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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-056390
Article Type:	Original research
Date Submitted by the Author:	14-Aug-2021
Complete List of Authors:	Cheng, Chung-Yi; Taipei Municipal Wan-Fang Hospital, Division of Nephrology, Department of Internal Medicine; Taipei Medical University, Department of Internal Medicine, School of Medicine, College of Medicine Kuo, Yi-Jie; Taipei Municipal Wan-Fang Hospital, Department of Orthopedic Surgery; Taipei Medical University, Department of Orthopedic Surgery, School of Medicine, College of Medicine, Taipei Medical University
Keywords:	Nephrology < INTERNAL MEDICINE, Chronic renal failure < NEPHROLOGY, Dialysis < NEPHROLOGY, Rheumatology < INTERNAL MEDICINE, Calcium & bone < DIABETES & ENDOCRINOLOGY

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4 **Cumulative Erythropoietin negatively correlated with bone mineral density in**
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7 **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

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4 patients on dialysis who received EPO treatments.
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8 **Results**

9
10 The mean age of the participants was 66.6 ± 11.1 years. A total of 99 (60%)
11 participants (41 male, 58 female) with a T-score of ≤ -2.5 were diagnosed as having
12 osteoporosis. Fifty-four (32.7%) participants with T-scores > -2.5 but < -1.0 were
13 diagnosed as having osteopenia. Osteoporotic participants received 1.61 ± 1.52
14 million) EPO units compared to nonosteoporotic participants, who received $1.01 \pm$
15 0.64 million units (EPO1 model), $P = 0.015$. The cumulative EPO dose negatively
16 correlated with the T-scores of participants ($P < 0.0001$).
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28 **Conclusion**

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

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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 fractures¹ and have reduced quality of life². Considering the increasing prevalence of CKD among aging populations, diagnosis and treatment of osteoporosis in patient with CKD deserve more attention.

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4 In patient with CKD, biochemical alterations resulting in vitamin D deficiency,
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6 hypocalcemia, hyperphosphatemia, and secondary hyperparathyroidism can cause
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8 deterioration of the cortical bone architecture, leading to reduced cortical density and
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10 increased cortical porosity earlier in the course of CKD than previously thought³.

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12 Osteoporosis is a decrease in bone mineral density (BMD). Dual-energy X-ray
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14 absorptiometry (DXA) is the most common method for measuring BMD and is
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16 considered the current gold standard for osteoporosis diagnosis. According to the
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18 World Health Organization (WHO) criteria, the standard BMD value (the average in
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20 young, healthy women) is a T-score of ≥ -1.0 . T-score values between -1.0 and -2.5
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22 are considered to indicate low bone density or osteopenia. A T-score of ≤ -2.5 is
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24 considered to indicate osteoporosis.
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31 More than 2 decades ago, the introduction of recombinant human Erythropoietin
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33 (EPO) in clinical practice completely altered the management of CKD. Treatment of
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35 renal anemia with EPO is now well established. The extensive use of EPO and its
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37 analogs (EPO-stimulating agents [ESAs]) for anemia correction has reduced the
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39 associated morbidity and improved functionality, exercise tolerance, cognitive
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41 function, and overall quality of life. However, over the last few years, much
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43 controversy has been raised over the possible risks of ESA therapy. Moreover,
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45 thorough investigation of the mechanism of action of EPO has revealed multiple
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47 biologic effects that extend beyond its erythropoietic effect and may have a favorable
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49 or sometimes unfavorable contribution to these outcomes.
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56 EPO acts on erythroid progenitor cells by binding to an EPO receptor (EPOR),
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58 promoting survival, proliferation, and differentiation⁴. Functioning EPOR is present in
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60 endothelial cells⁵, neurons⁶, skeletal muscle progenitor cells⁷, adipocytes⁵, and islets⁸,

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4 suggesting that EPO signaling exerts systemic regulation and interacts with
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6 nonerythroid cells through actions beyond erythropoiesis. Growing evidence from
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8 animal studies has demonstrated the critical role of EPO in regulating skeletal
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10 homeostasis^{9,10}. Moreover, recent evidence has also demonstrated that EPO resulted
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12 in reduced trabecular bone volume in a mouse model of diet-induced obesity¹¹.
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14 However, for humans, insufficient evidence exists on the role of EPO in mediating the
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16 bone microenvironment.
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21 This study aimed to assess the correlation between cumulative doses of EPO
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23 administration and the risk of osteoporosis in patients on chronic dialysis. Moreover,
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25 bone mass density in the femur and lumbar Spine of patients on dialysis was
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27 investigated, its correlation with some clinical and biochemical factors was
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29 determined.
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38 **Materials and methods**

39 **Patient and Public Involvement**

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42 Taipei Medical University, Wan Fang Hospital is a tertiary care hospital in Taipei. On
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44 average, there are 300 hemodialysis and 60 peritoneal dialysis patients under our
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46 maintenance renal replacement therapy program. Patients aged >20 years with end-
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48 stage renal disease and who were undergoing renal replacement therapy (hemodialysis
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50 or peritoneal dialysis) for more than one year were recruited. Patients on steroids,
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52 antiresorptive drugs (bisphosphonates), contraceptives, or calcitonin, and those who
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54 received parathyroidectomy were excluded from the study. Patients who did not
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4 initiate dialysis in our hospital were also excluded from the study due to the limitation
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6 in calculating cumulative EPO doses. Patients who were able to complete an
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8 interview were considered eligible. Of the 170 patients who gave consent, one died,
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10 three failed to undergo a DXA scan, and one DXA scan failed due to technical
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12 problems; the remaining 165 patients (74 males [44.8%] and 91 females [55.2%])
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14 completed the study, and their demographic data and biochemistry are summarized in
15
16 Table 1. Chronic renal failure of unknown etiology in six patients (3.6%) and was due
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18 to glomerulonephritis in 37 (22.4%), adult polycystic kidney disease in 7 (4.4%),
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20 diabetic nephropathy in 90 (54.5%), hypertensive nephrosclerosis in 24 (14.5%), and
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22 chronic tubulointerstitial nephritis in 1 (0.6%) patient. The mean duration of dialysis
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24 was 6.3 ± 5.4 years, and the number of hours of dialysis per week was 9.5–16.5 h, with
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26 a mean of 11.2 h. The dialysate calcium concentration was 2.5 meq/L in 30 patients,
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28 3.0 meq/L in 75 patients, and 3.5 meq/L in 60 patients.
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Table 1 Basic Characteristics of the Study Subjects and Univariate Analysis between Male and Female Dialysis Patients

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

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7 A detailed history of related risk factors (smoking, hypertension, diabetes, steroid
8 intake, and surgical menopause) was obtained from all patients, and medical records
9 were checked after consent was obtained. Baseline investigations were performed,
10 which included kidney function tests; determination of serum calcium, serum
11 phosphorus, intact parathyroid hormone, fasting glucose, and serum alkaline
12 phosphatase levels; liver function tests; complete blood counts; and determination of
13 lipid profiles.
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24 The DXA definition of osteoporosis and the bone mass criteria followed for its
25 diagnosis were adopted from the WHO definition of osteoporosis (1994). T-scores
26 were used for the evaluation of BMD and the definition of different stages of BMD
27 according to the WHO definition of osteoporosis. T-scores were obtained for the
28 femoral neck and L1 and L2.
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38 **EPO dose conversion**

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42 Patients receive either darbepoetin alfa (DPO) (Aranesp, Kyowa Hakko Kirin Co.,
43 Ltd.), epoetin beta (Recormon, Roche), or methoxy polyethylene glycol-epoetin beta
44 (Mircera, Roche) at our institution. EPO doses are administered according to the
45 patient's weekly hemoglobin levels. We maintain our patients' hemoglobin levels
46 between 10 and 12 g/dL. For conversion from EPO alfa to darbepoetin alfa, a fixed
47 conversion ratio of 200 IU EPO to 1 µg DPO was suggested by the manufacturer¹².
48 However, numerous studies have suggested that the conversion ratio should be 240–
49 400 IU of EPO and 1 µg of DPO¹³⁻¹⁵. In the current study, the cumulative dose of
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4 EPO received by the patient was calculated from the first day received EPO in our
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6 hospital until the DXA study date. We established three conversion doses of
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8 darbepoetin alpha (DPO) and methoxy polyethylene glycol-epoetin beta (Mircera) to
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10 calculate the statistical difference between patients with and without osteoporosis.
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12 EPO1 refers to the conversion of 1 µg of DPO/Mircera to 200 IU of EPO. EPO2
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14 refers to the conversion of 1 µg of DPO/Mircera to 300 IU of EPO. EPO3 refers to the
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16 conversion of 1 µg of DPO/Mircera to 400 IU of EPO.
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22 **Ethical approval**

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24 The study was approved by the Taipei Medical University Institutional Review Board
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26 for Human Experimentation. The accession number: TMU-IRB N202103059.
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30 **Statistical analysis**

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32 Data were expressed as mean ± standard deviation unless otherwise specified.
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34 Correlations between bone measurements and cumulative EPO doses were assessed
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36 using Pearson's correlation coefficients. Stepwise multiple regression analysis was
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38 used to investigate the relationships between bone measurements and biochemical
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40 markers or risk factors for bone diseases. The backward stepwise regression method
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42 was used to select variables in the multivariate analysis. The logarithmic scale of
43
44 EPOs was selected for multivariate analysis to avoid errors generated due to the
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46 collinearity of EPOs and Ln EPOs. Differences between the means of multiple
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48 subgroups were assessed using the Kruskal–Wallis test. An unpaired t-test or Mann–
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50 Whitney U test was used for continuous variables. SPSS version 25 (SPSS Inc.,
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52 Chicago, IL, USA) was used for analysis. A p-value of <0.05 was considered
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54 statistically significant.
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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	%
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

