

## Micropetrosis in hemodialysis patients

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### ARTICLE INFO

#### Keywords:

Micropetrosis  
Bone histomorphometry  
μCT  
Hemodialysis patients  
Fracture risk

### ABSTRACT

Micropetrosis develops as a result of stagnation of calcium, phosphorus and bone fluid, which appears as highly mineralized bone area in the osteocytic perilacunar/canalicular system regardless of bone turnover of the patients. And microcracks are predisposed to increase in these areas, which leads to increased bone fragility. However, micropetrosis of hemodialysis (HD) patients has not been discussed at all. Micropetrosis area per bone area (Mp.Ar/B.Ar) and osteocyte number per micropetrosis area (Ot.N/Mp.Ar) were measured in nine HD patients with renal hyperparathyroidism (Group I), twelve patients with hypoparathyroidism within 1 year after the treatment of renal hyperparathyroidism (Group II) and seven patients suffering from hypoparathyroidism for over two years (Group III). And bone mineral density (BMD) and tissue mineral density (TMD) were calculated using μCT to evaluate bone mineral content of iliac bone of the patients. These parameters were compared among the three groups. Only Mp.Ar/B.Ar was statistically greater in Group II and III compared to Group I in the parameters of bone mineral content and micropetrosis. However, the other parameters were not statistically different among the three groups. In long-term HD patients, BMD and TMD may be modified by the causes of renal insufficiency and the treatment of renal bone disease. We concluded that Mp.Ar/B.Ar was greater in patients with long-term hypoparathyroidism than both those with short-term hypoparathyroidism and with renal hyperparathyroidism. Special attention should be paid to avoid long-term hypoparathyroidism of the patients from the view point of increased fracture risk caused by increased micropetrosis area.

### 1. Introduction

Micropetrosis was defined and reported decades ago (Frost, 1960). It is described as small regions of highly mineralized bone that develop when the osteocytes die, and the osteocyte perilacunar/canalicular system fills with mineral (Schmidt et al., 2018; Carpentier et al., 2012; Busse et al., 2010). Perilacunar areas also may become highly mineralized. Micropetrotic bone is predisposed to developing microcracks in these highly mineralized areas, and the combination of

hypermineralization and microcracking are thought to be a cause of increased bone fragility (Frost, 1960).

Osteocyte apoptosis caused by microcracks triggers (Receptor activator of nuclear factor-kappa B ligand) RANKL production, initiates bone turnover and is important to maintain normal levels of mineralization and to avoid the accumulation of microcracks (Schmidt et al., 2018; Carpentier et al., 2012; Busse et al., 2010; Cabahug-Zuckerman et al., 2016; Burr, 2014; Kennedy et al., 2012; Herman et al., 2010; Emerton et al., 2010a; Cardoso et al., 2009; Seeman and Delmas, 2006;

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<https://doi.org/10.1016/j.bonr.2021.101150>

Received 3 September 2021; Received in revised form 6 November 2021; Accepted 15 November 2021

Available online 27 November 2021

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Burr et al., 1997). Bone turnover is important to maintain bone strength in hemodialysis (HD) patients, although the incidence of fracture is increased in dialysis patients regardless of bone turnover (Helal et al., 2010; Danese et al., 2006; Alem et al., 2000; Coco and Rush, 2000). Increased osteocyte death was demonstrated in HD patients treated with parathyroidectomy (PTX) for renal hyperparathyroidism (Yajima et al., 2010). It also is possible that osteocyte maturation is impaired in dialysis patients (Pereira et al., 2018). It was reported that the osteocyte number is inversely correlated with fracture risk in patients without chronic kidney disease (CKD) (Qiu et al., 2003). Therefore, it is possible that as osteocytes die (Yajima et al., 2010), micropetrotic areas accumulate within the bone of dialysis patients as well.

Fracture risk is increased in dialysis patients with long-term renal hyperparathyroidism (Malluche and Monier-Faugere, 1990). However, the mechanism for this increased fracture risk in dialysis patients is unknown.

