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ORIGINAL RESEARCH

Time-Dependent Effect of Anti-seizure Medications on Bone Metabolism in Patients with Epilepsy: A Cross-Sectional Study

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ABSTRACT

***Introduction*:** Patients with epilepsy (PWE) face an elevated risk of osteoporosis and bone fractures. This study aims to elucidate bone metabolic alterations in PWE and identify early detection biomarkers and contributing factors.

***Methods*:** This cross-sectional study analyzed PWE from the Epilepsy Clinical database stratified by anti seizure medication (ASM) exposure duration. We analyzed bone turnover markers (BTMs), including 25-hydroxy vitamin D, osteocalcin (OC), procollagen type 1 N-terminal propeptide (P1NP), β-crosslaps (β-CTX) and

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β-CTX/OC ratio. The effects of epilepsy and ASMs on bone metabolism were analyzed by XGBoost model and SHapley Additive exPlanations (SHAP). Finally, we analyzed the mediation analyses assessing inflammatory pathway contributions.

***Results*:** A total of 476 PWE were included in this study. Compared to ASM-naïve PWE, those receiving > 2 years of ASM therapy exhibited a reduced β-CTX/OC ratio ($p = 0.002$), while P1NP levels declined only after > 10 years of treatment ($p < 0.001$). Longitudinal data revealed a continued annual decline in the β-CTX/OC ratio during the 2-year follow-up period. After adjusting for confounders, longer ASM exposure duration was significantly correlated with decreased P1NP, β-CTX and β-CTX/OC ratio levels (β = – 1.74, 95% CI – 2.56 to – 0.92; $p < 0.001$). XGBoost-SHAP analysis identified valproic acid (VPA), oxcarbazepine (OXC) and history of status epilepticus as key contributors to β-CTX/OC ratio variability. Polytherapy had a more pronounced effect than monotherapy, particularly when levetiracetam was combined with VPA or OXC. Mediation analysis demonstrated that platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio mediate epilepsy/ASM-related bone metabolic alterations.

***Conclusion*:** PWE exhibit dynamic bone metabolic alterations. Following > 2 years of ASM therapy, osteoclast inhibition precedes the onset of osteoblast dysfunction. Prolonged ASM exposure eventually reduces bone formation markers, indicating progressive impairment of osteoblastic function and a concomitant decline in bone-forming capacity. Consequently, the β-CTX/OC ratio represents a pivotal early biomarker for monitoring bone health deterioration, demonstrating significant clinical utility.

Keywords: Boneturnovermarkers; β-Crosslaps/osteocalcin ratio; Epilepsy; Anti-seizure medications

Key Summary Points

Why carry out this study?

Patients with epilepsy (PWE) are known to have a significantly higher risk of developing osteoporosis and sustaining bone fractures compared to the general population. This elevated risk is frequently attributed to the long-term use of anti-seizure medications (ASMs), which are suspected to adversely affect bone metabolism

This study aimed to delineate the specific alterations in bone metabolism that occur in PWE. It sought to identify early biomarkers indicative of these changes and to investigate the contributing factors, and the potential mediating role of inflammation in epilepsy and ASM-related bone pathology

What was learned from the study?

Our findings demonstrate a dynamic, time-dependent progression of bone metabolic dysfunction in ASM-treated PWE. Osteoclastic activity (bone resorption), reflected by β-crosslaps (β-CTX) levels, is inhibited first, which is subsequently followed by a decline in osteoblastic function (bone formation), indicated by reduced procollagen type 1 N-terminal propeptide (P1NP)

This research could impact clinical practice by validating the β-CTX/osteocalcin ratio as a practical biomarker for routine monitoring of bone health in PWE. This enables earlier intervention strategies to prevent osteoporosis and ultimately to guide more personalized treatment approaches for epilepsy

INTRODUCTION

Epilepsy, a chronic neurological disorder characterized by recurrent abnormal neuronal discharges that result in transient central nervous system dysfunction, affects over 70 million individuals worldwide [1]. Current therapeutic strategies predominantly involve anti-seizure

medications (ASMs) to achieve seizure control and reduce seizure frequency. However, ASM administration induces systemic complications, including perturbations in bone metabolism. These medications, particularly enzyme-inducing ASMs (EIASMs) and valproic acid (VPA), reduce bone mineral density (BMD), accelerate osteoporosis and significantly increase fracture risk [2, 3]. Paradoxically, during the initial therapeutic phases, bone mineral density [4–6] and standard biochemical markers (vitamin D) frequently remain within normal reference ranges despite ongoing bone loss, potentially delaying clinical recognition of subclinical bone deterioration [7, 8]. Compounding this diagnostic challenge, epilepsy intrinsically alters bone remodeling processes [9], while bone-related symptoms typically manifest only after catastrophic fracture events occur. This diagnostic conundrum underscores the critical need for developing sensitive biomarkers capable of detecting early-stage bone microarchitectural changes prior [10].

Bone turnover markers (BTMs), comprising serum and urinary biomarkers reflecting osteoblastic and osteoclastic activities, provide dynamic insights into bone remodeling processes through quantification of bone formation and resorption markers [11]. The imbalance between bone formation and resorption plays a critical role in the development of osteoporosis. Measuring BTMs offers valuable insights into bone turnover dynamics and fracture risk [12]. Numerous studies confirm that specific BTM levels correlate with bone density change rates and future fracture risk. Organizations such as the International Osteoporosis Foundation emphasize BTMs' role as supplementary tools for predicting bone metabolism status and fracture risk [13]. Standard BMD assessment by dual-energy x-ray absorptiometry (DXA), while crucial, often lags behind ongoing metabolic bone disturbances, particularly in the initial phases of ASM therapy or epilepsy-related bone metabolism changes. Consequently, early detection of bone damage in patients with epilepsy (PWE) requires evaluating the relationship between bone formation and resorption. In specific clinical contexts—particularly for early risk stratification and rapid assessment of treatment response—BTMs may function as surrogate endpoints. This capability enables timely clinical interventions to prevent bone loss and fractures.

Bone metabolism abnormalities are increasingly recognized in PWE, posing significant clinical challenges. Early detection is crucial, as it provides a therapeutic window for timely intervention to improve outcomes. However, the underlying mechanisms linking epilepsy, ASMs, and bone health remain complex and multifaceted [14, 15]. While several potential mechanisms have been proposed, including the induction of cytochrome P450 enzymes leading to vitamin D deficiency [16], the influence of inflammatory cytokines and the role of oxidative stress [17], a comprehensive understanding of these interactions remains elusive. This knowledge gap highlights the need for further research to identify sensitive biomarkers for early detection of bone metabolic changes.

In this study, we address this gap by elucidating the interactions among epilepsy, ASMs and bone metabolism. We characterize changes in BMTs and identify candidate biomarkers for early detection of bone pathology in PWE. These data will provide a theoretical foundation for early clinical intervention protocols. Furthermore, we conducted inflammation-related mediation analyses to delineate hypothesized pathways linking ASMs, epilepsy and bone metabolic dysfunction.

