treatment with the risk of osteoporosis development. However, the present study was limited by its cross-sectional nature. It is difficult to establish the causal relationship between EPO accumulation and the risk of osteoporosis. A further longitudinal study is required to confirm the cause and effect of EPO in reducing BMD. Moreover, the present study involves a group of elderly participants. Our subgroup analysis showed that participants aged <65 years with osteoporosis did not receive a higher EPO dose

than participants aged >65 years with osteoporosis (r = −0.21, $P = 0.133$, data not shown).

In conclusion, we confirmed the importance of age and body weight as the risk factors affecting BMD in patients on hemodialysis. We found that the cumulative EPO dose has a negative correlation in dialysis patients' BMD. Elderly dialysis patients under long-term EPO treatment are at risk of developing osteoporosis. Managing anemia in this vulnerable population may consider other possible therapeutic strategies.

## Declarations

### Ethics approval and consent to participate

Taipei Medical University Institutional Review Board approved the study for Human Experimentation (TMU-eJIRB, N202103059).

### Data sharing

All free text entered will be published

### Competing interests

The authors declare that they have no competing interests.

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The present study has no funding available.

### Authors' contributions

Conceptualization, formal analysis, investigation, methodology, original draft writing, C-Y.C.; conceptualization, data curation, investigation, methodology, resources, and writing review and editing, Y-J-K.

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## Figure legends

**Figure 1** Descriptive plots of correlations between T-scores of L-spine, total right femur, total left femur, and cumulative Erythropoietin (EPO) dose received. EPO1, EPO2, and EPO3 represent three different dose conversion models. EPO1, 1 μg of darbepoietin/Mircera converts to 200 IU of EPO; EPO2, 1 μg of darbepoietin/Mircera converts to 300 IU of EPO; EPO3, 1 μg of darbepoietin/Mircera converts to 400 IU of EPO.

**Figure 2** Three different models of erythropoietin dose conversion versus bone mineral density among three different sites in dialysis patients with osteoporosis. Abbreviations: BMD, bone mineral density; EPO, erythropoietin; L1-L4, lumbar-spine 1 to 4; RFN, right femoral neck; RFT, total right femur; LFN, left femoral neck; LFT, total left femur.

**Figure 3** Receiver operating characteristic (ROC) curves of prediction models for cumulative erythropoietin dose converted to a logarithmic scale that incorporates patients with and without osteoporosis. AUC = area under the ROC curve; LogEPO1, the logarithmic scale of EPO1; LogEPO2, the logarithmic scale of EPO2; LogEPO3, the logarithmic scale of EPO3.

**Figure 1**

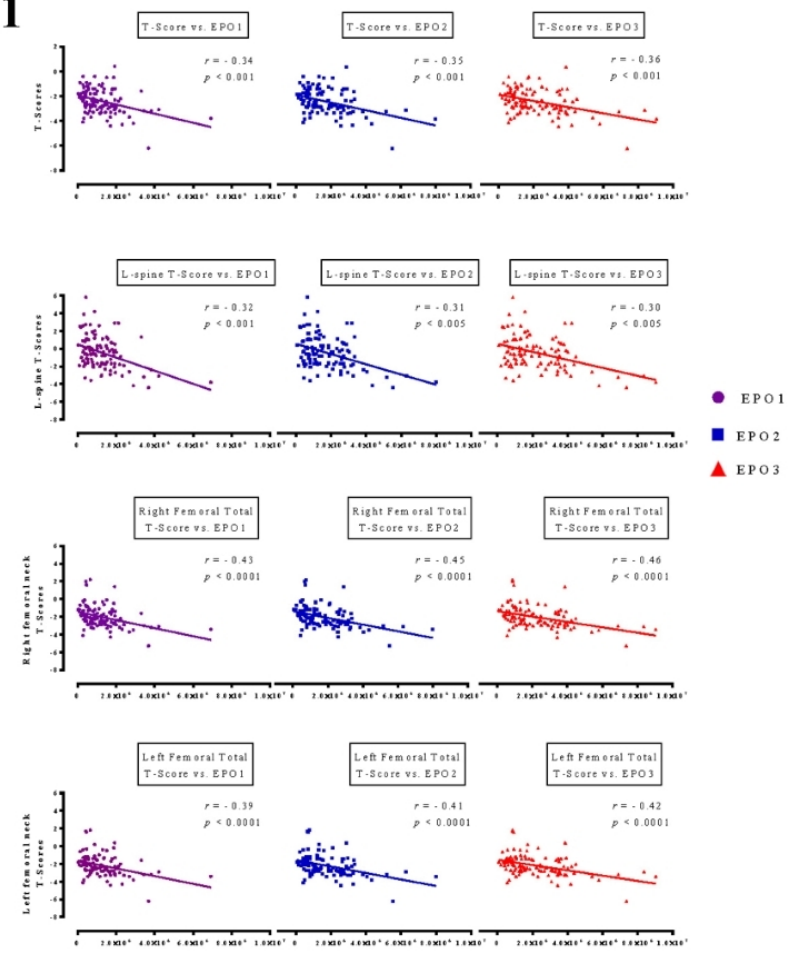


Figure 1 Descriptive plots of correlations between T-scores of L-spine, total right femur, total left femur, and cumulative Erythropoietin (EPO) dose received. EPO1, EPO2, and EPO3 represent three different dose conversion models. EPO1, 1 μg of darbepoietin/Mircera converts to 200 IU of EPO; EPO2, 1 μg of darbepoietin/Mircera converts to 300 IU of EPO; EPO3, 1 μg of darbepoietin/Mircera converts to 400 IU of EPO.

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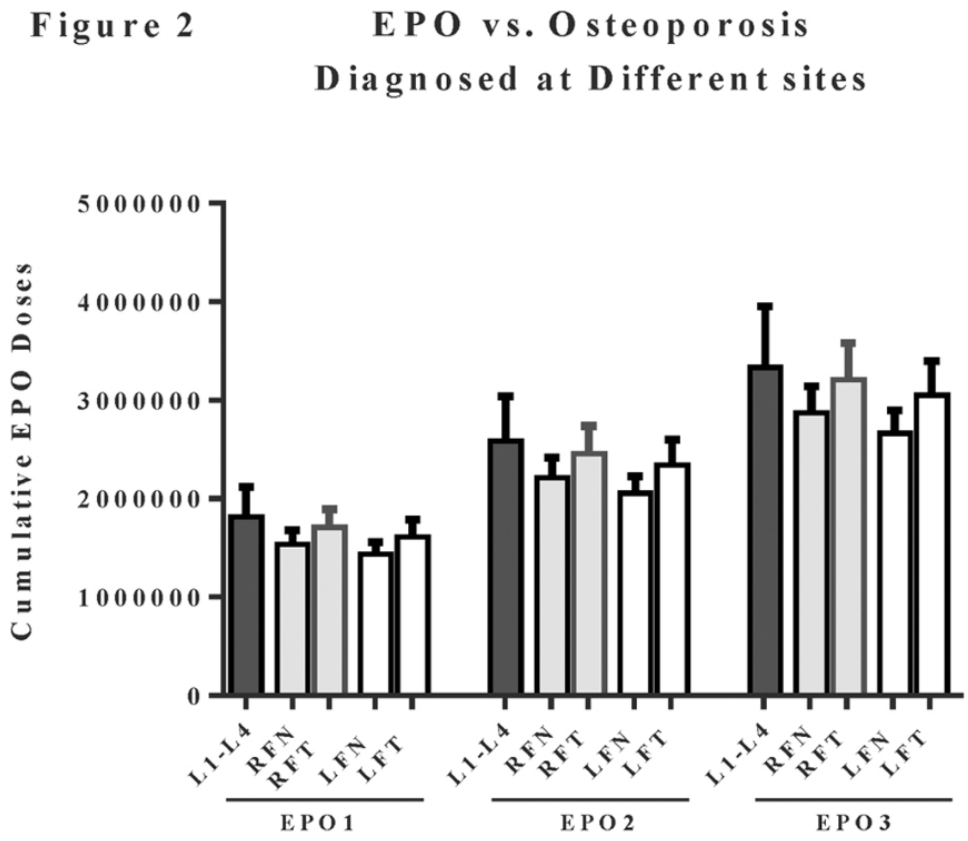


Figure 2 Three different models of erythropoietin dose conversion versus bone mineral density among three different sites in dialysis patients with osteoporosis. Abbreviations: BMD, bone mineral density; EPO, erythropoietin; L1-L4, lumbar-spine 1 to 4; RFN, right femoral neck; RFT, total right femur; LFN, left femoral neck; LFT, total left femur.

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**Figure 3**

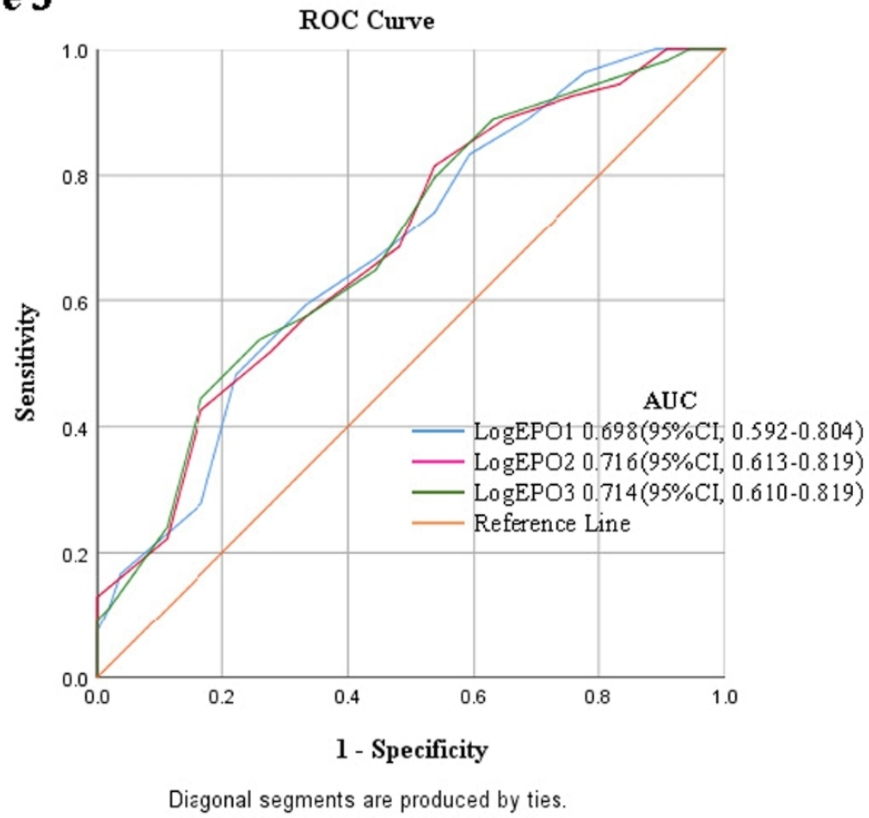


Figure 3 Receiver operating characteristic (ROC) curves of prediction models for cumulative erythropoietin dose converted to a logarithmic scale that incorporates patients with and without osteoporosis. AUC = area under the ROC curve; LogEPO1, the logarithmic scale of EPO1; LogEPO2, the logarithmic scale of EPO2; LogEPO3, the logarithmic scale of EPO3.

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# A Single-Center Cross-Sectional Study on the Impact of Cumulative Erythropoietin on Bone Mineral Density in Maintenance Dialysis Patients

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## Abstract

### Objectives

Numerous factors are associated with the risk of osteoporosis in chronic kidney disease (CKD) patients, including vitamin D deficiency, hypocalcemia, hyperphosphatemia, and secondary hyperparathyroidism. The present study aimed to assess the correlation between cumulative erythropoietin (EPO) doses and osteoporosis risk in patients on chronic dialysis. A further objective was to determine the bone mineral density (BMD) of patients undergoing dialysis and its correlation with specific clinical and biochemical factors.

### Setting

The study was undertaken at a tertiary care center within the southern region of the Taipei Metropolitan area.

### Participants

This cross-sectional study included 165 participants aged 41–90 years. Dual-energy X-ray absorptiometry was used to measure BMD. A total of 108 age- and sex-matched participants were selected for further analysis. Stepwise multiple regression analysis was used to investigate the relationship between bone measurements and bone diseases' risk factors.

### Primary and Secondary outcomes

The primary outcome of this study was to assess the T-scores of the participants who received dialysis for more than three months in our institution. The secondary outcome was using a receiver operating curve to predict osteoporosis development in patients on dialysis who received EPO treatments.

### Results

The mean age of the participants was 66.6 ± 11.1 years. A total of 99 (60%) participants (41 men, 58 women) were diagnosed as having osteoporosis. Fifty-four

(32.7%) participants with T-scores >−2.5 but <−1.0 were diagnosed as having osteopenia. Osteoporotic participants received 1.61 ± 1.52 million) EPO units compared to nonosteoporotic participants, who received 1.01 ± 0.64 million units (EPO1 model), $P = 0.015$. The cumulative EPO dose negatively correlated with the T-scores of participants ($P < 0.0001$).

**Conclusion**

On the basis of the results of the study, cumulative EPO doses show a negative correlation with BMD development in patients on chronic dialysis.

**Strengths and Limitations of this study**

- The present study presents a novel finding by elucidating the correlation of exogenous erythropoietin administration with the risk of reducing bone mineral density in the chronic dialysis Asian population.
- A sex and aged-match analysis increases the strength of the present study.
- The multivariate analysis identified the confounding factors to substantiate our study hypothesis.
- The present study is limited by a single-center experience on a relatively old age group of patients and a relatively small sample size of 165 patients.
- The study's retrospective nature is challenging to conclude the causal relationship between Erythropoietin and osteoporosis in dialysis patients.

## Introduction

Bone disease associated with chronic kidney disease (CKD) involves complicated biochemical and hormonal molecular interactions. In addition to bone abnormalities in patients with CKD–mineral bone disorders (CKD–MBDs), such as secondary hyperparathyroidism, osteomalacia, and adynamic bone disease, osteoporosis is another prevalent bone disease in patients with CKD. CKD patients with osteoporosis are at a higher risk of bone fractures[1] and have reduced quality of life[2]. Considering the increasing prevalence of CKD among aging populations, diagnosis and treatment of osteoporosis in a patient with CKD deserve more attention.

