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POST-TRANSPLANT BONE DISEASE: DIAGNOSTIC AND THERAPEUTIC PROCESS

Presentata da: Francesco Tondolo

Coordinatore Dottorato

Niccolò Daddi

Supervisore

Gaetano La Manna

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ABSTRACT

Background: Post-transplant bone disease (PTBD) represents an important cause of morbidity and mortality in kidney transplant recipients (KTRs): the pathogenesis is closely linked both to the pre-transplant and post-transplant period. Persistent hyperparathyroidism and the use of steroids are the most important risk factors for PTBD; bone turnover markers (BTMs) and their trend are a marker in fracture risk stratification. The aim of this study is to evaluate a cohort of de novo KTRs in an epidemiological, biochemical and instrumental way to identify patients with higher fracture risk and establish an early specific therapeutic approach.

Patients and Methods: Clinical, biochemical and instrumental data were measured at time 0 (from the third to the sixth month from KT) and time 1 (after 24 months from KT).

Results and Discussion: Thirty-two de novo KTRs were enrolled in the study with 21-31% of osteoporosis at different skeletal sites at time 0. BTMs represented a condition of high and low bone turnover for 16-22% and 6-19% of KTRs, respectively. An inverse correlation has been demonstrated between the values of densitometric tests and PTH value and cumulative dose of steroids. BMD change after KT was extremely variable and related to both cumulative dose of steroids and persistent hyperparathyroidism. Compared with patients with no change, patients with stability or gain of BMD had higher levels of BTMs and a greater decrease of BTMs from T0 to T1.

Conclusions: PTBD is one of the most important problems after KT with secondary hyperparathyroidism and steroids as key risk factors. BTMs and their change after KT are associated with BMD changes: the resolution of high bone turnover was responsible for BMD gain or stability. An evaluation of BTMs in the early post-transplant period could help to identify patients with higher fracture risk to early treat with anti-fracturative therapy.

INTRODUCTION

1.1 PTBD, CKD-MBD and Osteoporosis

Osteoporosis represents the main metabolic osteopathy in general population, and it is characterized by alterations both in *bone quality* (microarchitecture, turn-over and mineralization) and *bone density*; it's a chronic disease with a considerable social relevance associated with an increased risk of fracture and high morbidity and mortality rates [1, 2]. *Bone quality* represents the set of characteristics that contribute to bone's resistance or *bone strength*, but which are not included in the evaluation of *bone density* (bone mineral density) [3].

According to World Health Organisation (WHO) osteoporosis is a condition characterized by a reduction in bone mineral density, BMD, with T-score $\leq$ - 2.5 evaluated using densitometry (DXA) [4].

Bone disease is a frequent complication of chronic kidney disease (CKD) associated to severe complications in terms of morbidity and mortality; clinical, laboratoristics and instrumental alterations related to bone disease secondary to CKD should be defined more broadly as a clinical condition represented by *chronic kidney disease - Mineral and Bone Disorder (CKD-MBD).* This entity is the expression of the pathogenetic link among biochemical alterations, bone lesions and vascular disease (calcifications and early vascular sclerosis) [2]. Renal osteodystrophy represents bone involvement during CKD. Specifically, CKD-MBD is characterized by one or more of the following elements: alterations of main laboratory tests of mineral metabolism such as calcium, phosphorus, parathyroid hormone, vitamin D 25-OH; alterations of bone turnover and mineralization, volume or bone strength; vascular and soft tissue calcifications [1].

The clinical impact of CKD-MBD is represented by the high risk of fracture: fractures are very frequent and common in patients with CKD with an incidence from 2 to 14 times higher than general population; they occur 10-15 years earlier than in the rest of

general population of the same sex and they are associated to greater hospitalization and mortality [5].

Kidney Transplantation is the treatment of choice for patients with end-stage renal disease because it can reduce the risk of complications related to CKD-MBD (i.e. fracturative risk, cardiovascular disease and other different causes of mortality) [5]. However, kidney transplant recipients (KTRs) present a series of risk factors related to the pre-transplant phase, immunosuppressive therapy and complications related to the transplant itself that affect the long-term outcome of the patient and the graft. Post Transplant Bone Disease (PTBD) is one of the main causes of morbidity in KTRs and it is associated with high hospitalization costs, a high risk of fracture and a high mortality rate. This condition is present in about 15-50% of KTRs, depending on the case series considered [6].

1.2 PTBD: Physiopathology

The alterations in bone and mineral metabolism after kidney transplantation represent a continuation or an evolution of both the conservative phase of CKD and the dialysis replacement period. This condition represents the result of the pre-existing CKD-MBD in association with additional and peculiar factors of the post-transplant period such as immunosuppressive therapy, the recovery of kidney function, hypophosphoremia, alterations of the FGF-23-PTH-Vitamin D 25 OH axis [6]. In KTRs, different bone alterations coexist, such as osteoporotic lesions, high and low bone turnover lesions and those typical of post-transplantation and its specific risk factors [7]. The main risk factors for CKD-MBD and PTBD are shown in **Table 1**.

CKD-MBD	*PTBD*	
Hyperparathyrodism	Dialysis vintage	Hypomagnesemia
Vitamin D Deficiency	**Steroid cumulative dosage**	Diabetes
Chronic Inflammation	**Hyperparathyrodism**	High FGF-23 levels
Metabolic Acidosis	Positive history of fractures	Hypogonadism
Drugs (steroids, phosphate binders, CNI)	Drugs (CNI)	Postural instability
Factors related to general population	Smoking Habit	Age > 50 years

Table 1. *Main Risk Factors for CKD-MBD and PTBD (the most important ones are in **bold**)*

Therefore, it is possible to state that the pathogenesis of PTBD is multifactorial and concerns the alterations of mineral metabolism occurred in pre-transplantation period associated with post-transplant alterations related to immunosuppressive therapy, persistence of hyperparathyroidism and vitamin D deficiency [8]. The two main risk factors, with the greatest impact, of PTBD are hyperparathyroidism and steroid therapy.

Persistent hyperparathyroidism after kidney transplantation causes alterations that can involve both the cortical and trabecular bone depending on the levels and trend of the PTH itself: the result is a condition of reduced bone strength with an increased risk of fractures. The **action of glucocorticoids** is exerted at the level of the trabecular bone of the axial skeleton and is closely related to the cumulative dose of steroid; this drug has an inhibitory effect on osteoformation through apoptosis of osteoblasts and osteocytes and stimulation on the RANK/RANK-L (Receptor Activator of Nuclear Factor κ B) transmission pathway, altering bone structure [9]. According to these observations, authors have tried to propose steroid-free therapeutic schemes or with an important minimization of steroid therapy during the maintenance therapy: the goal was to reduce the risk of fractures in these patients and the potential other adverse effects of chronic steroid therapy. In a study published in 2012, *Nikkel and colleagues* analysed a cohort of KTRs treated with steroid-free immunosuppressive therapy regimens demonstrating a 31% reduction in fracture risk compared to the population treated with traditional immunosuppressive regimens [10]. Despite this consideration,

it is important to underline that the fracture risk persists consistently high even in patients with steroid-free therapeutic regimens, especially if we refer to the fracture risk of peripheral sites (the paradoxical effect of discontinuing steroid therapy in KTRs). In a study that enrolled 47 KTRs with steroid-therapy withdrawal on the third post-operative day, *Iyer and colleagues* demonstrated a stability of BMD at the lumbar and femoral level, but a simultaneous reduction of BMD at the ultra-distal radius after twelve months of KT: this paradoxical result seems to be associated with the catabolic effect of PTH [9]. The same results were obtained by *Evenepoel and colleagues* in a prospective, observational, 5-year follow-up study: authors demonstrated, in a cohort of 69 KTRs treated with a therapeutic regimen of steroid minimization, a significant reduction in BMD after one year only at the radial level (2.2%) compared to the other skeletal sites analysed; moreover, this alteration remained significant even 5 years after kidney transplantation [11].