MATERIALS AND METHODS

Study Population

The study is a retrospective analysis, whose data were extracted from the Epilepsy Clinical database of the Fourth Affiliated Hospital Zhejiang University School of Medicine. The database collects data from enrolled patients with epilepsy, including demographic characteristics, seizure semiology, frequency, laboratory examinations, anti-seizure medications and annual follow-up records of seizure control, treatment modifications and laboratory examinations. Data extraction covered the period from January 2019 to March 2024. Data quality control and supplementation were performed using the

hospital's electronic medical record system. The requirement for informed consent was waived because of the retrospective nature of the study. This study was approved by the hospital's Ethics Committee (approval number K2025016).

The inclusion criteria for this study were as follows: (1) aged ≥ 18 years; (2) the epilepsy diagnosis meets the criteria of the International League against Epilepsy in 2017; (3) complete data on bone metabolism. The exclusion criteria were as follows: (1) known pregnancy; (2) patients undergoing blood or abdominal dialysis; (3) patients with parathyroid disease, achondroplasia, rickets or other diseases known to affect bone metabolism (prostate cancer); (4) patients with insufficient data.

Healthy controls matched for age, sex and body mass index (BMI) were recruited in the physical examination center of the Fourth Affiliated Hospital Zhejiang University School of Medicine of the same period. The inclusion criteria were aged 18 years or older.

Data Collection

Data on the following aspects of the participants were collected by physicians and trained researchers: (1) demographic characteristics of the PWE, including age, height, weight, residence and marital status. Other potential confounders, such as hypertension, diabetes, malignancy, thyroid dysfunction, mental disorders, hyperpyretic convulsion and glucocorticoids use, were also collected; (2) epilepsy-related characteristics, including whether patients were taking ASMs and the detailed names of these medications, and frequency of seizures; (3) blood routine data. The calculations for the systemic immune inflammation index (SII), pan-immune inflammation value (PIV), platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) were done based on the blood routine data (SII = platelet count × neutrophil count/lymphocyte count; PIV = neutrophil count × monocyte count × platelet count/lymphocyte count; PLR = platelet count/lymphocyte count; NLR = neutrophil count/lymphocyte count) [18]; (4) bone turnover markers. The serum levels of 25-hydroxy vitamin D (25-OHD) were determined by chemiluminescence. The serum levels of osteocalcin (OC), procollagen type 1 N-terminal propeptide (P1NP) and β-crosslaps (β-CTX) were determined by chemiluminescence immunoassay, and the ratio of β-CTX to OC was calculated [19].

Statistical Analysis

The Kolmogorov-Smirnov test was used to test the data for normality. Normally distributed data were presented as mean ± standard deviation. The non-normally distributed data were presented as the median and interquartile range. Categorical variables were presented as count and frequency. Independent sample *t*-test, ANOVA test, Mann-Whitney *U* test and Kruskal-Wallis test were used to analyze the difference between the continuous variables. Chi-square and Fisher exact tests were used to analyze the difference between binary variable. We performed multiple imputations for missing BMI data. Initially, we conducted analysis comparing serum BMTs between PWE and healthy controls. Second, within the PWE, PWE were divided into five groups according to the ASM time and use (no medication; duration of medication ≤ 2 years; duration of medication 2–6 years; duration of medication 6–10 years; duration of medication > 10 years). We analyzed the differences in serum BMTs among the five groups.

Linear Regression Analysis

Linear regression analysis was performed to evaluate the association between ASM exposure duration and bone metabolism parameters. We adjusted the model for age, sex, BMI, residence, marital status, hypertension, diabetes, malignancy, thyroid dysfunction, mental disorders, hyperpyretic convulsion and glucocorticoids use. Additionally, we stratified participants by age, sex and BMI to examine subgroup associations between medication duration and bone metabolism parameters to validate the robustness of our findings.

Explainable Machine Learning Model

To investigate the specific effects and interactions of ASMs and epilepsy-related clinical factors, we employed the XGBoost machine learning algorithm for comprehensive analysis. As a powerful machine learning algorithm, XGBoost is widely used across various domains, including clinical medicine, to investigate the complex relationships between multifaceted exposures and outcomes as well as their reciprocal interactions. This algorithm provides not only high-accuracy risk predictions but also favorable interpretability by generating feature importance rankings. This capability aids in validating established clinical prior knowledge while potentially uncovering novel associations, thereby offering valuable clues for etiological exploration [20]. Recent research has demonstrated that SHapley Additive exPlanations (SHAP) can be used to visualize the results of machine learning models and thereby enhance our understanding of these models [21]. The combination of the machine learning model and the SHAP method can extract useful information from the machine learning model.

The XGBoost model was constructed by incorporating various features including frequency of seizures, forms of epileptic seizures, status epilepticus and ASM use, and all covariates. Bone metabolism indices served as the target variables. The dataset was divided into 70% for training and 30% for testing.

We employed SHAP to interpret the XGBoost model results. This included generating SHAP summary plots to: (1) visualize the influence of ASMs and epilepsy-related clinical factors on bone metabolism biomarkers; (2) assess interaction effects and combinatorial impacts between ASMs.

Mediation Analysis

To further investigate the relationship among epilepsy, ASMs and bone metabolism, we performed mediation analyses. In this study, we excluded patients with hematological disorders and those without contemporaneous routine blood markers. These analyses aimed to examine the potential mediating role of inflammatory mediators in the association among epilepsy, ASMs and bone metabolism.

All statistical analyses were conducted using SPSS 26.0 (IBM), R (version 4.4.1), and $p<0.05$ in two-sided test was set as the statistical significance level. To control the risk of false-positive results due to multiple hypothesis testing, Bonferroni correction was applied. The significance threshold was adjusted from $p<0.05$ to $p<0.005$ (0.05/10). Associations with p values between 0.05 and 0.005 were considered suggestive of a potential association [22].

RESULTS

Characteristics of Participants

The study comprised 476 PWE with bone metabolism alongside 166 healthy controls (Fig. SI1). The median age of the PWE was 33 (IQR 24.00–49.00) years, and the median BMI of the PWE was 22.25 (IQR 20.03–24.89). Sixty-five patients (13.7%) were not exposed to ASM, 240 patients (50.4%) were on monotherapy, and 171 patients (35.9%) were taking two or more ASMs. Among the treated patients, VPA was used in 173 individuals (42.1%), followed by levetiracetam (LEV) in 149 patients (36.2%) and oxcarbazepine (OXC) in 125 patients (30.4%). Lamotrigine (LTG) was administered to 67 (16.3%) patients, carbamazepine (CBZ) to 44 (10.7%) and other agents (including topiramate, phenytoin and phenobarbital) to 78 (19.0%). Among PWE, 105 (22.1%) had been seizure-free for over a year; 235 (49.4%) patients experienced seizures several times a year, 120 (25.2%) had seizures several times a month, and 16 (3.3%) patients had seizures several times a week. Notably, 59 (12.4%) patients experienced at least one episode of status epilepticus. No statistically significant differences were observed between the PWE group and the healthy control group regarding age ($p=0.129$), sex ($p=0.212$) or BMI ($p=0.956$) (Table 1).

