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ORIGINAL ARTICLE

Drugs associated with incident fragility fractures in kidney transplant recipients

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ABSTRACT

Background. The risk of fragility fractures is high in kidney transplant recipients, and steroids are reportedly a major cause. Other drugs known to induce fragility fractures have been studied in the general population but not in kidney transplant recipients. Here, we investigated the association between exposure over time to drugs that can injure bone (namely vitamin K antagonists, insulin, loop diuretics, proton pump inhibitors, opioids, selective serotonin reuptake inhibitors, antiepileptics and benzodiazepines) and incident fractures and changes over time in T-scores in this population.

Methods. A total of 613 consecutive kidney transplant recipients were included between 2006 and 2019. Drug exposures and incident fractures during the study period were comprehensively documented, and dual-energy X-ray absorptiometry was performed regularly. The data were analyzed using Cox proportional hazards models with time-dependent covariates and linear mixed models.

Results. Incident fractures occurred in 63 patients, giving a fracture incidence of 16.9 per 1000 person-years. Exposures to loop diuretics [hazard ratio (95% confidence interval) 2.11 (1.17–3.79)] and opioids [5.94 (2.14–16.52)] were associated with incident fractures. Exposure to loop diuretics was associated with a decrease over time in the T-score for the lumbar spine ($P = .022$) and for the wrist ($P = .028$).

Conclusions. This study suggests that the exposure to loop diuretics and opioids increases the risk of fracture in kidney transplant recipients.

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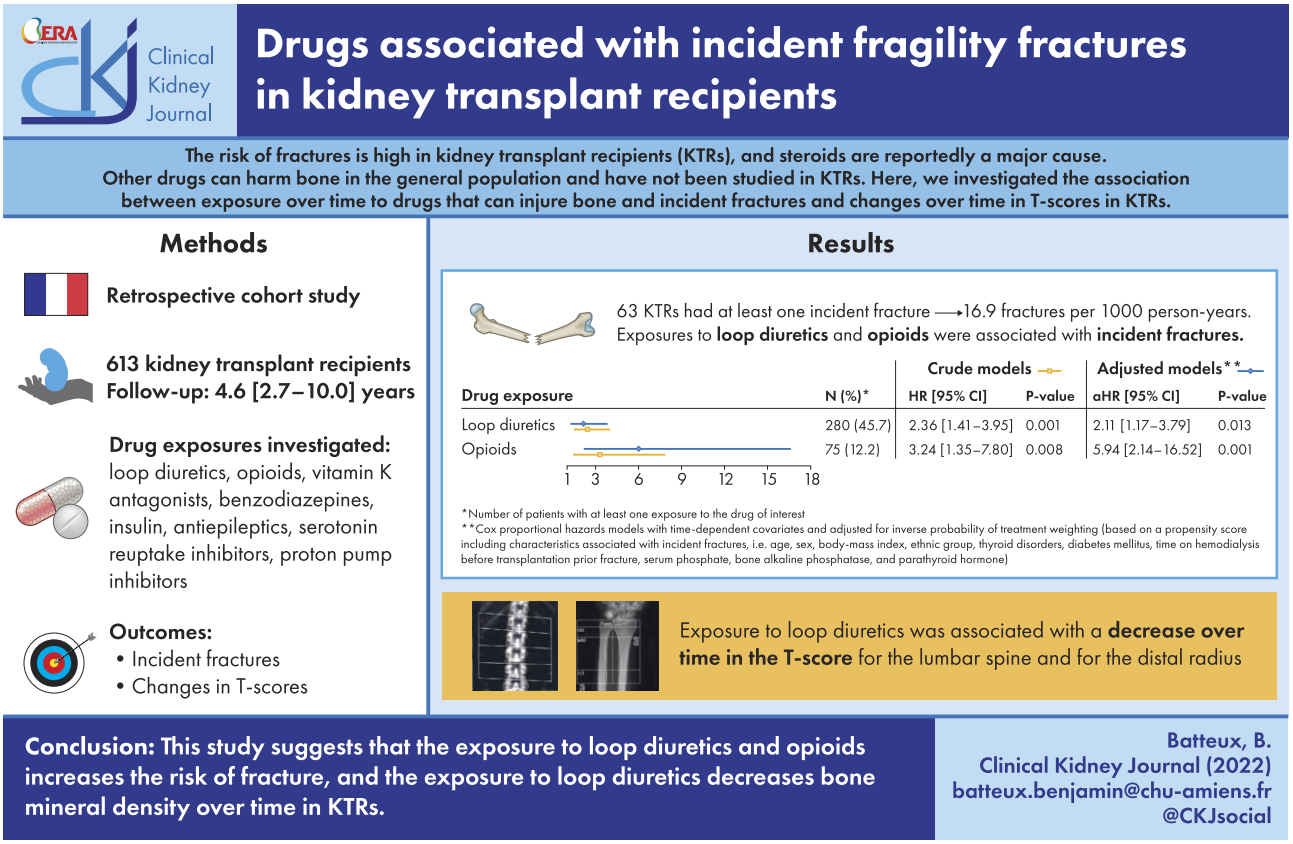
LAY SUMMARY

Kidney transplant recipients have an elevated risk of fragility fracture, which leads to higher morbidity and mortality rates. Several fracture risk factors have been identified, including steroid exposure. Other drugs known to induce fragility fractures have been studied in the general population but not in kidney transplant recipients. Here, we studied several classes of drugs that can induce fragility fractures and are likely to be frequently used in kidney transplant recipients, namely vitamin K antagonists, insulin, loop diuretics, proton pump inhibitors, opioids, selective serotonin reuptake inhibitors, antiepileptics and benzodiazepines. We found that exposures to loop diuretics, vitamin K antagonists and opioids were associated with prevalent osteoporotic fractures in our cohort of kidney transplant recipients. Our findings might prompt changes in practice and thus improved quality of life in this patient population.

GRAPHICAL ABSTRACT



Keywords: CKD-MBD, drugs, fracture, kidney transplantation

INTRODUCTION

Osteoporosis is a whole-body skeletal disease that alters the bone microarchitecture and increases the risk of a fracture [1]. Many risk factors for osteoporosis have been identified in the general population. Some are modifiable [such as smoking, alcohol consumption, sedentary lifestyle and low body mass index (BMI)] and some are not [e.g. sex, age, previous fractures, a family history of osteoporosis, endocrine diseases (Cushing's disease, hypogonadism, hyperparathyroidism and thyroid disorders) and chronic inflammatory diseases (particularly rheumatoid arthritis)] [2]. Furthermore, a number of drug classes are known to increase the risk of osteoporosis and thus fractures; they primarily include steroids, gonadotropin-releasing hormone analogs, anti-aromatases, androgen receptor blockers, thyroid hormones, proton pump inhibitors (PPIs), thiazolidinediones, vitamin K antagonists (VKAs), heparins, loop diuretics, antidepressants [particularly selective serotonin reuptake inhibitors (SSRIs)], benzodiazepines, protease inhibitors, nucleoside and nucleotide reverse transcriptase inhibitors, antiepileptics, insulin, and opioids [3].