We hypothesized that more regions of micropetrosis will be present in patients with long-term hypoparathyroidism than in those with long-term hyperparathyroidism, or than those who have become hypoparathyroid after PTX. The rationale is that bone turnover is reduced in patients with hypoparathyroidism, which will allow local accumulation of mineral as osteocytes die and lacunae and canaliculi are mineralized. In our previous study, the degree of mineralization by osteocytes was greater in HD patients compared to the normal subjects (Yajima et al., 2010; Yajima et al., 2019). If there are more micropetrotic regions in patients with end-stage renal disease, this could increase the fragility of the bone and lead to the increase in fractures in these patients (Milo-vanovic and Busse, 2020). This study investigated the relationship between the accumulation of local area of micropetrosis in the bone and the clinical features of patients with hyper- and hypoparathyroidism with end-stage renal disease who are receiving maintenance HD.

## 2. Materials and methods

### 2.1. Patients

Five female and 23 male HD patients (Age  $59.9 \pm 11.7$  years; HD duration  $14.2 \pm 7.6$  years) were assessed. These patients were divided into the three groups. Group I included patients with renal hyperparathyroidism ( $n = 9$ ; Age  $57.1 \pm 10.7$  years; HD duration  $20.4 \pm 6.1$  years; intact parathyroid hormone (iPTH) =  $847.1 \pm 350.4$  pg/mL). Group II included patients with post-parathyroidectomy hypoparathyroidism within one year after surgery or those with hypoparathyroidism after the 1-year treatment with cinacalcet hydrochloride (HCl) ( $n = 12$ ; Age  $56.4 \pm 11.4$  years; HD duration  $15.1 \pm 6.8$  years; iPTH =  $25.4 \pm 26.7$  pg/mL). Group III included patients with long-term ( $>3$  years) hypoparathyroidism ( $n = 7$ ; Age  $69.4 \pm 9.1$  years; HD duration  $9.1 \pm 8.5$  years; iPTH =  $35.7 \pm 23.0$  pg/mL).

The lower limit of the normal range of serum iPTH was set at 130 pg/mL because serum iPTH of HD patients should be maintained at 2–9 times the upper limit of the normal range of non-CKD subjects (10–65 pg/mL) (Barreto et al., 2008). The upper limit was set at 300 pg/mL because the HD patients with levels  $>300$  pg/mL were diagnosed with renal hyperparathyroidism and were treated with cinacalcet HCl (Yajima et al., 2008). These dialysis patients had not received anti-resorptive agents, including bisphosphonate, denosumab or romosozumab after initiating dialysis. The patients suffering from renal hyperparathyroidism were treated with parathyroidectomy or cinacalcet HCl and received bone biopsies before these treatments (Group I). The patients treated with parathyroidectomy did not receive vitamin D for at least two months before surgery in Group I. However, all six patients in Group II treated with parathyroidectomy received alfacalcidol ( $1\alpha$ -hydroxyvitamin D,  $2.0 \mu\text{g/day}$ , Chugai Pharma Co. Ltd., Tokyo, Japan) orally for four weeks after surgery. Three other patients in Group II who had not been parathyroidectomized, but were treated with cinacalcet HCl with or without concurrent maxacalcitol ( $1\alpha$ , 25-dihydroxy-22-

oxavitamin D<sub>3</sub>) (Chugai Pharma Co. Ltd., Tokyo, Japan) underwent bone biopsies one year after treatment. Therefore, vitamin D status was quite different in each patient after treatment. None of the patients in Group III received vitamin D sterols for at least one year to avoid low turnover bone disease caused by the long-term severe hypoparathyroidism.

Informed consent was obtained from all patients after they were provided with a detailed explanation of both the risks and possible outcomes of the bone biopsies and PTX. The procedure was conducted in accordance with the Declaration of Helsinki. The Institutional Review Board of Towa Hospital and its affiliated hospitals approved the study protocol.

### 2.2. Serum bone metabolism parameters

The serum levels of iPTH, tartrate resistant acid phosphatase (TRAP), total alkaline phosphatase (Total ALP), calcium (Ca) and phosphorus (P) were measured immediately before bone biopsies (Yajima et al., 2010; Yajima et al., 2019).