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

Variables	Mean \pm SD		<i>p</i> value
	Osteoporosis (<i>N</i> = 54)	Without Osteoporosis (<i>N</i> = 54)	
Age (years)	66.0 \pm 9.0	62.9 \pm 10.2	0.097
Men/women	28/26	28/26	1.0
Body mass index (kg/m ²)	23.0 \pm 4.0	24.0 \pm 3.0	0.142
Weight (kg)	59.7 \pm 11.7	62.6 \pm 10.6	0.176
Dialysis time	7.3 \pm 5.7	5.7 \pm 5.0	0.111
Calcium (mg/dL)	9.1 \pm 0.8	9.2 \pm 0.7	0.524
Phosphorus (mg/dL)	5.1 \pm 1.4	5.2 \pm 1.4	0.495
Parathyroid hormone (pg/mL)	418.0 \pm 419.5	329.2 \pm 307.0	0.212
Alkaline phosphatase(μ g/L)	102.8 \pm 47.9	96.6 \pm 57.6	0.240
Triglyceride (mg/dL)	195.9 \pm 139.2	197.9 \pm 144.6	0.941
Total Cholesterol (mg/dL)	148.6 \pm 40.3	155.1 \pm 30.9	0.355
Albumin (g/dL)	3.8 \pm 0.3	3.8 \pm 0.3	0.796
Fasting Glucose mg/dL)	138.8 \pm 69.5	163.0 \pm 84.4	0.106
Sodium (mmol/L)	136.5 \pm 3.2	136.4 \pm 3.6	0.844
Potassium (mmol/L)	4.4 \pm 0.8	4.5 \pm 0.8	0.287
Uric acid (mg/dL)	7.1 \pm 1.9	7.2 \pm 1.7	0.823
Hemoglobin (g/dL)	10.4 \pm 0.8	10.3 \pm 1.1	0.486
Ferritin	502.6 \pm 365.9	439.3 \pm 372.4	0.375
EPO1 (10 ⁶ units)	1.54 \pm 1.19	0.94 \pm 0.69	0.002
EPO2 (10 ⁶ units)	2.15 \pm 1.56	1.28 \pm 0.91	0.001
EPO3 (10 ⁶ units)	2.76 \pm 1.97	1.62 \pm 1.18	< 0.0001
T-score	-3.7 \pm 4.0	-1.6 \pm 0.6	< 0.0001
L-spine BMD	1.028 \pm 0.240	1.227 \pm 0.271	< 0.0001
Right femoral neck BMD	0.711 \pm 0.239	1.033 \pm 1.092	0.037
Left femoral neck BMD	0.664 \pm 0.147	0.889 \pm 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 measurement¹⁶. 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 populations¹⁹. 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).

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4 At our institution, EPO is administered based on the patient's weekly hemoglobin
5 levels. EPO doses received were positively correlated with patient dialysis duration.
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7 The longer the patient undergoes dialysis, the higher the dose of EPO the patient may
8 receive. However, no statistically significant differences in dialysis duration were
9 found between patients with osteoporosis and those without [$P = 0.762$ (unmatched),
10 $P = 0.111$ (age- and sex-matched)]. All three models, LnEPO1, LnEPO2, and
11 LnEPO3, showed significant differences in cumulative EPO in patients with
12 osteoporosis compared with those without (Table 5). A negative correlation was
13 observed between the total, lumbar, right femoral neck, and left femoral neck T-
14 scores and EPO dose (Figure 1). Although these results showed a low and negative
15 correlation between T-scores and EPO dose (Pearson's correlation coefficient r from
16 0.30 to 0.46), these data reached statistical significance ($P < 0.005$ to < 0.0001).
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25 Serum PTH is negatively associated with BMD measurements; cortical porosity
26 increased in patients with hyperparathyroidism²². Several studies have reported a
27 negative association between PTH levels and BMD measurements^{17,23,24}, whereas
28 others were unable to show this association²⁵⁻²⁷. In the present study, however, we
29 found a negative association between PTH levels and BMD measurements,
30 suggesting that other factors affect BMD in patients on hemodialysis. Forty-three
31 patients received active vitamin D treatment in the current study.
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38 Aluminum overload may be responsible for adynamic bone disease and osteomalacia.
39 At our institution, serum aluminum levels are measured annually in patients who have
40 undergone dialysis for >5 years. Our patients had no abnormally elevated serum
41 aluminum levels. Moreover, we did not perform a histological analysis of bone. Thus,
42 we cannot comment on the prevalence of adynamic bone disease and osteomalacia in
43 this population.
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50 The relationships between calcium intake, vitamin D supplementation, and
51 osteoporosis development remain controversial. One study has shown that oral 1α -
52 hydroxycalciferol treatment could prevent BMD loss in the Lumbar Spine in a study
53 of 165 male patients²⁸. All 165 patients were receiving calcium-containing phosphate
54 binders. Only ten patients received vitamin D supplements in the nonosteoporotic
55 group compared with 33 patients who received vitamin D supplements in the
56 osteoporotic group.
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4 The strengths of our study are the random sampling of the population and the high
5 accuracy of cumulative EPO treatment history collected. Participants with and
6 without osteoporosis were age- and sex-matched to examine the association of EPO
7 treatment with the risk of osteoporosis development. However, the present study was
8 limited by its cross-sectional nature. It is difficult to establish the causal relationship
9 between EPO cumulation and the risk of osteoporosis. A further longitudinal study is
10 required to confirm the cause and effect of EPO in reducing BMD. Moreover, the
11 present study involving a group of elderly participants. Our subgroup analysis showed
12 that participants aged <65 years with osteoporosis did not receive a higher EPO dose
13 than participants aged >65 years with osteoporosis ($r = -0.21$, $P = 0.133$, data not
14 shown).

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24 In conclusion, we confirmed the importance of age and body weight as the risk factors
25 affecting BMD in patients on hemodialysis. We found that the cumulative EPO dose
26 has a negative correlation in dialysis patients' BMD.
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30 31 32 **Declarations**

33 34 35 **Ethics approval and consent to participate**

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37 Taipei Medical University Institutional Review Board approved the study for
38 Human Experimentation.
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44 45 **Data sharing**

46 All data generated or analyzed during this study are included in this article; no
47 additional data is available.
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53 54 **Competing interests**

55 The authors declare that they have no competing interests.
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60 **Funding**

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4 The present study has no funding available.
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8 **Authors' contributions**

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10
11 YJK designed the study and helped in analyzing the data. CYC wrote the
12 manuscript and analyzed the data.
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16 **Acknowledgment**

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19 The authors gratefully acknowledge Professor Jin-Hua Chen of the Department of
20 Graduate Institute of Data Science, Taipei Medical University, for their advice on the
21 statistical analysis.
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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.

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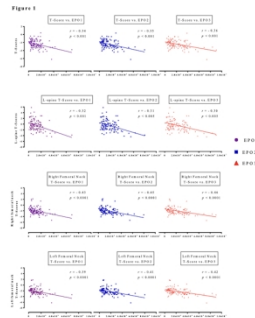


Figure 1 Descriptive plots of correlations between T-scores of L-spine, right femoral, left femoral, and cumulative Erythropoietin (EPO) dose received. EPO1, EPO2, and EPO3 represent three different dose conversion models. EPO1, 1 µg of darbepoietin/Mircera converts to 200 IU of EPO; EPO2, 1 µg of darbepoietin/Mircera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

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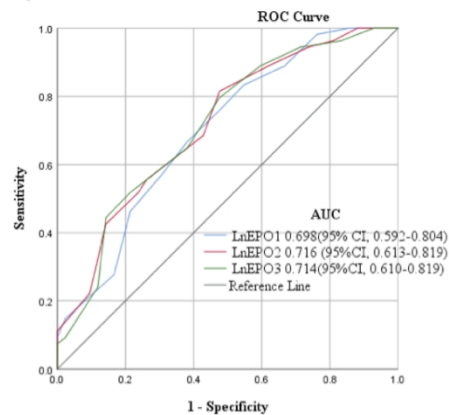
Figure 2

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.

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BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-056390.R1
Article Type:	Original research
Date Submitted by the Author:	27-Dec-2021
Complete List of Authors:	Cheng, Chung-Yi; Taipei Municipal Wan-Fang Hospital, Division of Nephrology, Department of Internal Medicine; Taipei Medical University, Department of Internal Medicine, School of Medicine, College of Medicine Kuo, Yi-Jie; Taipei Municipal Wan-Fang Hospital, Department of Orthopedic Surgery; Taipei Medical University, Department of Orthopedic Surgery, School of Medicine, College of Medicine, Taipei Medical University
Primary Subject Heading:	Renal medicine
Secondary Subject Heading:	Renal medicine, Geriatric medicine
Keywords:	Nephrology < INTERNAL MEDICINE, Chronic renal failure < NEPHROLOGY, Dialysis < NEPHROLOGY, Rheumatology < INTERNAL MEDICINE, Calcium & bone < DIABETES & ENDOCRINOLOGY

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4 **Cumulative Erythropoietin negatively correlated with bone mineral density in**
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7 **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 fractures¹ and have reduced quality of life². 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 thought³. 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

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4 analogs (EPO-stimulating agents [ESAs]) for anemia correction has reduced the
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6 associated morbidity and improved functionality, exercise tolerance, cognitive
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8 function, and overall quality of life. However, over the last few years, much
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10 controversy has been raised over the possible risks of ESA therapy. Moreover,
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12 thorough investigation of the mechanism of action of EPO has revealed multiple
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14 biologic effects that extend beyond its erythropoietic effect and may have a favorable
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16 or sometimes unfavorable contribution to these outcomes.
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21 EPO acts on erythroid progenitor cells by binding to an EPO receptor (EPOR),
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23 promoting survival, proliferation, and differentiation⁴. Functioning EPOR is present in
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25 endothelial cells⁵, neurons⁶, skeletal muscle progenitor cells⁷, adipocytes⁵, and islets⁸,
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27 suggesting that EPO signaling exerts systemic regulation and interacts with
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29 nonerythroid cells through actions beyond erythropoiesis. Growing evidence from
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31 animal studies has demonstrated the critical role of EPO in regulating skeletal
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33 homeostasis^{9,10}. Moreover, recent evidence has also demonstrated that EPO resulted
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35 in reduced trabecular bone volume in a mouse model of diet-induced obesity¹¹.
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37 However, for humans, insufficient evidence exists on the role of EPO in mediating the
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39 bone microenvironment.
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46 This study aimed to assess the correlation between cumulative doses of EPO
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48 administration and the risk of osteoporosis in patients on chronic dialysis. Moreover,
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50 bone mineral density in the femur and lumbar Spine of patients on dialysis was
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52 investigated, its correlation with some clinical and biochemical factors was
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54 determined.
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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,