The vast majority of kidney transplant recipients suffer from chronic kidney disease–mineral and bone disorders (CKD-MBD), as characterized by a range of histological bone lesions referred to collectively as renal osteodystrophy, which develops before transplantation, alters the bone microarchitecture and may increase the risk of fracture [4]. These lesions include osteoporosis, fibrous osteitis [characterized by a high degree of bone turnover and high circulating levels of parathyroid hormone (PTH)], and

adynamic bone disease (ABD, characterized by a low degree of bone turnover and low circulating levels of PTH) [5]. Furthermore, the most frequently reported risk factors for incident fragility fractures in kidney transplant recipients are older age, female sex, a low BMI at the time of transplantation, prior fracture, a low bone mineral density (BMD) at the time of transplantation, diabetes mellitus at the time of transplantation, a sedentary lifestyle, time on dialysis before transplantation, the urine protein-to-creatinine ratio at the time of transplantation, the etiology of CKD (namely glomerulonephritis and hypertension) and HLA-DR mismatch [6]. Steroids are the main drugs associated with the occurrence of fractures and the decrease in BMD in the first few years after kidney transplantation [7, 8]. In the general population, the use of other drugs might be associated with fractures, through a direct, weakening effect on bone or an indirect effect through hypotension and thus a greater risk of falls. However, with the exception of steroids, the drugs that might induce fragility fractures in kidney transplant recipients have not been studied.

Hence, the primary objective of the present study was to investigate the possible association between incident fragility fractures following kidney transplantation and exposure to drug classes that are (i) known to induce osteoporosis in the general population and (ii) frequently prescribed (in addition to steroids) after kidney transplantation (namely VKAs, insulin, loop diuretics, PPIs, opioids, SSRIs, antiepileptics and benzodiazepines). The secondary objective was to investigate the possible association between changes over time in T-scores and exposure to drug classes associated with incident fractures.

MATERIALS AND METHODS

Study design and participants

We performed a retrospective, longitudinal study of a cohort of adult patients (aged 18 years and over) having undergone kidney transplantation at Amiens University Medical Center (Amiens, France) between 1 January 2006 and 31 December 2019. The study end date was 31 October 2021, or (for kidney transplant recipient patients having resumed dialysis) the dialysis resumption date. On the transplantation day, the patients underwent an extensive laboratory workup including serum calcium, phosphate, 25(OH) vitamin D, osteocalcin, bone alkaline phosphatase (BAP) and PTH assays. The post-transplantation follow-up was standardized: extensive medical check-ups were performed 1 month and 4 months after transplantation and then annually. At each check-up, all medical events having occurred since the previous check-up were exhaustively documented. One month after transplantation, 1 year after transplantation and then every 2 years, each patient underwent a computed tomography (CT) scan of the abdomen and pelvis and dual-energy X-ray absorptiometry (DXA). The same bone densitometry system (Discovery System, Hologic Inc., Waltham, MA, USA) was used for all DXA measurements. Lastly, the patients could consult their usual nephrologist between scheduled study visits, if needed.

Inclusion criteria and the end of follow-up

Kidney transplant recipients were included if they had at least 1 year of dialysis-free follow-up after kidney transplantation. We considered that a minimum follow-up period of 1 year would be needed to observe the primary endpoint (fracture) and secondary endpoint (changes in DXA parameters), and that prolonged exposure to the drugs of interest would have an impact on bone health. Since the present study focused on kidney transplant recipients with a functional graft, resumption of dialysis in the year following transplantation was an exclusion criterion. For patients who resumed dialysis more than 1 year after transplantation of follow-up, the date of dialysis resumption was taken as the follow-up end date. In order to avoid issues in the statistical analyses, a patient having undergone kidney transplantation several times during the study period could not be included several times—only the first transplantation was considered, if it had happened more than a year before dialysis resumption. If the first transplantation did not meet these inclusion criteria, the next one that did was considered. Furthermore, patients having undergone kidney transplantation several times (including one or more transplantation prior to the study period and one or more transplantation during the study period) could be included. Using the same reasoning as above, these patients were included if a transplantation during the study period met the inclusion criteria (i.e. more than a year between the transplantation and the resumption of dialysis).

Collected data

The variables recorded at baseline included osteoporosis risk factors (age, sex, BMI, ethnic group, alcohol consumption, smoking, thyroid disorders, rheumatoid arthritis, other autoimmune/inflammatory diseases, diabetes mellitus, primary and secondary hyperparathyroidism, sedentary lifestyle, and previous vertebral and non-vertebral fractures), arterial hypertension, dyslipidemia, any history of cardiovascular events, data related to CKD and transplantation [the etiology of CKD, any previous transplantations, time on hemodialysis before transplantation, preemptive transplantation, peak panel-reactive antibodies (PRAs), donor-specific antibodies (DSAs), crossmatching results and induction therapy], and laboratory variables [serum calcium, phosphate, BAP, osteocalcin, PTH and 25(OH) vitamin D for all recipients, and creatinine for recipients having undergone preemptive transplantation only].

The variables recorded during the follow-up (from the transplantation date to the end date) included drug exposure, incident fractures and DXA data. Data on all prescription drugs (whether prescribed by a nephrologist or not) were extracted from the patient's medical records (a computerized prescription form, in the vast majority of cases): drug classes, and start and end dates for steroids, VKAs, insulin, loop diuretics, PPIs, opioids, SSRIs, antiepileptics, benzodiazepines, vitamin D, calcium and bisphosphonates. When a patient had received the same drug class intermittently during the follow-up, all periods of drug exposure were documented.