### 2.3. $\mu\text{CT}$ scanning evaluation and bone histomorphometry

#### 2.3.1. Bone biopsy

Tetracycline hydrochloride (Tc) (Japan Lederle, Tokyo, Japan) was administered for 2 or 3 days with an interlabel period of 8 to 10 days between double labels. A 3–4 day washout period was allowed before the bone biopsy. Transiliac bone biopsy specimens were obtained from the anterior iliac crest with a trephine having an inner diameter of 8 mm (Yajima et al., 2010; Yajima et al., 2008). Specimens were fixed in 70% ethanol, dehydrated, and stained with Villanueva solution. Next, specimens were embedded in methylmethacrylate and sections were cut on a Reichert-Jung microtome (model 2050; Finetech Scientific Instruments, Tokyo, Japan) (Yajima et al., 2008).

One co-author (A.I) measured the bone turnover parameters and micropetrosis parameters in cancellous bone by means of manual point counting according to the American Society for Bone and Mineral Research Histomorphometry Nomenclature Committee (Dempster et al., 2013).

#### 2.3.2. $\mu\text{CT}$ scanning evaluation

Bone mineral was evaluated by  $\mu\text{CT}$  scanning of the iliac bone biopsies (SkyScan 1176, Bruker, Kontich, Belgium).  $\mu\text{CT}$  scanning can only distinguish tissues by the degree of x-ray attenuation. Mineral absorbs more of the x-ray than soft tissue does, more highly mineralized areas appear brighter, and this is why  $\mu\text{CT}$  is useful for identifying micropetrotic areas. However,  $\mu\text{CT}$  cannot distinguish osteoid from bone marrow or other unmineralized tissues. Thus, histological data from biopsies are needed to make judgements about whether osteoid is increased.

Both bone mineral density (BMD;  $\text{g/cm}^3$ ) and tissue mineral density (TMD;  $\text{g/cm}^3$ ) of the bone samples were measured using  $\mu\text{CT}$  to evaluate bone mineral content of iliac bone in the HD patients.

BMD is the overall density of all tissue within the bone sample and includes the mineralized bone, osteoid tissue and bone marrow. It is theoretically the same value that would be obtained by measurements of weight/volume (Archimedes principle). BMD can be increased by having a greater volume of bone within the overall sample or by bone that is more highly mineralized (such as in micropetrosis). The unmineralized osteoid tissue will not noticeably change BMD. TMD is the density of only the mineralized bone tissue itself. Any tissue that is not highly mineralized (such as marrow or osteoid) is not a part of this measurement. The calculation of TMD requires a threshold be set that identifies each pixel as either bone (above the density threshold) or not bone (below the density threshold) based on the grayscale level. This creates a black and white (binary or segmented) image, in which the bone is white and everything else (marrow, osteoid, air, water) is black. TMD is the

density of only the areas of the sample that are white (bone) after the threshold is applied. The amount of bone in the sample is reported as bone volume/tissue volume (BV/TV).

### 2.3.3. Bone turnover parameters

Bone turnover parameters, including osteoclast surface (Oc.S/BS; %), eroded surface (ES/BS; %), Ob.S/BS (osteoblast surface; %), osteoid surface (OS/BS; %), and bone surface-referent bone formation rate (BFR/BS;  $\text{mm}^3/\text{mm}^2/\text{year}$ ) were measured.

### 2.4. Micropetrosis parameters and both BMD and TMD

Micropetrosis area/bone area (Mp.Ar/B.Ar; %); osteocyte number/micropetrosis area (N.Ot/Mp.Ar;  $\text{N}/\text{mm}^2$ ) were measured. Micropetrosis was defined as the lighter green compared to the darker green areas using fluorescent microscopy (Fig. 1). Both BMD and TMD were obtained to evaluate the presence of micropetrosis using  $\mu\text{CT}$ .

### 2.5. Correlation between bone turnover parameters and micropetrosis area

Correlations of bone turnover parameters, including serum levels of TRAP and total ALP and histomorphometric BFR/BS values with Mp.Ar/B.Ar were investigated to confirm whether micropetrosis area is associated with bone turnover markers in HD patients.