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3 hemoglobin; H.D., hemodialysis; K, potassium; LF-T BMD, total left femur bone mineral
4 density; L-spine BMD, lumbar-spine bone mineral density; Na, sodium; P, phosphorus;
5 intact PTH, intact parathyroid hormone; P.D., peritoneal dialysis; RF-T BMD, total right
6 femur bone mineral density; T.G., triglyceride; T-Chol, total cholesterol; yrs, years.
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12 A detailed history of related risk factors (smoking, hypertension, diabetes, steroid
13 intake, and surgical menopause) was obtained from all patients, and medical records
14 were checked after consent was obtained. Before initiating the dialysis session,
15 baseline investigations were performed at the patient's regular blood test session.
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17 Blood tests included kidney function tests, serum calcium, serum phosphorus, intact
18 parathyroid hormone, fasting glucose, serum alkaline phosphatase levels, liver
19 function tests, complete blood counts, ferritin, and determination of lipid profiles.
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30 The DXA definition of osteoporosis and the bone mass criteria followed for its
31 diagnosis were adopted from the WHO definition of osteoporosis (1994). T-scores
32 were used for the evaluation of BMD and the definition of different stages of BMD
33 according to the WHO definition of osteoporosis. T-scores were obtained for the
34 femoral necks and lumbar spines (L1-L4). The average of lumbar Spine BMD was to
35 evaluate the lumbar Spine T-score, use of three vertebrae if four cannot be used, and
36 two if three cannot be used for the diagnosis according to the (The International
37 Society for Clinical Densitometry, ISCD) guideline.¹² The lowest T-score among
38 femoral necks and lumbar spines was accounted for established osteoporosis. The T-
39 score Normative Database is calculated by using USA (Combined NHANES (ages 20-
40 30)/Lunar (ages 20-40) A.P. spine and Femur Reference Population).
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57 **EPO dose conversion**

58 Patients receive either darbepoetin alfa (DPO) (Aranesp, Kyowa Hakko Kirin Co.,
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4 Ltd.), epoetin beta (Recormon, Roche), or methoxy polyethylene glycol-epoetin beta
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6 (Mircera, Roche) at our institution. EPO doses are administered according to the
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8 patient's weekly hemoglobin levels. We maintain our patients' hemoglobin levels
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10 between 10 and 12 g/dl. For conversion from EPO alfa to darbepoetin alfa, a fixed
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12 conversion ratio of 200 IU EPO to 1 µg DPO was suggested by the manufacturer¹³.
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14 However, numerous studies have suggested that the conversion ratio should be 240–
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16 400 IU of EPO and 1 µg of DPO¹⁴⁻¹⁶. In the current study, the cumulative dose of
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18 EPO received by the patient was calculated from the first day received EPO in our
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20 hospital until the DXA study date. The patient might receive various combination of
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22 EPOs during their dialysis treatment in our institution. We established three
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24 conversion doses of darbepoetin alpha (DPO) and methoxy polyethylene glycol-
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26 epoetin beta (Mircera) to calculate the statistical difference between patients with and
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28 without osteoporosis. EPO1 refers to the conversion of 1 µg of DPO/Mircera to 200
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30 IU of EPO. EPO2 refers to the conversion of 1 µg of DPO/Mircera to 300 IU of EPO.
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32 EPO3 refers to the conversion of 1 µg of DPO/Mircera to 400 IU of EPO.
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40 **Ethical approval**

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42 The study was approved by the Taipei Medical University Institutional Review Board
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44 for Human Experimentation. The accession number: TMU-IRB N202103059.
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49 **Statistical analysis**

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

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

4 Total: the lowest T-score found among femoral necks and lumbar spines.

5 Abbreviations: BMD, bone mineral density; L.F. Neck, left femoral neck; L-spine,
6 lumbar-spine; R.F. Neck, right femoral neck.
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10 **Factors associated with reduced bone mineral density**

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13 In total, 165 patients with and without osteoporosis were evaluated for differences in
14 risk factors for and biochemical markers of bone diseases. These included all the
15 factors in Table 3, and individual variables were evaluated using Student's t-test.
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17 Independent variables that were analyzed and reached statistical significance ($P <$
18 0.05) are shown in Table 3. Age, body mass index (BMI), body weight (BW), serum
19 calcium, ferritin, and EPO doses show statistical differences between patients with
20 osteoporosis and patients without osteoporosis. Furthermore, 108 age- and sex-
21 matched patients were evaluated for differences in risk factors for and biochemical
22 markers of bone diseases. These included all the factors listed in Table 4. Cumulative
23 EPO dosage was significantly different in age- and sex-matched patients with
24 osteoporosis than nonosteoporotic patients on dialysis. All three EPO conversion
25 models showed similar and significant results. Three models of EPO dose conversion
26 were used to examine the association between EPO and T-scores of participants. The
27 statistical calculation process was repeated using different EPO dose models to avoid
28 collinearity. The results are shown in Figure 1. Pearson's correlation coefficient varied
29 between -0.30 and -0.46 , but p values were statistically significant.
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Table 3 The t-test analysis of chronic dialysis patients with and without osteoporosis

Variables	OS (<i>n</i> = 99)	Without OS (<i>n</i> = 66)	<i>p</i> 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 (<i>n</i> = 27)	Without OS (<i>n</i> = 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 (<i>n</i> = 48)	Without OS (<i>n</i> = 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 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 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; LogEPO3, 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 measurement¹⁷. 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 populations²⁰. 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).

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

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

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40 Serum PTH is negatively associated with BMD measurements; cortical porosity
41 increased in patients with hyperparathyroidism²³. Several studies have reported a
42 negative association between PTH levels and BMD measurements^{18,24,25}, whereas
43 others were unable to show this association²⁶⁻²⁸. In the present study, however, we
44 found a negative association between PTH levels and BMD measurements,
45 suggesting that other factors affect BMD in patients on hemodialysis. Forty-three
46 patients received active vitamin D treatment in the current study.

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53 Aluminum overload may be responsible for adynamic bone disease and osteomalacia.
54 At our institution, serum aluminum levels are measured annually in patients who have
55 undergone dialysis for >5 years. Our patients had no abnormally elevated serum
56 aluminum levels. Moreover, we did not perform a histological analysis of bone. Thus,
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4 we cannot comment on the prevalence of adynamic bone disease and osteomalacia in
5 this population.
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8 The relationships between calcium intake, vitamin D supplementation, and
9 osteoporosis development remain controversial. One study has shown that oral 1α -
10 hydroxycalciferol treatment could prevent BMD loss in the Lumbar Spine in a study
11 of 165 male patients²⁹. All 165 patients were receiving calcium-containing phosphate
12 binders. Only ten patients received vitamin D supplements in the nonosteoporotic
13 group compared with 33 patients who received vitamin D supplements in the
14 osteoporotic group.
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21 Clinical and molecular evidence suggests that chronic inflammation significantly
22 influences bone turnover.^{30,31} Uremic syndrome, hemodialysis, use of a catheter, and
23 persistent infection may contribute to the development of the inflammatory state in
24 CKD. In hemodialysis patients, inflammation has been associated with EPO
25 resistance mainly because the inflammatory state decreases the bone marrow response
26 to ESA, changing iron regulation through hepcidin upregulation and/or causing red
27 blood cell/erythrocyte hemolysis.³² In the current study, we had not studied the
28 inflammatory status among patients with/without osteoporosis. However, some
29 markers of inflammatory reaction had included in our laboratory study, including
30 WBC, platelets, ferritin, and albumin. Both platelet and white cell counts have been
31 implicated in playing an essential role in inflammatory reaction.^{33,34} Similarly, both
32 ferritin and albumin have also known as acute-phase proteins. WBC, platelets,
33 ferritin, and albumin have not shown statistical differences between osteoporotic and
34 non-osteoporotic patients in the age- and sex-matched model.
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46 The strengths of our study are the random sampling of the population and the high
47 accuracy of cumulative EPO treatment history collected. Participants with and
48 without osteoporosis were age- and sex-matched to examine the association of EPO
49 treatment with the risk of osteoporosis development. However, the present study was
50 limited by its cross-sectional nature. It is difficult to establish the causal relationship
51 between EPO accumulation and the risk of osteoporosis. A further longitudinal study
52 is required to confirm the cause and effect of EPO in reducing BMD. Moreover, the
53 present study involves a group of elderly participants. Our subgroup analysis showed
54 that participants aged <65 years with osteoporosis did not receive a higher EPO dose
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4 than participants aged >65 years with osteoporosis ($r = -0.21$, $P = 0.133$, data not
5 shown).
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8 In conclusion, we confirmed the importance of age and body weight as the risk factors
9 affecting BMD in patients on hemodialysis. We found that the cumulative EPO dose
10 has a negative correlation in dialysis patients' BMD. Elderly dialysis patients under
11 long-term EPO treatment are at risk of developing osteoporosis. Managing anemia in
12 this vulnerable population may consider other possible therapeutic strategies.
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20 21 **Declarations**

22 23 24 **Ethics approval and consent to participate**

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26 Taipei Medical University Institutional Review Board approved the study for
27 Human Experimentation (TMU-eJIRB, N202103059).
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33 34 **Data sharing**

35 All free text entered will be published
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40 41 **Competing interests**

42 The authors declare that they have no competing interests.
43
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47 48 **Funding**

49 The present study has no funding available.
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54 55 **Authors' contributions**

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4 Conceptualization, formal analysis, investigation, methodology, original draft
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6 writing, C-Y.C.; conceptualization, data curation, investigation, methodology,
7
8 resources, and writing review and editing, Y-J-K.
9