In CKD patients, biochemical alterations resulting in vitamin D deficiency, hypocalcemia, hyperphosphatemia, and secondary hyperparathyroidism can cause deterioration of the cortical bone architecture, leading to reduced cortical density and increased cortical porosity earlier in the course of CKD than previously thought[3]. Osteoporosis is a decrease in bone mineral density (BMD). Dual-energy X-ray absorptiometry (DXA) is the most common method for measuring BMD and is considered the current gold standard for osteoporosis diagnosis. According to the World Health Organization (WHO) criteria, the standard BMD value (the average in young, healthy women) is a T-score of $\geq -1.0$. T-score values between $-1.0$ and $-2.5$ are considered to indicate low bone density or osteopenia. A T-score of $\leq -2.5$ is considered to indicate osteoporosis.

More than two decades ago, the introduction of recombinant human Erythropoietin (EPO) in clinical practice completely altered CKD management. Treatment of renal anemia with EPO is now well established. The extensive use of EPO and its analogs

(EPO-stimulating agents [ESAs]) for anemia correction has reduced the associated morbidity and improved functionality, exercise tolerance, cognitive function, and overall quality of life. However, over the last few years, much controversy has been raised over the possible risks of ESA therapy. Moreover, a thorough investigation of the mechanism of action of EPO has revealed multiple biologic effects that extend beyond its erythropoietic effect and may have a favorable or sometimes unfavorable contribution to these outcomes.

EPO acts on erythroid progenitor cells by binding to an EPO receptor (EPOR), promoting survival, proliferation, and differentiation[4]. Functioning EPOR is present in endothelial cells[5], neurons[6], skeletal muscle progenitor cells[7], adipocytes[5], and islets[8], suggesting that EPO signaling exerts systemic regulation and interacts with nonerythroid cells through actions beyond erythropoiesis. Growing evidence from animal studies has demonstrated the critical role of EPO in regulating skeletal homeostasis[9,10]. Moreover, recent evidence has also demonstrated that EPO reduced trabecular bone volume in a mouse model of diet-induced obesity[11]. However, for humans, insufficient evidence exists on the role of EPO in mediating the bone microenvironment.

This study aimed to assess the correlation between cumulative doses of EPO administration and the risk of osteoporosis in patients on chronic dialysis. Moreover, bone mineral density in the femur and lumbar spine of patients on dialysis was investigated, its correlation with some clinical and biochemical factors was determined.

## Materials and methods

### Study design

A single center cross-sectional study

### Study population

Taipei Medical University, Wan Fang Hospital is a tertiary care hospital in Taipei. On average, there are 300 hemodialysis and 60 peritoneal dialysis patients under our maintenance renal replacement therapy program. Patients aged >20 years with end-stage renal disease and who were undergoing renal replacement therapy (hemodialysis or peritoneal dialysis) for more than one year were recruited. Patients on steroids, antiresorptive drugs (bisphosphonates), contraceptives, or calcitonin, and those who received parathyroidectomy were excluded from the study. Patients who did not initiate dialysis in our hospital were also excluded from the study due to the limitation in calculating cumulative EPO doses. Patients who were able to complete an interview were considered eligible. Of the 170 patients who gave consent, one died, three failed to undergo a DXA scan, and one DXA scan failed due to technical problems; the remaining 165 patients (74 males [44.8%] and 91 females [55.2%]) completed the study, and their demographic data and biochemistry are summarized in Table 1. The causes of chronic renal failure were diabetic nephropathy (DMN) (90 patients, 54.5%), chronic glomerulonephritis (37 patients, 22.4%), hypertensive nephrosclerosis (24 patients, 14.5%), adult polycystic kidney disease (7 patients, 4.4%), chronic renal failure of unknown etiology (6 patients, 3.6%), and chronic tubulointerstitial nephritis patient (1 patient, 0.6%). The mean duration of dialysis was 6.3±5.4 years, and the number of hours of dialysis per week was 9.5–16.5 h, with a

mean of 11.2 h. The dialysate calcium concentration was 2.5 meq/L in 30 patients, 3.0 meq/L in 75 patients, and 3.5 meq/L in 60 patients.

| Variables | Values ( N = 165) | Men ($n$ = 74) | Women ($n$ = 91) | $p$ value |
|---|---|---|---|---|
| Age (years) | 66.6 ± 11.1 | 66.9 ± 9.9 | 66.3 ± 12.0 | 0.519 |
| BMI (kg/m$^2$) | 23.4 ± 3.4 | 23.9 ± 3.2 | 22.8 ± 3.6 | 0.010* |
| BW (kg) | 59.4 ± 10.6 | 66.0 ± 9.2 | 54.1 ± 8.6 | < 0.0001**** |
| Dialysis vintage (yrs) | 6.3 ± 5.4 | 5.9 ± 5.2 | 6.1 ± 4.9 | 0.772 |
| Ca (mg/dl) | 9.1 ± 0.8 | 9.2 ± 0.7 | 9.0 ± 0.8 | 0.036* |
| P (mg/dl) | 5.0 ± 1.3 | 5.0 ± 1.3 | 5.1 ± 1.4 | 0.811 |
| Intact PTH (pg/ml) | 362.9 ± 364.3 | 343.0 ± 345.3 | 379.1 ± 380.2 | 0.508 |
| ALP (μg/l) | 97.2 ± 54.6 | 93.8 ± 53.0 | 100.0 ± 56.1 | 0.324 |
| TG (mg/dl) | 186.5 ± 131.9 | 182.5 ± 113.0 | 189.7 ± 145.9 | 0.937 |
| T-Chol (mg/dl) | 153.8 ± 34.9 | 141.3 ± 30.7 | 164.0 ± 34.9 | < 0.0001**** |
| Alb (g/dL) | 3.7 ± 0.4 | 3.8 ± 0.3 | 3.6 ± 0.4 | 0.0005*** |
| AC glucose (mg/dl) | 146.9 ± 70.3 | 148.1 ± 73.1 | 145.9 ± 68.4 | 0.907 |
| Na (mmol/l) | 136.1 ± 3.5 | 136.0 ± 3.5 | 136.3 ± 3.4 | 0.474 |
| K (mmol/l) | 4.4 ± 0.7 | 4.4 ± 0.8 | 4.4 ± 0.7 | 0.451 |
| Uric acid (mg/dl) | 6.9 ± 1.8 | 6.8 ± 1.8 | 7.0 ± 1.8 | 0.521 |
| Hb (g/dl) | 10.3 ± 0.9 | 10.4 ± 1.0 | 10.1 ± 0.8 | 0.093 |
| Ferritin (ng/ml) | 531.4 ± 426.9 | 442.9 ± 307.0 | 603.4 ± 493.9 | 0.008* |
| EPO1 (x10$^6$ units) | 1.38 ± 1.77 | 1.22 ± 1.38 | 1.51 ± 1.35 | 0.847 |
| EPO2 (x10$^6$ units) | 1.92 ± 1.80 | 1.63 ± 1.62 | 2.15 ± 1.91 | 0.414 |
| EPO3 (x10$^6$ units) | 2.45 ± 2.31 | 2.08 ± 2.01 | 2.76 ± 2.50 | 0.295 |
| T-score | -2.8 ± 2.6 | -2.5 ± 1.1 | -3.1 ± 3.3 | 0.291 |
| L-spine BMD (g/cm$^2$) | 1.093 ± 0.264 | 1.218 ± 0.247 | 0.991± 0.233 | < 0.0001**** |
| RF-T BMD (g/cm$^2$) | 0.769 ± 0.223 | 0.820 ± 0.247 | 0.728 ± 0.194 | 0.003** |
| LF-T BMD (g/cm$^2$) | 0.757 ± 0.228 | 0.817 ± 0.240 | 0.707 ± 0.207 | < 0.0001**** |
| HD/PD | 125/40 | 57/17 | 68/23 | 0.186 |
| DM | 97 | 48 | 49 | 0.012 |
| Hypertension | 148 | 68 | 80 | 0.175 |
| CHF | 23 | 8 | 15 | 0.213 |
| CAD | 44 | 23 | 21 | 0.189 |
| Vitamin D treatment | 35 | 14 | 21 | 0.531 |

**Table 1** Basic Characteristics of the Study Participants and Comparison between men and women

Abbreviations: A.C. glucose, fasting glucose; Alb, albumin; ALP, alkaline phosphatase; BMI, body mass index; B.W., body weight; CAD, coronary artery disease; Ca, calcium; CHF, congestive heart failure; D.M., diabetes mellitus; EPO, Erythropoietin; Hb, hemoglobin; H.D., hemodialysis; K, potassium; LF-T BMD, total left femur bone mineral density; L-spine BMD, lumbar-spine bone mineral density; Na, sodium; P, phosphorus; intact PTH, intact parathyroid hormone; P.D., peritoneal dialysis; RF-T BMD, total right femur bone mineral density; T.G., triglyceride; T-Chol, total cholesterol; yrs, years. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$, $^{****}p < 0.0001$.

A detailed history of related risk factors (smoking, hypertension, diabetes, steroid intake, and surgical menopause) was obtained from all patients, and medical records were checked after consent was obtained. The continuous medical records were available from January 2000 to December 2020. Before initiating the dialysis session, baseline investigations were performed at the patient's regular blood test session. Blood tests included kidney function tests, serum calcium, serum phosphorus, intact parathyroid hormone, fasting glucose, serum alkaline phosphatase levels, liver function tests, complete blood counts, ferritin, and determination of lipid profiles.

The DXA definition of osteoporosis and the bone mass criteria followed for its diagnosis were adopted from the WHO definition of osteoporosis (1994). T-scores were used for the evaluation of BMD and the definition of different stages of BMD according to the WHO definition of osteoporosis. T-scores were obtained for the femoral necks and lumbar spines (L1-L4). The average of lumbar spine BMD was to evaluate the lumbar spine T-score, use of three vertebrae if four cannot be used, and two if three cannot be used for the diagnosis according to the (The International

Society for Clinical Densitometry, ISCD) guideline.[12] The lowest T-score among femoral necks and lumbar spines was accounted for established osteoporosis. The T-score Normative Database is calculated by using USA (Combined NHANES (ages 20-30)/Lunar (ages 20-40) A.P. spine and Femur Reference Population).

**EPO dose conversion**

Patients receive either darbepoetin alfa (DPO) (Aranesp, Kyowa Hakko Kirin Co., Ltd.), epoetin beta (Recormon, Roche), or methoxy polyethylene glycol-epoetin beta (Mircera, Roche) at our institution. EPO doses are administered according to the patient's weekly hemoglobin levels. We maintain our patients' hemoglobin levels between 10 and 12 g/dl. For conversion from EPO alfa to darbepoetin alfa, a fixed conversion ratio of 200 IU EPO to 1 μg DPO was suggested by the manufacturer[13]. However, numerous studies have suggested that the conversion ratio be 240–400 IU of EPO and 1 μg of DPO[14-16]. In the current study, the cumulative dose of EPO received by the patient was calculated from the first day received EPO in our hospital until the DXA study date. The patient might receive various EPOs during their dialysis treatment in our institution. We established three conversion doses of darbepoetin alpha (DPO) and methoxy polyethylene glycol-epoetin beta (Mircera) to calculate the statistical difference between patients with and without osteoporosis. EPO1 refers to converting 1 μg of DPO/Mircera to 200 IU of EPO, EPO2 converting 1 μg of DPO/Mircera to 300 IU of EPO, and EPO3 converting 1 μg of DPO/Mircera to 400 IU of EPO.

**Ethical approval**

The study was approved by the Taipei Medical University Institutional Review Board for Human Experimentation. The accession number: TMU-IRB N202103059.

Informed consent was obtained from all subjects involved in the present study.

## Patient and Public Involvement

Patients and the public were not directly involved in this research. The nature of the anonymised records means individual participants could not be involved.

## Statistical analysis

Data were expressed as mean ± standard deviation unless otherwise specified. Pearson's correlation coefficients assessed correlations between bone measurements and cumulative EPO doses. Stepwise multiple regression analysis was used to investigate the relationships between bone measurements and biochemical markers or risk factors for bone diseases. The backward stepwise regression method was used to select variables in the multivariate analysis. Only a single log-transformed value of EPO was selected at every entry for multivariate analysis to avoid errors generated due to the collinearity of log EPOs. It means either log EPO1, log EPO2 or log EPO3 input into the multivariate analysis but not all three log EPOs entries. Differences between the means of multiple subgroups were assessed using the Kruskal–Wallis test. An unpaired t-test or Mann–Whitney U test was used for continuous variables. The chi-square test was used to compare frequencies between categorical variables. SPSS version 25 (SPSS Inc., Chicago, IL, USA) was used for analysis. A p-value of $<0.05$ was considered statistically significant.

## Results

### Bone mineral densitometry

Bone mineral densitometry measurements of the 165 patients are shown in Table 2. A good correlation was found between BMD measurements of the right and left femur ($r = 0.76$; $P < 0.0001$). However, lower correlation coefficients of BMD measurements were noted between lumbar spine values and right femoral neck ($r = 0.50$; $P < 0.0001$) and left femoral neck ($r = 0.54$; $P < 0.0001$) values, but they were still statistically significant. Ninety-nine patients with T-scores of ≤−2.5 were diagnosed with osteoporosis, and 54 patients with T-scores <−1.0 but > (−2.5 were diagnosed with osteopenia. Only twelve patients had T-scores of >−1.0.