### 2.6. Statistical analysis

Statistical analyses were conducted using JMP 13 (SAS Institute Inc., Cary, NC, USA). The parameters pertaining to serum bone metabolism, histomorphometric parameters of bone turnover and micropetrosis, and bone density parameters obtained by  $\mu\text{CT}$  were compared among Groups

I, II and III using ANOVA (Kolmogorov-Smirnov Test). *Post hoc* pairwise comparisons were made using Mann-Whitney non-parametric tests because of the small sample sizes and the comments regarding F-value were added below Table 3. However, Mann-Whitney non-parametric tests were performed only if *p* values were below 0.05 by the Kolmogorov-Smirnov Test. Bonferroni corrections were performed on the pairwise tests and differences were considered significant if *p* values were  $<0.05/3$  (0.017) (Tables 1, 2 and 3).

**Table 1**  
Serum bone metabolism parameters in Groups I, II and III.

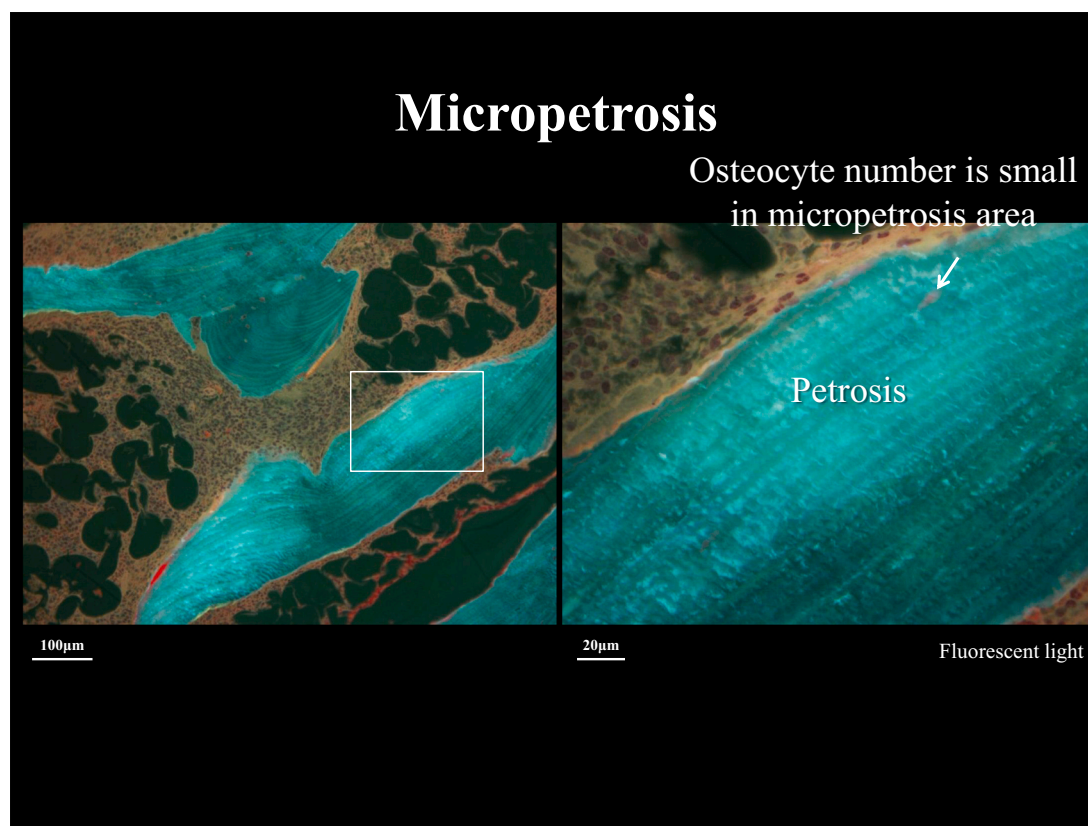
	Group I (n = 9)	Group II (n = 12)	<i>p</i> <sub>1</sub>	Group III (n = 7)	<i>p</i> <sub>2</sub>	<i>p</i> <sub>3</sub>
intact PTH (pg/mL)	874.1 ± 350.4	28.0 ± 28.3	<0.001	34.1 ± 22.9	0.001	(–)
TRAP (U/L)	19.7 ± 10.2	8.5 ± 3.0	0.002	7.6 ± 2.7	0.002	(–)
Total ALP (U/L)	590.3 ± 489.9	648.7 ± 559.3	(–)	168.5 ± 32.6	0.003	0.039
Ca (mg/dL)	9.8 ± 0.8	10.1 ± 1.3	(–)	9.4 ± 0.3	(–)	(–)
P (mg/dL)	5.1 ± 1.1	3.4 ± 1.5	(–)	4.1 ± 0.5	(–)	(–)