10 11 **Acknowledgment**

12
13
14 The authors gratefully acknowledge Professor Jin-Hua Chen of the Department of
15 Graduate Institute of Data Science, Taipei Medical University, for their advice on the
16 statistical analysis.
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20 21 22 **References**

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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

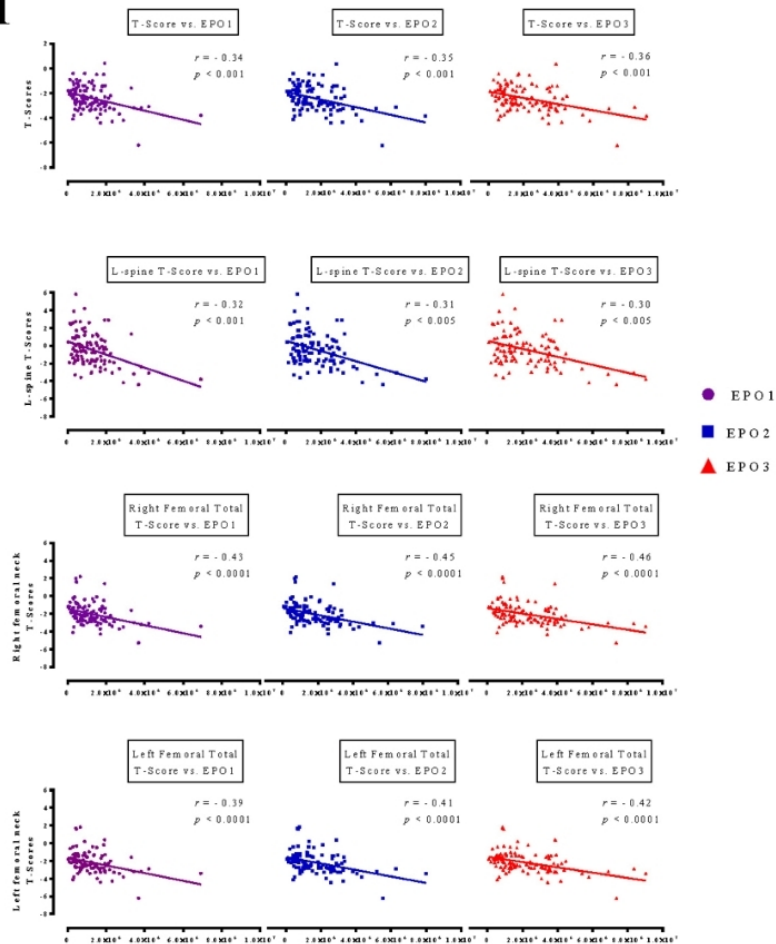


Figure 1 Descriptive plots of correlations between T-scores of L-spine, total right femur, total left femur, and cumulative Erythropoietin (EPO) dose received. EPO1, EPO2, and EPO3 represent three different dose conversion models. EPO1, 1 μ g of darbepoietin/Mircera converts to 200 IU of EPO; EPO2, 1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

76x76mm (300 x 300 DPI)

Figure 2 **EPO vs. Osteoporosis**
Diagnosed at Different sites

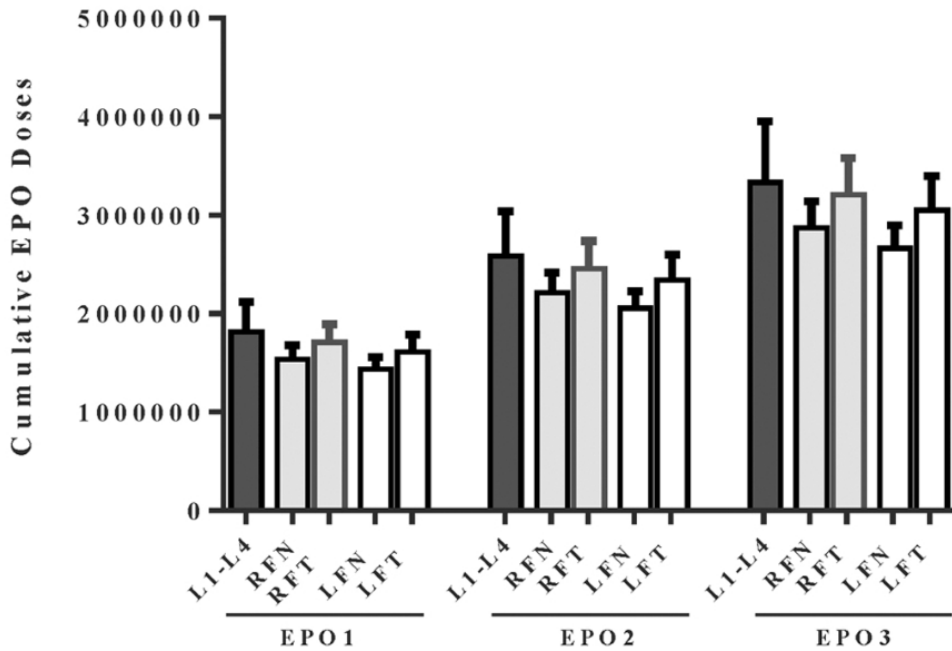
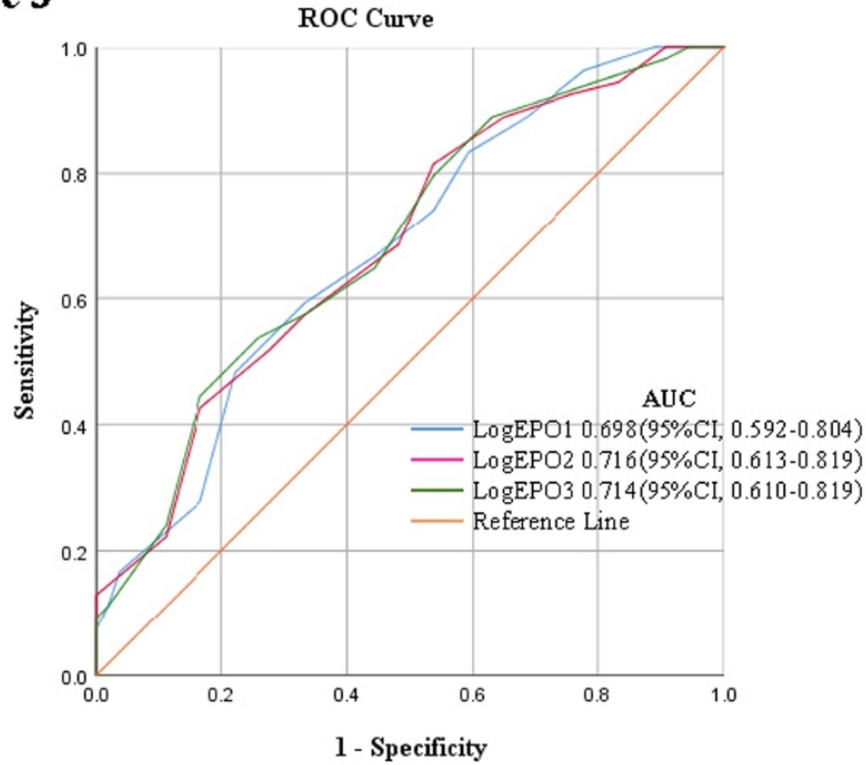


Figure 2 Three different models of erythropoietin dose conversion versus bone mineral density among three different sites in dialysis patients with osteoporosis. Abbreviations: BMD, bone mineral density; EPO, erythropoietin; L1-L4, lumbar-spine 1 to 4; RFN, right femoral neck; RFT, total right femur;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

76x76mm (300 x 300 DPI)

Figure 3

Diagonal segments are produced by ties.

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.

76x76mm (300 x 300 DPI)

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-056390.R2
Article Type:	Original research
Date Submitted by the Author:	22-Feb-2022
Complete List of Authors:	Cheng, Chung-Yi; Taipei Medical University, Division of Nephrology, Department of Internal Medicine, School of Medicine, College of Medicine; Taipei Medical University, Wan Fang Hospital, Division of Nephrology, Department of Internal Medicine Kuo, Yi-Jie; Taipei Medical University, Department of Orthopedic Surgery, School of Medicine, College of Medicine, Taipei Medical University; Taipei Medical University, Wan Fang Hospital, Department of Orthopedic Surgery
Primary Subject Heading:	Renal medicine
Secondary Subject Heading:	Renal medicine, Geriatric medicine
Keywords:	Nephrology < INTERNAL MEDICINE, Chronic renal failure < NEPHROLOGY, Dialysis < NEPHROLOGY, Rheumatology < INTERNAL MEDICINE, Calcium & bone < DIABETES & ENDOCRINOLOGY

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4 **A Single-Center Cross-Sectional Study on the Impact of Cumulative**
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7 **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 fractures¹ and have reduced quality of life². 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 thought³. 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 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

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4 (EPO-stimulating agents [ESAs]) for anemia correction has reduced the associated
5 morbidity and improved functionality, exercise tolerance, cognitive function, and
6 overall quality of life. However, over the last few years, much controversy has been
7 raised over the possible risks of ESA therapy. Moreover, a thorough investigation of
8 the mechanism of action of EPO has revealed multiple biologic effects that extend
9 beyond its erythropoietic effect and may have a favorable or sometimes unfavorable
10 contribution to these outcomes.
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21 EPO acts on erythroid progenitor cells by binding to an EPO receptor (EPOR),
22 promoting survival, proliferation, and differentiation⁴. Functioning EPOR is present in
23 endothelial cells⁵, neurons⁶, skeletal muscle progenitor cells⁷, adipocytes⁵, and islets⁸,
24 suggesting that EPO signaling exerts systemic regulation and interacts with
25 nonerythroid cells through actions beyond erythropoiesis. Growing evidence from
26 animal studies has demonstrated the critical role of EPO in regulating skeletal
27 homeostasis^{9,10}. Moreover, recent evidence has also demonstrated that EPO reduced
28 trabecular bone volume in a mouse model of diet-induced obesity¹¹. However, for
29 humans, insufficient evidence exists on the role of EPO in mediating the bone
30 microenvironment.
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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