1.3 Morphological alterations of the cortical and trabecular bone during PTBD

The main problem of PTBD, as well as of CKD-MBD, is represented by the possibility of identifying different morphological patterns. While this problem of classification is less relevant in primary osteoporosis, in the *nephrological* population it is necessary to distinguish these conditions because they require completely different approaches and treatments. Specifically, with the same densitometric data, it is possible to differentiate from a histological point of view, at least two main pictures based on the bone turnover that characterizes them:

- *High Bone Turnover*: *Osteitis fibrosa* characterized by increased remodelling processes with peritrabecular fibrosis; *mixed uremic osteodystrophy* associated with defective mineralization related to excessive PTH secretion.
- *Low Bone Turnover*: *adynamic bone disease* characterized by the absence of remodelling processes and extreme cellular poverty on resorption and apposition surfaces correlated with absolute or relative PTH deficiency; *osteomalacia* characterized by defective mineralization of osteoid matrix [12, 13].

These alterations are not static but dynamic along the course of chronic kidney disease, both in the pre-transplant and post-transplant phases [11].

1.4 PTBD and fracture risk

The close correlation among PTBD, fracture risk and negative outcomes is crucial in KTRs. The higher incidence of fractures in KTRs has been confirmed in literature: the main risk factors are represented by deceased donors, older age, history of fractures, episodes of rejection. The fracture risk in KTRs is higher when compared to the fracture risk of the general population with a negative history of non-vertebral fractures and to the risk of the population with advanced but not dialysis-dependent CKD. *Iseri and colleagues* analysed a cohort of 4190 KTRs and found a rate of fracture events greater than 7.4%: risk factors were female sex, older age, history of major fractures, cause of nephropathy, dialysis vintage and acute rejection. In the same article, authors state that patients with major fractures had a mortality rate from any cause of 78% when compared to those without a history of major fractures; moreover, in the sub-classification of type of fractures, femoral and vertebral fractures were associated with the worst mortality rate from any cause, when compared to peripheral fractures, which were in any case the most represented in the study [5]. The higher frequency of this type of fracture has no certain explanation in literature, although cyclosporine and immunosuppressive therapy have been identified in the past as an independent predictor of peripheral fractures.

1.5 Evaluation of Bone Density and Bone Quality in KTRs

PTBD consists of an alteration of bone strength: this is a combination of both bone density and bone quality. *Bone density* is assessed by densitometry, DXA, which aims to quantify BMD. *Bone quality* is evaluated by non-invasive methods (FRAX Score, Trabecular Bone Score, markers of bone turnover, high-resolution peripheral CT) and invasive methods (bone biopsy) [3]. These tools have been widely validated in general population and they play a fundamental role as predictive indices of fracture risk; in patient with CKD and in KTRs, as already reported, these methods are only partially useful.

1.5.1 FRAX Score

The FRAX Fracture Risk Assessment Tool is a validated score for general population used to manage osteoporosis: it estimates the fracture risk of a patient. It provides a ten-year fracture probability risk, and, therefore, this allows to evaluate the indication or not to a specific anti-fracturative treatment and to act on risk factors. The score considers different variables: *sex, age, weight, previous fracture, family history of fractures, cigarette smoking, corticosteroid use, rheumatoid arthritis, secondary osteoporosis, alcohol abuse*. Its useful in KTRs is widely debated: in a study of 458 patients, *Naylor and colleagues* established the low predictive capacity for fracture risk [14].

1.5.2 DXA, Dual x Ray Absorptiometry

The latest KDIGO 2020 focused on the management of Kidney Transplant Candidate, do not recommend the BMD assessment in the pre-transplant period [15]. For the post-transplant phase, KDIGO provide useful information especially for the first year; for the following post-transplant years, few tools have proven to be useful; specifically, KDIGO 2017 recommend assessing BMD in kidney transplant patients with risk factors for osteoporosis and for those the result of this evaluation may influence or modify the future therapeutic choice [16]. The dual x-ray absorptiometry (DXA) is an accurate, not expensive, non-invasive examination; it can assess (BMD) and predict the fracture risk in the general population and in CKD patients. For KTRs, literature data are doubtful [17]. In a Swedish study of 238 KTRs, authors demonstrated an increased fracture risk in KTRs with BMD in osteoporotic and osteopenia range compared to KTRs with normal BMD [18]. One of the main limitations of DXA is due to the inability to provide information about bone microarchitecture: BMD assessed by DXA, can only determine *bone density*; it doesn't provide information about *bone quality*.

1.5.3 Trabecular Bone Score

Trabecular bone score (TBS) is a recently validated score developed from lumbar DXA image; it's able to provide information about bone microarchitecture, regardless of BMD [19]. Data have established that a high TBS value (>1.370) is related with a

better bone architecture and bone strength than a lower TBS value (<1.370), related to an increased fracture risk, regardless of BMD value [20]. The validity and reliability of this score have also been demonstrated in KTRs: *Naylor et colleagues* stated, in a cohort of 327 patients, that low TBS value (<1.370) was related to an increased fracture risk, regardless of lumbar and femoral BMD value and of other risk factors. In another study, *Luckman and colleagues* examined the change in TBS in 47 kidney transplant patients over one year of follow-up: they detected a correlation between TBS and BMD at the lumbar, femoral and distal radial level. Specifically, while lumbar BMD reported values within the normal range (T Score > -1.0) both before transplantation and at 12 months after transplantation, TBS was able to identify a greater number of patients at high risk of fracture in both periods; this fact would confirm its predictive capacity, regardless of BMD [21].

1.5.4 Bone Turn Over Markers (BTMs)

The main problem of the previously considered instrumental investigations (DXA, Trabecular Bone Score, high resolution peripheral CT) is represented by the inability to give us information regarding bone turnover, a fundamental condition in the management and therapeutic process. Bone turnover markers (BTMs) were introduced in patients with osteoporosis to evaluate fracture risk, guide in therapeutic choice and monitor anti-fracturative treatment [22]. BTMs can be classified into *bone formation markers* and *bone resorption markers*; furthermore, another classification method considers the mechanism of production (cell derived or matrix derived) [23]. The most important BTMs are reported in **Table 2**.

Bone Formation Markers	**Bone Resorption Markers**
Bone-specific Alkaline Phosphatase	*Tartrate-resistant acid phosphatase isoform 5b*
Carboxylated Osteocalcin	*Carboxy Terminal Cross Linked Telopeptides of Type 1 Collagen*
Procollagen Type I N-terminal propeptide	

Table 2. *Bone Turn-Over markers.*

Among **bone formation BTMs**, we reported bone specific alkaline phosphatase (BALP), serum osteocalcin and procollagen type 1 aminoterminal propeptide (P1NP).

Bone Alkaline Phosphatase is secreted by osteoblasts and represents a direct indicator of the anabolic activity of bone; its serum values are not influenced by renal function and for this reason it can be considered an excellent indicator of new bone formation in nephropathic patients [24].

Osteocalcin is a 49 amino acid protein produced by mature osteoblasts; it plays a fundamental role in bone mineralization and in glucose homeostasis (ucOC undercarboxylated osteocalcin). The carboxylation of osteocalcin, a vitamin K-dependent activity, increases the affinity of osteocalcin itself with hydroxyapatite and, therefore, the carboxylated isoform, if elevated, is a marker of increased osteoformation [25]. The main problem of this biomarker is represented by the need to use kits capable of measuring the carboxylated isoform rather than the total form, but also by the mainly renal clearance of this peptide.

P1NP represents a marker of bone formation activity which does not appear to be directly secreted by osteoblasts but derives from the degradation of the N-terminal end of the type 1 collagen produced from osteoblasts [26]. Specifically, P1NP is initially produced as a trimeric form, subsequently degraded to a monomeric form in circulation: it is possible to dose both the total trimeric and monomeric form and the intact trimeric form, which, having a predominantly hepatic clearance, is not affected by the alteration of renal function [23].