Comparison of Bone Metabolism Between PWE and Healthy Control Group

Quantitative analysis revealed distinct bone metabolism patterns between PWE and the

Table 1 Population characteristics and comparison of participant characteristics grouped by patients with epilepsy

	Healthy controls *N* = 166	Patients with epilepsy *N*[a] = 476/411	*p* Value	No medication *N* = 65	Duration of medication ≤ 2 years *N* = 107	Duration of medication 2–6 years *N* = 133	Duration of medication 6–10 years *N* = 79	Duration of medication > 10 years *N* = 92	*p* Value
Characteristics									
Age, M (P25,P75)[b]	32 (29, 45)	33 (24, 49)	0.129	34 (25, 55)	35 (27, 53)	33 (26, 49)	25 (21, 39)	34 (26, 52)	**< 0.001**
Sex, male, *n* (%)	81 (48.8)	259 (54.4)	0.212	38 (58.5)	68 (63.6)	74 (55.6)	39 (49.4)	40 (43.5)	0.053
BMI (kg/m^2), M (P25,P75)	22.35 (20.73, 24.10)	22.25 (20.03, 24.89)	0.956	22.04 (19.75, 24.50)	22.49 (20.20, 25.40)	22.35 (19.99, 25.07)	21.56 (19.59, 25.54)	22.18 (20.55, 24.24)	0.873
Residence, *n* (%)									0.224
Village		176 (37.0)		24 (36.9)	40 (37.4)	54 (40.6)	20 (25.3)	38 (41.3)	
Towns		205 (43.0)		29 (44.6)	46 (43.0)	55 (41.4)	39 (49.4)	36 (39.1)	
City		95 (20.0)		12 (18.5)	21 (19.6)	24 (18.0)	20 (25.3)	18 (19.6)	
Marital status, *n* (%)									**0.001**
Married		305 (64.1)		47 (72.3)	75 (70.1)	92 (69.2)	35 (44.3)	56 (60.9)	
Widowed		2 (0.4)		0 (0.0)	1 (1.0)	1 (0.7)	0 (0.0)	0 (0.0)	
Divorced		2 (0.4)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.2)	
Never married		157 (33.0)		17 (26.2)	30 (27.9)	38 (28.6)	44 (55.7)	28 (30.4)	
Don't know		10 (2.1)		1 (1.5)	1 (1.0)	2 (1.5)	0 (0.0)	6 (6.5)	
Hypertension, *n* (%)		62 (13.0)		11 (16.9)	23 (21.5)	18 (13.5)	2 (2.5)	8 (8.8)	**0.002**
Diabetes, *n* (%)		28 (5.9)		4 (6.2)	11 (10.3)	8 (6.0)	1 (1.3)	4 (4.3)	0.126
Thyroid disease, *n* (%)		13 (2.7)		1 (1.5)	4 (3.7)	6 (4.5)	0 (0.0)	2 (2.2)	0.331

Table 1 continued

	Healthy controls	Patients with epilepsy	*p* Value	No medication	Duration of medication ≤ 2 years	Duration of medication 2–6 years	Duration of medication 6–10 years	Duration of medication > 10 years	*p* Value
	N = 166	*N*[a] = 476/411		*N* = 65	*N* = 107	*N* = 133	*N* = 79	*N* = 92	
Malignancy, *n* (%)		19 (4.0)		4 (6.2)	4 (3.7)	7 (5.3)	2 (2.5)	2 (2.2)	0.641
Mental disease, *n* (%)		36 (7.6)		4 (6.2)	4 (3.7)	9 (6.8)	6 (7.6)	13 (14.1)	0.084
Hyperpyretic convulsion, *n* (%)		33 (6.9)		4 (6.2)	4 (3.7)	8 (6.0)	6 (7.6)	11 (12.0)	0.233
Glucocorticoids, *n* (%)		7 (1.5)		2 (3.1)	3 (2.8)	1 (0.8)	0 (0.0)	1 (1.1)	0.369
Epilepsy and ASM									
Frequency of seizures, *n* (%)									0.098
No seizures for 1 year		105 (22.1)		5 (7.7)	13 (12.1)	36 (27.1)	26 (32.9)	25 (27.2)	
Annual seizures		235 (49.4)		51 (78.5)	61 (57.0)	65 (48.9)	30 (38.0)	28 (30.4)	
Monthly seizures		120 (25.2)		7 (10.7)	31 (29.0)	26 (19.5)	21 (26.6)	35 (38.1)	
Weekly seizures		16 (3.3)		2 (3.1)	2 (1.9)	6 (4.5)	2 (2.5)	4 (4.3)	
Status epilepticus, *n* (%)		59 (12.4)		10 (15.4)	16 (15.0)	12 (9.0)	6 (7.6)	15 (16.3)	0.236

Table 1 continued

	Healthy controls *N* = 166	Patients with epilepsy *N*[a] = 476/411	*p* Value	No medication *N* = 65	Duration of medication ≤ 2 years *N* = 107	Duration of medication 2–6 years *N* = 133	Duration of medication 6–10 years *N* = 79	Duration of medication > 10 years *N* = 92	*p* Value
Forms of epileptic seizures, *n* (%)									0.478
Focal onset (no tonic-clinic)		283 (59.5)		41 (63.1)	64 (59.8)	82 (61.7)	41 (51.9)	55 (59.8)	
Focal onset (tonic-clinic)		148 (31.1)		15 (23.1)	34 (31.8)	39 (29.3)	32 (40.5)	28 (30.4)	
Generalized onset		21 (4.4)		4 (6.2)	4 (3.7)	6 (4.5)	5 (6.3)	2 (2.2)	
Unknown onset		24 (5.0)		5 (7.6)	5 (4.7)	6 (4.5)	1 (1.3)	7 (7.6)	
Number of ASMs, *n* (%)									**< 0.001**
No medication		65 (13.7)		65 (100)	0	0	0	0	
Monotherapy		240 (50.4)		0	87 (81.3)	81 (60.9)	36 (45.6)	36 (39.1)	
Polytherapy		171 (35.9)		0	20 (18.7)	52 (39.1)	43 (54.4)	56 (60.9)	
VPA, *n* (%)[c]		173 (42.1)		NA	39 (36.4)	61 (45.9)	29 (36.7)	44 (47.8)	0.226
EIASM or VPA[d], *n* (%)[c]		187 (45.5)		NA	53 (49.5)	58 (43.6)	34 (43.0)	42 (45.7)	0.780
Bone metabolism									
OC (ng/ml), M (P25, P75)	17.35 (14.52, 19.63)	17.93 (14.00, 23.59)	0.069	18.01 (13.88, 24.29)	18.00 (13.22, 23.92)	17.93 (14.82, 23.84)	18.44 (14.93, 25.00)	17.34 (13.00, 21.00)	0.164