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4 Society for Clinical Densitometry, ISCD) guideline.¹² The lowest T-score among
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6 femoral necks and lumbar spines was accounted for established osteoporosis. The
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8 T-score Normative Database is calculated by using USA (Combined NHANES (ages
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10 20-30)/Lunar (ages 20-40) A.P. spine and Femur Reference Population).
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15 **EPO dose conversion**

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17 Patients receive either darbepoetin alfa (DPO) (Aranesp, Kyowa Hakko Kirin Co.,
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19 Ltd.), epoetin beta (Recormon, Roche), or methoxy polyethylene glycol-epoetin beta
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21 (Mircer, Roche) at our institution. EPO doses are administered according to the
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23 patient's weekly hemoglobin levels. We maintain our patients' hemoglobin levels
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25 between 10 and 12 g/dl. For conversion from EPO alfa to darbepoetin alfa, a fixed
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27 conversion ratio of 200 IU EPO to 1 µg DPO was suggested by the manufacturer¹³.
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29 However, numerous studies have suggested that the conversion ratio be 240–400 IU
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31 of EPO and 1 µg of DPO¹⁴⁻¹⁶. In the current study, the cumulative dose of EPO
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33 received by the patient was calculated from the first day received EPO in our hospital
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35 until the DXA study date. The patient might receive various EPOs during their
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37 dialysis treatment in our institution. We established three conversion doses of
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39 darbepoetin alpha (DPO) and methoxy polyethylene glycol-epoetin beta (Mircer) to
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41 calculate the statistical difference between patients with and without osteoporosis.
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43 EPO1 refers to converting 1 µg of DPO/Mircer to 200 IU of EPO, EPO2 converting
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45 1 µg of DPO/Mircer to 300 IU of EPO, and EPO3 converting 1 µg of DPO/Mircer
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47 to 400 IU of EPO.
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55 **Ethical approval**

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57 The study was approved by the Taipei Medical University Institutional Review Board
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59 for Human Experimentation. The accession number: TMU-IRB N202103059.
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4 Informed consent was obtained from all subjects involved in the present study.
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8 **Patient and Public Involvement**

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10 Patients and the public were not directly involved in this research. The nature of the
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12 anonymised records means individual participants could not be involved.
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16 **Statistical analysis**

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18 Data were expressed as mean \pm standard deviation unless otherwise specified.
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21 Pearson's correlation coefficients assessed correlations between bone measurements
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23 and cumulative EPO doses. Stepwise multiple regression analysis was used to
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25 investigate the relationships between bone measurements and biochemical markers or
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27 risk factors for bone diseases. The backward stepwise regression method was used to
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29 select variables in the multivariate analysis. Only a single log-transformed value of
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31 EPO was selected at every entry for multivariate analysis to avoid errors generated
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33 due to the collinearity of log EPOs. It means either log EPO1, log EPO2 or log EPO3
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35 input into the multivariate analysis but not all three log EPOs entries. Differences
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37 between the means of multiple subgroups were assessed using the Kruskal–Wallis
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39 test. An unpaired t-test or Mann–Whitney U test was used for continuous variables.
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41 The chi-square test was used to compare frequencies between categorical variables.
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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

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4 factors in Table 3, and individual variables were evaluated using Student's t-test.
5 Independent variables that were analyzed and reached statistical significance ($P <$
6 0.05) are shown in Table 3. Age, body mass index (BMI), body weight (B.W.), serum
7 calcium, ferritin, and EPO doses show statistical differences between patients with
8 osteoporosis and patients without osteoporosis. Furthermore, 108 age- and
9 sex-matched patients were evaluated for differences in risk factors for and
10 biochemical markers of bone diseases. These included all the factors listed in Table 4.
11 Cumulative EPO dosage was significantly different in age- and sex-matched patients
12 with osteoporosis than nonosteoporotic patients on dialysis. All three EPO conversion
13 models showed similar and significant results. Three models of EPO dose conversion
14 were used to examine the association between EPO and T-scores of participants. The
15 statistical calculation process was repeated using different EPO dose models to avoid
16 collinearity. The results are shown in Figure 1. Pearson's correlation coefficient varied
17 between -0.30 and -0.46 , but p values were statistically significant.
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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 (<i>n</i> = 54)	Without OS (<i>n</i> = 54)	<i>p</i> 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; LogEPO3, 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

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4 entered into the multivariate and age-sex model for analysis to avoid multicollinearity.
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7 **Role of erythropoietin use in osteoporosis development**

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

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25 This study found a moderate reduction in the mean BMD in this unselected
26 population of patients on chronic hemodialysis. The mean T-score of -2.17 in the
27 DXA measurement of the femoral neck implies that these patients had moderately less
28 favorable outcomes than age-matched controls. The mean T-score value found in the
29 present study is similar to several other studies that used the same bone mineral
30 density measurement¹⁷. Age and weight also emerged as important determinants of
31 BMD in our study. Age-related bone loss plays an essential role in the pathogenesis of
32 osteoporosis, and a negative association between age and BMD in female patients
33 with end-stage renal disease has been reported^{18,19}. The mean age of patients in these
34 two studies was 43 and 50.5 years, whereas, in our study, patients were older, with a
35 mean age of 66.6 years. With the number of older adults involved in the renal
36 replacement program increasing and with survival rates markedly improving,
37 age-related bone loss can be expected to become an increasingly important factor
38 causing bone disease in these patients.
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50 Moreover, evidence has revealed a positive correlation between weight and BMD in
51 healthy populations²⁰. The correlation between B.W. and BMD has been attributed to
52 bone formation stimulations through weight-bearing and adipose tissues' increased
53 peripheral conversion of adrenal androgens to estrogens. Two studies have reported a
54 positive association between BMI and BMD measurements^{21,22}. We showed a similar
55 association in our patients. Finally, we found a significant difference in cumulative
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4 EPO use in patients with osteoporosis compared with those without osteoporosis in
5 univariate and multivariate analyses (Table 5).
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8 EPO is administered based on the patient's weekly hemoglobin levels at our
9 institution. EPO doses received were positively correlated with patient dialysis
10 duration. The longer the patient undergoes dialysis, the higher the dose of EPO the
11 patient may receive. However, no statistically significant differences in dialysis
12 vintage were found between patients with osteoporosis and those without [$P = 0.762$
13 (unmatched), $P = 0.111$ (age- and sex-matched)]. All three models, logEPO1,
14 logEPO2, and logEPO3 showed significant differences in cumulative EPO in patients
15 with osteoporosis compared with those without (Table 5). A negative correlation was
16 observed between the total, lumbar, right femoral neck, and left femoral neck
17 T-scores and EPO dose (Figure 1). Although these results showed a low and negative
18 correlation between T-scores and EPO dose (Pearson's correlation coefficient r from
19 0.30 to 0.46), these data reached statistical significance ($P < 0.005$ to < 0.0001).
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30 Higher EPO dosages were administered in patients with lumbar spine osteoporosis
31 than patients with cortical bone osteoporosis (right or left femur). However, no
32 statistical significance was reached in the current study (Figure 1). Effects of
33 erythropoietin-induced bone loss had been demonstrated in experimental
34 mice.^{10,11} However, clinical evidence concerning EPO with bone mineral density is
35 lacking. Whether EPO exerts more trabecular bone loss or cortical bone loss remains
36 to be elucidated.
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43 Serum PTH is negatively associated with BMD measurements; cortical porosity
44 increased in patients with hyperparathyroidism²³. Several studies have reported a
45 negative association between PTH levels and BMD measurements^{18,24,25}, whereas
46 others were unable to show this association²⁶⁻²⁸. In the present study, however, we
47 found a negative association between PTH levels and BMD measurements,
48 suggesting that other factors affect BMD in patients on hemodialysis. Forty-three
49 patients received active vitamin D treatment in the current study.
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56 Aluminum overload may be responsible for adynamic bone disease and osteomalacia.
57 At our institution, serum aluminum levels are measured annually in patients who have
58 undergone dialysis for >5 years. Our patients had no abnormally elevated serum
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4 aluminum levels. Moreover, we did not perform a histological analysis of bone. Thus,
5 we cannot comment on the prevalence of adynamic bone disease and osteomalacia in
6 this population.
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10 The relationships between calcium intake, vitamin D supplementation and
11 osteoporosis development remain controversial. One study has shown that oral
12 1α -hydroxycalciferol treatment could prevent BMD loss in the Lumbar Spine in a
13 study of 165 male patients²⁹. All 165 patients were receiving calcium-containing
14 phosphate binders. Only ten patients received vitamin D supplements in the
15 nonosteoporotic group compared with 33 patients who received vitamin D
16 supplements in the osteoporotic group.
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23 Clinical and molecular evidence suggests that chronic inflammation significantly
24 influences bone turnover.^{30,31} Uremic syndrome, hemodialysis, use of a catheter, and
25 persistent infection may contribute to the development of the inflammatory state in
26 CKD. In hemodialysis patients, inflammation has been associated with EPO
27 resistance mainly because the inflammatory state decreases the bone marrow response
28 to ESA, changing iron regulation through hepcidin upregulation and/or causing red
29 blood cell/erythrocyte hemolysis.³² In the current study, we had not studied the
30 inflammatory status among patients with/without osteoporosis. However, some
31 markers of inflammatory reaction had included in our laboratory study, including
32 WBC, platelets, ferritin, and albumin. Both platelet and white cell counts have been
33 implicated in playing an essential role in inflammatory reaction.^{33,34} Similarly, both
34 ferritin and albumin have also known as acute-phase proteins. In the age- and
35 sex-matched model, WBC, platelets, ferritin, and albumin have not shown statistical
36 differences between osteoporotic and nonosteoporotic patients.
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48 The strengths of our study are the random sampling of the population and the high
49 accuracy of cumulative EPO treatment history collected. Participants with and
50 without osteoporosis were age- and sex-matched to examine the association of EPO
51 treatment with the risk of osteoporosis development. However, the present study was
52 limited by its cross-sectional nature. It is difficult to establish the causal relationship
53 between EPO accumulation and the risk of osteoporosis. A further longitudinal study
54 is required to confirm the cause and effect of EPO in reducing BMD. Moreover, the
55 present study involves a group of elderly participants. Our subgroup analysis showed
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4 that participants aged <65 years with osteoporosis did not receive a higher EPO dose
5 than participants aged >65 years with osteoporosis ($r = -0.21$, $P = 0.133$, data not
6 shown).
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10 In conclusion, we confirmed the importance of age and body weight as the risk factors
11 affecting BMD in patients on hemodialysis. We found that the cumulative EPO
12 negatively correlates with dialysis patients' BMD. Elderly dialysis patients under
13 long-term EPO treatment are at risk of developing osteoporosis. Managing anemia in
14 this vulnerable population may consider other possible therapeutic strategies.
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23 **Declarations**

24 **Ethics approval and consent to participate**

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28 Taipei Medical University Institutional Review Board approved the study for
29 Human Experimentation (TMU-eJIRB, N202103059).
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35 **Data sharing**

36 All free text entered will be published
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42 **Competing interests**

43
44 The authors declare that they have no competing interests.
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49 **Funding**

50
51 This research was funded by Taipei Medical University Hospital, Wan Fang
52 Hospital, and Taipei Medical University. Funding numbers: 106TMU-WFH-11,
53 106-eva-03, 108-wf-eva-31, and 108TMU-WFH-25.
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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.