Among **bone resorption BTMs**, we reported TRAP5b (Tartrate Resistant Acid Phosphate Isoform 5b) and β-CTX (C-Terminal telopeptides of type 1 collagen) [27].

TRAP5b is secreted by osteoclasts, a protein responsible for removing phosphate molecules from other structural proteins, modifying their function; this biomarker is not influenced by renal function and for this reason, even in patients on hemodialysis and peritoneal dialysis, it represents the main biomarker of bone remodeling [28].

B-CTX is derived from the action of degradation of type 1 collagen by cathepsin K, an enzyme produced by osteoclasts. Although it is influenced by renal clearance, it appears to be fundamental in the monitoring of some antiresorptive therapies, such as therapy with the monoclonal drug denosumab [23].

The role of BTMs has been widely discussed in literature: in a recent review published in 2017, *Vervloet and colleagues* highlight the close correlation between BTMs, morphological alterations at bone level, fracture risk and increased cardiovascular risk [28]. Despite these considerations, both in general and nephrological population, for none BTMs it is possible to define a specific clinical relevance and an absolute pathogenetic role; at the same time no one taken individually can provide us with unambiguous data on turnover. Although bone biopsy represents the gold standard in determining turnover, different authors tried to validate BTMs and to predict turnover status. We reported in **Table 3** two possible BTMS cut-off hypotheses according to *Salam* and *Jorgensen* data [29, 30] .

	High turnover		**Low turnover**	
	Salam	**Jørgensen**	**Salam**	**Jørgensen**
BALP, ug/L	> 31	> 33.7	< 21	< 24.2
Intact PINP, ng/mL	> 107	> 120.7	< 57	< 49.8
TRAP5b, U/L	> 4.6	> 5.05	< 4.6	< 3.44

Table 3. *Cut-off of BTMs values for the definition of high and low bone turnover according to Salam and Jorgensen.*

1.6 Bone biopsy

Bone biopsy with double-stained with tetracycline is the gold standard for differential diagnosis of PTBD, although in clinical practice it's not frequently performed [17]. According to KDIGO 2017, "*it is reasonable to consider a bone biopsy to guide treatment, specifically before the use of bisphosphonates due to the high incidence of adynamic bone disease*" [16]. Since in the KTRs it is possible to find a morphological pathway compatible with low turnover bone and because of the poor predictivity of PTH and others BTMs routinely used on bone turnover, the bone biopsy would seem

to play a fundamental role in the diagnostic process. Despite this consideration, bone biopsy has several limits: these include the potential sampling error (the bone neoformation is a cyclical process that includes phases of quiescence), the invasiveness, the expensiveness, the limited availability of specialized facilities, the difficulty of achieving repeatability [17].

1.7 Bone mineral density and bone turnover markers changes after kidney transplantation

BMD and BTMs changes after kidney transplantation have been extensively studied in literature with contrasting results. Until few years ago, most of the data reported in literature highlighted a loss of BMD in the first years after KT with a significant reduction in bone microstructure both at cortical and trabecular levels [6]. In more recent studies, it has been demonstrated that the main alterations, in the immediate post-transplant period, are determined at bone turnover with its reduction, regardless of the initial turnover status [31, 32]. A large variability in BMD changes after KT was observed with a group of KTRs exhibiting BMD loss, stability or gain [33]. As reported below, BTMs are released from the bone in the skeletal remodeling processes, and they could be used as non-invasive evaluation of skeletal bone turnover: bone turnover markers alterations after kidney transplantation correlate with BMD change but few data are reported in literature among the possibility to give information on later changes in BMD. There are multiple studies in literature that analyze the importance of BTMs and their trend, in patients with CKD and in KTRs: they are considered as a marker in fracture risk [34]. The importance of the trend of BTMs in KTRs was also demonstrated by *Evenepoel* in a study published in NDT in 2020: in this case all BTMs were reduced in the first year post-transplant with a statistical significance reached however only for TRAP5b; furthermore, in the subsequent follow-up up to 5 years, there was a stabilization of the main BTMs with only TRAP5b tending to increase [11].

AIM OF THE STUDY

The aim of this study is to *evaluate* a cohort of de novo kidney transplant recipients in an epidemiological, biochemical and instrumental way identifying patients with higher fracture risk and to *propose* a diagnostic screening scheme for PTBD improving the metabolic condition of KTRs. To address this question, this study aimed to analyze how BTMs and their trend may be related to changes in BMD after kidney transplantation.

PATIENTS AND METHODS

Study Population

We conducted a **prospective** and **observational** study. We analyzed a cohort of de novo KTRs referring to Nephrology, Dialysis and Renal Transplant Unit of IRCCS Policlinico Sant'Orsola Malpighi of Bologna during a follow-up period of two consecutive years. They were adult patients aged > 18 years who performed kidney transplant from January 1st, 2022, to June 30th, 2022, with regular nephrological follow-up. No further specific enrollment criteria were adopted. The only exclusion criterion was treatment with anti-resorptive therapy before kidney transplant until the first post-transplant year and the absence of a nephrological regular follow-up. No further changes to the regular follow-up path were performed.

Clinical Data

The main demographic data, comorbidities, therapies including details of mineral metabolism therapy and immunosuppressive therapy of the enrolled patients were identified. Demographic data included age at transplant, sex, underlying renal disease, duration of chronic kidney disease, menopause, living donor transplant, need for replacement treatment, dialysis vintage, body max index BMI. Among the comorbidities, diabetes and cigarette smoking. Data relating to therapies included the use and cumulative dosage of steroid therapy, the use of CNI calcineurin inhibitors, mycophenolic acid and mTOR inhibitors, the use of supplements with native vitamin D, active vitamin D and calcium, the use of calcimimetics. The value of iPTH before kidney transplant was collected too.

Patients underwent laboratoristics and instrumental exams both at time 0 (from the third to the sixth month from KT) and time 1 (after 24 months from KT).

Biochemical Analysis

Non fasting blood samples were collected at time 0 and time 1. Serum creatinine, total calcium corrects for albumin, phosphate, PTH, 25-OH vitamin D, bone-specific alkaline phosphatase (BALP), trimeric procollagen type I N-terminal propeptide (Intact PINP), tartate-resistant acid phosphatase isoform 5b (TRAP5b), serum

osteocalcin, beta-CTX were measured. The different biochemical parameters were evaluated with the standard techniques used at the Single Metropolitan Laboratory of Bologna.

Bone Densitometry and Trabecular Bone Score

Bone densitometry was performed at the lumbar spine (L1-L4) and proximal femur (both total and hip) by dual-energy X-ray absorptiometry (DXA) scan. The results were expressed both as the absolute value of BMD in g/cm2 and as a T-Score. Osteopenia was defined as a T-Score between -1 and -2.5, while osteoporosis was defined as a T-Score less than or equal to -2.5. Lumbar TBS trabecular bone score was extracted from DXA images using TBS iNsight v3.0 software. Referring to the work of *Naylor and colleagues*, TBS values higher than 1.370 were considered normal, while values lower than 1.370 were associated with high fracture risk.

Vertebral Morphometry

The presence, type and number of vertebral fractures were assessed with a lateral-lateral radiograph of the dorsal and lumbosacral spine performed at the same time as the densitometric examination. Among the data relating to morphometry, the presence of any type of fracture, the presence of wedge-shaped deformation fractures, the presence of femoral fractures, the presence of peripheral fractures, and the total number of fractures were reported.

According to the exams at time 0, patients were candidate or not to supportive therapy (vitamin D supplementation, calcio-mimetic, VDRA) according to the clinical practice.