Fractures were documented from medical records and from CT scans of the abdomen and pelvis. Previous fractures were defined as those having occurred before transplantation. Thus, if a fracture was reported in the patient's medical records before transplantation and/or the last CT scan performed before transplantation, it was classified as a previous fracture. Incident fractures were defined as those having occurred after transplantation. Thus, if a fracture was reported in a patient's medical records (from each annual, extensive medical check-up, each intermediate medical consultation and each hospital stay) during the follow-up period or was detected on CT scans after transplantation (performed 1 month, 1 year and then every 2 years after transplantation), it was classified as an incident fracture. Non-vertebral fractures (most of which were symptomatic) were exclusively documented from medical records. Since vertebral fractures can have mild symptoms and therefore not always

detected, they were documented from both CT scans and medical records. Hence, vertebral fractures observed on CT scans were detected in two ways. First, radiology reports were reviewed; fractures detected and reported by radiologists were systematically reported in medical records. Secondly, the last CT scan performed before transplantation and all the CT scans performed after transplantation were reviewed by three rheumatologists (B.B., A.S. and C.P.). These CT scans were reviewed because they were prescribed as morphologic assessments for the transplantation and then as assessments for the detection of neoplasia of the abdomen and native kidneys, rather than as a screen for a vertebral fracture, and it is well known that radiologists frequently miss or do not report vertebral fractures when imaging is requested for a different clinical indication [9]. There were no disagreements between the three rheumatologists with regard to the detection of vertebral fractures on the CT scans, and there were no chronological ambiguities (i.e. the three rheumatologists always agreed on whether a vertebral fracture have occurred before or after transplantation).

T-scores for the lumbar spine (from vertebra L1 to vertebra L4), total hip and wrist (i.e. the distal radius) were collected from the DXA measurements performed 1 month, 1 year and then every 2 years after transplantation. Unfortunately, DXA data were not available between 2006 and 2010; this prevented us from evaluating the study's secondary endpoints in a portion of the study population. Unlike the T-scores, Z-scores were not well documented in the medical records, and we did not have direct access to DXA data. It would have been more appropriate to study Z-scores than T-scores—especially for changes over time—because the Z-score is the number of standard deviations (SDs) between the subject's BMD value and the mean value for adults of the same sex and age. The T-score is the number of SDs between the subject's BMD value and the average value for 20-year-old adults of the same sex. Hence, in non-menopausal women and men younger than 50 years old, the Z-score should be used [1, 10]. Given the absence of Z-score data, we nevertheless studied the T-score data.

Assays of serum PTH, 25(OH) vitamin D, bone alkaline phosphatases and osteocalcin

Serum PTH levels were evaluated in a chemiluminescent whole PTH immunoassay (ADVIA Centaur PTH from Siemens Healthcare Diagnostics SAS; intra-assay coefficient of variation: <2.05%; inter-assay coefficient of variation: <4.04%; limit of detection: 4.6 pg/mL), according to the manufacturer's instructions. Serum 25(OH) vitamin D was assayed using an ELISA (assay kit total Vitamin D-ADVIA Centaur from Siemens Healthcare Diagnostics SAS; intra-assay coefficient of variation: <9.79%; inter-assay coefficient of variation: <7.32%; limit of detection: 4.20 ng/mL). Bone alkaline phosphatase and osteocalcin were assayed using the Liaison-XL system from DiaSorin SA (intra-assay coefficients of variation: <2.16% and <4.99% for serum bone alkaline phosphatase and osteocalcin, respectively; inter-assay coefficients of variation: <4.19% and <5.33%, respectively; limit of detection: 3.0 μg/L and 1.5 ng/mL, respectively). The assay methods were the same throughout the study period.

Immunosuppressive regimens

Since March 2016, kidney transplant recipients with a low immunological risk (defined as a peak panel-reactive antibody <20%) being treated in the Department of Nephrology at Amiens University Medical Center have discontinued their corticosteroid treatment on Day 7 post-transplantation. The treatment consisted of a bolus of methylprednisolone (500 mg) at the time of transplantation, followed by oral prednisolone (20 mg/day for 4 days and then 10 mg/day for 3 days). The patients had received induction therapy with basiliximab and maintenance therapy with mycophenolate mofetil plus tacrolimus or mycophenolate mofetil plus cyclosporine, depending on the risk of developing type 2 diabetes mellitus or exacerbating preexisting diabetes. These patients could resume corticosteroid therapy for various reasons. Before March 2016, all patients (regardless of the immunological risk) received the same long-term corticosteroid treatment, consisting of a bolus of methylprednisolone (500 mg) at the time of transplantation and then oral prednisolone (20 mg/day, with a gradual dose reduction to between 5 and 10 mg/day by Month 3).

Statistical methods

In a descriptive analysis, categorical variables were expressed as the number (percentage), and continuous variables were expressed as the mean ± SD, the median [interquartile range (IQR)], or the median (range), depending on the data distribution. The Shapiro–Wilk test was used to determine whether or not data were normally distributed. In bivariate analyses comparing groups (i.e. with an incident fracture or not), continuous variables were compared using Student's *t*-test or a Wilcoxon's rank sum test (depending on the data distribution), and categorical variables were compared using a chi-square test or Fisher's exact test. Univariate Cox proportional hazards models were also used to identify factors associated with incident fractures (Supplementary data, Table S1).

The proportion of missing data was very low (0.8%) for baseline serum PTH, osteocalcin, BAP and 25(OH) vitamin D. In order to identify patients with ABD, we performed multiple imputation for these variables with the fully conditional specification method (10 datasets and 10 iterations), using the patients' characteristics in Table 1 [11]. The data patterns suggested that the assumption whereby data were missing at random was plausible.

Cox proportional hazards models with time-dependent covariates were used to assess the associations between incident fractures and drug exposure. We chose these models because drug exposure could be discontinuous. Hence, in simple analyses, a Cox proportional hazards model was built for each drug class of interest and the corresponding exposure periods. The survival time was right-censored if the event (the fracture) was not observed in patients still being followed up when the data were collected, those lost to follow-up, those having resumed dialysis after 1 year of follow-up or deceased patients. In multivariable analyses, each previous model had been fitted with inverse probability treatment weighting (IPTW) based on a propensity score (PS). The rationale for using Cox proportional hazards models fitted with IPTW (rather than osteoporosis and/or fracture risk factors) is that the number of events (fractures) per predictor variable (osteoporosis and/or fracture risk factors and drugs of interest) was below 10; this would have caused overfitting in the latter model. Another advantage of IPTW is that the probability distribution for receiving a given drug was similar in the weighted population. The PS was calculated using a logistic regression model. Treatment assignment (the use or not of each drug of interest) was regressed against baseline variables associated with fractures ($P < .2$) and against variables associated with the use of each drug of interest ($P < .2$); it is advisable to include covariates with prognostic value (i.e. those

Table 1. Baseline characteristics of the study population.