**Table 2** Results of bone mineral densitometry measurements of patients on dialysis

| | BMD (g/cm$^2$) | T-score (SD) | Osteopenia | | Osteoporosis | |
|---|---|---|---|---|---|---|
| | | | N | % | N | % |
| L-spine | 1.093 ± 0.264 | -0.67 ± 1.85 | 54 | 32.7 | 27 | 16.4 |
| RF Neck | 0.769 ± 0.223 | -2.17 ± 1.27 | 74 | 44.8 | 51 | 30.9 |
| RF Total | 0.842 ± 0.225 | -1.72 ± 1.31 | 68 | 41.2 | 48 | 29.1 |
| LF Neck | 0.757 ± 0.228 | -2.31 ± 1.24 | 77 | 46.7 | 53 | 32.1 |
| LF Total | 0.839 ± 0.231 | -1.78 ± 1.29 | 72 | 43.6 | 54 | 32.7 |
| Total | - | -2.62 ± 1.14 | 54 | 32.7 | 99 | 60 |

Osteopenia: T-score < −1.0 but > −2.5; Osteoporosis: T-score ≤ −2.5
Total: the lowest T-score found among femoral necks and lumbar spines.
Abbreviations: BMD, bone mineral density; L.F. Neck, left femoral neck; L-spine, lumbar-spine; R.F. Neck, right femoral neck.

### Factors associated with reduced bone mineral density

In total, 165 patients with and without osteoporosis were evaluated for differences in risk factors for and biochemical markers of bone diseases. These included all the

factors in Table 3, and individual variables were evaluated using Student's t-test. Independent variables that were analyzed and reached statistical significance ($P < 0.05$) are shown in Table 3. Age, body mass index (BMI), body weight (B.W.), serum calcium, ferritin, and EPO doses show statistical differences between patients with osteoporosis and patients without osteoporosis. Furthermore, 108 age- and sex-matched patients were evaluated for differences in risk factors for and biochemical markers of bone diseases. These included all the factors listed in Table 4. Cumulative EPO dosage was significantly different in age- and sex-matched patients with osteoporosis than nonosteoporotic patients on dialysis. All three EPO conversion models showed similar and significant results. Three models of EPO dose conversion were used to examine the association between EPO and T-scores of participants. The statistical calculation process was repeated using different EPO dose models to avoid collinearity. The results are shown in Figure 1. Pearson's correlation coefficient varied between −0.30 and −0.46, but *p* values were statistically significant.

**Table 3** The clinical and laboratory characteristics of patients with and without

| Variables | OS ($n$ = 99) | Without OS ($n$ = 66) | $p$ value |
|---|---|---|---|
| Age (years) | 70.0 ± 9.9 | 61.4 ± 10.8 | < 0.0001**** |
| Men/women | 41/58 | 33/33 | 0.278 |
| BMI (kg/m$^2$) | 22.7 ± 3.5 | 24.1 ± 3.2 | 0.009** |
| BW (kg) | 58.2 ± 14.6 | 62.7 ± .10.4 | 0.040* |
| Dialysis vintage (years) | 6.3 ± 5.5 | 6.1 ± 5.2 | 0.762 |
| Ca (mg/dL) | 9.0 ± 0.8 | 9.2 ± 0.7 | 0.028* |
| P (mg/dL) | 5.0 ± 1.4 | 5.2 ± 1.4 | 0.227 |
| Intact PTH (pg/mL) | 367.7 ± 398.2 | 353.4 ± 310.9 | 0.805 |
| ALP (μg/L) | 99.6 ± 54.8 | 93.1 ± 54.5 | 0.456 |
| TG (mg/dL) | 187.8 ± 128.8 | 183.7 ± 137.2 | 0.843 |
| T-Chol (mg/dL) | 154.2 ± 36.9 | 153.4 ± 31.6 | 0.884 |
| Alb (g/dL) | 3.7 ± 0.4 | 3.7 ± 0.3 | 0.184 |
| AC Glucose mg/dL) | 147.1 ± 71.6 | 153.0 ± 80.3 | 0.618 |
| Na (mmol/L) | 136.1 ± 3.4 | 136.1 ± 3.7 | 0.905 |
| K (mmol/L) | 4.3 ± 0.7 | 4.5 ± 0.8 | 0.201 |
| Uric acid (mg/dL) | 6.8 ± 1.8 | 7.0 ± 1.8 | 0.627 |
| Hb (g/dL) | 10.3 ± 0.8 | 10.2 ± 1.1 | 0.383 |
| WBC (/μl) | 7090 ± 636.7 | 6366 ± 199.5 | 0.365 |
| Platelet ( x10$^3$/μl) | 182.50 ± 6.30 | 179.20 ± 7.08 | 0.732 |
| Ferritin (ng/ml) | 592.7 ± 45.03 | 439.4 ± 36.51 | 0.023* |
| EPO1 (10$^6$ units) | 1.61 ± 1.52 | 1.01 ± 0.64 | 0.015* |
| EPO2 (10$^6$ units) | 2.23 ± 1.93 | 1.42 ± 0.92 | 0.013* |
| EPO3 (10$^6$ units) | 2.82 ± 2.45 | 1.87 ± 1.22 | 0.039* |
| T-score | -3.3 ± 0.78 | -1.5 ± 0.6 | < 0.0001**** |
| L-spine BMD | 1.012 ± 0.232 | 1.214 ± 0.264 | < 0.0001**** |
| RF-T BMD | 0.770 ± 0.025 | 0.952 ± 0.015 | < 0.0001**** |
| LT-T BMD | 0.749 ± 0.021 | 0.979 ± 0.024 | < 0.0001**** |
| HD/PD | 79/20 | 46/20 | 0.140 |
| DM | 58 | 39 | 0.949 |
| Hypertension | 88 | 60 | 0.676 |
| CHF | 17 | 6 | 0.148 |
| CAD | 27 | 17 | 0.829 |

osteoporosis

T-scores represents the lowest value among the three areas of BMD measurements. Abbreviations: A.C. glucose, fasting glucose; Alb, albumin; ALP, alkaline phosphatase; BMI, body mass index; B.W., body weight; CAD, coronary artery disease; Ca, calcium; CHF, congestive heart failure; D.M., diabetes mellitus; EPO, Erythropoietin; Hb, hemoglobin; H.D., hemodialysis; K, potassium; LF-T BMD, total left femur bone mineral density; L-spine BMD, lumbar-spine bone mineral density; Na, sodium; O.S., osteoporosis; P, phosphorus; intact PTH, intact parathyroid hormone; P.D., peritoneal dialysis; RF-T BMD, total right femur bone mineral density; T.G., triglyceride; T-Chol, total cholesterol; WBC, white cell count. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$.

**Erythropoietin dosage associated with osteoporosis among three different sites of bone mineral density measurement**

Significantly higher EPO dosages were found among osteoporotic participants using BMD measured from lumbar spines, right total, and left total femur (Table 4a-4c). However, no statistical difference was found on the cumulative EPO doses (all three models) using different sites to diagnose osteoporosis (Figure 2).

**Table 4a** Association of cumulative dose of erythropoietin with L-spine BMD

| L-Spine | OS ($n$ = 27) | Without OS ( $n$ = 138) | p Value |
|---|---|---|---|
| M/F | 6/21 | 68/70 | |
| BMD | 0.95±0.20 | 1.14±0.26 | $0.001^{***}$ |
| EPO1 | 1.82±1.57 | 1.22±1.13 | $0.020^{*}$ |
| EPO2 | 2.59±2.35 | 1.71±1.41 | $0.010^{**}$ |
| EPO3 | 3.34±3.21 | 2.19±1.76 | $0.009^{**}$ |

Abbreviations: BMD, bone mineral density; EPO, Erythropoietin; L-spine, lumbar-spine; O.S., osteoporosis. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$.

**Table 4b** Association of cumulative dose of Erythropoietin with the total right femur BMD

| Right femur total | OS ( $n$ = 48 ) | Without OS ( $n$ = 117 ) | p Value |
|---|---|---|---|
| M/F | 15/33 | 59/58 | |
| BMD | 0.71±0.17 | 0.90±0.22 | $< 0.0001^{****}$ |

| | | | |
|---|---|---|---|
| EPO1 | 1.71±1.29 | 1.15±1.17 | 0.008** |
| EPO2 | 2.46±1.92 | 1.61±1.43 | 0.002** |
| EPO3 | 3.21±2.61 | 2.04±1.75 | 0.001*** |

Abbreviations: BMD, bone mineral density; EPO, Erythropoietin; L-spine, lumbar-spine; O.S., osteoporosis. ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0001$.

**Table 4c** Association of cumulative dose of Erythropoietin with the total left femur BMD

| Left femur total | OS ( $n = 54$ ) | Without OS ( $n = 111$ ) | p Value |
|---|---|---|---|
| M/F | 18/36 | 56/55 | |
| BMD | 0.71±0.18 | 0.90±0.23 | < 0.0001**** |
| EPO1 | 1.61±1.30 | 1.17±1.17 | 0.028* |
| EPO2 | 2.34±1.91 | 1.62±1.42 | 0.007** |
| EPO3 | 3.05±2.57 | 2.05±1.75 | 0.004** |

Abbreviations: BMD, bone mineral density; EPO, Erythropoietin; L-spine, lumbar-spine; O.S., osteoporosis. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

**Factors associated with osteoporosis in patients on dialysis**

Table 5 shows clinical factors associated with osteoporosis in age- and sex-matched chronic dialysis patients. All three EPO conversion models show significant cumulative EPO use among osteoporotic dialysis patients than nonosteoporotic dialysis patients. Table 6 shows factors associated with osteoporosis in patients on dialysis after different statistical models were applied. The univariate analysis results showed a statistically significant difference in age, BMI, ferritin's log-transformed value (logFerritin), and cumulative EPO's log-transformed value (logEPO) in osteoporotic patients compared to those without osteoporosis. Backward stepwise logistic regression was used to select multiple variables. Age, sex, B.W., BMI, hemoglobin, logFerritin, and a single entry of logEPO were selected as variables to enter the logistic regression model. In addition to age, ferritin, and EPO, both hemoglobin and B.W. were significantly different between patients with and without

osteoporosis. In the age- and sex-matched multivariate analysis model, the

| Variables | OS ($n$ = 54) | Without OS (n = 54) | $p$ value |
|---|---|---|---|
| Age (years) | 66.0 ± 9.0 | 62.9 ± 10.2 | 0.097 |
| Men/women | 28/26 | 28/26 | 1.0 |
| BMI (kg/m$^2$) | 23.0 ± 4.0 | 24.0 ± 3.0 | 0.142 |
| BW (kg) | 59.7 ± 11.7 | 62.6 ± .10.6 | 0.176 |
| Dialysis vintage (yrs) | 7.3 ± 5.7 | 5.7 ± 5.0 | 0.111 |
| Ca (mg/dL) | 9.1 ± 0.8 | 9.2 ± 0.7 | 0.524 |
| P (mg/dL) | 5.1 ± 1.4 | 5.2 ± 1.4 | 0.495 |
| Intact PTH (pg/mL) | 418.0 ± 419.5 | 329.2 ± 307.0 | 0.212 |
| ALP (μg/L) | 102. 8 ± 47.9 | 96.6 ± 57.6 | 0.240 |
| TG (mg/dL) | 195.9 ± 139.2 | 197.9 ± 144.6 | 0.941 |
| T-Chol (mg/dL) | 148.6 ± 40.3 | 155.1 ± 30.9 | 0.355 |
| Alb (g/dL) | 3.8 ± 0.3 | 3.8 ± 0.3 | 0.796 |
| AC Glucose mg/dL) | 138.8 ± 69.5 | 163.0 ± 84.4 | 0.106 |
| Na (mmol/L) | 136.5 ± 3.2 | 136.4 ± 3.6 | 0.844 |
| K (mmol/L) | 4.4 ± 0.8 | 4.5 ± 0.8 | 0.287 |
| Uric acid (mg/dL) | 7.1 ± 1.9 | 7.2 ± 1.7 | 0.823 |
| Hb (g/dL) | 10.4 ± 0.8 | 10.3 ± 1.1 | 0.486 |
| WBC (/μl) | 7595 ± 1142 | 6518 ± 231.3 | 0.357 |
| Platelet ( x10$^3$/μl) | 178.89 ± 7.79 | 183.37 ± 9.76 | 0.721 |
| Ferritin | 502.6 ± 365.9 | 439.3 ± 372.4 | 0.375 |
| EPO1 (x10$^6$ units) | 1.54 ± 1.19 | 0.94 ± 0.69 | 0.002*** |
| EPO2 (x10$^6$ units) | 2.15 ± 1.56 | 1.28 ± 0.91 | 0.001** |
| EPO3 (x10$^6$ units) | 2.76 ± 1.97 | 1.62 ± 1.18 | < 0.0001**** |
| T-score | -3.7 ± 4.0 | -1.6 ± 0.6 | < 0.0001**** |
| L-spine BMD | 1.029 ± 0.033 | 1.227 ± 0.037 | < 0.0001**** |

log-transformed EPOs are the only significant factors associated with osteoporosis.

**Table 5** Age- and sex-matched t-test analysis of chronic dialysis patient with and without osteoporosis

| | | | |
|---|---|---|---|
| RF-T BMD | 0.775 ± 0.033 | 0.962 ± 0.020 | < 0.0001**** |
| LF-T BMD | 0.737 ± 0.022 | 0.974 ± 0.026 | < 0.0001**** |
| HD/PD | 41/13 | 41/13 | - |
| DM | 29 | 35 | 0.244 |
| Hypertension | 45 | 49 | 0.256 |
| CHF | 10 | 6 | 0.283 |
| CAD | 15 | 14 | 0.830 |

Abbreviations: A.C. glucose, fasting glucose; Alb, albumin; ALP, alkaline phosphatase; BMI, body mass index; B.W., body weight; CAD, coronary artery disease; Ca, calcium; CHF, congestive heart failure; D.M., diabetes mellitus; EPO, Erythropoietin; Hb, hemoglobin; H.D., hemodialysis; K, potassium; LF-T BMD, total left femur bone mineral density; L-spine BMD, lumbar-spine bone mineral density; Na, sodium; OS, osteoporosis; P, phosphorus; intact PTH, intact parathyroid hormone; P.D., peritoneal dialysis; RF-T BMD, total right femur bone mineral density; T.G., triglyceride; T-Chol, total cholesterol; WBC, white cell count. ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0001$.