Intact PTH = intact parathyroid hormone, TRAP = tartrate-resistant acid phosphatase.

Total ALP = total alkaline phosphatase, Ca = calcium, P = phosphorus.

*P*<sub>1</sub> = Group I vs. Group II, *P*<sub>2</sub> = Group I vs. Group III, *P*<sub>3</sub> = Group II vs. Group III.

\* If *F* values are below 0.05, *p* values were calculated.



**Fig. 1.** Micropetrosis was detected as the lighter green areas compared to the darker green ones using fluorescent microscopy. Osteocyte number was smaller in micropetrosis areas.

**Table 2**

Bone histomorphometric parameters in Groups I, II and III.

	Group I (n = 9)	Group II (n = 12)	P <sub>1</sub>	Group III (n = 7)	P <sub>2</sub>	P <sub>3</sub>
Oc.S/BS (%)	4.7 ± 3.9	0.3 ± 0.6	<0.001	0.7 ± 0.9	0.015	(–)
ES/BS (%)	24.5 ± 8.9	4.4 ± 4.7	<0.001	3.7 ± 4.2	0.002	(–)
Ob.S/BS (%)	24.2 ± 10.7	6.6 ± 9.0	0.002	2.5 ± 4.2	0.002	(–)
OS/BS (%)	49.1 ± 16.5	55.9 ± 32.2	(–)	17.4 ± 19.2	0.021	(–)
BFR/BS (mm <sup>3</sup> /mm <sup>2</sup> /year)	0.034 ± 0.014	0.005 ± 0.005	0.022	0.002 ± 0.004	0.022	(–)

Oc.S/BS = osteoclast surface; ES/BS = eroded surface; Ob.S/BS = osteoblast surface; OS/BS = osteoid surface.

BFR/BS = bone formation rate normalized to bone surface.

P<sub>1</sub> = Group I vs. Group II, P<sub>2</sub> = Group I vs. Group III, P<sub>3</sub> = Group II vs. Group III.

\* If *F* values are below 0.05, *p* values were calculated.

**Table 3**

BMD and TMD values measured using micro CT and micropetrosis parameters obtained by bone histomorphometry in Groups I, II and III.

	Group I (n = 9)	Group II (n = 12)	P <sub>1</sub>	Group III (n = 7)	P <sub>2</sub>	P <sub>3</sub>
BMD (g/cm <sup>3</sup> )	0.189 ± 0.139	0.218 ± 0.122	(–)	0.156 ± 0.075	(–)	(–)
TMD (g/cm <sup>3</sup> )	0.860 ± 0.040	0.882 ± 0.057	(–)	0.924 ± 0.066	(–)	(–)
Mp.Ar/B.Ar (%)	16.1 ± 16.9	27.6 ± 20.7	n. s.	50.7 ± 15.7	0.006	0.013
N.Ot/Mp.Ar (N/mm <sup>2</sup> )	66.7 ± 77.7	86.3 ± 82.3	(–)	50.9 ± 51.3	(–)	(–)

BMD = bone mineral density, TMD = tissue mineral density,

Mp.Ar/B.Ar = micropetrosis area per bone area, N.Ot/Mp.Ar = number of osteocytes per micropetrosis area.

P<sub>1</sub> = Group I vs. Group II, P<sub>2</sub> = Group I vs. Group III, P<sub>3</sub> = Group II vs. Group III.

