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# Single-centre cross-sectional study on the impact of cumulative erythropoietin on bone mineral density in maintenance dialysis patients

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## ABSTRACT

**Objectives** Numerous factors are associated with the risk of osteoporosis in patients with chronic kidney disease, including vitamin D deficiency, hypocalcaemia, hyperphosphataemia and secondary hyperparathyroidism. This 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 centre 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-matched 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 3 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 units EPO compared with 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

- ► This 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-matched and aged-match analysis increases the strength of this study.
- ► The multivariate analysis identified the confounding factors to substantiate our study hypothesis.
- ► This study is limited by a single-centre 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. Patients with CKD with osteoporosis are at a higher risk of bone fractures[1] and have reduced quality of life.[2] Considering the increasing prevalence of CKD among ageing populations, diagnosis and treatment of osteoporosis in a patient with CKD deserve more attention.

In patients with CKD, biochemical alterations resulting in vitamin D deficiency, hypocalcaemia, hyperphosphataemia and secondary hyperparathyroidism can cause deterioration of the cortical bone architecture, leading to reduced cortical density and increased cortical porosity earlier in the course of CKD than previously thought.[3] Osteoporosis is a decrease in bone mineral density (BMD). Dual-energy X-ray absorptiometry (DXA) is the most common method for measuring BMD and is considered the current gold standard for osteoporosis diagnosis. According to the WHO criteria, the

Table 1 Basic characteristics of the study participants and comparison between men and women

| Variables | Values (N=165) | Men (n=74) | Women (n=91) | P value |
|---|---|---|---|---|
| Age (years) | 66.6±11.1 | 66.9±9.9 | 66.3±12.0 | 0.519 |
| BMI (kg/m$^2$) | 23.4±3.4 | 23.9±3.2 | 22.8±3.6 | 0.010* |
| BW (kg) | 59.4±10.6 | 66.0±9.2 | 54.1±8.6 | <0.0001**** |
| Dialysis vintage (years) | 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/L) | 103±9 | 104±10 | 101±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 |

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

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 anaemia with EPO is now well established. The extensive use of EPO and its analogues (EPO-stimulating agents (ESAs)) for anaemia correction has reduced the associated morbidity and improved functionality, exercise tolerance, cognitive function and overall quality of life. However, over the last few years, much controversy has been raised over the possible risks of ESA therapy. Moreover, a thorough investigation of the mechanism of action of EPO has revealed multiple biological effects that extend beyond its EPO effect and may have a favourable or sometimes unfavourable contribution to these outcomes.

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

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

| | | | Osteopenia | | Osteoporosis | |
|---|---|---|---|---|---|---|
| | BMD (g/cm$^2$) | T-score (SD) | 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.
BMD, bone mineral density; L-spine, lumbar-spine; R.F. Neck, right femoral neck; L.F. Neck, left femoral neck.

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

This study aimed to assess the correlation between cumulative doses of EPO administration and the risk of osteoporosis in patients on chronic dialysis. Moreover, BMD 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-centre cross-sectional study.

### Study population

Taipei Medical University, Wan Fang Hospital is a tertiary care hospital in Taipei. On average, there are 300 haemodialysis and 60 peritoneal dialysis patients under our maintenance renal replacement therapy programme. Patients aged >20 years with end-stage renal disease and who were undergoing renal replacement therapy (hamodialysis or peritoneal dialysis) for more than 1 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 summarised 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 aetiology (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 hours, with a mean of 11.2 hours. 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.

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

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

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±0.10.4 | 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/L) | 103±8 | 102±11 | 0.383 |
| WCC (x10$^9$/L) | 7.090±0.637 | 6.366±0.200 | 0.365 |
| Platelet (x10$^9$/L) | 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.
*P<0.05, **p<0.01, ****p<0.0001.
A.C, glucose, fasting glucose; Alb, albumin; ALP, alkaline phosphatase; BMI, body mass index; B.W, body weight; Ca, calcium; CAD, coronary artery disease; CHF, congestive heart failure; D.M, diabetes mellitus; EPO, erythropoietin; Hb, haemoglobin; H.D, haemodialysis; intact PTH, intact parathyroid hormone; 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; P.D, peritoneal dialysis; RF-T BMD, total right femur bone mineral density; T-Chol, total cholesterol; T.G, triglyceride; WCC, white cell count.

### EPO dose conversion

Patients receive either darbepoetin alfa (DPO) (Aranesp, Kyowa Hakko Kirin), 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 haemoglobin levels. We maintain our patients' haemoglobin levels between 100 and 120 g/L. For conversion from EPO alfa to DPO, a fixed conversion ratio of 200 IU EPO to 1 µg DPO was suggested by the manufacturer.[13] However, numerous studies have suggested that the conversion ratio be 240–400 IU of EPO and 1 µg of DPO.[14–16] In this 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

**Table 4** 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** |

*P<0.05, **p<0.01, ***p<0.005.
BMD, bone mineral density; EPO, Erythropoietin; L-spine, lumbar-spine; O.S, osteoporosis.