Table 1 continued

	Healthy controls *N* = 166	Patients with epilepsy *N*[a] = 476/411	*p* Value	No medication *N* = 65	Duration of medication ≤ 2 years *N* = 107	Duration of medication 2–6 years *N* = 133	Duration of medication 6–10 years *N* = 79	Duration of medication > 10 years *N* = 92	*p* Value
P1NP (ng/ml), M (P25, P75)	49.90 (41.20, 61.77)	55.09 (39.38, 75.15)	**0.025**	57.21 (45.37, 79.10)	57.45 (43.44, 83.20)	55.18 (38.99, 75.88)	57.50 (41.82, 83.21)	45.70 (34.61, 59.98)	**< 0.001**
β-CTX (pg/ml), M (P25, P75)	276.80 (175.05, 394.88)	380.45 (241.43, 599.00)	**< 0.001**	493.00 (315.75, 661.50)	458.80 (273.40, 627.90)	375.00 (263.85, 608.00)	339.00 (202.00, 647.00)	286.75 (173.75, 453.10)	**< 0.001**
25-OHD (ng/ml), M (P25, P75)	18.45 (15.19, 23.39)	19.18 (14.48, 25.19)	0.780	20.50 (14.11, 24.55)	19.71 (15.97, 25.37)	19.18 (14.71, 27.30)	17.40 (12.70 22.04)	19.31 (14.29, 25.32)	**0.025**
β-CTX/OC, M (P25, P75)	16.75 (10.68, 22.29)	21.11 (14.17, 29.18)	**< 0.001**	26.60 (18.73, 37.17)	22.33 (16.68, 31.35)	21.24 (14.70, 27.54)	19.04 (12.02, 27.50)	17.29 (10.34, 24.73)	**< 0.001**

Bold values denote statistical significance at $p < 0.05$.

BMI body mass index, *ASM* anti-seizure medication, *EIASM* enzyme inducing anti-seizure medication, *VPA* valproic acid, *OC* osteocalcin, *P1NP* procollagen type 1 N-terminal propeptide, *β-CTX* β-crosslaps, *25-OHD* 25-hydroxy vitamin D

[a]*N* (number)

[b]Y (year), M, median, P, percent

[c]The number of people taking the ASMs was 411

[d]The only medications taken are EIASM or VPA

healthy control group. The bone formation marker (P1NP) ($Z = -2.2$, $p = 0.025$), the bone resorption marker (β-CTX) ($Z = -6.0$, $p < 0.001$) and the β-CTX/OC ratio ($Z = -5.7$, $p < 0.001$) were all significantly elevated in PWE compared to controls. No significant differences were observed in OC ($p = 0.069$) or 25-OHD levels ($p = 0.780$) between the groups (Table 1).

Comparison of Bone Metabolism Among PWE with Different Durations of Medication

To evaluate durations of ASM on bone metabolism, PWE were stratified into five subgroups based on ASM exposure duration. There were statistically significant differences in age ($H = 23.2$, $p < 0.001$), marital status ($H = 18.2$, $p = 0.001$) and hypertension comorbidity prevalence ($\chi^2(1) = 16.9$, $p = 0.002$). Intergroup variations in bone metabolic parameters were observed across the five PWE subgroups, including P1NP ($H = 23.1$, $p < 0.001$), β-CTX ($H = 29.9$, $p < 0.001$), 25-OHD ($H = 11.1$, $p = 0.025$) and β-CTX/OC ratio ($H = 31.6$, $p < 0.001$) (Table 1). Bonferroni method was used to correct the significance results. Comparative analysis of treatment duration subgroups revealed no significant differences in BTM profiles between PWE who did not take medication and those with ≤ 2 years of ASM exposure. Compared to PWE who did not take medication, the β-CTX/OC ratio showed divergence at > 2 years of ASM exposure ($Z = -3.1$, $p = 0.002$, Fig. 1a), while P1NP levels exhibited marked reduction after > 10 years of continuous therapy ($Z = -3.5$, $p < 0.001$) (Fig. 1d).

Therapeutic regimen stratification analysis revealed differential patterns in bone metabolic alterations. Compared with PWE who did not take medication, both monotherapy and polytherapy groups demonstrated significantly reduced β-CTX/OC ratios after 6-year ASM exposure (monotherapy: $Z = -3.0$, $p = 0.002$; polytherapy: $Z = -3.7$, $p < 0.001$, Fig. 1e), with P1NP

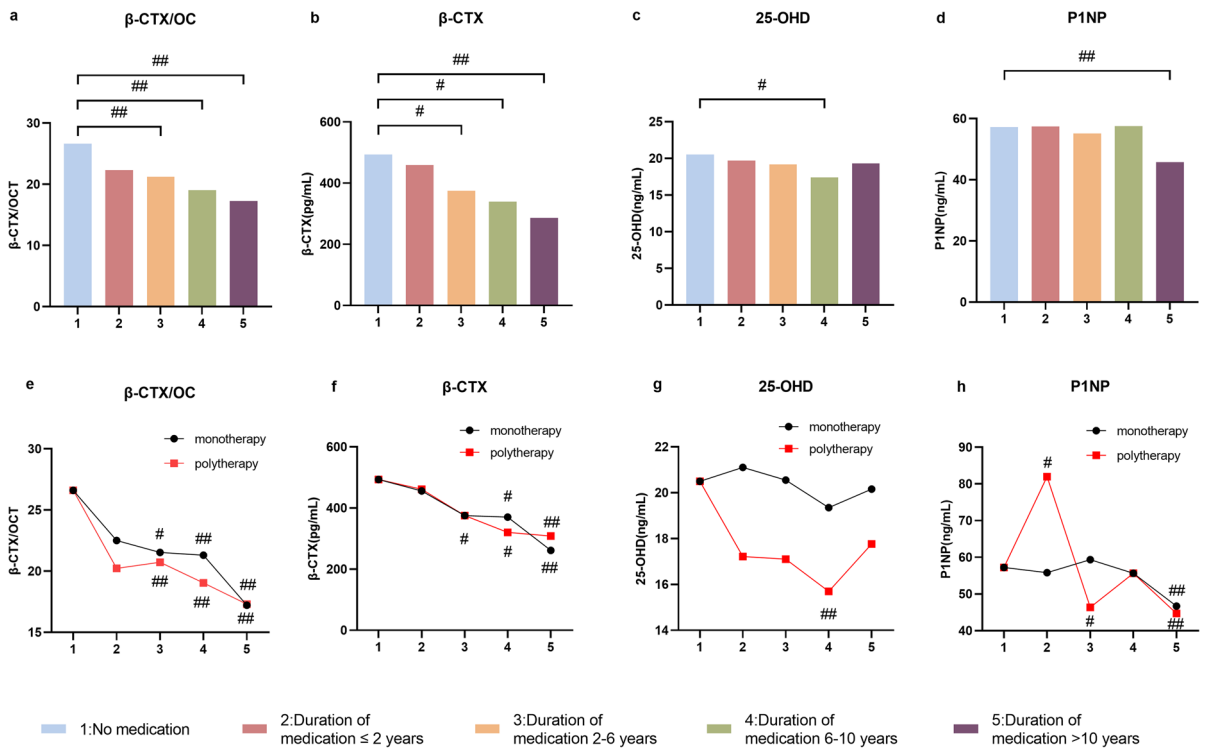

Fig. 1 Comparison of the levels of various bone metabolism markers among the five groups. Bonferroni: $^{\#}p < 0.05$. $^{\#\#}p < 0.005$. *OC* osteocalcin, *P1NP* procollagen type 1 N-terminal propeptide, *β-CTX* β-crosslaps, *25-OHD* 25-hydroxy vitamin D

decline emerging after > 10 years of treatment (monotherapy: $Z = -2.9$, $p = 0.003$; polytherapy: $Z = -3.0$, $p = 0.003$, Fig. 1h). Notably, the polytherapy group exhibited accelerated 25-OHD depletion during the 6–10 year treatment ($Z = -3.2$, $p = 0.001$). No significant 25-OHD alterations were observed in the monotherapy group ($p = 0.575$) (Fig. 1g).