**Table 6** Factors associated with osteoporosis in dialysis patients of different statistical models

| | Univariate model | | Multivariate model | | Age-sex matched model | |
|---|---|---|---|---|---|---|
| | p Values | OR (95% C.I.) | p values | OR (95% C.I.) | p values | OR (95% C.I.) |
| Age (yrs) | < 0.0001**** | 1.08(1.05- 1.12) | 0.001*** | 1.07(1.03 - 1.12) | - | - |
| Sex | 0.278 | 0.71(0.38- 1.32) | 0.759 | 1.21(0.37 - 3.96) | - | - |
| BW (kg) | 0.053 | 0.97(0.95- 1.00) | 0.010* | 0.95(0.92 - 0.99) | 0.766 | 0.99(0.93 - 1.06) |
| BMI(kg/m$^2$) | 0.012* | 0.88(0.80- 0.97) | 0.065 | 0.95(0.74 - 1.20) | 0.461 | 0.92(0.75 - 1.14) |
| Hb (g/dl) | 0.508 | 1.13(0.80- 1.60) | 0.022* | 1.76(1.08 - 2.85) | 0.197 | 1.41(0.84 - 2.36) |
| LogFerritin | 0.003*** | 1.20(1.06- 1.36) | 0.033* | 2.96(1.09 - 8.03) | 0.656 | 1.30(0.42 - 4.03) |
| LogEPO1 | 0.007** | 1.08(1.02- 1.13) | 0.005** | 4.25(1.56 - 11.56) | 0.002*** | 9.11(2.18 - 38.0) |
| LogEPO2 | 0.007** | 1.07(1.02- 1.13) | 0.008** | 4.70(1.50 - 14.76) | 0.002*** | 10.61(2.43 - 46.4) |
| LogEPO3 | 0.007** | 1.07(1.02- 1.13) | 0.007** | 4.85(1.54 - 15.29) | 0.002*** | 11.32(2.52 - 50.9) |

Abbreviations: BMI, body mass index; LogFerritin, logarithmic scale Ferritin; LogEPO1, logarithmic scale EPO1; LogEPO2, logarithmic scale, LogEPO2; LogPO3, logarithmic scale EPO3. Multivariate model represents a stepwise backward logistic regression model of the unmatched individuals. The age-sex matched model represents a stepwise backward logistic regression model of the age- and sex-matched individuals. Only a single LogEPO

entered into the multivariate and age-sex model for analysis to avoid multicollinearity.

**Role of erythropoietin use in osteoporosis development**

A receiver operating curve was generated to assess the area under the curve (AUC) to predict the risk of osteoporosis in patients on dialysis receiving cumulative EPO doses. A logarithmic scale was used to examine all three EPO dose conversion models and the development of osteoporosis. The AUC varied between 0.698 and 0.714 and showed moderate utility in predicting osteoporosis development in patients on dialysis (Figure 3).

## Discussion

This study found a moderate reduction in the mean BMD in this unselected population of patients on chronic hemodialysis. The mean T-score of -2.17 in the DXA measurement of the femoral neck implies that these patients had moderately less favorable outcomes than age-matched controls. The mean T-score value found in the present study is similar to several other studies that used the same bone mineral density measurement[17]. Age and weight also emerged as important determinants of BMD in our study. Age-related bone loss plays an essential role in the pathogenesis of osteoporosis, and a negative association between age and BMD in female patients with end-stage renal disease has been reported[18,19]. The mean age of patients in these two studies was 43 and 50.5 years, whereas, in our study, patients were older, with a mean age of 66.6 years. With the number of older adults involved in the renal replacement program increasing and with survival rates markedly improving, age-related bone loss can be expected to become an increasingly important factor causing bone disease in these patients.

Moreover, evidence has revealed a positive correlation between weight and BMD in healthy populations[20]. The correlation between B.W. and BMD has been attributed to bone formation stimulations through weight-bearing and adipose tissues' increased peripheral conversion of adrenal androgens to estrogens. Two studies have reported a positive association between BMI and BMD measurements[21,22]. We showed a similar association in our patients. Finally, we found a significant difference in cumulative

EPO use in patients with osteoporosis compared with those without osteoporosis in univariate and multivariate analyses (Table 5).

EPO is administered based on the patient's weekly hemoglobin levels at our institution. EPO doses received were positively correlated with patient dialysis duration. The longer the patient undergoes dialysis, the higher the dose of EPO the patient may receive. However, no statistically significant differences in dialysis vintage were found between patients with osteoporosis and those without [$P = 0.762$ (unmatched), $P = 0.111$ (age- and sex-matched)]. All three models, logEPO1, logEPO2, and logEPO3 showed significant differences in cumulative EPO in patients with osteoporosis compared with those without (Table 5). A negative correlation was observed between the total, lumbar, right femoral neck, and left femoral neck T-scores and EPO dose (Figure 1). Although these results showed a low and negative correlation between T-scores and EPO dose (Pearson's correlation coefficient $r$ from 0.30 to 0.46), these data reached statistical significance ($P < 0.005$ to $< 0.0001$).

Higher EPO dosages were administered in patients with lumbar spine osteoporosis than patients with cortical bone osteoporosis (right or left femur). However, no statistical significance was reached in the current study (Figure 1). Effects of erythropoietin-induced bone loss had been demonstrated in experimental mice.[10,11]However, clinical evidence concerning EPO with bone mineral density is lacking. Whether EPO exerts more trabecular bone loss or cortical bone loss remains to be elucidated.

Serum PTH is negatively associated with BMD measurements; cortical porosity increased in patients with hyperparathyroidism[23]. Several studies have reported a negative association between PTH levels and BMD measurements[18,24,25], whereas others were unable to show this association[26-28]. In the present study, however, we found a negative association between PTH levels and BMD measurements, suggesting that other factors affect BMD in patients on hemodialysis. Forty-three patients received active vitamin D treatment in the current study.

Aluminum overload may be responsible for adynamic bone disease and osteomalacia. At our institution, serum aluminum levels are measured annually in patients who have undergone dialysis for >5 years. Our patients had no abnormally elevated serum

aluminum levels. Moreover, we did not perform a histological analysis of bone. Thus, we cannot comment on the prevalence of adynamic bone disease and osteomalacia in this population.

The relationships between calcium intake, vitamin D supplementation and osteoporosis development remain controversial. One study has shown that oral 1$\alpha$-hydroxycalciferol treatment could prevent BMD loss in the Lumbar Spine in a study of 165 male patients[29]. All 165 patients were receiving calcium-containing phosphate binders. Only ten patients received vitamin D supplements in the nonosteoporotic group compared with 33 patients who received vitamin D supplements in the osteoporotic group.

Clinical and molecular evidence suggests that chronic inflammation significantly influences bone turnover.[30,31]Uremic syndrome, hemodialysis, use of a catheter, and persistent infection may contribute to the development of the inflammatory state in CKD. In hemodialysis patients, inflammation has been associated with EPO resistance mainly because the inflammatory state decreases the bone marrow response to ESA, changing iron regulation through hepcidin upregulation and/or causing red blood cell/erythrocyte hemolysis.[32]In the current study, we had not studied the inflammatory status among patients with/without osteoporosis. However, some markers of inflammatory reaction had included in our laboratory study, including WBC, platelets, ferritin, and albumin. Both platelet and white cell counts have been implicated in playing an essential role in inflammatory reaction.[33,34]Similarly, both ferritin and albumin have also known as acute-phase proteins. In the age- and sex-matched model, WBC, platelets, ferritin, and albumin have not shown statistical differences between osteoporotic and nonosteoporotic patients.

The strengths of our study are the random sampling of the population and the high accuracy of cumulative EPO treatment history collected. Participants with and without osteoporosis were age- and sex-matched to examine the association of EPO treatment with the risk of osteoporosis development. However, the present study was limited by its cross-sectional nature. It is difficult to establish the causal relationship between EPO accumulation and the risk of osteoporosis. A further longitudinal study is required to confirm the cause and effect of EPO in reducing BMD. Moreover, the present study involves a group of elderly participants. Our subgroup analysis showed

that participants aged <65 years with osteoporosis did not receive a higher EPO dose than participants aged >65 years with osteoporosis (r = −0.21, $P = 0.133$, data not shown).

In conclusion, we confirmed the importance of age and body weight as the risk factors affecting BMD in patients on hemodialysis. We found that the cumulative EPO negatively correlates with dialysis patients' BMD. Elderly dialysis patients under long-term EPO treatment are at risk of developing osteoporosis. Managing anemia in this vulnerable population may consider other possible therapeutic strategies.

## Declarations

### Ethics approval and consent to participate

Taipei Medical University Institutional Review Board approved the study for Human Experimentation (TMU-eJIRB, N202103059).

### Data sharing

All free text entered will be published

### Competing interests

The authors declare that they have no competing interests.

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**Figure legends**

**Figure 1** Descriptive plots of correlations between T-scores of L-spine, total right femur, total left femur, and cumulative Erythropoietin (EPO) dose received. EPO1, EPO2, and EPO3 represent three different dose conversion models. EPO1, 1 μg of darbepoietin/Mircera converts to 200 IU of EPO; EPO2, 1 μg of darbepoietin/Mircera converts to 300 IU of EPO; EPO3, 1 μg of darbepoietin/Mircera converts to 400 IU of EPO.

**Figure 2** Three different models of erythropoietin dose conversion versus bone mineral density among three different sites in dialysis patients with osteoporosis. Abbreviations: BMD, bone mineral density; EPO, Erythropoietin; L1-L4, lumbar-spine 1 to 4; RFN, right femoral neck; RFT, total right femur; LFN, left femoral neck; LFT, total left femur.

**Figure 3** Receiver operating characteristic (ROC) curves of prediction models for cumulative erythropoietin dose transformed to a log value that incorporates patients with and without osteoporosis. AUC = area under the ROC curve; LogEPO1, the log-transformed value of EPO1; LogEPO2, the log-transformed value of EPO2; LogEPO3, the log-transformed value of EPO3.

**Figure 1**

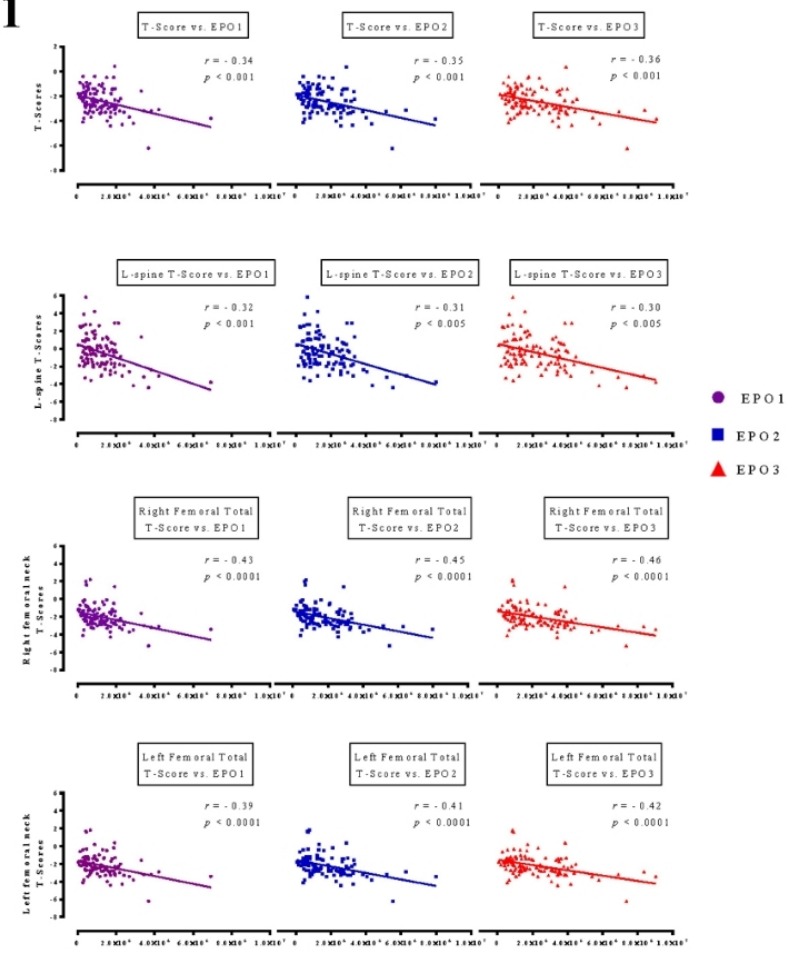


Figure 1 Descriptive plots of correlations between T-scores of L-spine, total right femur, total left femur, and cumulative Erythropoietin (EPO) dose received. EPO1, EPO2, and EPO3 represent three different dose conversion models. EPO1, 1 μg of darbepoietin/Mircera converts to 200 IU of EPO; EPO2, 1 μg of darbepoietin/Mircera converts to 300 IU of EPO; EPO3, 1 μg of darbepoietin/Mircera converts to 400 IU of EPO.