\* If *F* values are below 0.05, *p* values were calculated.

### 3. Results

#### 3.1. Serum bone metabolism parameters

Serum levels of TRAP, a marker of bone resorption, were significantly greater in Group I compared to both Groups II and III (Table 1). Serum levels of Total ALP, a marker of bone formation, were lower in Group III than in Groups I and II. Serum Ca levels were not different among the three groups because a large dose of Ca gluconate, Ca carbonate and vitamin D sterols was administered to the patients in Group II after PTX to maintain serum Ca levels. Serum P levels were significantly lower after parathyroidectomy as presented previously (Groups II vs. I) (Yajima et al., 2010; Yajima et al., 2019). However, P levels in those with long-term hypoparathyroidism (Group III) did not differ significantly from the other two groups.

#### 3.2. Bone histomorphometry

##### 3.2.1. Bone turnover parameters

The histomorphometric resorption parameters, Oc.S/BS and ES/BS were significantly lower in both Groups II and III than in Group I. Ob.S/BS also was lower in Groups II and Group III than in Group I, suggesting an overall lower bone turnover rate in Groups II and III. This is further reflected by lower BFR/BS values in Groups II and III (Yajima et al., 2019; Yajima et al., 2008). OS/BS was higher in Groups I and II compared to Group III (Table 2).

#### 3.3. Micropetrosis parameters and both BMD and TMD

Mp.Ar/B.Ar values were greater in Group III than in the other Groups, but not statistically different between Groups I and II. Neither BMD nor TMD values were statistically different among the Groups (Table 3).

#### 3.4. Correlation between bone turnover parameters and micropetrosis area

Serum levels of TRAP were associated negatively with Mp.Ar/B.Ar ( $r^2 = 0.233$ ,  $p = -0.027$ ). Serum levels of total ALP were not significantly associated with Mp.Ar/B.Ar ( $r^2 = 0.103$ ,  $p = 0.128$ ), but BFR/BS values were associated negatively with Mp.Ar/B.Ar ( $r^2 = 0.363$ ,  $p = -0.017$ ).

### 4. Discussion

Scarcity of reports on micropetrosis in subjects with end-stage renal disease prompted us to investigate it in HD patients according to their clinical features because fracture risk is high in these patients (Frost, 1960; Carpentier et al., 2012; Busse et al., 2010; Milovanovic and Busse, 2020; Misof et al., 2019; Jansz et al., 2020; Sidibé et al., 2018; Fusaro et al., 2010; Inaba et al., 2005). As previously reported, the osteocyte lacunae are mineralized gradually after osteocyte death due to the reduction of serum PTH and estrogen withdrawal in these patients (Yajima et al., 2010; Yajima et al., 2019; Yajima et al., 2018a; Emerton et al., 2010b; Tomkinson et al., 1997). Calcium, phosphorus and bone fluid stagnate in the canaliculi, which induces development of micropetrosis by allowing mineralization in the osteocytic perilacunar/canalicular system of these CKD patients (Fig. 1) (Frost, 1960; Schmidt et al., 2018; Carpentier et al., 2012; Busse et al., 2010; Yajima et al., 2010; Yajima et al., 2019; Milovanovic and Busse, 2020; Yajima et al., 2018b; Rolvien et al., 2018; Shah et al., 2017; Qing et al., 2012; Baylink et al., 1971). Hypermineralized lacunae and tetracycline labeling have been shown in osteocyte lacunae and on lacunar walls in CKD and non-CKD subjects (Yajima et al., 2010; Yajima et al., 2019; Milovanovic and Busse, 2020; Misof et al., 2019; Yajima et al., 2018a; Yajima et al., 2018b; Rolvien et al., 2018; Shah et al., 2017; Qing et al., 2012; Baylink et al., 1971). This creates local pockets of highly mineralized bone within which microcracks can initiate more easily. This is supported by recent evidence of microcrack accumulation in association with highly mineralized areas of bone devoid of osteocytes in non-CKD subjects (Shah et al., 2017; Burr, 2019; Hokugo et al., 2010; Burr and Allen, 2009; Burr et al., 2003).