Additionally, for longitudinal assessment of the bone metabolism, we identified PWE with > 2 years of follow-up data from our clinical database. Among them, 44 PWE completed both annual follow-ups. Measurements revealed marked deviations from baseline β-CTX levels (year 1: $Z = -2.1$, $p = 0.044$; year 2: $Z = -2.8$, $p = 0.005$) and the β-CTX/OC ratios (year 1: $Z = -2.9$, $p = 0.003$; year 2: $Z = -4.2$, $p < 0.001$), with both parameters declining progressively over the follow-up period (Fig. SI2).

Linear Regression Analysis and Subgroup Analysis

Linear regression analysis evaluating ASM exposure duration and bone metabolic parameters revealed consistent negative correlations. Univariate analysis revealed that longer ASM exposure duration correlated with lower levels of P1NP (β = – 2.98, 95% CI – 5.89 to – 0.08; $p = 0.044$), β-CTX (β = – 42.61, 95% CI – 61.48 to – 23.74; $p < 0.001$) and β-CTX/OC ratio levels (β = – 2.30, 95% CI – 3.13 to – 1.48; $p < 0.001$) in patients with epilepsy. Subsequent multivariable-adjusted analysis incorporating age, sex, BMI, residence, marital status, hypertension, diabetes, malignancy, thyroid dysfunction, mental disorders, hyperpyretic convulsion and glucocorticoid use as covariates maintained statistical significance for these associations (P1NP: β = – 3.54, 95% CI – 6.37 to – 0.72; $p = 0.014$; β-CTX: β = – 37.97, 95% CI – 56.89 to – 19.06; $p < 0.001$; β-CTX/OC: β = – 1.74, 95% CI – 2.56 to – 0.92; $p < 0.001$) (Table 2).

Subgroup analyses stratified by age, sex and BMI demonstrated that longer ASM exposure duration correlated with lower levels of β-CTX/OC ratio across all population strata (β range: – 1.67 to – 3.12, all $p < 0.05$). Notably, accelerated P1NP attenuation was specifically observed in two high-risk subgroups: patients aged ≥ 50 years (β = – 3.63, 95% CI – 6.68 to – 0.58; $p = 0.020$) and females (β = – 5.33, 95% CI – 8.18 to – 2.48; $p < 0.001$, Table 3).

XGBoost Model and SHAP

We chose the XGBoost model to assess the relationship of epilepsy and ASMs to the β-CTX/OC ratio and then used SHAP to interpret the XGBoost results. The SHAP feature importance plot quantified the global feature importance for β-CTX/OC ratio variability (Fig. 2a). VPA demonstrated the strongest association, followed by OXC and LEV, indicating differential osteometabolic impacts of specific ASMs in descending order of effect magnitude. Regarding epileptic diseases, the occurrence of status epilepticus and

Table 2 Linear regression analysis for the association between ASM exposure duration and bone metabolism

	Unadjusted β (95% CI)	*p* Value	Adjusted β (95% CI)	*p* Value
OC	– 0.31 (– 0.94, 0.31)	0.325	– 0.47 (– 1.06, 0.13)	0.122
P1NP	– 2.98 (– 5.89, – 0.08)	**0.044**	– 3.54 (– 6.37, – 0.72)	**0.014**
β-CTX	– 42.61 (– 61.48, – 23.74)	**< 0.001**	– 37.97 (– 56.89, – 19.06)	**< 0.001**
25-OHD	– 0.40 (– 1.04, 0.23)	0.213	0.04 (– 0.58, 0.65)	0.901
β-CTX/OC	– 2.30 (– 3.13, – 1.48)	**< 0.001**	– 1.74 (– 2.56, – 0.92)	**< 0.001**

Bold values denote statistical significance at $p < 0.05$

The adjustment model adjusts for age, sex, BMI, residence, marital status, hypertension, diabetes, malignancy, thyroid dysfunction, mental disorders, hyperpyretic convulsion and glucocorticoids use

OC osteocalcin, *P1NP* procollagen type 1 N-terminal propeptide, *β-CTX* β-crosslaps, *25-OHD* 25-hydroxy vitamin D

Table 3 Subgroup analysis of medication duration

Variable	OC		P1NP		β-CTX		25-OHD		β-CTX/OC	
	β (95% CI)	*p* Value	*β* (95% CI)	*p* Value	*β* (95% CI)	*p* Value	*β* (95% CI)	*p* Value	*β* (95% CI)	*p* Value
Sex										
Male	0.57 (− 0.39, 1.52)	0.073	0.29 (− 4.56, 5.13)	0.907	− 26.22 (− 53.80, 1.37)	0.062	− 0.23 (− 1.12, 0.66)	0.614	− 2.46 (− 3.66, − 1.27)	**< 0.001**
Female	− 1.06 (− 1.84, − 0.27)	**0.008**	− 5.33 (− 8.18, − 2.48)	**< 0.001**	− 48.77 (− 73.37, − 24.16)	**< 0.001**	− 0.24 (− 1.13, 0.65)	0.595	− 1.67 (− 2.79, − 0.55)	**0.004**
Age (years)										
≥ 50	− 0.11 (− 1.17, 0.94)	0.834	− 3.63 (− 6.68, − 0.58)	**0.020**	− 33.46 (− 66.15, − 0.77)	**0.045**	− 0.71 (− 2.31, 0.90)	0.386	− 1.81 (− 3.53, − 0.08)	**0.040**
< 50	− 0.47 (− 1.22, 0.29)	0.225	− 3.21 (− 6.94, 0.52)	0.091	− 45.47 (− 68.34, − 22.59)	**< 0.001**	− 0.09 (− 0.71, 0.52)	0.765	− 2.33 (− 3.26, − 1.40)	**< 0.001**
BMI										
> 25	0.16 (− 0.88, 1.20)	0.758	− 1.33 (− 5.35, 2.69)	0.514	− 39.58 (− 71.73, − 7.43)	**0.016**	− 1.14 (− 2.68, 0.41)	0.147	− 3.12 (− 4.65, − 1.59)	**< 0.001**
≤ 25	− 0.45 (− 1.19, 0.29)	0.231	− 3.45 (− 7.00, 0.09)	0.056	− 43.54 (− 66.00, − 21.07)	**< 0.001**	− 0.20 (− 0.88, 0.49)	0.567	− 2.08 (− 3.05, − 1.11)	**< 0.001**