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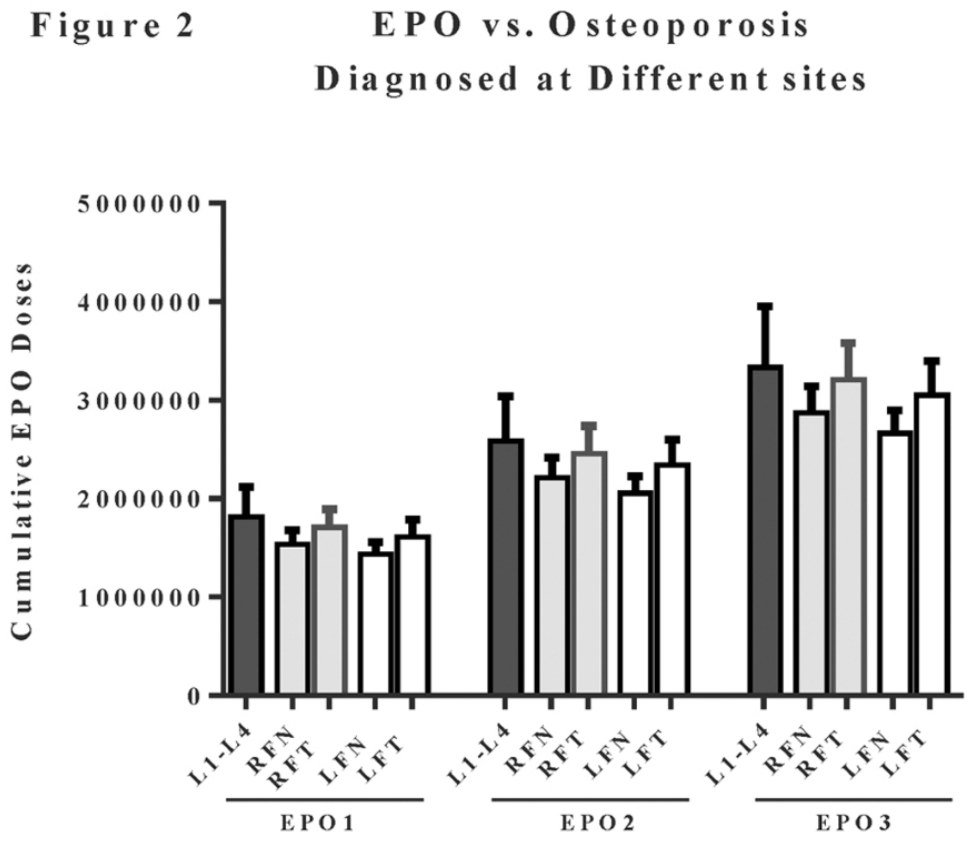


Figure 2 Three different models of erythropoietin dose conversion versus bone mineral density among three different sites in dialysis patients with osteoporosis. Abbreviations: BMD, bone mineral density; EPO, erythropoietin; L1-L4, lumbar-spine 1 to 4; RFN, right femoral neck; RFT, total right femur; LFN, left femoral neck; LFT, total left femur.

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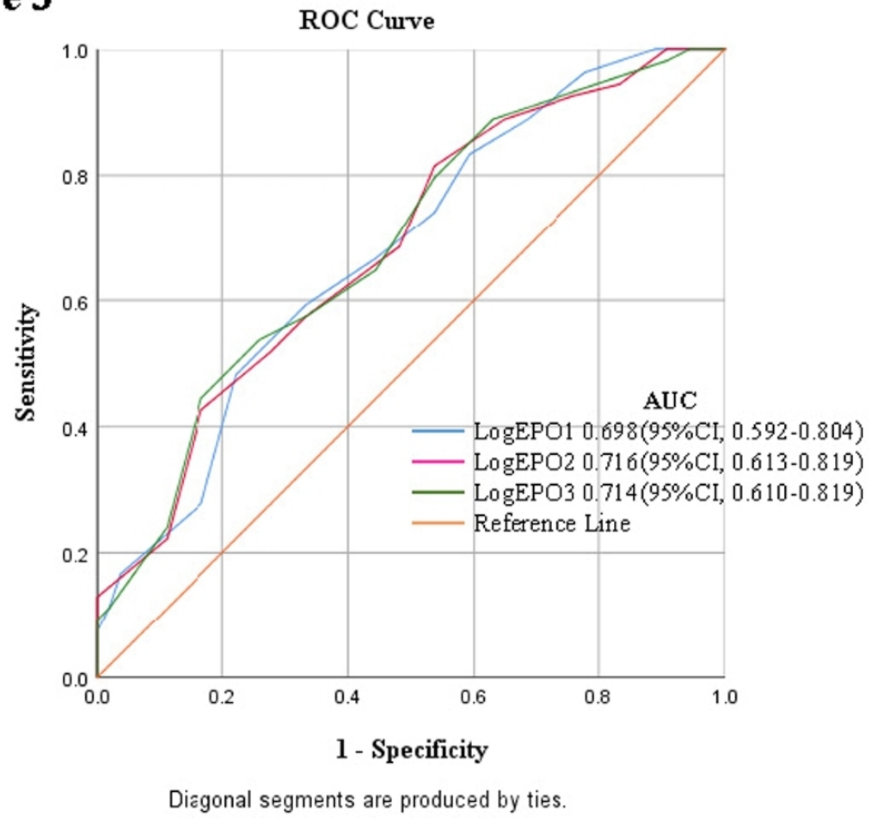


Figure 3 Receiver operating characteristic (ROC) curves of prediction models for cumulative erythropoietin dose transformed to a log value that incorporates patients with and without osteoporosis. AUC = area under the ROC curve; LogEPO1, the log-transformed value of EPO1; LogEPO2, the log-transformed value of EPO2; LogEPO3, the log-transformed value of EPO3.

76x76mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

| | Item No | Recommendation | Page No |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 3-4 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 5-6 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 6 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 7 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 7-10 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants | 7-10 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 7-11 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 7-12 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 7 |
| Study size | 10 | Explain how the study size was arrived at | 7-9 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 11-12 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 11-12 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 11-12 |
| | | (*c*) Explain how missing data were addressed | 11-12 |
| | | (*d*) If applicable, describe analytical methods taking account of sampling strategy | 11-12 |
| | | (*e*) Describe any sensitivity analyses | 11-12 |
| **Results** | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 10,12 |
| | | (b) Give reasons for non-participation at each stage | 12 |
| | | (c) Consider use of a flow diagram | - |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 10, 12 |
| | | (b) Indicate number of participants with missing data for each variable of interest | - |

| | | | |
|---|---|---|---|
| Outcome data | 15* | Report numbers of outcome events or summary measures | 12-19 |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 12-19 |
| | | (*b*) Report category boundaries when continuous variables were categorized | 12-19 |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | 12-19 |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 12-19 |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 20 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 22 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 20-22 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 20-23 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 23 |

*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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# A Single-Center Cross-Sectional Study on the Impact of Cumulative Erythropoietin on Bone Mineral Density in Maintenance Dialysis Patients

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# A Single-Center Cross-Sectional Study on the Impact of Cumulative Erythropoietin on Bone Mineral Density in Maintenance Dialysis Patients

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## Abstract

### Objectives

Numerous factors are associated with the risk of osteoporosis in chronic kidney disease (CKD) patients, including vitamin D deficiency, hypocalcemia, hyperphosphatemia, and secondary hyperparathyroidism. The present study aimed to assess the correlation between cumulative erythropoietin (EPO) doses and osteoporosis risk in patients on chronic dialysis. A further objective was to determine the bone mineral density (BMD) of patients undergoing dialysis and its correlation with specific clinical and biochemical factors.

### Setting

The study was undertaken at a tertiary care center within the southern region of the Taipei Metropolitan area.

### Participants

This cross-sectional study included 165 participants aged 41–90 years. Dual-energy X-ray absorptiometry was used to measure BMD. A total of 108 age- and sex-matched participants were selected for further analysis. Stepwise multiple regression analysis was used to investigate the relationship between bone measurements and bone diseases' risk factors.

### Primary and Secondary outcomes

The primary outcome of this study was to assess the T-scores of the participants who received dialysis for more than three months in our institution. The secondary outcome was using a receiver operating curve to predict osteoporosis development in patients on dialysis who received EPO treatments.

### Results

The mean age of the participants was 66.6 ± 11.1 years. A total of 99 (60%) participants (41 men, 58 women) were diagnosed as having osteoporosis. Fifty-four

(32.7%) participants with T-scores >−2.5 but <−1.0 were diagnosed as having osteopenia. Osteoporotic participants received 1.61 ± 1.52 million) EPO units compared to nonosteoporotic participants, who received 1.01 ± 0.64 million units (EPO1 model), $P = 0.015$. The cumulative EPO dose negatively correlated with the T-scores of participants ($P < 0.0001$).

**Conclusion**

On the basis of the results of the study, cumulative EPO doses show a negative correlation with BMD development in patients on chronic dialysis.

**Strengths and Limitations of this study**

- The present study presents a novel finding by elucidating the correlation of exogenous erythropoietin administration with the risk of reducing bone mineral density in the chronic dialysis Asian population.
- A sex and aged-match analysis increases the strength of the present study.
- The multivariate analysis identified the confounding factors to substantiate our study hypothesis.
- The present study is limited by a single-center experience on a relatively old age group of patients and a relatively small sample size of 165 patients.
- The study's retrospective nature is challenging to conclude the causal relationship between Erythropoietin and osteoporosis in dialysis patients.

## Introduction

Bone disease associated with chronic kidney disease (CKD) involves complicated biochemical and hormonal molecular interactions. In addition to bone abnormalities in patients with CKD–mineral bone disorders (CKD–MBDs), such as secondary hyperparathyroidism, osteomalacia, and adynamic bone disease, osteoporosis is another prevalent bone disease in patients with CKD. CKD patients with osteoporosis are at a higher risk of bone fractures[1] and have reduced quality of life[2]. Considering the increasing prevalence of CKD among aging populations, diagnosis and treatment of osteoporosis in a patient with CKD deserve more attention.

In CKD patients, biochemical alterations resulting in vitamin D deficiency, hypocalcemia, hyperphosphatemia, and secondary hyperparathyroidism can cause deterioration of the cortical bone architecture, leading to reduced cortical density and increased cortical porosity earlier in the course of CKD than previously thought[3]. Osteoporosis is a decrease in bone mineral density (BMD). Dual-energy X-ray absorptiometry (DXA) is the most common method for measuring BMD and is considered the current gold standard for osteoporosis diagnosis. According to the World Health Organization (WHO) criteria, the standard BMD value (the average in young, healthy women) is a T-score of $\geq -1.0$. T-score values between $-1.0$ and $-2.5$ are considered to indicate low bone density or osteopenia. A T-score of $\leq -2.5$ is considered to indicate osteoporosis.

More than two decades ago, the introduction of recombinant human Erythropoietin (EPO) in clinical practice completely altered CKD management. Treatment of renal anemia with EPO is now well established. The extensive use of EPO and its analogs

(EPO-stimulating agents [ESAs]) for anemia correction has reduced the associated morbidity and improved functionality, exercise tolerance, cognitive function, and overall quality of life. However, over the last few years, much controversy has been raised over the possible risks of ESA therapy. Moreover, a thorough investigation of the mechanism of action of EPO has revealed multiple biologic effects that extend beyond its erythropoietic effect and may have a favorable or sometimes unfavorable contribution to these outcomes.

EPO acts on erythroid progenitor cells by binding to an EPO receptor (EPOR), promoting survival, proliferation, and differentiation[4]. Functioning EPOR is present in endothelial cells[5], neurons[6], skeletal muscle progenitor cells[7], adipocytes[5], and islets[8], suggesting that EPO signaling exerts systemic regulation and interacts with nonerythroid cells through actions beyond erythropoiesis. Growing evidence from animal studies has demonstrated the critical role of EPO in regulating skeletal homeostasis[9,10]. Moreover, recent evidence has also demonstrated that EPO reduced trabecular bone volume in a mouse model of diet-induced obesity[11]. However, for humans, insufficient evidence exists on the role of EPO in mediating the bone microenvironment.

This study aimed to assess the correlation between cumulative doses of EPO administration and the risk of osteoporosis in patients on chronic dialysis. Moreover, bone mineral density in the femur and lumbar spine of patients on dialysis was investigated, its correlation with some clinical and biochemical factors was determined.

## Materials and methods

### Study design

A single center cross-sectional study

### Study population

Taipei Medical University, Wan Fang Hospital is a tertiary care hospital in Taipei. On average, there are 300 hemodialysis and 60 peritoneal dialysis patients under our maintenance renal replacement therapy program. Patients aged >20 years with end-stage renal disease and who were undergoing renal replacement therapy (hemodialysis or peritoneal dialysis) for more than one year were recruited. Patients on steroids, antiresorptive drugs (bisphosphonates), contraceptives, or calcitonin, and those who received parathyroidectomy were excluded from the study. Patients who did not initiate dialysis in our hospital were also excluded from the study due to the limitation in calculating cumulative EPO doses. Patients who were able to complete an interview were considered eligible. Of the 170 patients who gave written consent, one died, three failed to undergo a DXA scan, and one DXA scan failed due to technical problems; the remaining 165 patients (74 males [44.8%] and 91 females [55.2%]) completed the study, and their demographic data and biochemistry are summarized in Table 1. The causes of chronic renal failure were diabetic nephropathy (DMN) (90 patients, 54.5%), chronic glomerulonephritis (37 patients, 22.4%), hypertensive nephrosclerosis (24 patients, 14.5%), adult polycystic kidney disease (7 patients, 4.4%), chronic renal failure of unknown etiology (6 patients, 3.6%), and chronic tubulointerstitial nephritis patient (1 patient, 0.6%). The mean duration of dialysis was 6.3±5.4 years, and the number of hours of dialysis per week was 9.5–16.5 h, with a

mean of 11.2 h. The dialysate calcium concentration was 2.5 meq/L in 30 patients, 3.0 meq/L in 75 patients, and 3.5 meq/L in 60 patients.