Acknowledgment

The authors gratefully acknowledge Professor Jin-Hua Chen of the Department of Graduate Institute of Data Science, Taipei Medical University, for their advice on the statistical analysis.

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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

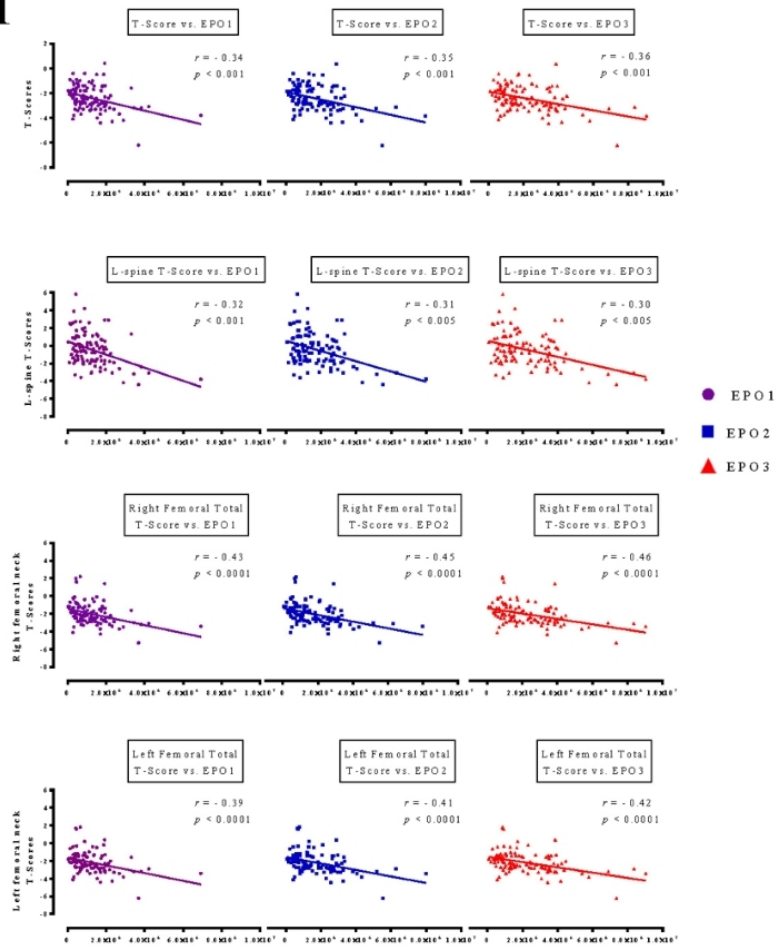


Figure 1 Descriptive plots of correlations between T-scores of L-spine, total right femur, total left femur, and cumulative Erythropoietin (EPO) dose received. EPO1, EPO2, and EPO3 represent three different dose conversion models. EPO1, 1 µg of darbepoietin/Mircera converts to 200 IU of EPO; EPO2, 1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

76x76mm (300 x 300 DPI)

Figure 2 **EPO vs. Osteoporosis**
Diagnosed at Different sites

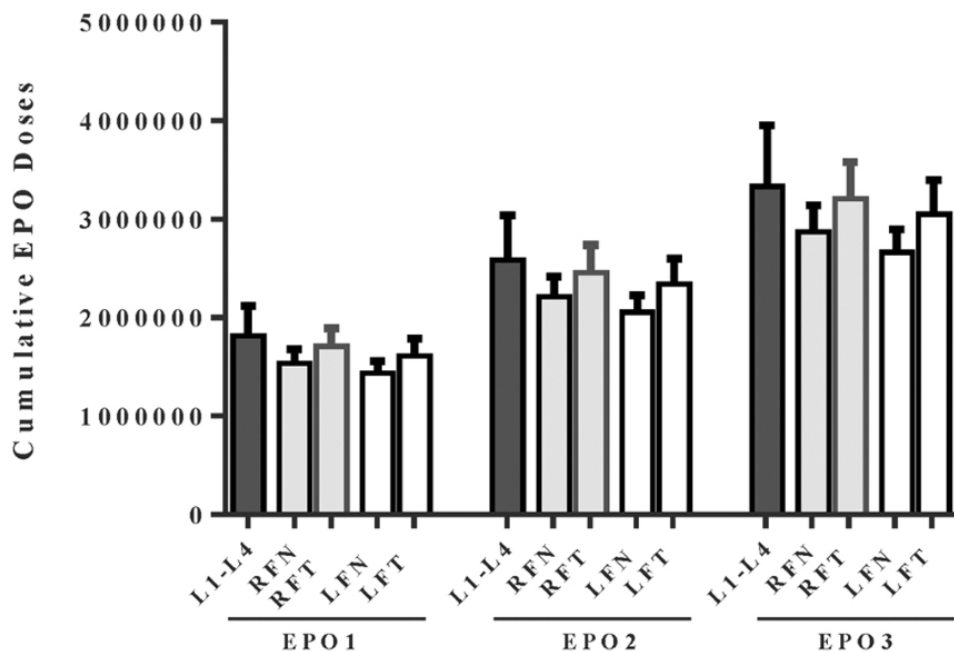
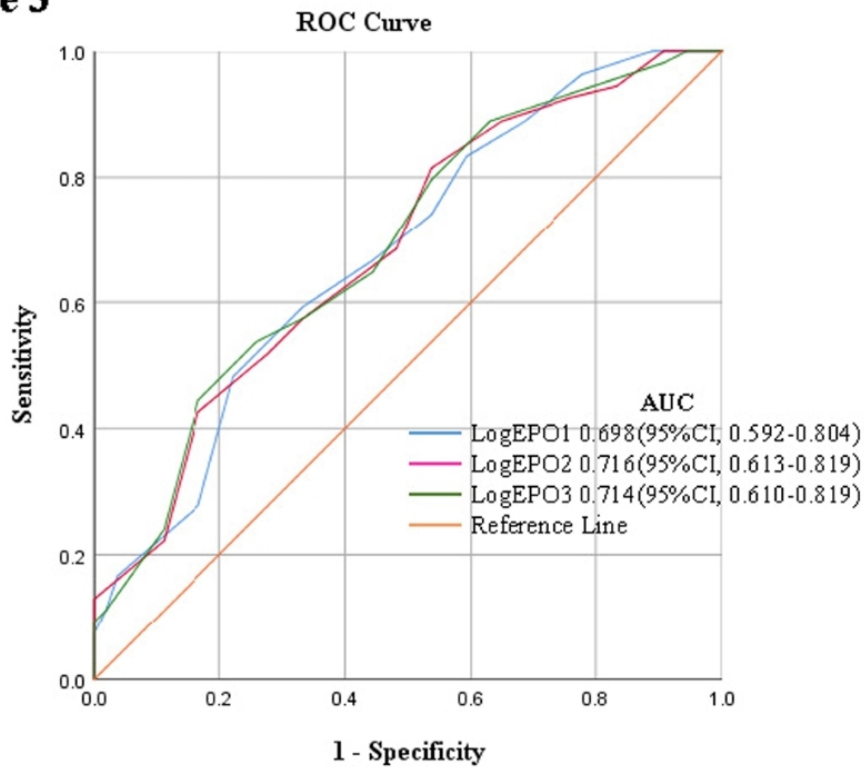


Figure 2 Three different models of erythropoietin dose conversion versus bone mineral density among three different sites in dialysis patients with osteoporosis. Abbreviations: BMD, bone mineral density; EPO, erythropoietin; L1-L4, lumbar-spine 1 to 4; RFN, right femoral neck; RFT, total right femur;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

76x76mm (300 x 300 DPI)

Figure 3

Diagonal segments are produced by ties.

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.