Statistical Analysis

Continuous variables are given as mean with SD (±SD) if normally distributed or median with interquartile range if skewed. Dichotomous and categorical variables are given as number and proportion (%). Between-group differences were evaluated by Student's t test, Wilcoxon signed-rank test, or Pearson's chi-squared test, respectively. A change in DXA BMD > 2.5% was considered a clinically relevant change. It should be noted that the study was carried out as an applicative thesis with a pre-eminent

educational purpose of scientific research. This thesis does not require the opinion of the Ethics Committee since the research concerns a study in which the data collected guarantees the absolute anonymity of the respondent already at the source.

RESULTS

Epidemiological and Clinical Data

We enrolled 32 patients with kidney transplants from both living and deceased donors, 48% and 52% respectively. They performed kidney transplants from January 1st, 2022, to June 30th, 2022. The main baseline characteristics are reported in **Table 4**.

EPIDEMIOLOGICAL DATA	**PATIENTS (32)**
Age (years)	**52.9 ± 10.8**
Living/Deceased KT (%)	48 % - 52 %
Sex M (%)	44 %
Menopause (%)	33 %
BMI (Kg/m^2)	**25.7 ± 3.7**
CKD duration (months)	**103 (53.3 – 197.3)**
Smoking Habit (%)	13 %
Diabetes (%)	19 %
Cumulative Steroid Dose (mg)	**3325 (2622 – 3995)**
Dialisys vintage (months)	**37 (14.3 – 65)**

Table 4 *Epidemiological Data*

The mean age was 52.9 ± 10.8 years with 56% of female patients; of these, 33% were in menopause. The mean BMI was 25.7 ± 3.7 Kg/ m^2. 19% of patients had diabetes, while 13% of patients had a smoking habit. The median duration of chronic kidney disease was 103 months.

The cause of CKD and kidney transplantation was autosomal dominant polycystic kidney disease (ADPKD) in 10.1% of cases, IgA Nephropathy in 6.8% of cases, a Congenital Abnormalities of Kidney and Urinary Tract (CAKUT) in 14.4%, another glomerular disease in 18.1%, Alport Disease in 1.5%, diabetic nephropathy in 10.3%, an unknown cause in 38.8% of cases. At the time of kidney transplantation, 81.3% of patients had a history of hemodialysis or peritoneal replacement treatment with a median dialysis vintage of 37 months.

The immunosuppressive therapy was represented by standard regimens (steroids + CNIs + anti-metabolite) in 90% of KTRs; specifically, a median cumulative steroid dose of 3.3 grams was observed at time 0; furthermore, for all patients on a steroid-free immunosuppressive therapeutic regimen, suspension occurred on the seventh post-operative day. Among the CKD-MBD therapy, no patient was treated with anti-resorptive or anabolic therapy: native and active vitamin D supplementation was used in 85% and 51% respectively; the calcium-mimetic drug was used in 16% of patients.

Biochemical Data

The main biochemical data at Time 0 (from the third to the sixth month from KT) was reported in **Table 5**. The mean renal function of the enrolled patients was 1.4 mg/dL with a corresponding mean eGFR of 61.2 ml/min; the post-transplant hyperparathyroidism was well controlled by the ongoing therapy as demonstrated by the PTH and calcium levels. The median value of PTH before kidney transplantation was 249 pg/ml. The main markers of bone turnover that could be measured at the LUM Metropolitan Single Laboratory were represented by bone alkaline phosphatase, total osteocalcin, β-cross laps, intact PINP and TRAP5b.

Biochemical Data	**PATIENTS (32)**
Creatinine (mg/dL)	1.4 ± 0.6
eGFR (ml/min)	61.2 ± 21.0
Calcium (mg/dL)	9.7 ± 0.5
Phosphorus (mg/dL)	2.9 ± 0.5
25-OH, Vit D3 (ng/mL)	25.0 ± 6.9
iPTH pre-transplant (pg/mL)	249 (197.8 – 320)
iPTH (pg/mL)	147.5 (101.8 – 173.3)
Bone Alkaline Phosphatase (microg/L)	19.1 (14.7 – 25.9)
Total Osteocalcin (ng/mL)	44.4 (24.1 – 66.6)
β-CTX (ng/mL)	0.556 (0.444 – 0.777)
Intact PINP (ng/mL)	80.9 (71.5 – 148.1)
TRAP5b (U/L)	4.9 (3.4 – 6.3)

Table 5 *Biochemical Data at Time 0*

We reported the correlation between PTH values at T0 and other BTMs values in **Figure 1**.

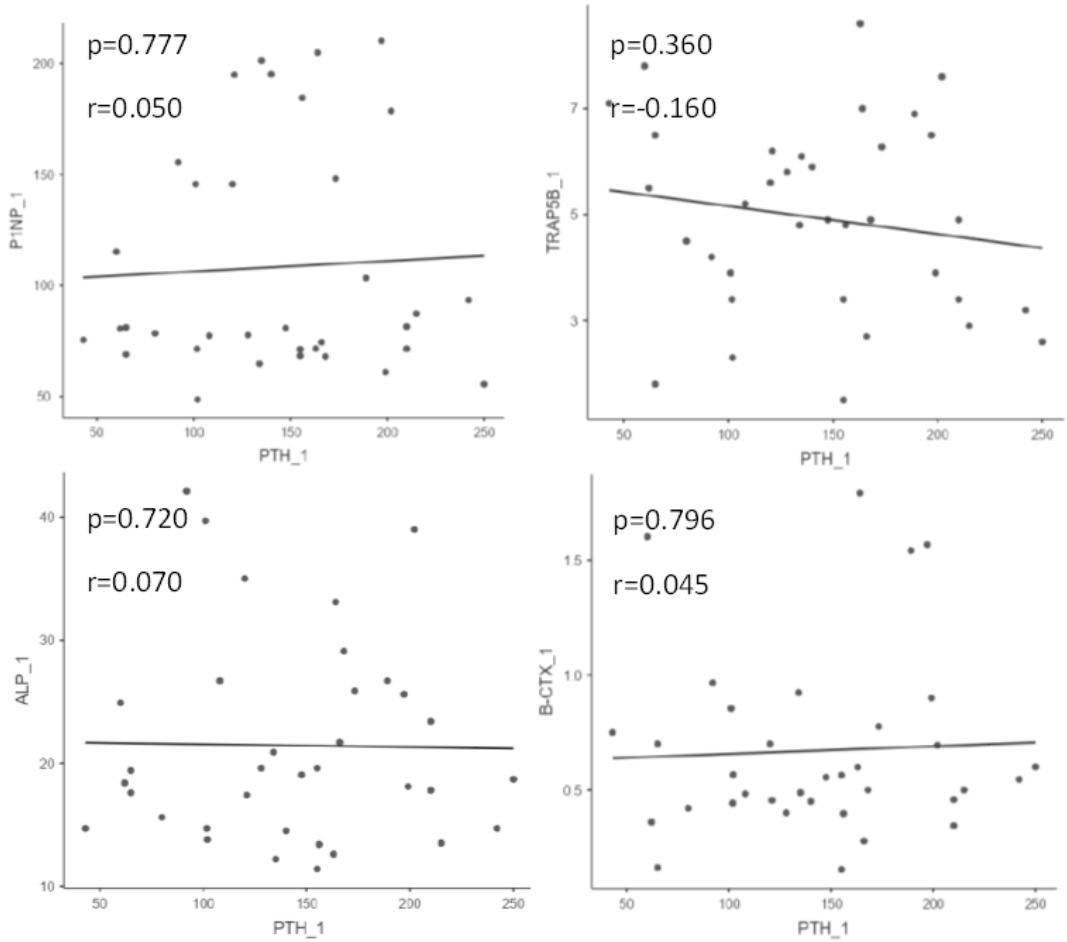

Figure 1 *Correlation between PTH and Bone Turn Over Markers at Time 0*

Therefore, we have divided our cohort according to the entity of bone turnover starting from literature's cut-off of BTMs (*Jorgensen et al.)* [30]: data were reported in **Table 6**. In our cohort, the value of BTMs represented a condition of high bone turnover (two markers above the cutoffs) for 16-22% of KTRs depending on couple of BTMs considered, while a condition of low bone turnover (two markers under the cutoffs) was observed in 6-19% of patients.