**Table 1** Basic Characteristics of the Study Participants and Comparison between men and women

| Variables | Values ( N = 165) | Men (*n* = 74) | Women (*n* = 91) | *p* value |
|---|---|---|---|---|
| Age (years) | 66.6 ± 11.1 | 66.9 ± 9.9 | 66.3 ± 12.0 | 0.519 |
| BMI (kg/m$^2$) | 23.4 ± 3.4 | 23.9 ± 3.2 | 22.8 ± 3.6 | 0.010* |
| BW (kg) | 59.4 ± 10.6 | 66.0 ± 9.2 | 54.1 ± 8.6 | < 0.0001**** |
| Dialysis vintage (yrs) | 6.3 ± 5.4 | 5.9 ± 5.2 | 6.1 ± 4.9 | 0.772 |
| Ca (mg/dl) | 9.1 ± 0.8 | 9.2 ± 0.7 | 9.0 ± 0.8 | 0.036* |
| P (mg/dl) | 5.0 ± 1.3 | 5.0 ± 1.3 | 5.1 ± 1.4 | 0.811 |
| Intact PTH (pg/ml) | 362.9 ± 364.3 | 343.0 ± 345.3 | 379.1 ± 380.2 | 0.508 |
| ALP (μg/l) | 97.2 ± 54.6 | 93.8 ± 53.0 | 100.0 ± 56.1 | 0.324 |
| TG (mg/dl) | 186.5 ± 131.9 | 182.5 ± 113.0 | 189.7 ± 145.9 | 0.937 |
| T-Chol (mg/dl) | 153.8 ± 34.9 | 141.3 ± 30.7 | 164.0 ± 34.9 | < 0.0001**** |
| Alb (g/dL) | 3.7 ± 0.4 | 3.8 ± 0.3 | 3.6 ± 0.4 | 0.0005*** |
| AC glucose (mg/dl) | 146.9 ± 70.3 | 148.1 ± 73.1 | 145.9 ± 68.4 | 0.907 |
| Na (mmol/l) | 136.1 ± 3.5 | 136.0 ± 3.5 | 136.3 ± 3.4 | 0.474 |
| K (mmol/l) | 4.4 ± 0.7 | 4.4 ± 0.8 | 4.4 ± 0.7 | 0.451 |
| Uric acid (mg/dl) | 6.9 ± 1.8 | 6.8 ± 1.8 | 7.0 ± 1.8 | 0.521 |
| Hb (g/dl) | 10.3 ± 0.9 | 10.4 ± 1.0 | 10.1 ± 0.8 | 0.093 |
| Ferritin (ng/ml) | 531.4 ± 426.9 | 442.9 ± 307.0 | 603.4 ± 493.9 | 0.008* |
| EPO1 (x10$^6$ units) | 1.38 ± 1.77 | 1.22 ± 1.38 | 1.51 ± 1.35 | 0.847 |
| EPO2 (x10$^6$ units) | 1.92 ± 1.80 | 1.63 ± 1.62 | 2.15 ± 1.91 | 0.414 |
| EPO3 (x10$^6$ units) | 2.45 ± 2.31 | 2.08 ± 2.01 | 2.76 ± 2.50 | 0.295 |
| T-score | -2.8 ± 2.6 | -2.5 ± 1.1 | -3.1 ± 3.3 | 0.291 |
| L-spine BMD (g/cm$^2$) | 1.093 ± 0.264 | 1.218 ± 0.247 | 0.991± 0.233 | < 0.0001**** |
| RF-T BMD (g/cm$^2$) | 0.769 ± 0.223 | 0.820 ± 0.247 | 0.728 ± 0.194 | 0.003** |
| LF-T BMD (g/cm$^2$) | 0.757 ± 0.228 | 0.817 ± 0.240 | 0.707 ± 0.207 | < 0.0001**** |
| HD/PD | 125/40 | 57/17 | 68/23 | 0.186 |
| DM | 97 | 48 | 49 | 0.012 |
| Hypertension | 148 | 68 | 80 | 0.175 |
| CHF | 23 | 8 | 15 | 0.213 |
| CAD | 44 | 23 | 21 | 0.189 |
| Vitamin D treatment | 35 | 14 | 21 | 0.531 |

Abbreviations: A.C. glucose, fasting glucose; Alb, albumin; ALP, alkaline phosphatase; BMI, body mass index; B.W., body weight; CAD, coronary artery disease; Ca, calcium; CHF, congestive heart failure; D.M., diabetes mellitus; EPO, Erythropoietin; Hb, hemoglobin; H.D., hemodialysis; K, potassium; LF-T BMD, total left femur bone mineral density; L-spine BMD, lumbar-spine bone mineral density; Na, sodium; P, phosphorus; intact PTH, intact parathyroid hormone; P.D., peritoneal dialysis; RF-T BMD, total right femur bone mineral density; T.G., triglyceride; T-Chol, total cholesterol; yrs, years. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$, $^{****}p < 0.0001$.

A detailed history of related risk factors (smoking, hypertension, diabetes, steroid intake, and surgical menopause) was obtained from all patients, and medical records were checked after consent was obtained. The continuous medical records were available from January 2000 to December 2020. Before initiating the dialysis session, baseline investigations were performed at the patient's regular blood test session. Blood tests included kidney function tests, serum calcium, serum phosphorus, intact parathyroid hormone, fasting glucose, serum alkaline phosphatase levels, liver function tests, complete blood counts, ferritin, and determination of lipid profiles.

The DXA definition of osteoporosis and the bone mass criteria followed for its diagnosis were adopted from the WHO definition of osteoporosis (1994). T-scores were used for the evaluation of BMD and the definition of different stages of BMD according to the WHO definition of osteoporosis. T-scores were obtained for the femoral necks and lumbar spines (L1-L4). The average of lumbar spine BMD was to evaluate the lumbar spine T-score, use of three vertebrae if four cannot be used, and two if three cannot be used for the diagnosis according to the (The International Society for Clinical Densitometry, ISCD) guideline.[12] The lowest T-score among femoral necks and lumbar spines was accounted for established osteoporosis. The T-

score Normative Database is calculated by using USA (Combined NHANES (ages 20-30)/Lunar (ages 20-40) A.P. spine and Femur Reference Population).

**EPO dose conversion**

Patients receive either darbepoetin alfa (DPO) (Aranesp, Kyowa Hakko Kirin Co., Ltd.), epoetin beta (Recormon, Roche), or methoxy polyethylene glycol-epoetin beta (Mircera, Roche) at our institution. EPO doses are administered according to the patient's weekly hemoglobin levels. We maintain our patients' hemoglobin levels between 10 and 12 g/dl. For conversion from EPO alfa to darbepoetin alfa, a fixed conversion ratio of 200 IU EPO to 1 μg DPO was suggested by the manufacturer[13]. However, numerous studies have suggested that the conversion ratio be 240–400 IU of EPO and 1 μg of DPO[14-16]. In the current study, the cumulative dose of EPO received by the patient was calculated from the first day received EPO in our hospital until the DXA study date. The patient might receive various EPOs during their dialysis treatment in our institution. We established three conversion doses of darbepoetin alpha (DPO) and methoxy polyethylene glycol-epoetin beta (Mircera) to calculate the statistical difference between patients with and without osteoporosis. EPO1 refers to converting 1 μg of DPO/Mircera to 200 IU of EPO, EPO2 converting 1 μg of DPO/Mircera to 300 IU of EPO, and EPO3 converting 1 μg of DPO/Mircera to 400 IU of EPO.

**Ethical approval**

The study was approved by the Taipei Medical University Institutional Review Board for Human Experimentation. The accession number: TMU-IRB N202103059. Written informed consent was obtained from all subjects involved in the present study.

## Patient and Public Involvement

Patients and the public were not directly involved in this research. The nature of the anonymised records means individual participants could not be involved.

## Statistical analysis

Data were expressed as mean ± standard deviation unless otherwise specified. Pearson's correlation coefficients assessed correlations between bone measurements and cumulative EPO doses. Stepwise multiple regression analysis was used to investigate the relationships between bone measurements and biochemical markers or risk factors for bone diseases. The backward stepwise regression method was used to select variables in the multivariate analysis. Only a single log-transformed value of EPO was selected at every entry for multivariate analysis to avoid errors generated due to the collinearity of log EPOs. It means either log EPO1, log EPO2 or log EPO3 input into the multivariate analysis but not all three log EPOs entries. Differences between the means of multiple subgroups were assessed using the Kruskal–Wallis test. An unpaired t-test or Mann–Whitney U test was used for continuous variables. The chi-square test was used to compare frequencies between categorical variables. SPSS version 25 (SPSS Inc., Chicago, IL, USA) was used for analysis. A p-value of $<0.05$ was considered statistically significant.

## Results

### Bone mineral densitometry

Bone mineral densitometry measurements of the 165 patients are shown in Table 2. A good correlation was found between BMD measurements of the right and left femur ($r = 0.76$; $P < 0.0001$). However, lower correlation coefficients of BMD measurements were noted between lumbar spine values and right femoral neck ($r = 0.50$; $P < 0.0001$) and left femoral neck ($r = 0.54$; $P < 0.0001$) values, but they were still statistically significant. Ninety-nine patients with T-scores of ≤−2.5 were diagnosed with osteoporosis, and 54 patients with T-scores <−1.0 but > (−2.5 were diagnosed with osteopenia. Only twelve patients had T-scores of >−1.0.

**Table 2** Results of bone mineral densitometry measurements of patients on dialysis

| | BMD (g/cm$^2$) | T-score (SD) | Osteopenia | | Osteoporosis | |
|---|---|---|---|---|---|---|
| | | | N | % | N | % |
| L-spine | 1.093 ± 0.264 | -0.67 ± 1.85 | 54 | 32.7 | 27 | 16.4 |
| RF Neck | 0.769 ± 0.223 | -2.17 ± 1.27 | 74 | 44.8 | 51 | 30.9 |
| RF Total | 0.842 ± 0.225 | -1.72 ± 1.31 | 68 | 41.2 | 48 | 29.1 |
| LF Neck | 0.757 ± 0.228 | -2.31 ± 1.24 | 77 | 46.7 | 53 | 32.1 |
| LF Total | 0.839 ± 0.231 | -1.78 ± 1.29 | 72 | 43.6 | 54 | 32.7 |
| Total | - | -2.62 ± 1.14 | 54 | 32.7 | 99 | 60 |

Osteopenia: T-score < −1.0 but > −2.5; Osteoporosis: T-score ≤ −2.5
Total: the lowest T-score found among femoral necks and lumbar spines.
Abbreviations: BMD, bone mineral density; L.F. Neck, left femoral neck; L-spine, lumbar-spine; R.F. Neck, right femoral neck.

### Factors associated with reduced bone mineral density

In total, 165 patients with and without osteoporosis were evaluated for differences in risk factors for and biochemical markers of bone diseases. These included all the factors in Table 3, and individual variables were evaluated using Student's t-test. Independent variables that were analyzed and reached statistical significance ($P <$

0.05) are shown in Table 3. Age, body mass index (BMI), body weight (B.W.), serum calcium, ferritin, and EPO doses show statistical differences between patients with osteoporosis and patients without osteoporosis. Furthermore, 108 age- and sex-matched patients were evaluated for differences in risk factors for and biochemical markers of bone diseases. These included all the factors listed in Table 4. Cumulative EPO dosage was significantly different in age- and sex-matched patients with osteoporosis than nonosteoporotic patients on dialysis. All three EPO conversion models showed similar and significant results. Three models of EPO dose conversion were used to examine the association between EPO and T-scores of participants. The statistical calculation process was repeated using different EPO dose models to avoid collinearity. The results are shown in Figure 1. Pearson's correlation coefficient varied between −0.30 and −0.46, but *p* values were statistically significant.

**Table 3** The clinical and laboratory characteristics of patients with and without osteoporosis

| Variables | OS ($n$ = 99) | Without OS ($n$ = 66) | $p$ value |
|---|---|---|---|
| Age (years) | 70.0 ± 9.9 | 61.4 ± 10.8 | < 0.0001**** |
| Men/women | 41/58 | 33/33 | 0.278 |
| BMI (kg/m$^2$) | 22.7 ± 3.5 | 24.1 ± 3.2 | 0.009** |
| BW (kg) | 58.2 ± 14.6 | 62.7 ± .10.4 | 0.040* |
| Dialysis vintage (years) | 6.3 ± 5.5 | 6.1 ± 5.2 | 0.762 |
| Ca (mg/dL) | 9.0 ± 0.8 | 9.2 ± 0.7 | 0.028* |
| P (mg/dL) | 5.0 ± 1.4 | 5.2 ± 1.4 | 0.227 |
| Intact PTH (pg/mL) | 367.7 ± 398.2 | 353.4 ± 310.9 | 0.805 |
| ALP (μg/L) | 99.6 ± 54.8 | 93.1 ± 54.5 | 0.456 |
| TG (mg/dL) | 187.8 ± 128.8 | 183.7 ± 137.2 | 0.843 |
| T-Chol (mg/dL) | 154.2 ± 36.9 | 153.4 ± 31.6 | 0.884 |
| Alb (g/dL) | 3.7 ± 0.4 | 3.7 ± 0.3 | 0.184 |
| AC Glucose mg/dL) | 147.1 ± 71.6 | 153.0 ± 80.3 | 0.618 |
| Na (mmol/L) | 136.1 ± 3.4 | 136.1 ± 3.7 | 0.905 |
| K (mmol/L) | 4.3 ± 0.7 | 4.5 ± 0.8 | 0.201 |
| Uric acid (mg/dL) | 6.8 ± 1.8 | 7.0 ± 1.8 | 0.627 |
| Hb (g/dL) | 10.3 ± 0.8 | 10.2 ± 1.1 | 0.383 |
| WBC (/μl) | 7090 ± 636.7 | 6366 ± 199.5 | 0.365 |
| Platelet ( x10$^3$/μl) | 182.50 ± 6.30 | 179.20 ± 7.08 | 0.732 |
| Ferritin (ng/ml) | 592.7 ± 45.03 | 439.4 ± 36.51 | 0.023* |
| EPO1 (10$^6$ units) | 1.61 ± 1.52 | 1.01 ± 0.64 | 0.015* |
| EPO2 (10$^6$ units) | 2.23 ± 1.93 | 1.42 ± 0.92 | 0.013* |
| EPO3 (10$^6$ units) | 2.82 ± 2.45 | 1.87 ± 1.22 | 0.039* |
| T-score | -3.3 ± 0.78 | -1.5 ± 0.6 | < 0.0001**** |
| L-spine BMD | 1.012 ± 0.232 | 1.214 ± 0.264 | < 0.0001**** |
| RF-T BMD | 0.770 ± 0.025 | 0.952 ± 0.015 | < 0.0001**** |
| LT-T BMD | 0.749 ± 0.021 | 0.979 ± 0.024 | < 0.0001**** |
| HD/PD | 79/20 | 46/20 | 0.140 |
| DM | 58 | 39 | 0.949 |
| Hypertension | 88 | 60 | 0.676 |
| CHF | 17 | 6 | 0.148 |
| CAD | 27 | 17 | 0.829 |

T-scores represents the lowest value among the three areas of BMD measurements. Abbreviations: A.C. glucose, fasting glucose; Alb, albumin; ALP, alkaline phosphatase; BMI, body mass index; B.W., body weight; CAD, coronary artery disease; Ca, calcium; CHF, congestive heart failure; D.M., diabetes mellitus; EPO, Erythropoietin; Hb, hemoglobin; H.D., hemodialysis; K, potassium; LF-T BMD, total left femur bone mineral density; L-spine BMD, lumbar-spine bone mineral density; Na, sodium; O.S., osteoporosis; P, phosphorus; intact PTH, intact parathyroid hormone; P.D., peritoneal dialysis; RF-T BMD, total right femur bone mineral density; T.G., triglyceride; T-Chol, total cholesterol; WBC, white cell count. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$.