Bold values denote statistical significance at $p < 0.05$

BMI body mass index, *OC* osteocalcin, *P1NP* procollagen type 1 N-terminal propeptide, *β-CTX* β-crosslaps, *25-OHD* 25-hydroxy vitamin D

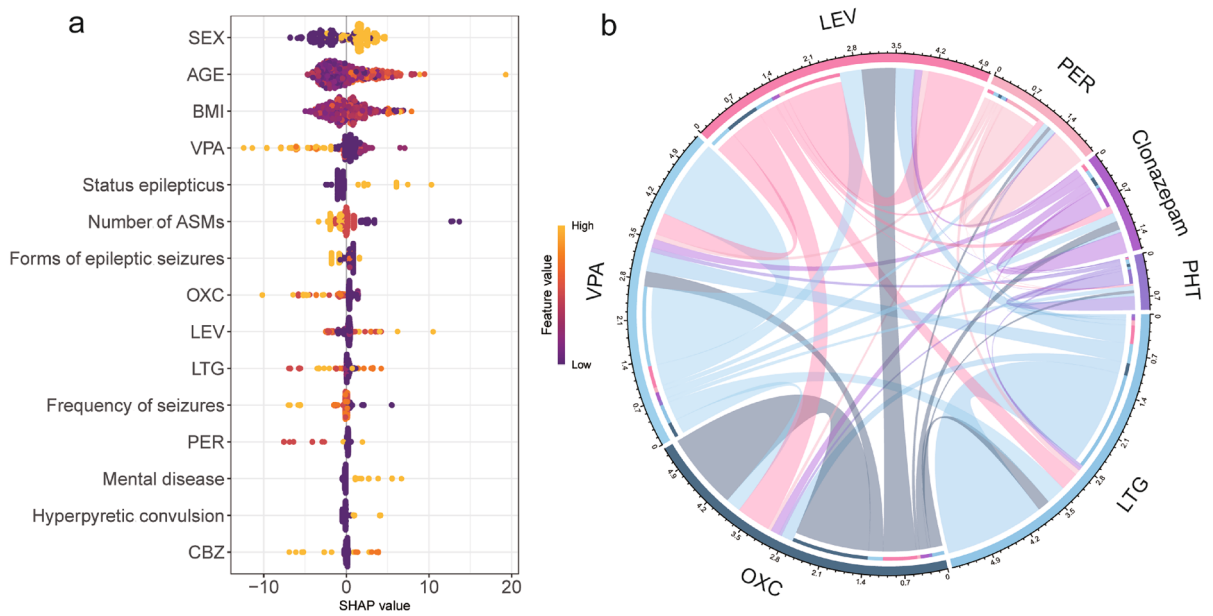

Fig. 2 **a** The SHAP plots for the effect of each feature on XGBoost output. **b** Interaction effects between features used in XGBoost model. *BMI* body mass index, *VPA* valproic acid, *OXC* oxcarbazepine, *CBZ* carbamazepine, *LEV* levetiracetam, *LTG* lamotrigine, *PHT* phenytoin, *PER* pirfenidone

the form of epilepsy were the most important factors.

According to SHAP analysis, the β-CTX/OC ratio in patients treated with VPA showed an initial increase for < 2 years of treatment, followed by a subsequent decrease after 2 years. The most pronounced decline was observed in patients who had been on VPA for > 6 years. Patients receiving OXC demonstrated reductions in the β-CTX/OC ratio following both 2 and 10 years of treatment (Fig. SI3).

SHAP interaction analysis uncovered pharmacodynamic synergism impacting β-CTX/OC homeostasis. Combination therapy with LEV and OXC demonstrated the most pronounced effect on β-CTX/OC ratios, followed by the combination of LEV and VPA (Fig. 2b).

Mediation Analysis for Associations of Epilepsy with β-CTX/OC Ratio

To further verify the relationship between inflammatory factors in epilepsy and bone metabolism, we conducted mediation analyses. The PIV mediated 12.21% ($p=0.010$) of ASM polytherapy's total effect on β-CTX/OC elevation, with an equivalent mediation proportion (12.21%, $p=0.002$) observed for status epilepticus. Comparatively, NLR demonstrated stronger mediation effects: 24.17% ($p=0.027$) of ASM polytherapy's impact and 29.10% ($p=0.011$) of status epilepticus's effects on β-CTX/OC ratios. Notably, NLR mediated 15.90% ($p=0.012$) of the effect of LTG on β-CTX/OC ratios. Polytherapy and LTG monotherapy demonstrated significant negative indirect effects. This suggests that a part of their action may be achieved by mitigating inflammatory responses, thereby reducing the β-CTX/OC ratio. In contrast, status epilepticus exhibited a positive mediating effect (Fig. 3).

DISCUSSION

This study evaluated bone metabolic in PWE and identified key clinical determinants. Compared to healthy controls, PWE showed significantly elevated serum levels of the bone formation marker P1NP and the resorption marker β-CTX, along with an increased β-CTX/