	High Bone Turn Over Cut-Off	High Bone Turn Over	Low Bone Turn Over Cut-Off	Low Bone Turn Over
BALP	> 33.7 µg/L	5/32 (16%)	< 24.2 µg/L	22/32 (69%)
Intact P1NP	> 120.7 ng/ml	10/32 (31%)	< 49.8 ng/ml	2/32 (6%)
TRAP5b	> 5.05 U/L	14/32 (44%)	< 3.44 U/L	9/32 (28%)
BALP + iP1NP	As above	5/32 (16%)	As above	2/32 (6%)
BALP + TRAP5b	As above	3/32 (9%)	As above	6/32 (19%)
iP1NP + TRAP5b	As above	7/32 (22%)	As above	2/32 (6%)

Table 6 *High bone turnover and Low bone turnover KTRs percentage at time 0*

Bone Strength: Bone Mineral Density and Trabecular Bone Score

At Time 0, the prevalence of osteoporosis (T-Score ≤ -2.5) was 21-31% at different skeletal sites. At the lumbar level (L1-L4), 37% of patients were in the range of osteopenia, while 28% were affected by osteoporosis; at the femoral level, 50% and 31% of patients were affected by osteopenia and osteoporosis respectively. The specific areal bone mineral density values at lumbar spine, femoral neck, total hip and the corresponding T-Score in the different skeletal districts are shown in **Table 7.**

The mean value of Trabecular Bone Score (TBS) was 1.290 ± 0.124; according to *Naylor et al.*, 72% presented a degraded TBS. Among these patients, 39% were in the range of osteoporosis, 30.5% in the range of osteopenia and 30.5% had a normal areal bone mineral density. Specifically, the mean TBS value in patients with lumbar osteoporosis and osteopenia was 1.187 ± 0.127 and 1.329 ± 0.123 respectively: a statistically significant difference was found between the TBS of patients with normal bone density and patients with both osteopenia p=0.02 and osteoporosis p=0.0001. The mean TBS values of patients with osteoporosis and osteopenia at the femoral level (femoral neck) were 1.246 ± 0.174 and 1.301 ± 0.099 with a statistically significant correlation with the femoral osteoporosis and osteopenia.

Bone Strenght	PATIENTS (32)
Lumbar T-Score	-1.6 (-0.6 – -2.5)
aBMD L1-L4 (g/cm²)	0.940 (0.819 – 1.040)
Total Hip T-Score	-1.6 (-0.9 – -2.4)
aBMD Total Hip (g/cm²)	0.897 (0.709 – 0.970)
Femoral Neck T-Score	-1.8 (-1.4 – -2.5)
aBMD Femoral Neck (g/cm²)	0.855 (0.678 – 0.954)
Trabecular Bone Score	1.290 ± 0.124

Table 7 *Bone Strenght Data at Time 0*

A direct correlation has been demonstrated between the T-Score values at the femoral site and at the lumbar level with the trabecular bone score values, as reported in **Figure 2.**

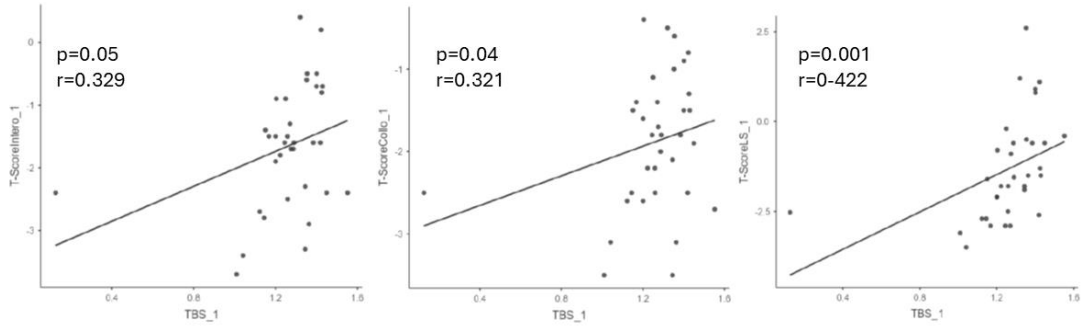

Figure 2. *Correlation between Trabecular Bone Score and Lumbar and Femoral T-Score at Time 0.*

Correlation between BMD-TBS and baseline characteristics

T-Score values both at lumbar and femoral levels correlated in a statistically significant manner with female sex (p=0.006), age at kidney transplant (p=0.004), CKD duration (p=0.003) and dialysis vintage (p=0.0031). We did not observe statistical correlation for TBS.

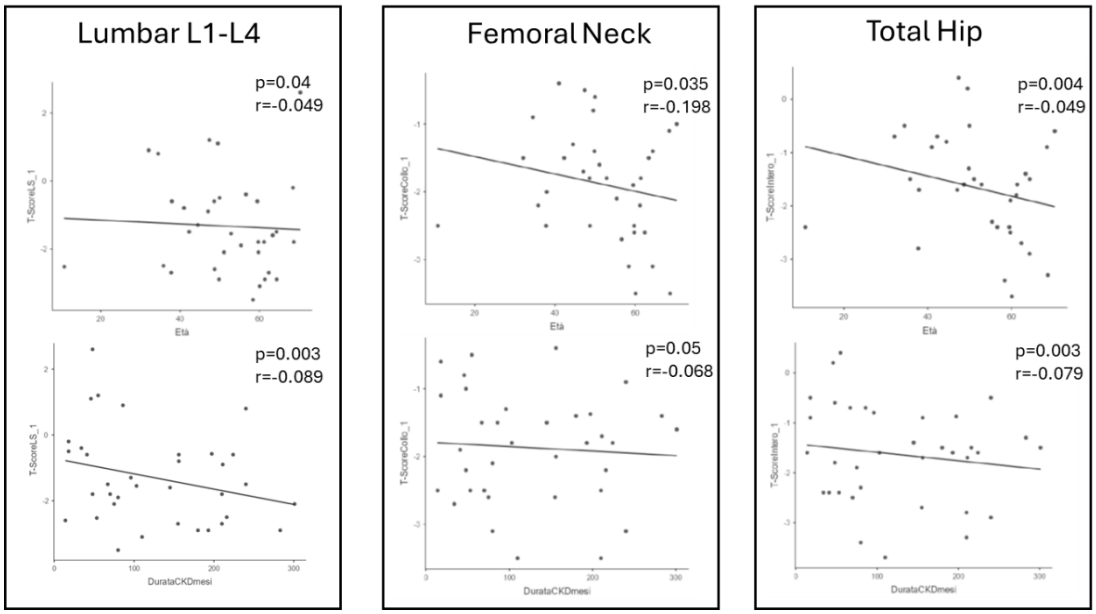

Figure 3 *Correlation between Lumbar and Femoral T-Score with age at kidney transplant and CKD duration at time 0.*

Therefore, we considered the cumulative steroid dosage at time 0: the cumulative steroid dose value reflected the dose of steroid in the immediate post-transplant period and the possible use of steroid before the transplant (immuno-mediated kidney disease). According to our data, we observed a statistical correlation between cumulative steroid dosage and lumbar T-Score, while no statistical correlation was demonstrated with femoral T-Score and Trabecular Bone Score (p=0.060) as reported in **Figure 4**.