**Erythropoietin dosage associated with osteoporosis among three different sites of bone mineral density measurement**

Significantly higher EPO dosages were found among osteoporotic participants using BMD measured from lumbar spines, right total, and left total femur (Table 4a-4c). However, no statistical difference was found on the cumulative EPO doses (all three models) using different sites to diagnose osteoporosis (Figure 2).

**Table 4a** Association of cumulative dose of erythropoietin with L-spine BMD

| L-Spine | OS ($n$ = 27) | Without OS ( $n$ = 138) | p Value |
|---|---|---|---|
| M/F | 6/21 | 68/70 | |
| BMD | 0.95±0.20 | 1.14±0.26 | 0.001*** |
| EPO1 | 1.82±1.57 | 1.22±1.13 | 0.020* |
| EPO2 | 2.59±2.35 | 1.71±1.41 | 0.010** |
| EPO3 | 3.34±3.21 | 2.19±1.76 | 0.009** |

Abbreviations: BMD, bone mineral density; EPO, Erythropoietin; L-spine, lumbar-spine; O.S., osteoporosis. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$.

**Table 4b** Association of cumulative dose of Erythropoietin with the total right femur BMD

| Right femur total | OS ( $n$ = 48 ) | Without OS ( $n$ = 117 ) | p Value |
|---|---|---|---|
| M/F | 15/33 | 59/58 | |
| BMD | 0.71±0.17 | 0.90±0.22 | < 0.0001**** |
| EPO1 | 1.71±1.29 | 1.15±1.17 | 0.008** |
| EPO2 | 2.46±1.92 | 1.61±1.43 | 0.002** |

| | | | |
|---|---|---|---|
| EPO3 | 3.21±2.61 | 2.04±1.75 | 0.001*** |

Abbreviations: BMD, bone mineral density; EPO, Erythropoietin; L-spine, lumbar-spine; O.S., osteoporosis. ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0001$.

**Table 4c** Association of cumulative dose of Erythropoietin with the total left femur BMD

| Left femur total | OS ( $n = 54$ ) | Without OS ( $n = 111$ ) | p Value |
|---|---|---|---|
| M/F | 18/36 | 56/55 | |
| BMD | 0.71±0.18 | 0.90±0.23 | < 0.0001**** |
| EPO1 | 1.61±1.30 | 1.17±1.17 | 0.028* |
| EPO2 | 2.34±1.91 | 1.62±1.42 | 0.007** |
| EPO3 | 3.05±2.57 | 2.05±1.75 | 0.004** |

Abbreviations: BMD, bone mineral density; EPO, Erythropoietin; L-spine, lumbar-spine; O.S., osteoporosis. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

**Factors associated with osteoporosis in patients on dialysis**

Table 5 shows clinical factors associated with osteoporosis in age- and sex-matched chronic dialysis patients. All three EPO conversion models show significant cumulative EPO use among osteoporotic dialysis patients than nonosteoporotic dialysis patients. Table 6 shows factors associated with osteoporosis in patients on dialysis after different statistical models were applied. The univariate analysis results showed a statistically significant difference in age, BMI, ferritin's log-transformed value (logFerritin), and cumulative EPO's log-transformed value (logEPO) in osteoporotic patients compared to those without osteoporosis. Backward stepwise logistic regression was used to select multiple variables. Age, sex, B.W., BMI, hemoglobin, logFerritin, and a single entry of logEPO were selected as variables to enter the logistic regression model. In addition to age, ferritin, and EPO, both hemoglobin and B.W. were significantly different between patients with and without osteoporosis. In the age- and sex-matched multivariate analysis model, the log-transformed EPOs are the only significant factors associated with osteoporosis.

**Table 5** Age- and sex-matched t-test analysis of chronic dialysis patient with and without osteoporosis

| Variables | OS ($n$ = 54) | Without OS (n = 54) | $p$ value |
|---|---|---|---|
| Age (years) | 66.0 ± 9.0 | 62.9 ± 10.2 | 0.097 |
| Men/women | 28/26 | 28/26 | 1.0 |
| BMI (kg/m$^2$) | 23.0 ± 4.0 | 24.0 ± 3.0 | 0.142 |
| BW (kg) | 59.7 ± 11.7 | 62.6 ± .10.6 | 0.176 |
| Dialysis vintage (yrs) | 7.3 ± 5.7 | 5.7 ± 5.0 | 0.111 |
| Ca (mg/dL) | 9.1 ± 0.8 | 9.2 ± 0.7 | 0.524 |
| P (mg/dL) | 5.1 ± 1.4 | 5.2 ± 1.4 | 0.495 |
| Intact PTH (pg/mL) | 418.0 ± 419.5 | 329.2 ± 307.0 | 0.212 |
| ALP (μg/L) | 102. 8 ± 47.9 | 96.6 ± 57.6 | 0.240 |
| TG (mg/dL) | 195.9 ± 139.2 | 197.9 ± 144.6 | 0.941 |
| T-Chol (mg/dL) | 148.6 ± 40.3 | 155.1 ± 30.9 | 0.355 |
| Alb (g/dL) | 3.8 ± 0.3 | 3.8 ± 0.3 | 0.796 |
| AC Glucose mg/dL) | 138.8 ± 69.5 | 163.0 ± 84.4 | 0.106 |
| Na (mmol/L) | 136.5 ± 3.2 | 136.4 ± 3.6 | 0.844 |
| K (mmol/L) | 4.4 ± 0.8 | 4.5 ± 0.8 | 0.287 |
| Uric acid (mg/dL) | 7.1 ± 1.9 | 7.2 ± 1.7 | 0.823 |
| Hb (g/dL) | 10.4 ± 0.8 | 10.3 ± 1.1 | 0.486 |
| WBC (/μl) | 7595 ± 1142 | 6518 ± 231.3 | 0.357 |
| Platelet ( x10$^3$/μl) | 178.89 ± 7.79 | 183.37 ± 9.76 | 0.721 |
| Ferritin | 502.6 ± 365.9 | 439.3 ± 372.4 | 0.375 |
| EPO1 (x10$^6$ units) | 1.54 ± 1.19 | 0.94 ± 0.69 | 0.002*** |
| EPO2 (x10$^6$ units) | 2.15 ± 1.56 | 1.28 ± 0.91 | 0.001** |
| EPO3 (x10$^6$ units) | 2.76 ± 1.97 | 1.62 ± 1.18 | < 0.0001**** |
| T-score | -3.7 ± 4.0 | -1.6 ± 0.6 | < 0.0001**** |
| L-spine BMD | 1.029 ± 0.033 | 1.227 ± 0.037 | < 0.0001**** |
| RF-T BMD | 0.775 ± 0.033 | 0.962 ± 0.020 | < 0.0001**** |
| LF-T BMD | 0.737 ± 0.022 | 0.974 ± 0.026 | < 0.0001**** |
| HD/PD | 41/13 | 41/13 | - |
| DM | 29 | 35 | 0.244 |
| Hypertension | 45 | 49 | 0.256 |
| CHF | 10 | 6 | 0.283 |

| | | | |
|---|---|---|---|
| CAD | 15 | 14 | 0.830 |

Abbreviations: A.C. glucose, fasting glucose; Alb, albumin; ALP, alkaline phosphatase; BMI, body mass index; B.W., body weight; CAD, coronary artery disease; Ca, calcium; CHF, congestive heart failure; D.M., diabetes mellitus; EPO, Erythropoietin; Hb, hemoglobin; H.D., hemodialysis; K, potassium; LF-T BMD, total left femur bone mineral density; L-spine BMD, lumbar-spine bone mineral density; Na, sodium; OS, osteoporosis; P, phosphorus; intact PTH, intact parathyroid hormone; P.D., peritoneal dialysis; RF-T BMD, total right femur bone mineral density; T.G., triglyceride; T-Chol, total cholesterol; WBC, white cell count. $^{**}$ $p < 0.01$, $^{***}$ $p < 0.005$, $^{****}$ $p < 0.0001$.

**Table 6** Factors associated with osteoporosis in dialysis patients of different statistical models

| | Univariate model | | Multivariate model | | Age-sex matched model | |
|---|---|---|---|---|---|---|
| | p Values | OR (95% C.I.) | p values | OR (95% C.I.) | p values | OR (95% C.I.) |
| Age (yrs) | < 0.0001$^{****}$ | 1.08(1.05- 1.12) | 0.001$^{***}$ | 1.07(1.03 - 1.12) | - | - |
| Sex | 0.278 | 0.71(0.38- 1.32) | 0.759 | 1.21(0.37 - 3.96) | - | - |
| BW (kg) | 0.053 | 0.97(0.95- 1.00) | 0.010$^{*}$ | 0.95(0.92 - 0.99) | 0.766 | 0.99(0.93 - 1.06) |
| BMI(kg/m$^2$) | 0.012$^{*}$ | 0.88(0.80- 0.97) | 0.065 | 0.95(0.74 - 1.20) | 0.461 | 0.92(0.75 - 1.14) |
| Hb (g/dl) | 0.508 | 1.13(0.80- 1.60) | 0.022$^{*}$ | 1.76(1.08 - 2.85) | 0.197 | 1.41(0.84 - 2.36) |
| LogFerritin | 0.003$^{***}$ | 1.20(1.06- 1.36) | 0.033$^{*}$ | 2.96(1.09 - 8.03) | 0.656 | 1.30(0.42 - 4.03) |
| LogEPO1 | 0.007$^{**}$ | 1.08(1.02- 1.13) | 0.005$^{**}$ | 4.25(1.56 - 11.56) | 0.002$^{***}$ | 9.11(2.18 - 38.0) |
| LogEPO2 | 0.007$^{**}$ | 1.07(1.02- 1.13) | 0.008$^{**}$ | 4.70(1.50 - 14.76) | 0.002$^{***}$ | 10.61(2.43 - 46.4) |
| LogEPO3 | 0.007$^{**}$ | 1.07(1.02- 1.13) | 0.007$^{**}$ | 4.85(1.54 - 15.29) | 0.002$^{***}$ | 11.32(2.52 - 50.9) |

Abbreviations: BMI, body mass index; LogFerritin, logarithmic scale Ferritin; LogEPO1, logarithmic scale EPO1; LogEPO2, logarithmic scale, LogEPO2; LogPO3, logarithmic scale EPO3. Multivariate model represents a stepwise backward logistic regression model of the unmatched individuals. The age-sex matched model represents a stepwise backward logistic regression model of the age- and sex-matched individuals. Only a single LogEPO entered into the multivariate and age-sex model for analysis to avoid multicollinearity.

**Role of erythropoietin use in osteoporosis development**

A receiver operating curve was generated to assess the area under the curve (AUC) to predict the risk of osteoporosis in patients on dialysis receiving cumulative EPO

doses. A logarithmic scale was used to examine all three EPO dose conversion models and the development of osteoporosis. The AUC varied between 0.698 and 0.714 and showed moderate utility in predicting osteoporosis development in patients on dialysis (Figure 3).

**Discussion**

This study found a moderate reduction in the mean BMD in this unselected population of patients on chronic hemodialysis. The mean T-score of -2.17 in the DXA measurement of the femoral neck implies that these patients had moderately less favorable outcomes than age-matched controls. The mean T-score value found in the present study is similar to several other studies that used the same bone mineral density measurement[17]. Age and weight also emerged as important determinants of BMD in our study. Age-related bone loss plays an essential role in the pathogenesis of osteoporosis, and a negative association between age and BMD in female patients with end-stage renal disease has been reported[18,19]. The mean age of patients in these two studies was 43 and 50.5 years, whereas, in our study, patients were older, with a mean age of 66.6 years. With the number of older adults involved in the renal replacement program increasing and with survival rates markedly improving, age-related bone loss can be expected to become an increasingly important factor causing bone disease in these patients.

Moreover, evidence has revealed a positive correlation between weight and BMD in healthy populations[20]. The correlation between B.W. and BMD has been attributed to bone formation stimulations through weight-bearing and adipose tissues' increased peripheral conversion of adrenal androgens to estrogens. Two studies have reported a positive association between BMI and BMD measurements[21,22]. We showed a similar association in our patients. Finally, we found a significant difference in cumulative EPO use in patients with osteoporosis compared with those without osteoporosis in univariate and multivariate analyses (Table 5).

EPO is administered based on the patient's weekly hemoglobin levels at our institution. EPO doses received were positively correlated with patient dialysis duration. The longer the patient undergoes dialysis, the higher the dose of EPO the

patient may receive. However, no statistically significant differences in dialysis vintage were found between patients with osteoporosis and those without [$P = 0.762$ (unmatched), $P = 0.111$ (age- and sex-matched)]. All three models, logEPO1, logEPO2, and logEPO3 showed significant differences in cumulative EPO in patients with osteoporosis compared with those without (Table 5). A negative correlation was observed between the total, lumbar, right femoral neck, and left femoral neck T-scores and EPO dose (Figure 1). Although these results showed a low and negative correlation between T-scores and EPO dose (Pearson's correlation coefficient $r$ from 0.30 to 0.46), these data reached statistical significance ($P < 0.005$ to $< 0.0001$).

Higher EPO dosages were administered in patients with lumbar spine osteoporosis than patients with cortical bone osteoporosis (right or left femur). However, no statistical significance was reached in the current study (Figure 1). Effects of erythropoietin-induced bone loss had been demonstrated in experimental mice.[10,11]However, clinical evidence concerning EPO with bone mineral density is lacking. Whether EPO exerts more trabecular bone loss or cortical bone loss remains to be elucidated.

Serum PTH is negatively associated with BMD measurements; cortical porosity increased in patients with hyperparathyroidism[23]. Several studies have reported a negative association between PTH levels and BMD measurements[18,24,25], whereas others were unable to show this association[26-28]. In the present study, however, we found a negative association between PTH levels and BMD measurements, suggesting that other factors affect BMD in patients on hemodialysis. Forty-three patients received active vitamin D treatment in the current study.