Baseline characteristics	Total, N = 613	No-fracture group, N = 550	Fracture group, N = 63	P-value	Imputed data (%), N = 613
Osteoporosis risk factors					
Age (years)	49.9 ± 13.2	48.9 ± 13.2	58.3 ± 10.5	<.001	0
Sex					0
Female	226 (36.9)	195 (35.5)	31 (49.2)	.045	
Male	387 (63.1)	355 (64.5)	32 (50.8)		
BMI (kg/m²)	25.5 ± 4.2	25.4 ± 4.2	26.2 ± 4.3	.150	0
Ethnic group					0
Caucasian	574 (93.6)	511 (92.9)	63 (100)	.092	
Black	37 (6.0)	37 (6.7)	0		
Other	2 (0.3)	0	0		
Alcohol consumption	48 (7.8)	44 (8.0)	4 (6.3)	.830	0
Smoking					0
Never	315 (51.4)	278 (50.5)	37 (58.7)	.069	
Past	171 (27.9)	151 (27.3)	20 (31.7)		
Current	127 (20.7)	121 (22.0)	6 (9.5)		
Thyroid disorders	35 (5.7)	25 (4.5)	10 (15.9)	<.001	0
Diabetes mellitus	93 (15.2)	82 (14.9)	11 (17.5)	.727	0
Rheumatoid arthritis	6 (1.0)	5 (0.9)	1 (1.6)	1.000	0
Other autoimmune diseases	32 (5.2)	28 (5.1)	4 (6.3)	.900	0
Sedentary lifestyle	125 (20.4)	108 (19.6)	17 (27.0)	.228	0
Previous fracture[a]	79 (12.9)	65 (11.8)	14 (22.2)	.033	0
Vertebral[a]	25 (4.1)	14 (2.5)	11 (17.5)	<.001	0
Non-vertebral[a]	59 (9.6)	52 (9.5)	7 (11.1)	.844	0
Type of baseline renal osteodystrophy					
Adynamic bone disease	95 (15.5)	83 (15.1)	12 (19.0)	.523	0
Other renal osteodystrophy	518 (84.5)	467 (84.9)	51 (81.0)		0
Other clinical data					
Arterial hypertension	524 (85.5)	469 (85.3)	55 (87.3)	.807	0
Dyslipidemia	336 (54.8)	298 (54.2)	38 (60.3)	.428	0
History of cardiovascular event	154 (25.1)	136 (24.7)	18 (28.6)	.608	0
CKD and kidney transplantation data					
Etiology of CKD				.830	0
Glomerulonephritis	178 (29.0)	164 (29.8)	14 (22.2)		
Hereditary disease	114 (18.7)	98 (17.7)	16 (25.4)		
Polycystic kidney disease	106 (17.3)	91 (16.5)	15 (23.8)		
Renal and urinary tract malformations	70 (11.4)	61 (11.1)	9 (14.3)		
Hypertensive kidney disease	43 (7.0)	38 (6.9)	5 (7.9)		
Diabetic kidney disease	51 (8.3)	46 (8.4)	5 (7.9)		
Interstitial nephritis	26 (4.2)	24 (4.4)	2 (3.2)		
Vascular nephropathy	28 (4.6)	24 (4.4)	4 (6.3)		
Indeterminate	72 (11.7)	67 (12.2)	5 (7.9)		
Other	31 (5.1)	28 (5.1)	3 (4.8)		
Time on dialysis before transplantation (months)	(N′ = 588) 27.5 [16.6–46.7]	(N′ = 529) 26.7 [16.6–45.9]	(N′ = 59) 34.1 [17.0–57.3]	.238	0
Previous kidney transplantation	79 (12.9)	69 (12.5)	10 (15.9)	.584	0
Preemptive transplantation	25 (4.1)	21 (3.8)	4 (6.3)	.531	0
Peak PRAs					0
<20%	497 (80.9)	441 (80.2)	55 (87.3)	.576	
20%–80%	81 (13.2)	75 (13.6)	6 (9.5)		
>80%	35 (5.7)	33 (6.0)	2 (3.2)		
DSAs					0
No	598 (97.6)	537 (97.6)	61 (96.8)	.919	
Previous	8 (1.3)	7 (1.3)	1 (1.6)		
Current	7 (1.1)	6 (1.1)	1 (1.6)		
Positive crossmatch	15 (2.4)	14 (2.5)	1 (1.6)	.971	0
Cinacalcet use before transplantation	128 (20.9)	118 (21.5)	10 (15.9)	.385	0
Deceased donor	571 (93.1)	511 (92.9)	60 (95.2)	.667	0

Table 1. Continued

Baseline characteristics	Total, $N = 613$	No-fracture group, $N = 550$	Fracture group, $N = 63$	P-value	Imputed data (%), $N = 613$
Laboratory data at the time of transplantation					
Serum calcium (RVs: 84.0–104.4 mg/L)	92.6 ± 8.0	92.6 ± 8.4	93.8 ± 8.0	.323	0
Serum phosphate (RVs: 24.8–44.9 mg/L)	45.0 ± 14.9	45.3 ± 14.6	42.5 ± 15.5	.192	0
Bone alkaline phosphatase (RVs: 5.5–24.6 µg/L)	12.3 [8.4–19.8]	12.4 [8.5–19.85]	11.9 [7.9–18.9]	.011	13.7
Osteocalcin (RVs: 4.6–65.4 ng/mL)	146 [48.9–333.0]	151 [25.0–336.0]	120 [38.8–321.0]	<.001	20.7
PTH (RVs: 18.5–88.0 pg/mL)	177 [26.0–470.0]	190.2 [30.0–475.0]	84 [13.85–356.0]	<.001	16.2
25(OH) vitamin D (RVs: 30.0–80.0 ng/L)	30.9 [19.0–41.0]	31 [19–40.6]	28.75 [17.0–45.0]	.482	6.7
Serum creatinine in patients with preemptive	($N' = 25$)	($N' = 21$)	($N' = 4$)	.008	0
transplantation (RVs: 6.0–11.0 mg/L)	61.6 ± 15.4	64.7 ± 14.9	46.8 ± 7.9		
Induction therapy					
Basiliximab	381 (62.2)	340 (61.8)	41 (65.1)	.713	0
Thymoglobulin	225 (36.7)	204 (37.1)	21 (33.3)	.654	0
IVIg	17 (2.8)	16 (2.9)	1 (1.6)	.841	0
Drugs of interest used after transplantation					
Steroids[b]	613 (100)	550 (100)	63 (100)	NA	0
VKAs[b]	105 (17.1)	92 (16.7)	13 (20.6)	.424	0
Insulin[b]	116 (18.9)	107 (19.5)	9 (14.3)	.393	0
Loop diuretics[b]	280 (45.7)	240 (43.6)	40 (63.5)	.004	0
PPIs[b]	591 (96.4)	530 (96.4)	61 (96.8)	1.000	0
Opioids[b]	75 (12.2)	67 (12.2)	8 (12.7)	1.000	0
SSRIs[b]	43 (7.0)	38 (6.9)	5 (7.9)	.923	0
Antiepileptics[b]	70 (11.4)	63 (11.5)	7 (11.1)	1.000	0
Benzodiazepines[b]	220 (35.9)	193 (35.1)	27 (42.9)	.281	0
Vitamin D[b]	572 (93.3)	514 (93.5)	58 (92.1)	.879	0
Calcium[b]	277 (45.2)	247 (44.9)	30 (47.6)	.745	0
Bisphosphonates[b]	126 (20.6)	105 (19.1)	21 (33.3)	.013	0

Continuous variables are quoted as the mean ± SD or the median [interquartile range], depending on the data distribution, and categorical variables are quoted as the number (percentage).