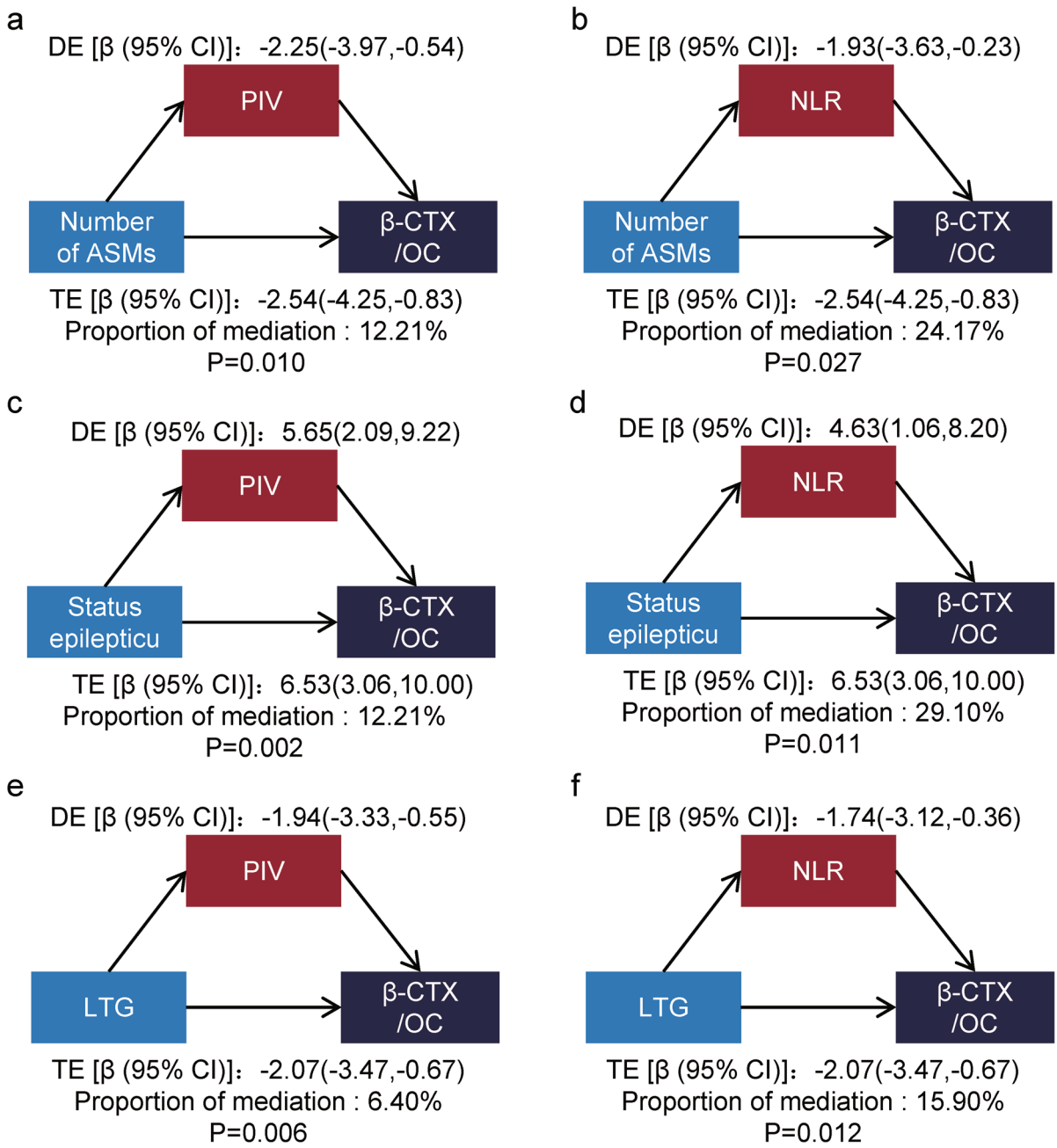

Fig. 3 Mediation analysis for associations of epilepsy and ASM with β-CTX/OC ratio. **e** Linear regression of LTG and PIV ($p > 0.05$). *TE* total effect, *DE* direct effect, *OC* osteocalcin, *β-CTX* β-crosslaps, *ASMs* anti-seizure medications, *PIV* pan-immune inflammation value, *NLR* neutrophil-to-lymphocyte ratio, *LTG* lamotrigine

OC ratio. Compared to PWE who did not take medication, the β-CTX/OC ratio decreased after 2 years of ASM therapy, whereas P1NP levels declined only after 10 years. The β-CTX/OC ratio demonstrated utility as an early biomarker for microarchitectural bone deterioration. Using an XGBoost-SHAP integrated machine learning approach, we identified three primary drivers of bone metabolic disturbance: VPA exposure, OXC exposure and history of status epilepticus.

Polytherapy regimens—particularly combinations of LEV with OXC or VPA—imposed greater detrimental effects on bone metabolism than monotherapy. Mediation analysis further revealed that systemic inflammation (reflected by the PIV and NLR indices) partially mediated the effects of both ASM polytherapy and status epilepticus history on the β-CTX/OC ratio.

Our study found that compared to healthy controls, PWE exhibited elevation in bone turnover markers (P1NP↑, β-CTX↑, β-CTX/OC ratio↑). This suggests that the PWE population as a whole is in a state of heightened bone turnover, potentially linked to epilepsy itself [9], early-stage neuroinflammation or ASM effects [23, 24]. However, this generalized elevation may obscure the differential effects of ASM exposure duration on bone metabolism. To address this, we performed a stratified analysis of PWE based on ASM treatment duration. This analysis revealed that compared to PWE who did not take medication, in the patient group receiving ASM therapy for > 2 years, the β-CTX/OC ratio showed a progressive decline while P1NP levels remained stable. This decrease in the ratio was primarily driven by a reduction in serum β-CTX levels (a marker of bone resorption activity)[11], indicating suppressed bone resorption. Bone formation, as reflected by stable P1NP levels, appeared unaffected at this stage. Furthermore, longitudinal follow-up analysis (mean ASM exposure approximately 4 years) demonstrated that the β-CTX/OC ratio progressively decreased over time. This temporal pattern further confirms a time-dependent association between longer ASM exposure and a declining ratio, strengthening the reliability of our cross-sectional findings. Crucially, in the patient group receiving ASM treatment for > 10 years, we observed a significant decrease in P1NP levels. P1NP is a key marker of bone formation, reflecting type I collagen synthesis and osteoblast activity [25]. This decline clearly indicates diminished osteoblast activity and reduced bone formation. Considering the known negative impact of ASMs on bone mineral density [3–5], this late decline in P1NP signifies a substantial deterioration in bone metabolic status. This deterioration leads to progressive bone loss and a significantly increased fracture risk. Notably, the progressive decline in the β-CTX/OC ratio is an earlier signal, suggesting that the impact of ASMs on bone metabolism (particularly on the resorption side) initiates relatively early in the treatment course. Therefore, monitoring changes in the β-CTX/OC ratio holds promise as a key biomarker for screening early adverse ASM-related effects on bone metabolism. Conversely, the decline in P1NP among long-term users (especially > 10 years) signals a higher risk of bone loss and underscores the urgent need for proactive bone health management interventions.

Subgroup analysis showed that longer ASM exposure was consistently associated with reduced β-CTX/OC ratios across age and sex groups, suggesting that the β-CTX/OC ratio dynamics were independent of age and sex. In addition, subgroup analyses identified high-risk subgroups requiring intensified monitoring, demonstrating that age ≥ 50 years and female sex significantly potentiated the inverse association between treatment duration and P1NP. Importantly, the consistency of findings across monotherapy and polytherapy cohorts suggests class-wide ASM effects on bone metabolism. In this study, VPA and LEV were the most frequently prescribed antiseizure medications, a finding that aligns with the prescribing trends reported in large-scale Chinese studies [26]. Nonetheless, subtle differences in the specific prescription rates were observed. Such variations may stem from differences in patient demographics across medical institutions, regional prescribing habits or the extent of adherence to clinical guidelines. Consistent with prior evidence [7, 8], newer generation antiepileptic drugs are considered among the safest for bone health, exerting complex yet minimal effects on vitamin D and calcium homeostasis. This favorable profile is attributed to their lack of induction of the cytochrome P450 system and their non-involvement in vitamin D catabolism mediated by the vitamin D receptor—pathways frequently implicated in bone loss associated with conventional antiepileptic drugs [27]. Similarly, clinical pediatric studies have similarly reported comparable vitamin D status to controls [28]. In alignment with these studies, with the exception of patients on long-term polytherapy, most groups did not experience vitamin D decline,

reinforcing the idea that observed vitamin D deficiencies primarily reflected cumulative ASM exposure rather than epilepsy pathophysiology.