Aluminum overload may be responsible for adynamic bone disease and osteomalacia. At our institution, serum aluminum levels are measured annually in patients who have undergone dialysis for >5 years. Our patients had no abnormally elevated serum aluminum levels. Moreover, we did not perform a histological analysis of bone. Thus, we cannot comment on the prevalence of adynamic bone disease and osteomalacia in this population.

The relationships between calcium intake, vitamin D supplementation and osteoporosis development remain controversial. One study has shown that oral 1$\alpha$-

hydroxycalciferol treatment could prevent BMD loss in the Lumbar Spine in a study of 165 male patients[29]. All 165 patients were receiving calcium-containing phosphate binders. Only ten patients received vitamin D supplements in the nonosteoporotic group compared with 33 patients who received vitamin D supplements in the osteoporotic group.

Clinical and molecular evidence suggests that chronic inflammation significantly influences bone turnover.[30,31]Uremic syndrome, hemodialysis, use of a catheter, and persistent infection may contribute to the development of the inflammatory state in CKD. In hemodialysis patients, inflammation has been associated with EPO resistance mainly because the inflammatory state decreases the bone marrow response to ESA, changing iron regulation through hepcidin upregulation and/or causing red blood cell/erythrocyte hemolysis.[32]In the current study, we had not studied the inflammatory status among patients with/without osteoporosis. However, some markers of inflammatory reaction had included in our laboratory study, including WBC, platelets, ferritin, and albumin. Both platelet and white cell counts have been implicated in playing an essential role in inflammatory reaction.[33,34]Similarly, both ferritin and albumin have also known as acute-phase proteins. In the age- and sex-matched model, WBC, platelets, ferritin, and albumin have not shown statistical differences between osteoporotic and nonosteoporotic patients.

The strengths of our study are the random sampling of the population and the high accuracy of cumulative EPO treatment history collected. Participants with and without osteoporosis were age- and sex-matched to examine the association of EPO treatment with the risk of osteoporosis development. However, the present study was limited by its cross-sectional nature. It is difficult to establish the causal relationship between EPO accumulation and the risk of osteoporosis. A further longitudinal study is required to confirm the cause and effect of EPO in reducing BMD. Moreover, the present study involves a group of elderly participants. Our subgroup analysis showed that participants aged <65 years with osteoporosis did not receive a higher EPO dose than participants aged >65 years with osteoporosis ($r = -0.21$, $P = 0.133$, data not shown).

In conclusion, we confirmed the importance of age and body weight as the risk factors affecting BMD in patients on hemodialysis. We found that the cumulative EPO negatively correlates with dialysis patients' BMD. Elderly dialysis patients under long-term EPO treatment are at risk of developing osteoporosis. Managing anemia in this vulnerable population may consider other possible therapeutic strategies.

## Declarations

### Ethics approval and consent to participate

Taipei Medical University Institutional Review Board approved the study for Human Experimentation (TMU-eJIRB, N202103059).

### Data sharing

All free text entered will be published

### Competing interests

The authors declare that they have no competing interests.

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## Figure legends

**Figure 1** Descriptive plots of correlations between T-scores of L-spine, total right femur, total left femur, and cumulative Erythropoietin (EPO) dose received. EPO1, EPO2, and EPO3 represent three different dose conversion models. EPO1, 1 μg of darbepoietin/Mircera converts to 200 IU of EPO; EPO2, 1 μg of darbepoietin/Mircera converts to 300 IU of EPO; EPO3, 1 μg of darbepoietin/Mircera converts to 400 IU of EPO.

**Figure 2** Three different models of erythropoietin dose conversion versus bone mineral density among three different sites in dialysis patients with osteoporosis. Abbreviations: BMD, bone mineral density; EPO, Erythropoietin; L1-L4, lumbar-spine 1 to 4; RFN, right femoral neck; RFT, total right femur; LFN, left femoral neck; LFT, total left femur.

**Figure 3** Receiver operating characteristic (ROC) curves of prediction models for cumulative erythropoietin dose transformed to a log value that incorporates patients with and without osteoporosis. AUC = area under the ROC curve; LogEPO1, the log-transformed value of EPO1; LogEPO2, the log-transformed value of EPO2; LogEPO3, the log-transformed value of EPO3.

**Figure 1**

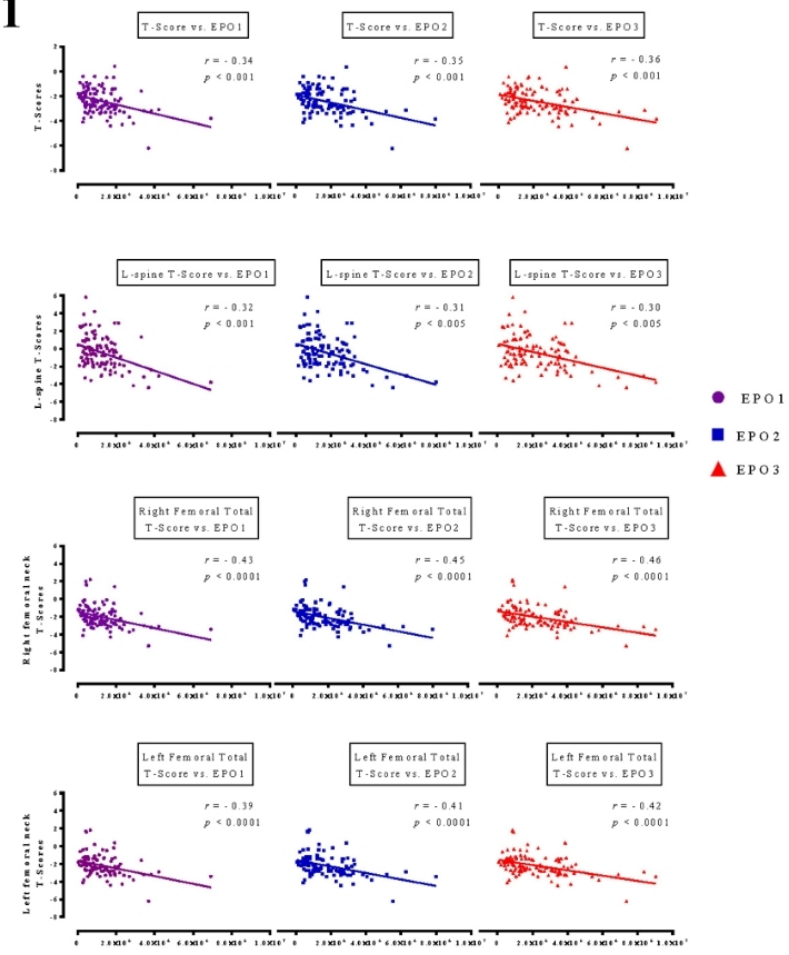


Figure 1 Descriptive plots of correlations between T-scores of L-spine, total right femur, total left femur, and cumulative Erythropoietin (EPO) dose received. EPO1, EPO2, and EPO3 represent three different dose conversion models. EPO1, 1 μg of darbepoietin/Mircera converts to 200 IU of EPO; EPO2, 1 μg of darbepoietin/Mircera converts to 300 IU of EPO; EPO3, 1 μg of darbepoietin/Mircera converts to 400 IU of EPO.

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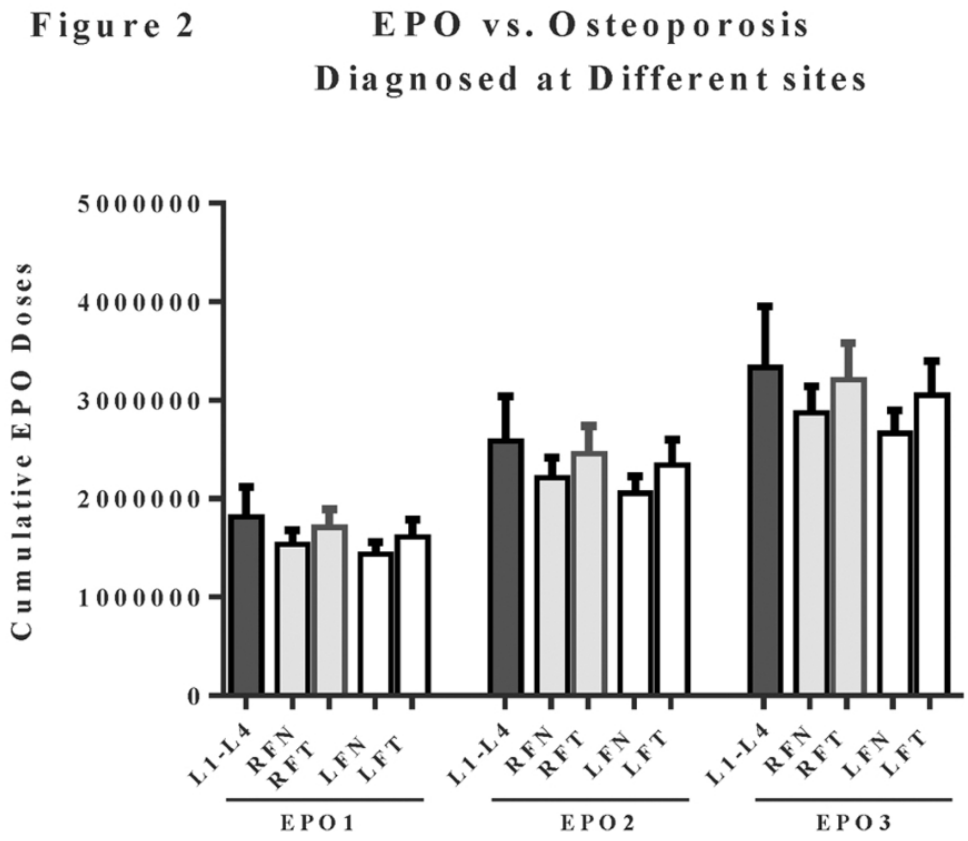


Figure 2 Three different models of erythropoietin dose conversion versus bone mineral density among three different sites in dialysis patients with osteoporosis. Abbreviations: BMD, bone mineral density; EPO, erythropoietin; L1-L4, lumbar-spine 1 to 4; RFN, right femoral neck; RFT, total right femur; LFN, left femoral neck; LFT, total left femur.

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**Figure 3**

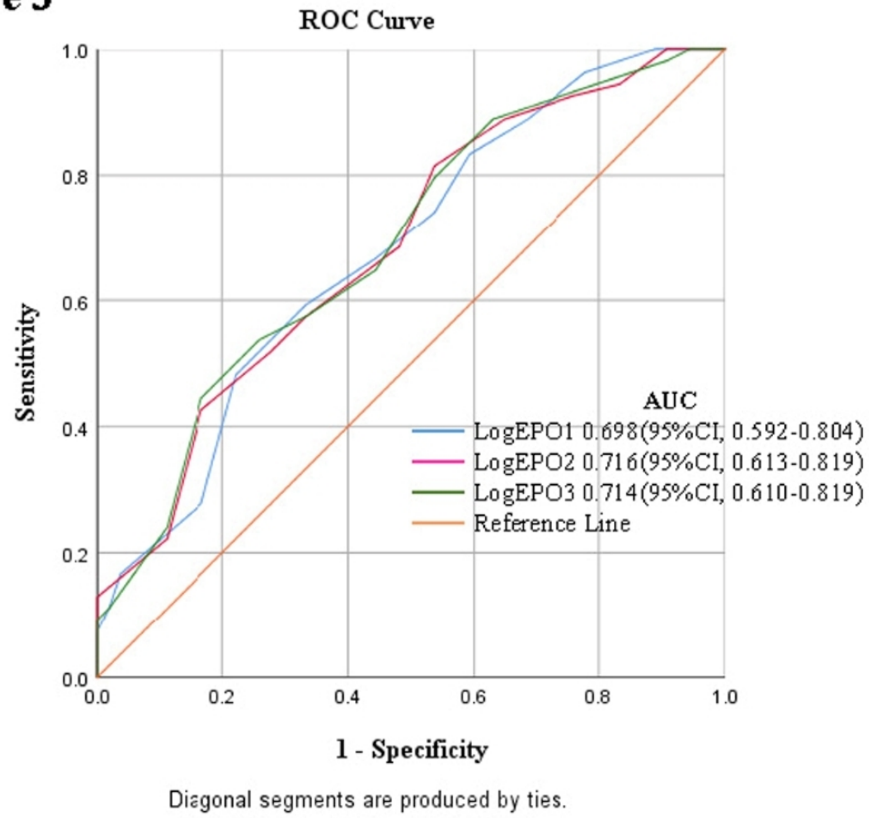


Figure 3 Receiver operating characteristic (ROC) curves of prediction models for cumulative erythropoietin dose transformed to a log value that incorporates patients with and without osteoporosis. AUC = area under the ROC curve; LogEPO1, the log-transformed value of EPO1; LogEPO2, the log-transformed value of EPO2; LogEPO3, the log-transformed value of EPO3.

76x76mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

| | Item No | Recommendation | Page No |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 3-4 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 5-6 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 6 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 7 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 7-10 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants | 7-10 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 7-11 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 7-12 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 7 |
| Study size | 10 | Explain how the study size was arrived at | 7-9 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 11-12 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 11-12 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 11-12 |
| | | (*c*) Explain how missing data were addressed | 11-12 |
| | | (*d*) If applicable, describe analytical methods taking account of sampling strategy | 11-12 |
| | | (*e*) Describe any sensitivity analyses | 11-12 |
| **Results** | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 10,12 |
| | | (b) Give reasons for non-participation at each stage | 12 |
| | | (c) Consider use of a flow diagram | - |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 10, 12 |
| | | (b) Indicate number of participants with missing data for each variable of interest | - |

| | | | |
|---|---|---|---|
| Outcome data | 15* | Report numbers of outcome events or summary measures | 12-19 |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 12-19 |
| | | (*b*) Report category boundaries when continuous variables were categorized | 12-19 |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | 12-19 |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 12-19 |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 20 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 22 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 20-22 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 20-23 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 23 |

*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.