Because osteocyte signaling is important to bone remodeling (Busse et al., 2010; Cabahug-Zuckerman et al., 2016; Burr, 2014; Kennedy et al., 2012; Herman et al., 2010; Emerton et al., 2010a; Cardoso et al., 2009; Seeman and Delmas, 2006; Emerton et al., 2010b; Vahidi et al., 2021), pockets of bone without viable osteocytes will not remodel, permitting increased mineralization and preventing any repair of damage that initiates in these regions. Therefore, dialysis patients with low turnover bone disease may have higher fracture risk because of increased local mineralization and the accumulation of microdamage in these areas. However, whether the micropetrotic bone itself is associated with increased bone fragility in HD patients has not been investigated.

Both TRAP and BFR/BS were negatively associated with Mp.Ar/B.Ar, suggesting a low bone turnover state that may lead to unremodeled bone resulting in more pockets of micropetrosis. Total ALP was not significantly associated with Mp.Ar/B.Ar, probably because ALP levels transiently increased after PTX (Yajima et al., 2010; Yajima et al., 2019). The use of serum bone markers to predict precise bone turnover during or soon after the treatment of bone disease at that time in HD patients may not provide a completely accurate picture of turnover (Yajima et al., 2010; Yajima et al., 2019; Yajima et al., 2008; Yajima et al., 2018a).

BMD and TMD were measured using  $\mu$ CT to evaluate bone mineral



content of iliac bone in HD patients because micropetrotic bone is highly mineralized (Milovanovic and Busse, 2020; Fusaro et al., 2010; Rolvien et al., 2018; Shah et al., 2017; Qing et al., 2012). The presence of ~80 nm to 3 µm wide, distinctly faceted, magnesium whilockite  $[\text{Ca}_{18}\text{Mg}_2(\text{HPO}_4)_2(\text{PO}_4)_2]$  crystals within the lacunae and could alter local nanomechanical properties (Shah et al., 2017). Therefore, we hypothesized that BMD and TMD would be higher in HD patients with long-term hypoparathyroidism (Group III). However, neither BMD nor TMD were statistically different among the three groups even though micropetrotic areas measured histomorphometrically were significantly greater in patients with long-term hypoparathyroidism (Group III). However, because most of the bone is not micropetrotic, the larger areas of normally mineralized bone will mask the presence of localized micropetrosis. Bone volume and texture of the patients are changed by age and disease-complications. They may be modified by vitamin D administration, treatment of renal bone disease, including parathyroidectomy, and by drugs administered before and after the commencement of HD therapy (Yajima et al., 2010; Yajima et al., 2019; Yajima et al., 2008; Yajima et al., 2018a; Yajima et al., 2013; Nakashima et al., 2004; Nishi et al., 2005). Hutchison et al. investigated iliac bone samples before initiating the first dialysis treatment for CKD patients. They found that high turnover bone disease, including osteitis fibrosa, was common in pre-dialysis patients (Hutchison et al., 1993). Hernandez et al. reported that the more common cause of renal insufficiency was diabetes in the pre-dialysis patients, who suffered from adynamic low turnover bone disease (Hernandez et al., 1994). It appears that bone is not sensitive to PTH in pre-dialysis patients, but treatment with maintenance dialysis improves the response to PTH in CKD patients. Maintenance dialysis and associated medications reduce serum phosphorus levels and lowers serum PTH (Nishi et al., 2005; Slatopolsky et al., 1996).