**Table 5** 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*** |

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

various EPOs during their dialysis treatment in our institution. We established three conversion doses of 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.

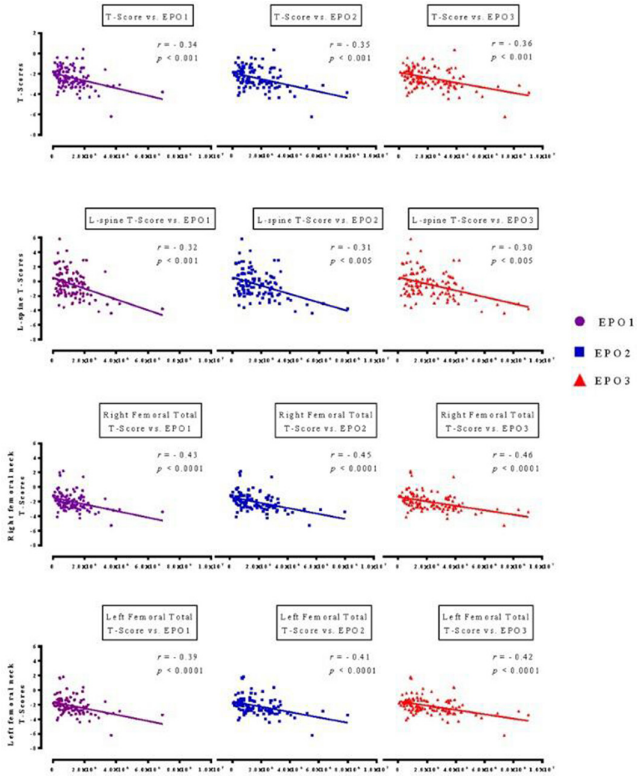


**Figure 1** Descriptive plots of correlations between T-scores of L-spine, total right femur, total left femur and cumulative erythropoietin (EPO) dose received. EPO1, EPO2 and EPO3 represent three different dose conversion models. EPO1, 1 µg of darbepoietin/Mircera converts to 200 IU of EPO; EPO2,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

## 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±SD 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 $\chi^2$ test was used to compare frequencies between categorical variables. SPSS V.25 (SPSS) was used for analysis. A p<0.05 was considered statistically significant.

**Table 6** 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** |

*P<0.05, **p<0.01, ****p<0.0001.
BMD, bone mineral density; EPO, erythropoietin; L-spine, lumbar-spine; O.S, osteoporosis.

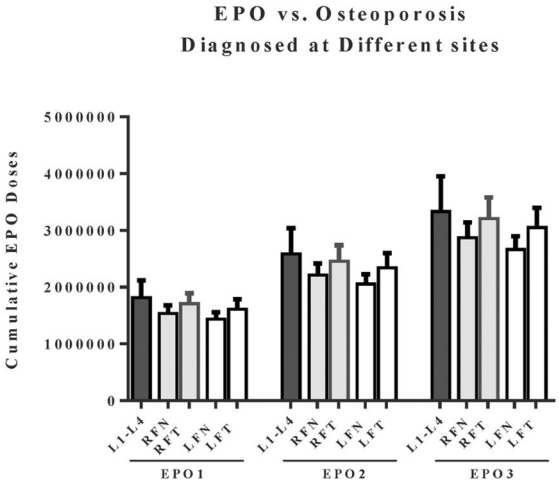


**Figure 2** Three different models of EPO dose conversion versus bone mineral density among three different sites in dialysis patients with osteoporosis. L1–L4, lumbar-spine 1–4; LFN, left femoral neck; LFT, total left femur; RFN, right femoral neck; RFT, total right femur.

## 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 12 patients had T-scores of >−1.0.

### Factors associated with reduced BMD

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 analysed and reached statistical significance (p<0.05) are shown in table 3. Age, body mass index (BMI), body weight (B.W.), serum calcium, ferritin and EPO doses show statistical differences between patients with osteoporosis and patients without osteoporosis. Furthermore, 108 age-matched 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-matched and sex-matched patients with osteoporosis than nonosteoporotic patients on dialysis. All three EPO conversion models showed similar and significant results. Three models of EPO dose conversion were used to examine the association between EPO and T-scores of participants. The statistical calculation process was repeated using different EPO dose models to avoid collinearity. The results are shown in figure 1. Pearson's correlation coefficient varied between −0.30 and −0.46, but p values were statistically significant.

### EPO dosage associated with osteoporosis among three different sites of BMD measurement

Significantly higher EPO dosages were found among osteoporotic participants using BMD measured from lumbar spines, right total and left total femur (tables 4–6). However, no statistical difference was found on the cumulative EPO doses (all three models) using different sites to diagnose osteoporosis (figure 2).