[a]A given patient may have had more than one prior fractures.

[b]The number of patients with at least one period of exposure to the corresponding drug.

IVIg, intravenous immunoglobulins; RV: reference values.

related to outcomes, i.e. fractures) and confounding covariates (i.e. those related to the use of the drugs of interest) [12]. Thus, 12 PSs were produced—one for each drug of interest (steroids, VKAs, insulin, loop diuretics, PPIs, opioids, SSRIs, antiepileptics, benzodiazepines, vitamin D, calcium and bisphosphonates). The PSs were balanced by excluding counterfactuals, when required. Next, the PS distributions were evaluated using a kernel density plot (Supplementary data, Fig. S1). Lastly, the balance of each PS was assessed by examining the standardized mean differences (Supplementary data, Figs S2–S13). Next, Cox proportional hazards models with IPTW and time-dependent covariates were built for each drug of interest. We always checked that the models' validity conditions (and the proportional hazards assumption in particular) were met. The main limitation of this approach is that only one drug class of interest can be included per model and so only one PS per drug class can be calculated; this prevented us from building a single Cox proportional hazards model for fitting all drug classes with a significant hazard ratio (HR). Hence, in a sensitivity analysis, we built a Cox proportional hazards model with drugs as time-dependent covariates fitted for the more relevant osteoporotic fracture risks ($P < .05$ in the bivariate comparison of facture and no-fracture groups) and the drugs of interest with a significant HR ($P < .05$) in simple Cox proportional hazards models. In order to limit the number of variable included in this model and to take account of the renal osteodystrophy, patients with PTH < 150 pg/mL (positive predictive value for ABD: 97%) and BAP < 10 ng/mL (which can further bolster the diagnosis of ABD, as it is 100% sensitive and 93.7% specific) were classified as having ABD [13].

Linear mixed models (LMMs) were built to assess the influence of drugs [with a significant HR ($P < .05$) in simple Cox proportional hazards models] on changes over time in T-scores. A multivariable LMM was built for each of the three T-score measurement sites (lumbar spine, total hip and wrist). For each model, within-individual variance and between-individual variance were estimated using an unstructured matrix with random intercepts and slopes. To check on the relevance of using these random effects, we calculating the restricted maximum likelihood for models with two, one or none of the effects. The most relevant osteoporosis risk factors (age, sex, BMI, smoking, alcohol consumption, thyroid disorders, previous fracture, rheumatoid arthritis, other inflammatory autoimmune diseases, diabetes mellitus, sedentary lifestyle and the type of renal osteodystrophy) and drugs with a significant HR ($P < .05$) in simple Cox proportional hazards models were considered as fixed effects in the models. Furthermore, interaction terms (time × each drug of interest) were created to assess whether changes over time in T-scores differed significantly as a function of drug exposure (defined as the duration of exposure to a given drugs between two documented DXA measurements). Thus, patients were included in these analyses if they had a DXA measurement 1 month after kidney transplantation and another during the follow-up period. We checked that the conditions for a valid LMMs were met, namely the normal distribution of

residuals, the absence of multicollinearity, the normal distribution of random variable variances, and the independence between random variables and residuals. The LMM results are expressed as the t-value, which is the ratio between the coefficient of the regression line and the standard error of the regression coefficient. This value is used to calculate the significance of the observed difference with respect to the degrees of freedom, i.e. the *P*-value (the probability associated with the observed difference that determines whether or not there is a significant difference).

Given their long bone half-life of bisphosphonates and their prolonged post-treatment effectiveness [14, 15], we considered that these drugs were effective for 1 year after discontinuation.

Age was included as a binary variable in the multivariable Cox model. The age cut-off (52.9) was determined using a receiver operating characteristic curve (sensitivity: 61.3%; specificity: 73.0%; area under the curve: 70.6%; Supplementary data, Fig. S14).

All analyses were performed using R software (version 4.0.5, R Foundation for Statistical Computing, Vienna, Austria).

Ethical aspects

In line with the French legislation on retrospective analyses of routine clinical practice, patients were not required to give their informed consent. On admission to hospital, however, patients could refuse the use of their medical data for research purposes. The study protocol was approved by an institutional committee with competency for studies not requiring approval by an investigational review board and was registered with the French National Data Protection Commission (Commission Nationale de l'Informatique et des Libertés, Paris, France; reference: PI2019_843_0055).

RESULTS

Study population

Six hundred and thirteen consecutive patients having received a kidney transplant between 1 January 2006 and 13 February 2019 met the inclusion criteria and so were included in the present study. Of these, 387 (63.1%) were men. The mean ± SD age of the study population at the time of transplantation was 49.9 ± 13.2 years. The most common indication for kidney transplantation was glomerulonephritis (29.0%). Twenty-five (4.1%) recipients had a preemptive transplantation and 588 (95.9%) were on dialysis before transplantation with a median time [IQR] on dialysis of 27.5 [16.6–46.7] months before transplantation. The mean ± SD serum creatinine level (which is only relevant for preemptive transplants) was 61.6 ± 15.4 mg/L for the 25 recipients concerned (Table 1).

The median [IQR] length of follow-up was 4.6 [2.7–10.0] years. Of the 613 recipients, 17.1% were exposed to VKAs, with 18.9% exposed to insulin, 45.7% to loop diuretics, 96.4% to PPIs, 12.2% to opioids, 7.0% to SSRIs, 11.4% to antiepileptics and 35.9% to benzodiazepines; 93.3% were treated with vitamin D, 45.2% were treated with calcium and 20.6% were treated with bisphosphonates. In line with our immunosuppressive regimens, all recipients were exposed to steroids. In univariate analyses (not considering the duration or moment of exposure), the use of loop diuretics (43.6% in the no-fracture group vs 63.5% in the fracture group, $P = .004$) and the use of bisphosphonates (19.1% in the no-fracture group vs 33.3% in the fracture group, $P = .013$) were significantly associated with prevalent fractures (Table 1).