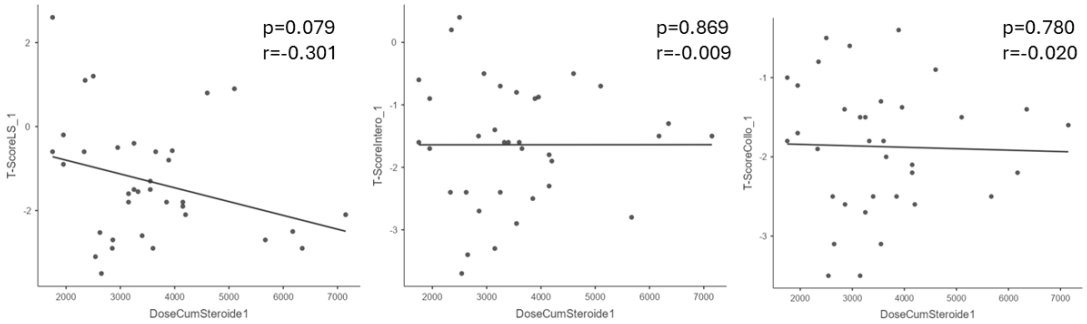

Figure 4 *Correlation between cumulative steroid dose and bone mineral density at Time 0.*

Then, the PTH value before kidney transplantation and PTH value at time 0 were related to bone mineral density and trabecular bone score: a statistical inverse correlation was demonstrated between PTH and lumbar and femoral bone mineral density.

Changes in Bone Turnover Markers

The main biochemical data at Time 1 (after 2 years from kidney transplantation) are reported in **Table 8**. The mean renal function of the enrolled patients was 1.3 mg/dL with a corresponding mean eGFR of 64.3 ml/min; overall, median values of the bone turnover markers and PTH decreased from T0 to T1.

Biochemical Data	T0	T1	p Value
Creatinine (mg/dL)	1.4 ± 0.6	1.3 ± 0.4	0.292
eGFR (ml/min)	61.2 ± 21.0	64.3 ± 16.6	0.349
Calcium (mg/dL)	9.7 ± 0.5	9.5 ± 0.5	**0.029**
Phosphorus (mg/dL)	2.9 ± 0.5	2.9 ± 0.5	0.907
25-OH, Vit D3 (ng/mL)	25.0 ± 6.9	27.4 ± 9.0	0.159
iPTH (pg/mL)	147.5 (101.8 – 173.3)	122.5 (82.8 – 144.3)	**0.042**
BALP (µg/L)	19.1 (14.7 – 25.9)	18.2 (12.1 – 23.6)	**0.007**
Total Osteocalcin (ng/mL)	44.4 (24.1 – 66.6)	20.8 (14.2 – 42.4)	**0.001**
β-CTX (ng/ml)	0.556 (0.444 – 0.777)	0.390 (0.176 – 0.729)	**0.005**
Intact PINP (ng/ml)	80.9 (71.5 – 148.1)	53.4 (35.1 – 89.5)	**0.001**
TRAP5b (U/L)	4.9 (3.4 – 6.3)	3.5 (2.3 – 4.7)	**0.005**

Table 8 *Biochemical Data at Time 0 and Time 1*

Changes in Bone Mineral Density and Trabecular Bone Score

As reported previously, at time of kidney transplantation (T0) the prevalence of osteoporosis was 21-31% at different skeletal sites. After two years from kidney transplantation (T1), the prevalence of osteoporosis was 38-56% at different skeletal sites. A significant decrease in BMD was detected at the lumbar spine, femoral neck

and total hip, as reported in **Table 9 and Figure 4**. The same result was observed for Trabecular Bone Score.

Bone Strenght	**T0**	**T1**	**p Value**
Lumbar T-Score	-1.6 (-0.6 – -2.5)	-1.9 (-0.8 – -2.7)	**0.001**
aBMD L1-L4 (g/cm²)	0.940 (0.819 – 1.040)	0.876 (0.783 – 1.032)	**0.001**
Total Hip T-Score	-1.6 (-0.9 – -2.4)	-1.7 (-1.2 – -2.3)	**0.001**
aBMD Total Hip (g/cm²)	0.897 (0.709 – 0.976)	0.871 (0.679 – 0.955)	**0.001**
Femoral Neck T-Score	-1.8 (-1.4 – -2.5)	-1.9 (-1.6 – -2.6)	**0.001**
aBMD Femoral Neck (g/cm²)	0.855 (0.678 – 0.954)	0.835 (0.654 – 0.933)	**0.001**
Trabecular Bone Score	1.290 ± 0.124	1.260 ± 0.111	**0.001**

Table 9 *Bone Strenght at Time 0 and Time 1*

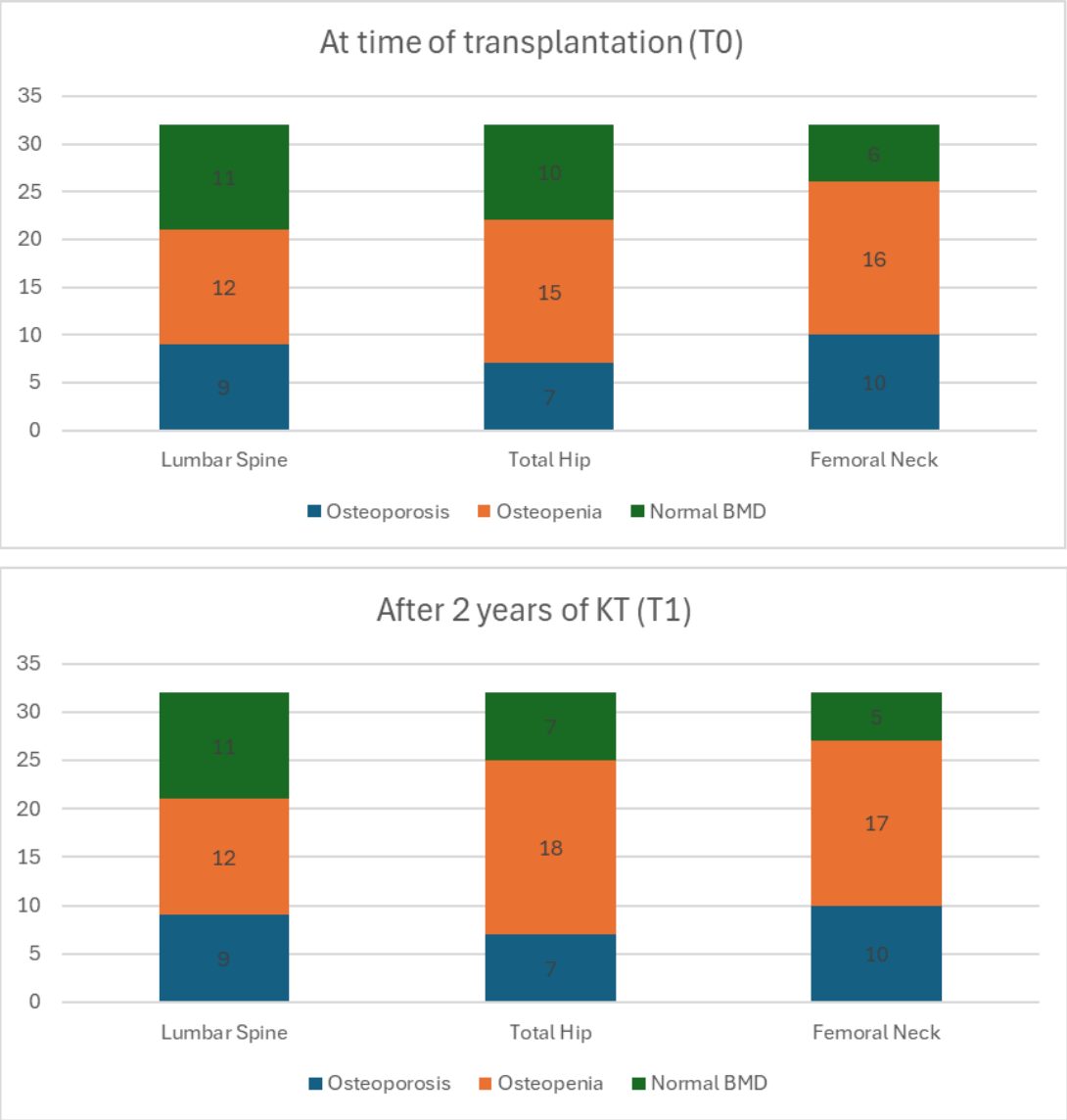

Figure 4. *Prevalence of osteoporosis and osteopenia at time 0 and time 1.*

Relationship between changes in Bone Mineral Density and clinical/biochemical data

A change in DXA BMD ≥ 2.5% from baseline was considered a clinically relevant change; in our cohort of KTRs, a BMD decrease ≥ 2.5% was seen in 59% (19/32) of patients at the lumbar spine, 47% (15/32) at the total hip and in 41% (13/32) at the femoral neck.