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-056390.R3
Article Type:	Original research
Date Submitted by the Author:	03-Mar-2022
Complete List of Authors:	Cheng, Chung-Yi; Taipei Medical University, Division of Nephrology, Department of Internal Medicine, School of Medicine, College of Medicine; Taipei Medical University, Wan Fang Hospital, Division of Nephrology, Department of Internal Medicine Kuo, Yi-Jie; Taipei Medical University, Department of Orthopedic Surgery, School of Medicine, College of Medicine, Taipei Medical University; Taipei Medical University, Wan Fang Hospital, Department of Orthopedic Surgery
Primary Subject Heading:	Renal medicine
Secondary Subject Heading:	Renal medicine, Geriatric medicine
Keywords:	Nephrology < INTERNAL MEDICINE, Chronic renal failure < NEPHROLOGY, Dialysis < NEPHROLOGY, Rheumatology < INTERNAL MEDICINE, Calcium & bone < DIABETES & ENDOCRINOLOGY

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4 **A Single-Center Cross-Sectional Study on the Impact of Cumulative**

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7 **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 fractures¹ and have reduced quality of life². 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 thought³. 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 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

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4 (EPO-stimulating agents [ESAs]) for anemia correction has reduced the associated
5 morbidity and improved functionality, exercise tolerance, cognitive function, and
6 overall quality of life. However, over the last few years, much controversy has been
7 raised over the possible risks of ESA therapy. Moreover, a thorough investigation of
8 the mechanism of action of EPO has revealed multiple biologic effects that extend
9 beyond its erythropoietic effect and may have a favorable or sometimes unfavorable
10 contribution to these outcomes.
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22 EPO acts on erythroid progenitor cells by binding to an EPO receptor (EPOR),
23 promoting survival, proliferation, and differentiation⁴. Functioning EPOR is present in
24 endothelial cells⁵, neurons⁶, skeletal muscle progenitor cells⁷, adipocytes⁵, and islets⁸,
25 suggesting that EPO signaling exerts systemic regulation and interacts with
26 nonerythroid cells through actions beyond erythropoiesis. Growing evidence from
27 animal studies has demonstrated the critical role of EPO in regulating skeletal
28 homeostasis^{9,10}. Moreover, recent evidence has also demonstrated that EPO reduced
29 trabecular bone volume in a mouse model of diet-induced obesity¹¹. However, for
30 humans, insufficient evidence exists on the role of EPO in mediating the bone
31 microenvironment.
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56 This study aimed to assess the correlation between cumulative doses of EPO
57 administration and the risk of osteoporosis in patients on chronic dialysis. Moreover,
58 bone mineral density in the femur and lumbar spine of patients on dialysis was
59 investigated, its correlation with some clinical and biochemical factors was
60 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

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

women

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6 Abbreviations: A.C. glucose, fasting glucose; Alb, albumin; ALP, alkaline phosphatase;
7 BMI, body mass index; B.W., body weight; CAD, coronary artery disease; Ca, calcium;
8 CHF, congestive heart failure; D.M., diabetes mellitus; EPO, Erythropoietin; Hb,
9 hemoglobin; H.D., hemodialysis; K, potassium; LF-T BMD, total left femur bone mineral
10 density; L-spine BMD, lumbar-spine bone mineral density; Na, sodium; P, phosphorus;
11 intact PTH, intact parathyroid hormone; P.D., peritoneal dialysis; RF-T BMD, total right
12 femur bone mineral density; T.G., triglyceride; T-Chol, total cholesterol; yrs, years. * $p <$
13 0.05, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0001$.
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21 A detailed history of related risk factors (smoking, hypertension, diabetes, steroid
22 intake, and surgical menopause) was obtained from all patients, and medical records
23 were checked after consent was obtained. The continuous medical records were
24 available from January 2000 to December 2020. Before initiating the dialysis session,
25 baseline investigations were performed at the patient's regular blood test session.
26 Blood tests included kidney function tests, serum calcium, serum phosphorus, intact
27 parathyroid hormone, fasting glucose, serum alkaline phosphatase levels, liver
28 function tests, complete blood counts, ferritin, and determination of lipid profiles.
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41 The DXA definition of osteoporosis and the bone mass criteria followed for its
42 diagnosis were adopted from the WHO definition of osteoporosis (1994). T-scores
43 were used for the evaluation of BMD and the definition of different stages of BMD
44 according to the WHO definition of osteoporosis. T-scores were obtained for the
45 femoral necks and lumbar spines (L1-L4). The average of lumbar spine BMD was to
46 evaluate the lumbar spine T-score, use of three vertebrae if four cannot be used, and
47 two if three cannot be used for the diagnosis according to the (The International
48 Society for Clinical Densitometry, ISCD) guideline.¹² The lowest T-score among
49 femoral necks and lumbar spines was accounted for established osteoporosis. The T-
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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 manufacturer¹³. However, numerous studies have suggested that the conversion ratio be 240–400 IU of EPO and 1 µg of DPO¹⁴⁻¹⁶. 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 \pm 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 ($P <$

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4 0.05) are shown in Table 3. Age, body mass index (BMI), body weight (B.W.), serum
5 calcium, ferritin, and EPO doses show statistical differences between patients with
6 osteoporosis and patients without osteoporosis. Furthermore, 108 age- and sex-
7 matched patients were evaluated for differences in risk factors for and biochemical
8 markers of bone diseases. These included all the factors listed in Table 4. Cumulative
9 EPO dosage was significantly different in age- and sex-matched patients with
10 osteoporosis than nonosteoporotic patients on dialysis. All three EPO conversion
11 models showed similar and significant results. Three models of EPO dose conversion
12 were used to examine the association between EPO and T-scores of participants. The
13 statistical calculation process was repeated using different EPO dose models to avoid
14 collinearity. The results are shown in Figure 1. Pearson's correlation coefficient varied
15 between -0.30 and -0.46 , but p values were statistically significant.
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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***
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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
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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; LogEPO3, 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

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4 doses. A logarithmic scale was used to examine all three EPO dose conversion models
5 and the development of osteoporosis. The AUC varied between 0.698 and 0.714 and
6 showed moderate utility in predicting osteoporosis development in patients on dialysis
7 (Figure 3).
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10 11 12 13 **Discussion**

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16 This study found a moderate reduction in the mean BMD in this unselected
17 population of patients on chronic hemodialysis. The mean T-score of -2.17 in the
18 DXA measurement of the femoral neck implies that these patients had moderately less
19 favorable outcomes than age-matched controls. The mean T-score value found in the
20 present study is similar to several other studies that used the same bone mineral
21 density measurement¹⁷. Age and weight also emerged as important determinants of
22 BMD in our study. Age-related bone loss plays an essential role in the pathogenesis of
23 osteoporosis, and a negative association between age and BMD in female patients
24 with end-stage renal disease has been reported^{18,19}. The mean age of patients in these
25 two studies was 43 and 50.5 years, whereas, in our study, patients were older, with a
26 mean age of 66.6 years. With the number of older adults involved in the renal
27 replacement program increasing and with survival rates markedly improving, age-
28 related bone loss can be expected to become an increasingly important factor causing
29 bone disease in these patients.
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41 Moreover, evidence has revealed a positive correlation between weight and BMD in
42 healthy populations²⁰. The correlation between B.W. and BMD has been attributed to
43 bone formation stimulations through weight-bearing and adipose tissues' increased
44 peripheral conversion of adrenal androgens to estrogens. Two studies have reported a
45 positive association between BMI and BMD measurements^{21,22}. We showed a similar
46 association in our patients. Finally, we found a significant difference in cumulative
47 EPO use in patients with osteoporosis compared with those without osteoporosis in
48 univariate and multivariate analyses (Table 5).
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55 EPO is administered based on the patient's weekly hemoglobin levels at our
56 institution. EPO doses received were positively correlated with patient dialysis
57 duration. The longer the patient undergoes dialysis, the higher the dose of EPO the
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4 patient may receive. However, no statistically significant differences in dialysis
5 vintage were found between patients with osteoporosis and those without [$P = 0.762$
6 (unmatched), $P = 0.111$ (age- and sex-matched)]. All three models, logEPO1,
7 logEPO2, and logEPO3 showed significant differences in cumulative EPO in patients
8 with osteoporosis compared with those without (Table 5). A negative correlation was
9 observed between the total, lumbar, right femoral neck, and left femoral neck T-
10 scores and EPO dose (Figure 1). Although these results showed a low and negative
11 correlation between T-scores and EPO dose (Pearson's correlation coefficient r from
12 0.30 to 0.46), these data reached statistical significance ($P < 0.005$ to < 0.0001).
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20 Higher EPO dosages were administered in patients with lumbar spine osteoporosis
21 than patients with cortical bone osteoporosis (right or left femur). However, no
22 statistical significance was reached in the current study (Figure 1). Effects of
23 erythropoietin-induced bone loss had been demonstrated in experimental
24 mice.^{10,11} However, clinical evidence concerning EPO with bone mineral density is
25 lacking. Whether EPO exerts more trabecular bone loss or cortical bone loss remains
26 to be elucidated.
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33 Serum PTH is negatively associated with BMD measurements; cortical porosity
34 increased in patients with hyperparathyroidism²³. Several studies have reported a
35 negative association between PTH levels and BMD measurements^{18,24,25}, whereas
36 others were unable to show this association²⁶⁻²⁸. In the present study, however, we
37 found a negative association between PTH levels and BMD measurements,
38 suggesting that other factors affect BMD in patients on hemodialysis. Forty-three
39 patients received active vitamin D treatment in the current study.
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46 Aluminum overload may be responsible for adynamic bone disease and osteomalacia.
47 At our institution, serum aluminum levels are measured annually in patients who have
48 undergone dialysis for >5 years. Our patients had no abnormally elevated serum
49 aluminum levels. Moreover, we did not perform a histological analysis of bone. Thus,
50 we cannot comment on the prevalence of adynamic bone disease and osteomalacia in
51 this population.
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57 The relationships between calcium intake, vitamin D supplementation and
58 osteoporosis development remain controversial. One study has shown that oral 1α -
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4 hydroxycalciferol treatment could prevent BMD loss in the Lumbar Spine in a study
5 of 165 male patients²⁹. All 165 patients were receiving calcium-containing phosphate
6 binders. Only ten patients received vitamin D supplements in the nonosteoporotic
7 group compared with 33 patients who received vitamin D supplements in the
8 osteoporotic group.
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13 Clinical and molecular evidence suggests that chronic inflammation significantly
14 influences bone turnover.^{30,31} Uremic syndrome, hemodialysis, use of a catheter, and
15 persistent infection may contribute to the development of the inflammatory state in
16 CKD. In hemodialysis patients, inflammation has been associated with EPO
17 resistance mainly because the inflammatory state decreases the bone marrow response
18 to ESA, changing iron regulation through hepcidin upregulation and/or causing red
19 blood cell/erythrocyte hemolysis.³² In the current study, we had not studied the
20 inflammatory status among patients with/without osteoporosis. However, some
21 markers of inflammatory reaction had included in our laboratory study, including
22 WBC, platelets, ferritin, and albumin. Both platelet and white cell counts have been
23 implicated in playing an essential role in inflammatory reaction.^{33,34} Similarly, both
24 ferritin and albumin have also known as acute-phase proteins. In the age- and sex-
25 matched model, WBC, platelets, ferritin, and albumin have not shown statistical
26 differences between osteoporotic and nonosteoporotic patients.
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38 The strengths of our study are the random sampling of the population and the high
39 accuracy of cumulative EPO treatment history collected. Participants with and
40 without osteoporosis were age- and sex-matched to examine the association of EPO
41 treatment with the risk of osteoporosis development. However, the present study was
42 limited by its cross-sectional nature. It is difficult to establish the causal relationship
43 between EPO accumulation and the risk of osteoporosis. A further longitudinal study
44 is required to confirm the cause and effect of EPO in reducing BMD. Moreover, the
45 present study involves a group of elderly participants. Our subgroup analysis showed
46 that participants aged <65 years with osteoporosis did not receive a higher EPO dose
47 than participants aged >65 years with osteoporosis ($r = -0.21$, $P = 0.133$, data not
48 shown).
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4 In conclusion, we confirmed the importance of age and body weight as the risk factors
5 affecting BMD in patients on hemodialysis. We found that the cumulative EPO
6 negatively correlates with dialysis patients' BMD. Elderly dialysis patients under
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8 long-term EPO treatment are at risk of developing osteoporosis. Managing anemia in
9
10 this vulnerable population may consider other possible therapeutic strategies.
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16 **Declarations**