Among users of drugs of interest, the median [IQR] duration of exposure was 51.2 [23.0–99.5] months for steroids, 27.2 [9.4–48.5] months for VKAs, 35.5 [15.5–68.8] months for insulin, 16.1 [4.0–42.4] months for loop diuretics, 35.6 [12.9–71.9] months for PPIs, 4.8 [1.5–20.0] months for opioids, 26.3 [12.4–48.0] months for SSRIs, 21.7 [7.8–48.0] months for antiepileptics, 12.3 [3.0–38.9] months for benzodiazepines, 44.1 [21.9–85.8] months for vitamin D, 19.0 [6.0–49.6] months for calcium and 30.3 [18.1–60.8] months for bisphosphonates.

Sixty-three (10.3%) patients had at least one incident fracture, seven (1.1%) patients had at least two incident fractures during the study, one patient had three different incident fractures and one patient had six incident fractures at various times. Overall, 34 (5.5%) patients had at least one vertebral fracture, and 38 (6.2%) had at least one non-vertebral fracture [including 7 (1.1%) hip fractures]. The median [IQR] time to the first fracture was 4.0 [1.8–5.1] years. The fracture incidence was 16.9 per 1000 person-years.

Of the 613 recipients, 79 (12.9%) experienced at least one fracture prior to transplantation, and 10 (1.6%) experienced two or more fractures prior to transplantation. Overall, 25 (4.1%) recipients had a previous vertebral facture, and 59 (9.6%) had a previous non-vertebral fracture [including 4 (0.6%) hip fractures].

The baseline characteristics significantly associated with incident fractures were older age ($P < .001$), female sex ($P = .045$), thyroid disorders ($P < .001$), previous fracture ($P = .033$; vertebral fractures in particular, $P < .001$), serum BAP [11.9 (7.9–18.9) µg/L in the fracture group vs 12.4 (8.5–19.85) µg/L in the no-fracture group, $P = .011$], serum osteocalcin [120 (38.8–321.0) ng/mL in the fracture group vs 151 (25.0–336.0) ng/mL in the no-fracture group, $P < .001$], serum PTH [84 (13.85–356.0) pg/mL in the fracture group vs 190.2 (30.0–475.0) pg/mL in the no-fracture group, $P < .001$] and serum creatinine (69.0 ± 24.4 mg/L in the fracture group vs 79.9 ± 27.4 mg/L in the no-fracture group, $P = .001$) (Table 1).

Forty-two of the 63 patients with an incident fracture had DXA data in the year before or after the fracture; these patients were more likely to have osteopenia at the lumbar spine (46.3%), total hip (72.2%) or any site (47.6%), and osteoporosis at the wrist (35.9%) (Fig. 1).

In a subgroup of recipients ($n = 433$) for whom DXA data at 1 month after transplantation was available, DXA data was available for 429 recipients (including 38 patients with a fracture) at the lumbar spine, 380 recipients (including 30 patients with a fracture) at the total hip and 413 recipients (including 37 patients with a fracture) at the wrist. One month after transplantation, the median (range) T-score at the lumbar spine was associated with incident fractures [−1.1 (−3.8; +2.4) in the fracture group vs −0.6 (−4.6; +2.9) in the no-fracture group, $P = .047$], as well as T-score at total hip [−1.3 (−2.7; +1.5) in the fracture group vs −1.0 (−3.9; +2.6) in the no-fracture group, $P = .010$] and T-score at wrist [−1.2 (−5.5; +2.2) in the fracture group vs −0.8 (−5.4; +3.2) in the no-fracture group, $P = .015$].

Associations between incident fractures and drug exposure

In simple Cox proportional hazards models with drug exposures as time-dependent covariates, exposures to VKAs {HR [95% confidence interval (CI)] 2.23 (1.17–4.25)}, loop diuretics [2.36 (1.41–3.95)] and opioids [3.24 (1.35–7.80)] were significantly associated with incident fractures, whereas the use of steroids [0.64 (0.25–1.62)] or bisphosphonates [1.62 (0.85–3.10)] was not (Fig. 2).

In Cox proportional hazards models adjusted for the PS (one PS per drug class and thus one adjusted Cox proportional

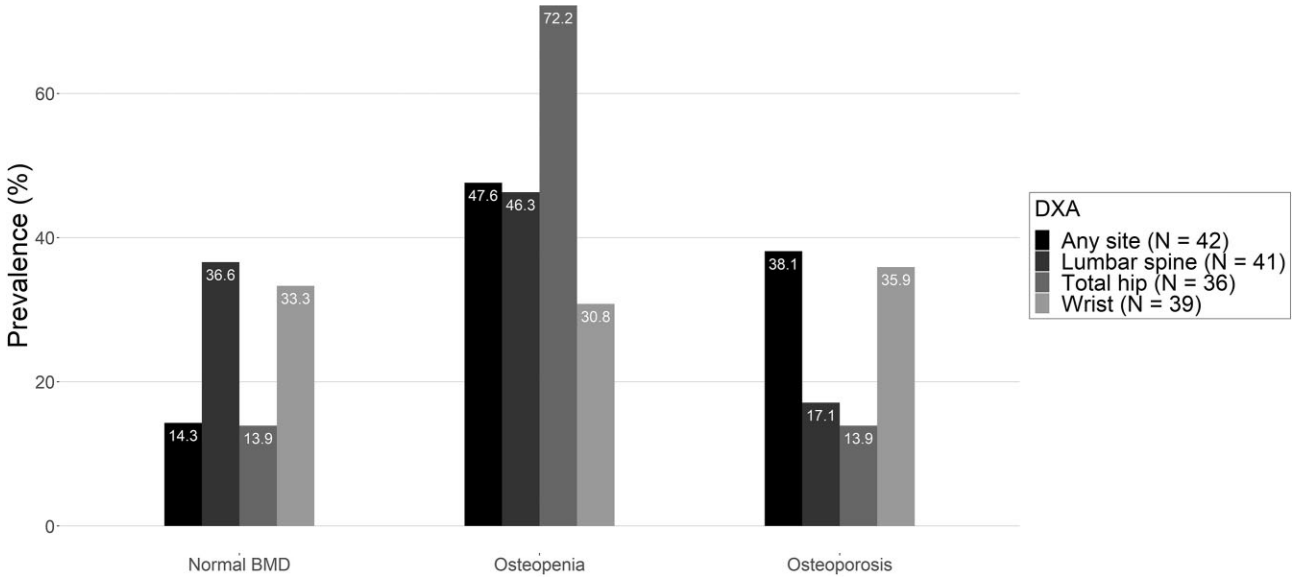


Figure 1: Prevalence of normal BMD, osteopenia and osteoporosis for the lumbar spine, total hip, wrist or at any site in patients with an incident fracture and data on a DXA measured in the 12 months before or after the fracture.

hazards model per drug class), exposures to loop diuretics [HR (95% CI) 2.11 (1.17–3.79)] and opioids [5.94 (2.14–16.52)] were still significantly associated with incident fractures. In contrast, exposure to VKAs showed a non-significant trend after this adjustment [1.94 (0.93–4.01)] (Fig. 2).