Considering BMD change from T1 to T0, there not was correlation between serum creatinine and eGFR at time 1 and BMD change at the femoral and lumbar site.

Therefore, a negative correlation between cumulative dose of steroids at time 0 and BMD change was observed both at femoral spine and lumbar site (**Figure 5).**

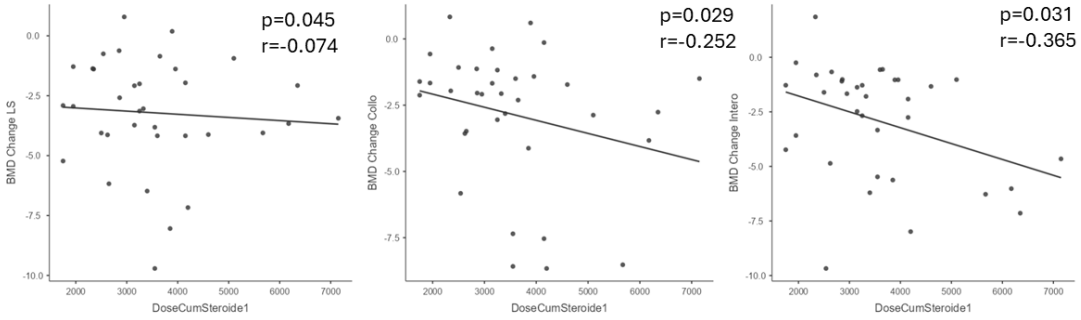

Figure 5 *Correlation between BMD change and cumulative steroid dose.*

Then, we demonstrated a correlation between BMD change and pre-transplant PTH and PTH at time 0 (p=0.04)

Starting from a cut-off of 2.5%, we divided the cohort into 2 depending on the gain/stable or loss of bone mineral density from T0 to T1 and we analyzed PTH and BTMs both as absolute value and trend. According to the skeletal site considered, we reported the results in the **Table 10 and Table 11:** patients who gained BMD at the lumbar spine and femoral site had higher levels of bone turnover markers at the time of transplantation; at the same time, these patients presented a greater decrease of biomarkers from T0 to T1. For PTH no significant correlations were found between the two BMD groups.

Lumbar Site	L1-L4 BMD LOSS (N=19)	L1-L4 BMD GAIN or STABLE (N=13)	p Value
iPTH (pg/mL)	156 (115 – 167)	121 (101 – 189)	0.192
BALP (microg/L)	18.1 (14.2 – 20.7)	24.9 (17.4 – 26.7)	**0.049**
β-CTX (ng/ml)	0.489 (0.380 – 0.601)	0.701 (0.547 – 0.924)	**0.042**
Intact PINP (ng/ml)	75.5 (70.2 – 118.5)	103.4 (78.5 – 145.6)	**0.029**
Δ iPTH	-33 (-73 – 8)	-7 (-34 – 21)	0.159
Δ BALP	-2.1 (-4.9 – 0.4)	-6 (-14.8 – 0.1)	**0.034**
Δβ-CTX	-0.017 (-0.193 –0.132)	-0.201 (-0.387 –0.084)	**0.007**
Δ Intact PINP	-15.3 (-49.1 – -5.5)	-23.7 (-57.7 – -11.2)	**0.042**

Table 10. *BTMs at T0 and ΔBTMs in BMD loss and BMD gain/stable patients at lumbar site.*

Total Hip Site	BMD LOSS (N=15)	BMD GAIN or STABLE (N=17)	p Value
iPTH (pg/mL)	155 (72.5 – 182.5)	155 (131 – 176.5)	0.453
BALP (microg/L)	17.8 (14.7 – 20.7)	19.6 (16.0 – 26.2)	0.124
β-CTX (ng/ml)	0.547 (0.383 – 0.726)	0.565 (0.454 – 0.813)	**0.042**
Intact PINP (ng/ml)	75.5 (71.4 – 81.3)	145.6 (73.1 – 195.0)	**0.029**
Δ iPTH	-13 (-63 – 13.5)	-12 (-53 – 4.5)	0.159
Δ BALP	-2.1 (-5.6 – 0.4)	-3.8 (-8.5 – 0.3)	**0.031**
Δβ-CTX	-0.094 (-0.353 –0.038)	-0.114 (-0.319 –0.146)	**0.021**
Δ Intact PINP	-15.3 (-47.7 – -2.6)	-23.7 (-112.5 – -15.5)	**0.001**

Table 11. *BTMs at T0 and ΔBTMs in BMD loss and BMD gain/stable patients at femoral site.*

DISCUSSION

In our study population, areal bone mineral density (aBMD) is lower both at the lumbar and femoral site. This decrease is linked to partially degraded trabecular bone score (TBS) values, which suggest a higher risk of fractures. These factors show a negative correlation with hyperparathyroidism and the cumulative dose of steroids. Additionally, we observe connections with bone turnover markers and certain clinical characteristics of the KTRs, such as age at kidney transplant and duration of CKD. The degree of change of aBMD after kidney transplantation depends on cumulative dose of steroid and both BTMs level at kidney transplant and their trend. Bone turnover markers at basal level are considered as a marker in fracture risk stratification after kidney transplant.

Among the 32 KTRs in our cohort, according to WHO criteria, we found that 37% had osteopenia and 28% had osteoporosis at the lumbar level. At the femoral neck, 50% had osteopenia and 31% had osteoporosis. These findings align with existing literature, which reports that the incidence of osteoporosis in kidney transplant patients ranges from 15% to 50% [6, 11].

In our cohort, the mean value of TBS is 1.290; specifically, 72% of patients had a value indicative of high fracture risk, lower than the threshold value identified by *Naylor and colleagues* of 1.370 [20, 35]. The TBS is an indicator that evaluates the variations in the gray level of the pixels in the DXA image of the lumbar spine, providing indirect information on the trabecular microarchitecture. The information regarding bone quality provided by TBS is not necessarily comparable to that given by bone densitometry: specifically, TBS can include patients with normal bone mineral density or osteopenia in a high fracture risk category [21]. Among patients with degraded TBS (< 1.350) in our cohort, 30.5% had a normal areal bone mineral density at the lumbar and femoral levels: the values detected by densitometry, taken individually, do not provide complete information about the severity of bone disease in the kidney transplant patient and for this reason integration with other parameters, including TBS, is particularly useful [36]. TBS has been shown to predict fractures independently of

bone mineral density, even in transplant patients [20, 21, 37]. Reduced bone mineral density and degraded TBS are closely related to an increased fracture risk in kidney transplant recipients [32].

Given the significance of bone disease in KTRs, it's crucial to analyze the key risk factors that contribute to post-transplant bone disease (PTBD). This analysis helps us to identify and treat these factors. PTBD is influenced by both pre-transplant and immediate post-transplant risk factors. In the pre-transplant phase, important risk factors include a history of chronic kidney disease, the type of underlying nephropathy, associated immunosuppressive therapies (e.g. steroids), and the duration of any replacement treatments. These factors overlap with those commonly seen in the general population [2, 8]. As reported in the literature, even in our population, bone mineral density values are lower in female with a longer history of chronic replacement dialysis treatment [17]. Dialysis vintage and the resulting metabolic changes from chronic kidney disease-mineral and bone disorder (CKD-MBD) have a significant negative impact on bone mineral density in our cohort too [38].