There were several limitations to this study. First, the sample size is small. However, it is difficult to obtain a large sample of HD patients in whom iliac biopsies can be performed. Second, although we can speculate that more micropetrotic bone may increase bone fragility, we were not able to perform mechanical testing on the iliac crest biopsies once they had been prepared for histomorphometry. Therefore, we do not know whether this creates greater fragility. Third, we measured micropetrosis only in trabecular bone in this study. Cortical thinning is common and often severe in CKD patients (Burr, 2018; Nickolas et al., 2013; Yajima et al., 2007a; Schober et al., 1998; Tanizawa et al., 1999), making it difficult to find micropetrosis in cortical bone in dialysis patients. Therefore, we measured micropetrosis in only trabecular bone in this study. Further research regarding differences in micropetrosis between trabecular bone and cortical bone is needed because bone texture and turnover are quite different in these areas. In addition, there may be the significantly different responses to treatments in cortical and trabecular bone (Feher et al., 2010; Burr, 2010; Yajima et al., 2007b). Moreover, cortical bone is the most important element regarding bone strength (Singleton et al., 2021; Ferretti et al., 1995). Fourth, we did not measure serum osteocalcin levels of the patients because it is unclear whether osteocalcin is important in mineralization in dialysis patients (Ducy et al., 1996).

The study shows that micropetrosis is more common in HD patients with long-term hypoparathyroidism compared to those with renal hyperparathyroidism and those after treatment with parathyroidectomy or cinacalcet HCl for renal hyperparathyroidism. On the other hand, it was revealed that fracture risk is significantly decreased after parathyroidectomy and after conservative therapy with cinacalcet HCl. In these treatments, it is possible bone mineral density is significantly increased if alfacalcidol is administered after parathyroidectomy or maxacalcitol is administered during the treatment with cinacalcet HCl (Rudser et al., 2007; Moe et al., 2015). Whether decreased fracture risk soon after these treatments is associated with micropetrosis is unknown although Mp.Ar/B-Ar was not different between Group I and Group II (Table 3). In addition, fracture risk is increased in dialysis patients with

both high PTH and low PTH although Mp.Ar/B-Ar was significantly different between Group I and Group III (Danese et al., 2006). It is possible that micropetrosis is one of many factors, that influences fracture risk in dialysis patients. Thus, it is likely that low turnover disease is associated with increased micropetrosis area in HD patients as indicated by the correlation between bone turnover parameters and micropetrosis area. However, changes in BMD and TMD values obtained by µCT were not different among these three groups in this clinical research. The failure to find differences in bone density parameters suggests that the increased fragility in these patients is related to some aspects of bone quality. More numerous pockets of micropetrosis could reflect this change in quality. This is the first report of micropetrosis in dialysis patients. Therefore, further basic and clinical research is needed to consider the pathophysiology of micropetrosis in CKD subjects, and the effects of clinical treatment.

## CRedit authorship contribution statement

1, Aiji Yajima: Management of the patients, parathyroidectomy, bone biopsies, measurement of the bone histomorphometric parameters and their interpretation, measurement of BMD and TMD measured using µCT, validation of the data, taking image using fluorescent microscopy, and formal analyses of all data. 2, Ken Tsuchiya: Management of the data and formal analyses of the data. 3, David B. Burr: Methodology, formal analyses of the data, validation of the data, validation of BMD and TMD measured using µCT, and proofreading of the manuscript. 4, Masaaki Inaba: Analyses and interpretation of the data. 5, Taro Murata: Validation of the data and formal analyses of the data. 6, Masaki Nakamura: Validation of the data and formal analyses of the data. 7, Yoshihiro Tominaga: Parathyroidectomy, 8, Tatsuhiko Tanizawa: The interpretation of the histomorphometric parameters and proofreading of the manuscript. 9, Takashi Nakayama: Bone biopsies. 10, Akemi Ito: Measurement of the bone histomorphometric parameters and their interpretation. 11, Kosaku Nitta: Management of the data, interpretation of all data and proofreading of the manuscript.

All authors made substantial contributions to the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

## Declaration of competing interest

Aiji Yajima, Ken Tsuchiya, David B. Burr, Taro Murata, Masaki Nakamura, Masaaki Inaba, Yoshihiro Tominaga, Tatsuhiko Tanizawa, Takashi Nakayama, Akemi Ito and Kosaku Nitta state that they have no conflicts of interest and do not receive any specific financial supports.

## Acknowledgements

We would like to express our appreciation for the understanding of the Towa Hospital Director, Dr. Tsunamasa Inou and Professor Dr. Haruki Kume at Department of Urology, Graduate School of Medicine, The University of Tokyo.

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