In multivariable Cox proportional hazards model including exposures to VKAs, loop diuretics and opioids as time-dependent covariates and baseline characteristics associated with incident fractures (with $P < .05$), exposures to VKAs [HR (95% CI) 1.84 (1.02–3.49)], loop diuretics [1.56 (1.04–2.57)] and opioids [3.05 (1.19–7.80)] were independently associated with incident fractures, as were age above 52.9 years [3.60 (1.94–6.59)] and thyroid disorders [2.69 (1.25–5.80)]. Moreover, a history of fracture tended to be associated with incident fractures [1.78 (0.95–3.34)] (Fig. 3).

Associations between changes over time in the T-scores and drug exposures

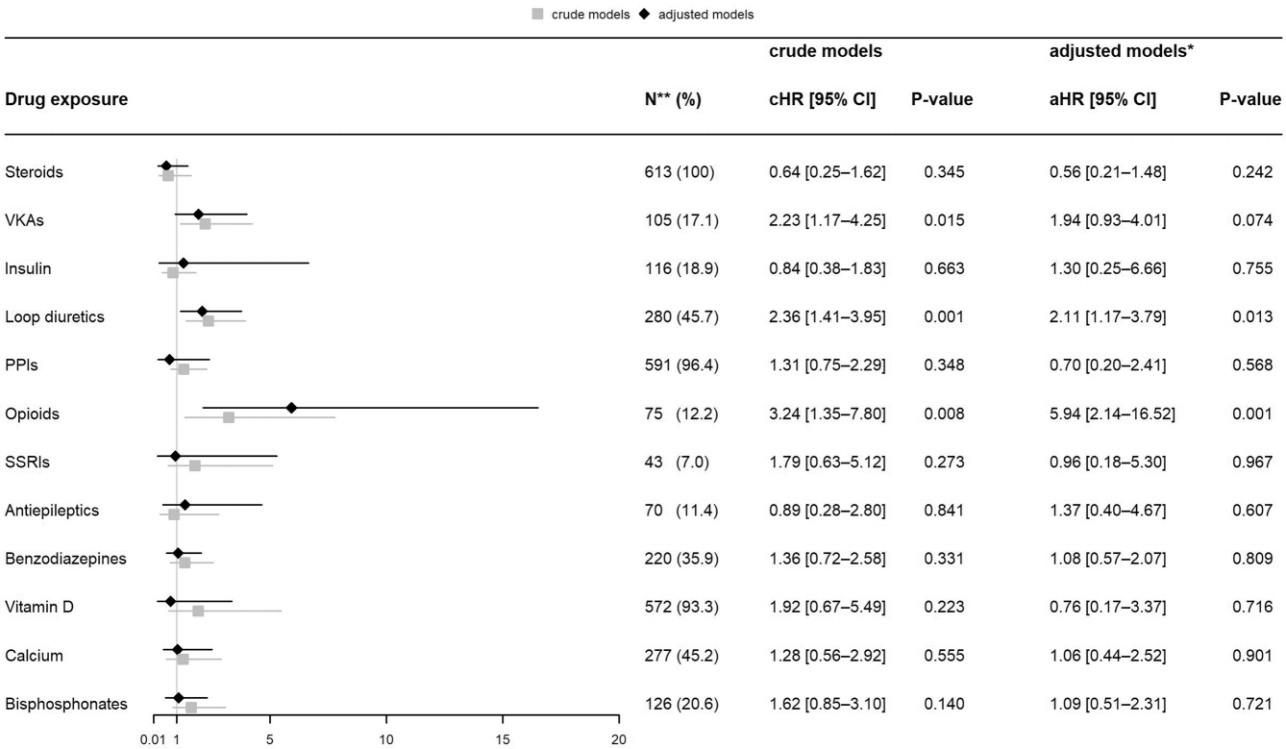
The numbers of patients with DXA data 1 month after transplantation and at least once thereafter were 411 (67.0%) for the spine, 359 (58.6%) for the total hip and 387 (63.1%) for the wrist.

In the multivariable LMMs including loop diuretic, VKA, opioid, steroid and bisphosphonate exposure times and baseline osteoporosis risk factors, exposure to loop diuretics was significantly and negatively correlated with changes over time in the T-score for the lumbar spine (t-value = −2.288, $P = .022$) and for the wrist (t-value = −2.195, $P = .028$, Table 2). Incorporation of the interaction term (time × loop diuretics) as a fixed effect showed that in the absence of loop diuretic exposure, the T-scores increased significantly more for the lumbar spine (t-value = 2.056, $P = .040$) and for the wrist (t-value = 1.992, $P = .047$). Opioid or VKA exposures did not influence changes over time in the T-score. The baseline osteoporosis risk factors that were significantly and negatively correlated with changes over time in T-scores were age (t-value = −2.688, $P = .007$) for the lumbar spine, male sex (t-value = −3.884, $P < .001$) and ABD (t-value = −2.007, $P = .045$) for the wrist, thyroid disorders (t-value = −2.912, $P = .004$) for the total hip and BMI ($P < .001$) for all three sites.

DISCUSSION

Knowledge of the modifiable factors associated with the fracture risk after kidney transplantation is essential for the choice of an appropriate treatment. After kidney transplantation, the recipients have a high drug therapy burden [16]. Some of these drugs have significant effects on bone loss and the fracture risk. The main finding of the present study of a longitudinal cohort of kidney transplant recipients was that exposures to loop diuretics, VKAs and opioids were associated with incident fragility fractures. To the best of our knowledge, the present study is the first to have addressed the risk of fragility fractures induced by drugs other than steroids after kidney transplantation. Furthermore, exposure to loop diuretic was significantly and negatively correlated with changes over time in the T-score.