Extended ASM exposure in PWE is associated with reduced BMD [3, 29, 30], consistent with our results. Our results demonstrate that in PWE, the β-CTX/OC ratio exhibits significant alterations early in ASM therapy (within > 2 years). Furthermore, this association persists after adjusting for clinical confounders and remains statistically robust across the entire PWE. Therefore, we selected the β-CTX/OC ratio as a key biomarker to investigate the associations among epilepsy, various ASMs and bone metabolism. In terms of ASM, EIASMs and VPA exhibit stronger impacts on bone metabolism [31, 32]. Our findings confirm that VPA and OXC alter the β-CTX/OC ratio, reinforcing their contribution to worsening bone metabolic imbalances. We also observed that LEV coadministered with VPA or OXC potentiated β-CTX/OC ratio disturbances. While LEV monotherapy demonstrates minimal bone toxicity [33], its interactions with VPA or OXC require vigilant monitoring. We hypothesize that a previously unrecognized pharmacokinetic or pharmacodynamic interaction between these drugs could exacerbate their individual impacts on bone metabolism. Although clinical studies support an association between low BMD and ASMs, epilepsy's intrinsic pathophysiological burden on bone remains incompletely characterized. Animal research indicates that status epilepticus impairs bone mass and mineralization, weakening structural integrity and elevating fracture risk [15]. Our study supports these findings that PWE with status epilepticus history exhibited accelerated bone resorption, necessitating bone health management alongside status epilepticus control.

The mechanisms underlying epilepsy and the impact of ASMs on bone health constitute a multifactorial framework involving interconnected biological pathways [34]. Animal studies have indicated that ASMs may influence bone metabolism via liver enzyme-independent pathways, including direct cellular toxicity [17]. With the continuous updating of ASM and the in-depth study of hormone metabolism in PWE, it is necessary to consider the impact of other mechanisms on bone. Research has demonstrated a significant correlation among increased seizure frequency, status epilepticus and elevated levels of inflammatory markers such as C-reactive protein, NLR and PLR, establishing epilepsy-associated inflammation as a pathophysiological feature [18, 35]. Moreover, there is growing evidence that sustained inflammation disrupts bone remodeling homeostasis, which may be associated with age-related oxidative stress and activation of the immune system [36]. Activated B cells enhance osteoclastogenesis in inflammatory milieus, while neutrophil infiltration sustains pro-osteolytic microenvironments [37]. It has been shown that systemic inflammation biomarkers are inversely associated with lumbar BMD and predict osteoporosis incidence [38, 39]. Therefore, inflammatory factors play roles in both initiating and perpetuating osteoporosis [40]. Our study suggests that in the mediation analysis, the PIV and NLR participated in mediating the effects of ASM polytherapy and status epilepticus history on bone turnover indices, which can induce bone changes. Studies suggest that status epilepticus can enhance neuroinflammatory responses [18], which in turn indirectly promote bone resorption, whereas ASMs may indirectly suppress the bone-resorptive process via their neuroinflammatory-modulating effects [41]. These findings provide a mechanistic scaffold for investigating epilepsy-ASM-bone interactions.

This study examines bone remodeling dynamics in PWE, highlighting the clinical relevance of the β-CTX/OC ratio as a biomarker for bone metabolism. We incorporated variables such as seizure frequency and duration of ASM therapy among PWE, applying XGBoost-SHAP machine learning frameworks to visualize and interpret our findings effectively. Despite these advantages, the limitations of this study should be considered when interpreting our results. First, while our data suggest an association between osteoclast inhibition and osteoblast dysfunction in PWE and it is biologically plausible that the former precedes the latter, this temporal sequence is inferred from cross-sectional data and cannot establish causality. Future longitudinal studies are needed to confirm this proposed relationship over time. Second, the main purpose of this study is to describe the BTM

characteristics of patients with epilepsy. Therefore, we only measured biochemical markers of bone metabolism and did not measure BMD. BTMs are auxiliary predictors of bone loss and fracture risk in general and they remain surrogate endpoints. Third, lifestyle factors influencing bone metabolism were not assessed. Finally, while we made efforts to adjust for potential confounders in our analyses, there may still be other unadjusted factors (vitamin D supplementation or anti-osteoporotic drugs). While use of such medications was likely low in this study, their potential influence, especially in long-term ASM users, cannot be ruled out and may attenuate the observed associations. Future prospective, longitudinal studies that incorporate direct BMD measurements and detailed assessments of lifestyle factors (e.g., physical activity, sun exposure, dietary calcium intake) are essential to validate our findings.

CONCLUSION

This study elucidates the dynamic evolution of bone metabolism in PWE. ASM therapy progressively reduces the β-CTX/OC ratio, indicating gradual suppression of bone resorption. During extended treatment periods, the bone formation marker P1NP remains stable, suggesting sustained osteoblastic activity. However, after exceeding 10 years of continuous ASM exposure, patients exhibit significantly declined P1NP levels, reflecting progressive impairment of osteoblastic function. Consequently, the β-CTX/OC ratio serves as a sensitive early biomarker for detecting emerging bone metabolic abnormalities in PWE. Building on these findings, we propose to investigate vitamin D supplementation in PWE with either vitamin D deficiency or altered bone metabolism indices. This research aims to clarify intervention-induced changes in bone metabolism and optimize clinical bone health management strategies.

Author Contributions. Conceptualization: Jiajia Fang, Xiuying Chen, Bofei Chen. Data collection, and interpretation of data: Bofei Chen, Jiahui Guo, Zhiruo Qiu, Beibei Shen, Yi Shi, Huali Luo, Lina Jiang. Drafting of the manuscript: Bofei Chen. Critical revision of the manuscript for important intellectual content: Jiajia Fang, Xiuying Chen. Supervision: Yi Wang, Lei Chen, Ping Su. Statistical analysis: Bofei Chen, Jiahui Guo. All authors critically reviewed the manuscript, approved the final version, and take responsibility for the accuracy and integrity of this work.

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Data Availability. The data supporting this study's findings are available from the corresponding author upon reasonable request.

Declarations

Conflicts of Interest. Bofei Chen, Jiahui Guo, Zhiruo Qiu, Beibei Shen, Yi Shi, Huali Luo, Lina Jiang, Yi Wang, Lei Chen, Ping Su, Xiuying Chen and Jiajia Fang declare that they have no conflict of interest. Two authors have changed their affiliation since the work described in the article was carried out. Author Beibei Shen was affiliated with the Fourth Affiliated Hospital of School of Medicine, and International School of Medicine, International Institutes of Medicine, Zhejiang University, during the study and is now with The First Affiliated Hospital of Huzhou University (Huzhou First People's Hospital). Author Yi Shi was affiliated with the Fourth Affiliated Hospital of School of Medicine and International School of Medicine, International Institutes of Medicine, Zhejiang University, during the study and is now with Ningbo University Affiliated Yangming Hospital, Yuyao People's Hospital. The affiliation at the time of the study is reflected on the title page.

Ethical Approval. This study only involved the collection and analysis of retrospective data that had already been generated and recorded in the course of routine clinical practice. This study

was approved by the hospital Ethics Committee (approval number K2025016). Foremost, we thank all patients and study participants.

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