In post-transplant period, two most important risk factors for PTBD are represented by immunosuppressive therapy (the cumulative steroid dose) and the condition of persistent hyperparathyroidism. The value of PTH before kidney transplant is important too.

Regarding immunosuppressive therapy, in our cohort 90% of KTRs assumed standard regimen (steroids + CNIs + antimetabolite), while 10% had a steroid free immunosuppressive regimen (suspension of steroid on the seventh post-operative day). The median cumulative dose of steroid was 3.3 grams at time 0: the cumulative dose of steroid is correlated with reduced T-Score values at both the femoral and lumbar levels, but also with low TBS values. These results, in our study, were confirmed only at the lumbar level. The mechanism by which steroid therapy is responsible for the reduction of bone mineral density has been widely studied in the literature: steroids have both a direct effect increasing the expression of the RANK-L pathway with activation of the osteoclastic pathway and an indirect effect reducing in muscle mass and altering the pulsatility of the PTH [2, 39]. The greater correlation of

steroid therapy with reduced bone mineral density in a site does not have a unique explanation: the different composition in terms of trabecular bone and cortical bone in the different skeletal segments examined could be partly an explanation [3].

A second and important risk factor for PTBD is represented by persistent secondary hyperparathyroidism. In our cohort the degree of hyperparathyroidism appeared to be well controlled with medical therapy: specifically, the median PTH value was 147.5 pg/ml with an average calcemia of 9.7 ± 0.5 mg/dl. These values are representative of one of the main goals of the management of PTBD: the control of hyperparathyroidism and calcemia must start already in the CKD-MBD phase and must also continue in the post-transplant period to reduce the fracture risk [40]. In our cohort, patients with hyperparathyroidism present reduced values of bone mineral density and TBS; the inverse correlation between the PTH values and the instrumental data, also confirmed in the literature, is related to the increased bone remodeling induced by the PTH itself. This hormone is responsible for an induction of bone resorption both at the trabecular and cortical level which is premature compared to the increased mineralization. Persistent hyperparathyroidism is associated with a reduction in bone mineral density and a progressive increase in cortical porosity with a consequent increased risk of fracture. Specifically, PTH has a predominantly anabolic role at the trabecular level and a catabolic role at the cortical level with an effect, in both cases, dependent on the severity of the hyperPTH; in case of moderate hyperparathyroidism, bone turnover is increased with a consensual increase in cortical porosity and endocortical resorption, reducing cortical thickness [41].

The evaluation of PTH and its trend is part of a broader and more general analysis of bone turnover in KTRs. In recent years the role of other markers of bone turnover (P1NP, TRAP5b, B-CTX) has progressively increased; no BTMs taken individually can uniquely and precisely identify the type of turnover present in KTRs [42]. The possibility of associating PTH with the dosage of other BTMs plays a fundamental role in the evaluation of the patient's bone strength, fracture risk and possible therapeutic choices [41]. The correlation between PTH values and other BTMs was analyzed in our cohort: therefore, a tight association between bone resorption and bone formation is validated in literature [32]. Although bone biopsy represents the gold

standard for identifying bone turnover, bone turnover markers can help to suggest patients' turnover. *Salam and colleagues* reported the diagnostic performance of a panel of BTMs in patients with CKD: BALP, intact P1NP, TRAP5b were particularly able to discriminate low turnover [29]. *Evenepoel and colleagues* analyzed the diagnostic accuracy of BTMs in a cohort of CKD patients (119 KTRs were considered): in their analysis, authors demonstrated a very good discriminatory ability for both high and low bone turnover for all biomarkers. Compared to PTH, intact P1NP was a significantly better predictor of high turnover and TRAP5b was a better predictor for low turnover. With two markers above the cutoffs, positive predictive values up towards 90% were seen for high bone turnover [30]. In our cohort, the value of BTMs represented a condition of high bone turnover (two markers above the cutoffs) for 16-22% of KTRs depending on couple of BTMs considered, while a condition of low bone turnover (two markers under the cutoffs) was observed in 6-19% of patients. Therefore, the condition of normal bone turnover is the most common phenotype at time 0 as reported and confirmed in literature [30]. BTMs can play an additional role in association with instrumental imaging and possibly bone biopsy, to identify a specific bone pattern and opt for a correct therapeutic choice [41].

A negative correlation between aBMD and markers of bone formation and resorption has found ample confirmation in the literature [28, 41].

In the second part of the study we analyzed the modification of baseline, biochemical and instrumental characteristics from T0 (from the third to the sixth month after kidney transplantation) to T1 (after two years from kidney transplantation): the aim was to analyze the association between BMD and BTMs changes after kidney transplantation. In our cohort, median values of the bone turnover markers and PTH decreased in a statistically significant way after kidney transplantation ($p < 0.05$). A significant decrease in BMD of at least 2.5% was detected at the lumbar spine, femoral neck and total hip too after kidney transplantation. Nevertheless, BMD changes after KT were highly variable: BMD loss was seen in 59% (19 KTRs) and 47% (15 KTRs) at the lumbar spine and total hip, respectively. BMD stability or gain was seen in 41% (13 KTRs) and 53% (17 KTRs) at the lumbar spine and total hip, respectively. This

condition of extreme variability in post-transplant bone mineral density has been observed in several cohorts of patients in literature [9, 33].

In our cohort, patients with stability or gain of BMD (both at lumbar spine and femoral site) presented higher levels of BTMs (P1NP, BALP, β-CTX) at time 0 and a greater decrease from T0 to T1 than patients with BMD loss. These data were confirmed in literature: it is possible to hypotize that the resolution of the condition of a high bone turnover state is responsible of BMD gain in these patients (the presence of bone biopsy could confirm this condition as reported in literature) [43]. According to our data, PTH value and PTH trend from T0 to T1 were not related to a condition of gain or loss of bone mineral density: these data are not confirmed in literature. *Jorgensen and colleagues* demontrated that patients who experienced BMD loss during the first post-transplant year had lower levels of PTH at time of transplantation, with an increase in bone turnover markers during the first post-transplant year [43]. In a post hoc analysis of the POSTOP trial, authors demonstrated that change in levels of BSAP and P1NP after 3 months inversely correlated with the variation of BMD of the hip after 6 months; at the lumbar spine this result was observed only for BALP [44].

As reported previously, the cumulative dose of steroids was an important risk factor for PTBD: a negative correlation between cumulative dose of steroids at time 0 and BMD change was observed both at the lumbar and femoral site.

The aim of this study is to evaluate a cohort of de novo kidney transplant recipients in an epidemiological, biochemical and instrumental way. In literature it is difficult to identify a precise diagnostic scheme aimed at investigating both the bone quality and the bone density of kidney transplant recipients; in this study a screening scheme for PTBD is proposed with the aim of improving the outcome, identifying KTRs with higher fracture risk and establishing a possible specific therapeutic approach. This study has both merits and limitations that need to be mentioned. The merits of this study are represented by the prospective and observational characteristics and the absence of confounding factors represented by previous anti-fracturative therapies. An important limitation is given by the small number of patients enrolled in the study; although it is a well-distributed population in terms of sex, age, duration of chronic kidney disease and dialysis age, it is necessary to implement the numbers to obtain

more significant results. A further limitation is given by the impossibility of measuring the biomarkers of bone turnover at the time of kidney transplant (T0 in our study corresponds to a period from the third month and the sixth month from kidney transplant). Therefore, no patients performed bone biopsy, and we did not consider the clinical outcome represented by bone fractures (due to the small follow-up period).

In conclusion, PTBD is one of the most important problems after kidney transplant with secondary hyperparathyroidism and the use of steroids as key risk factors. The levels of BTMs and their change after KT are associated to BMD changes: an evaluation of BTMs in the early post-transplant period could help to identify patients with a greater possibility to gain or loss bone mineral density (to candidate to a CKD-MBD therapy modification in a personalised way).

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