17 18 19 **Ethics approval and consent to participate**

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22 Taipei Medical University Institutional Review Board approved the study for
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24 Human Experimentation (TMU-eJIRB, N202103059).
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29 **Data sharing**

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31 All free text entered will be published
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36 **Competing interests**

37
38 The authors declare that they have no competing interests.
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43 **Funding**

44
45 This research was funded by Taipei Medical University Hospital, Wan Fang
46
47 Hospital, and Taipei Medical University. Funding numbers: 106TMU-WFH-11, 106-
48
49 eva-03, 108-wf-eva-31, and 108TMU-WFH-25.
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54 **Authors' contributions**

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4 Conceptualization, formal analysis, investigation, methodology, original draft
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6 writing, C-Y.C.; conceptualization, data curation, investigation, methodology,
7
8 resources, and writing review and editing, Y-J-K.
9

10 11 **Acknowledgment**

12
13
14 The authors gratefully acknowledge Professor Jin-Hua Chen of the Department of
15 Graduate Institute of Data Science, Taipei Medical University, for their advice on the
16 statistical analysis.
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20 21 **References**

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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

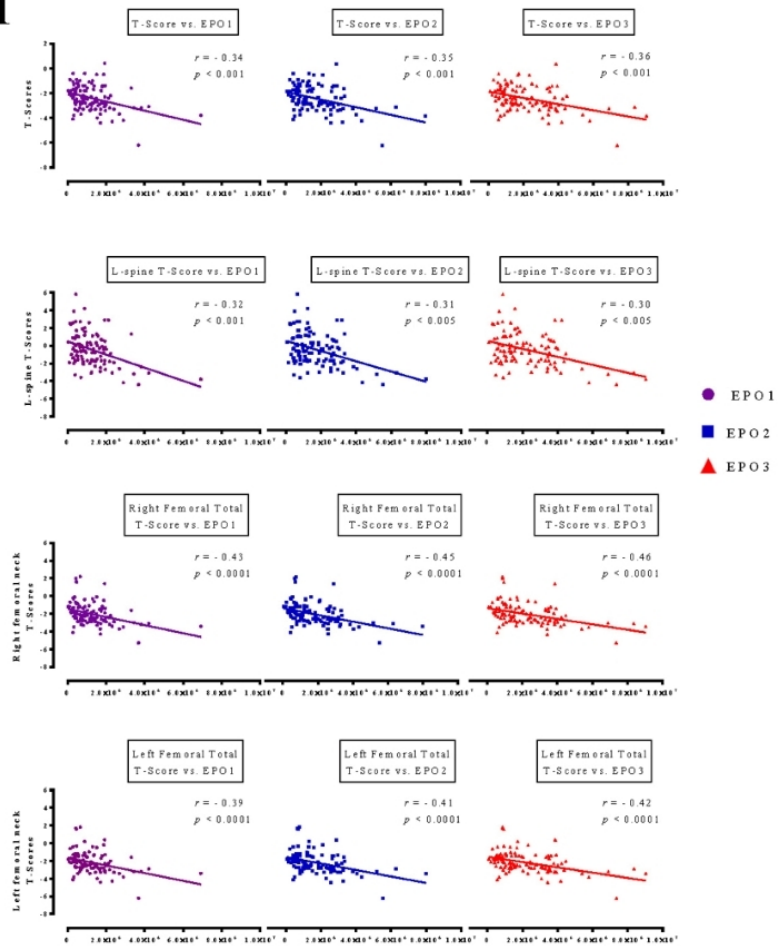


Figure 1 Descriptive plots of correlations between T-scores of L-spine, total right femur, total left femur, and cumulative Erythropoietin (EPO) dose received. EPO1, EPO2, and EPO3 represent three different dose conversion models. EPO1, 1 µg of darbepoietin/Mircera converts to 200 IU of EPO; EPO2, 1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

76x76mm (300 x 300 DPI)

Figure 2 **EPO vs. Osteoporosis**
Diagnosed at Different sites

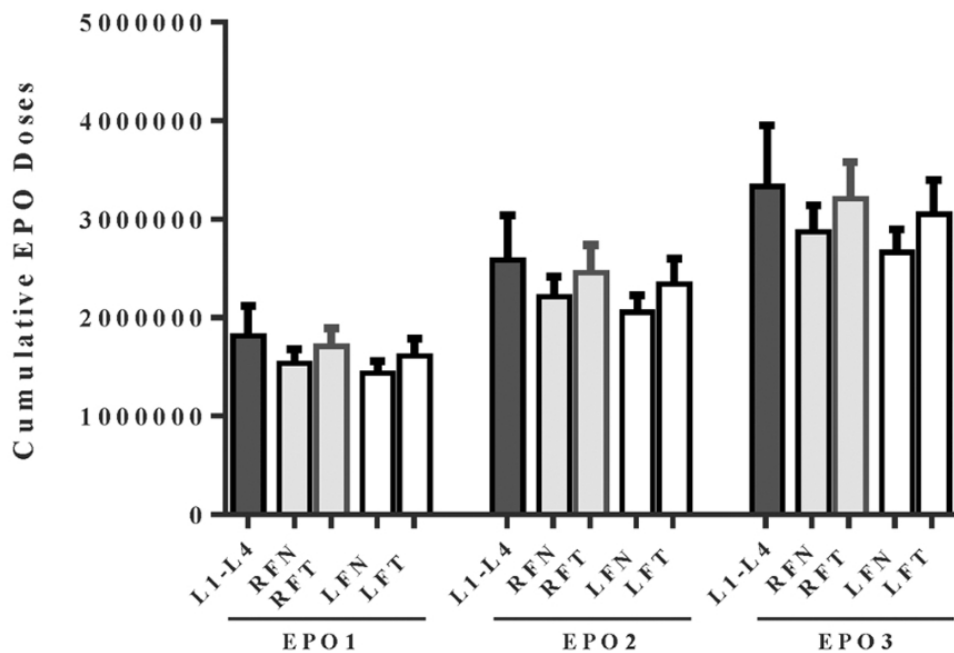
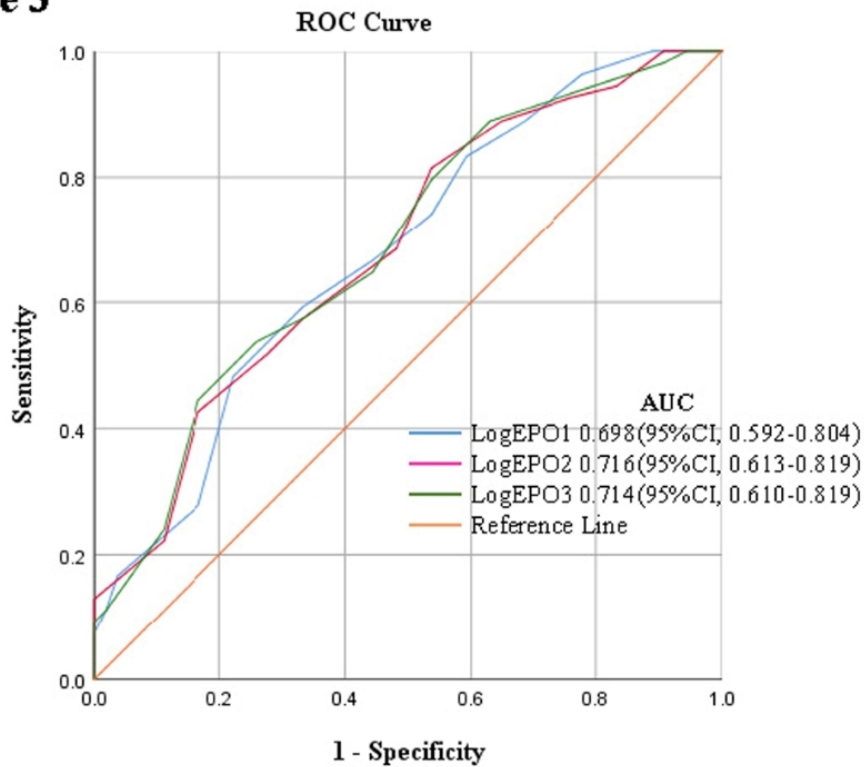


Figure 2 Three different models of erythropoietin dose conversion versus bone mineral density among three different sites in dialysis patients with osteoporosis. Abbreviations: BMD, bone mineral density; EPO, erythropoietin; L1-L4, lumbar-spine 1 to 4; RFN, right femoral neck; RFT, total right femur;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

76x76mm (300 x 300 DPI)

Figure 3

Diagonal segments are produced by ties.

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.