### Factors associated with osteoporosis in patients on dialysis

Table 7 shows clinical factors associated with osteoporosis in age-matched 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 8 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 with those without osteoporosis. Backward stepwise logistic regression was used to select multiple variables. Age, sex, B.W., BMI, haemoglobin, 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 haemoglobin and B.W. were significantly different between patients with and without osteoporosis. In the age-matched and sex-matched multivariate analysis model, the log-transformed EPOs are the only significant factors associated with osteoporosis.

### Role of EPO 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 haemodialysis. The mean T-score of −2.17 in the DXA measurement of the femoral neck implies that these patients had moderately less favourable outcomes than age-matched controls. The mean T-score value found in this study is similar to several other studies that used the same BMD measurement.[17] Age and weight also emerged as important determinants of BMD in our study. Age-related bone loss plays an essential role in the pathogenesis

Table 7 Age-matched 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±0.10.6 | 0.176 |
| Dialysis vintage (years) | 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/L) | 104±8 | 103±11 | 0.486 |
| WCC (x10$^9$/L) | 7.595±1.142 | 6.518±0.231 | 0.357 |
| Platelet (x10$^9$/L) | 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 |
| CA | 15 | 14 | 0.830 |

****p<0.005, ****p<0.0001.
A.C, glucose, fasting glucose; Alb, albumin; ALP, alkaline phosphatase; BMI, body mass index; B.W, body weight; Ca, calcium; CAD, coronary artery disease; CHF, congestive heart failure; D.M, diabetes mellitus; EPO, Erythropoietin; Hb, haemoglobin; H.D, haemodialysis; intact PTH, intact parathyroid hormone; 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; P.D, peritoneal dialysis; RF-T BMD, total right femur bone mineral density; T-Chol, total cholesterol; T.G, triglyceride; WCC, white cell count.

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 programme increasing and with survival rates markedly improving, age-related bone loss can be expected to become an increasingly important factor causing bone disease in these patients.

Moreover, evidence has revealed a positive correlation between weight and BMD in healthy populations.[20] The correlation between B.W. and BMD has been attributed to bone formation stimulations through weight-bearing and adipose tissues' increased peripheral conversion of adrenal androgens to estrogens. Two studies have

Table 8 Factors associated with osteoporosis in dialysis patients of different statistical models

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

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

reported a positive association between BMI and BMD measurements.[21 22] We showed a similar association in our patients. Finally, we found a significant difference in cumulative EPO use in patients with osteoporosis compared with those without osteoporosis in univariate and multivariate analyses (table 7).

EPO is administered based on the patient's weekly haemoglobin levels at our institution. EPO doses received were positively correlated with patient dialysis duration.

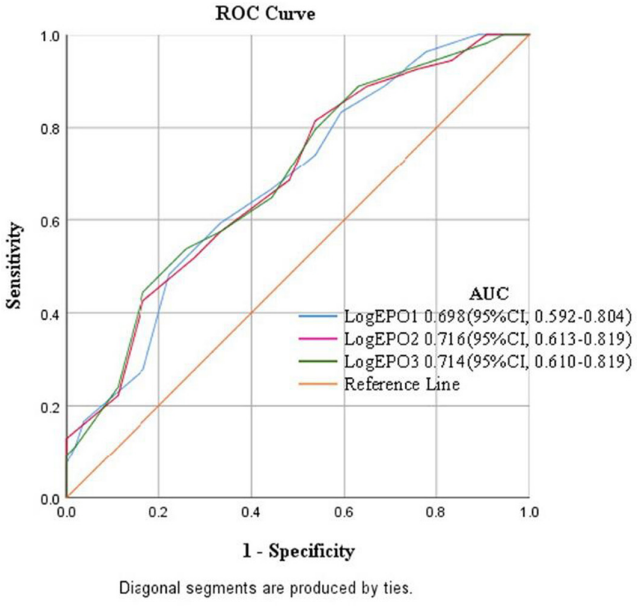


Figure 3 Receiver operating characteristic (ROC) curves of prediction models for cumulative erythropoietin dose transformed to a log value that incorporates patients with and without osteoporosis. AUC, area under the curve; LogEPO1, the log-transformed value of EPO1; LogEPO2, the log-transformed value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

The longer the patient undergoes dialysis, the higher the dose of EPO the patient may receive. However, no statistically significant differences in dialysis vintage were found between patients with osteoporosis and those without (p=0.762 (unmatched), p=0.111 (age matched and sex matched)). All three models, logEPO1, logEPO2 and logEPO3 showed significant differences in cumulative EPO in patients with osteoporosis compared with those without (table 7). A negative correlation was observed between the total, lumbar, right femoral neck and left femoral neck T-scores and EPO dose (figure 1). Although these results showed a low and negative correlation between T-scores and EPO dose (Pearson's correlation coefficient $r$ from 0.30 to 0.46), these data reached statistical significance (p<0.005 to<0.0001).

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

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

Aluminium overload may be responsible for adynamic bone disease and osteomalacia. At our institution, serum

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

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

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

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

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

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