Our various analyses suggested that kidney transplant recipients treated with loop diuretics have a greater fracture risk and a lower BMD. In the general population, loop diuretics are known to harm bone and induce fractures [17, 18]. The greater risk of fracture in patients treated with loop diuretics was highlighted in Rejnmark *et al.*'s population-based pharmaco-epidemiologic case–control study of 258 810 patients [odds ratio (95% CI) 1.16 (1.10–1.23)] [19] and, more recently, in Bokrantz *et al.*'s longitudinal study of a cohort of 59 246 patients [HR (95% CI) 1.23 (1.11–1.35)] [20]. One possible explanation for this elevated risk is that loop diuretics increase renal calcium excretion and thus affect calcium homeostasis and bone metabolism. Rejnmark *et al.* studied the effects of loop diuretics and thiazide diuretics on calcium homeostasis, calcitropic hormones and bone markers in postmenopausal women with lumbar spine osteopenia [21]. Compared with the thiazide diuretic group, renal calcium excretion and the serum PTH, $1,25(OH)_2$ vitamin D and osteocalcin levels were significantly greater and the serum BAP level was significantly lower in the loop diuretic group. Other possible (indirect) explanations for the elevated fracture risk with loop diuretics



Drug exposure	N** (%)	crude models cHR [95% CI]	P-value	adjusted models* aHR [95% CI]	P-value
Steroids	613 (100)	0.64 [0.25–1.62]	0.345	0.56 [0.21–1.48]	0.242
VKAs	105 (17.1)	2.23 [1.17–4.25]	0.015	1.94 [0.93–4.01]	0.074
Insulin	116 (18.9)	0.84 [0.38–1.83]	0.663	1.30 [0.25–6.66]	0.755
Loop diuretics	280 (45.7)	2.36 [1.41–3.95]	0.001	2.11 [1.17–3.79]	0.013
PPIs	591 (96.4)	1.31 [0.75–2.29]	0.348	0.70 [0.20–2.41]	0.568
Opioids	75 (12.2)	3.24 [1.35–7.80]	0.008	5.94 [2.14–16.52]	0.001
SSRIs	43 (7.0)	1.79 [0.63–5.12]	0.273	0.96 [0.18–5.30]	0.967
Antiepileptics	70 (11.4)	0.89 [0.28–2.80]	0.841	1.37 [0.40–4.67]	0.607
Benzodiazepines	220 (35.9)	1.36 [0.72–2.58]	0.331	1.08 [0.57–2.07]	0.809
Vitamin D	572 (93.3)	1.92 [0.67–5.49]	0.223	0.76 [0.17–3.37]	0.716
Calcium	277 (45.2)	1.28 [0.56–2.92]	0.555	1.06 [0.44–2.52]	0.901
Bisphosphonates	126 (20.6)	1.62 [0.85–3.10]	0.140	1.09 [0.51–2.31]	0.721

Figure 2: Crude and adjusted analyses of incident fractures as a function of drug exposure (in Cox proportional hazards models with time-dependent covariates). A Cox proportional hazards model was built for each drug class used as time-dependent covariate and then adjusted against a specific PS for each drug class. *Baseline characteristics associated with incident fractures (with $P < .2$; namely age, sex, BMI, ethnic group, thyroid disorders, prior fracture, serum phosphate, BAP and PTH) were included in all PSs. Furthermore, other baseline characteristics associated ($P < .2$) with the use of each drug class were included in the respective PSs, i.e. diabetes mellitus, basiliximab, previous transplantation and peak PRAs for the steroid PS; arterial hypertension and history of cardiovascular events for the VKA PS; diabetes mellitus, dyslipidemia, sedentary lifestyle and history of cardiovascular disease for the insulin PS; time on hemodialysis before transplantation, serum calcium, diabetes mellitus and etiology of CKD for the loop diuretic PS; alcohol consumption, other autoimmune disease and basiliximab for the PPI PS; alcohol consumption and peak PRAs for the opioid PS; etiology of CKD for the SSRI PS; diabetes mellitus, history of cardiovascular disease, sedentary lifestyle and etiology of CKD for the antiepileptic PS; history of cardiovascular disease, crossmatching results and peak PRAs for the benzodiazepine PS; alcohol consumption, DSAs, peak PRAs and dyslipidemia for the vitamin D PS; serum calcium, 25(OH) vitamin D, rheumatoid arthritis, dyslipidemia, IVIg and etiology of CKD for the calcium PS; 25(OH) vitamin D, arterial hypertension, dyslipidemia, sedentary lifestyle and peak PRAs for the bisphosphonate PS. **Number of patients with at least one exposure to the drug of interest.

relate to peripheral edema, heart failure, the decrease in blood volume and the induction of arterial hypotension, and thus a greater risk of falls [22]. However, the latter explanation can be challenged; several studies have found that by decreasing renal calcium excretion [21], thiazide diuretics were significantly associated with a lower serum osteocalcin level [23], greater BMD [23] and a lower fracture risk (even though thiazide diuretics also induce arterial hypotension) [20]. However, other studies did not find a significantly greater BMD [24] or a significantly lower fracture risk [25] in patients taking thiazide diuretics. In kidney transplant recipients, further clinical studies are necessary to determine whether the use of thiazide diuretics is preferable to loop diuretics with respect to fracture risk.

We found that VKA exposure tended to be associated with incident fractures; this has already been observed in the general population, in a time-dependent manner [26, 27]. One possible explanation for this finding is that the use of VKAs results in under-carboxylation of osteocalcin, which cannot then incorporate calcium into the ground substance. In fact, carboxylated osteocalcin contains three residues of gammacarboxyglutamic acid formed by the vitamin K-dependent posttranslational modification of glutamic acid residues [28]. We did not observe an association between VKA exposure and low BMD; this is generally in line with the results of prospective studies of older women [29] and older men [30] not having undergone kidney transplantation. Only one case–control study of 70 patients evidenced low BMD in non-transplanted patients on long-term warfarin therapy [31]. In the general population, meta-analyses have shown that the fracture risk in lower in patients taking direct oral anticoagulants than in patients taking VKAs [32, 33]. Here again, in kidney transplant recipients, further clinical studies are necessary to compare the risk of fracture in VKA users and in direct oral anticoagulant users.

Lastly, we found that opioid exposure was associated with incident fractures in our cohort of kidney transplant recipients, which is in line with the literature data on the general population [34–36]. One possible explanation is that the opioids' effects on the central nervous system (including sedation and dizziness) increase the risk of falls [34]. Moreover, opioids inhibit (through mu-opioid receptors) the secretion of gonadotropin-releasing hormone and reduce the release of follicle-stimulating hormone and luteinizing hormone, leading to secondary hypogonadism and low testosterone levels. In addition to this central mechanism of action, opioids stimulate 5-alpha-reductase—the enzyme that breaks down testosterone into dihydrotestosterone—and thus further reduce the bioavailability of testosterone in the serum [37]. Hence, several studies have evidenced low BMD in opioids users; however, these were

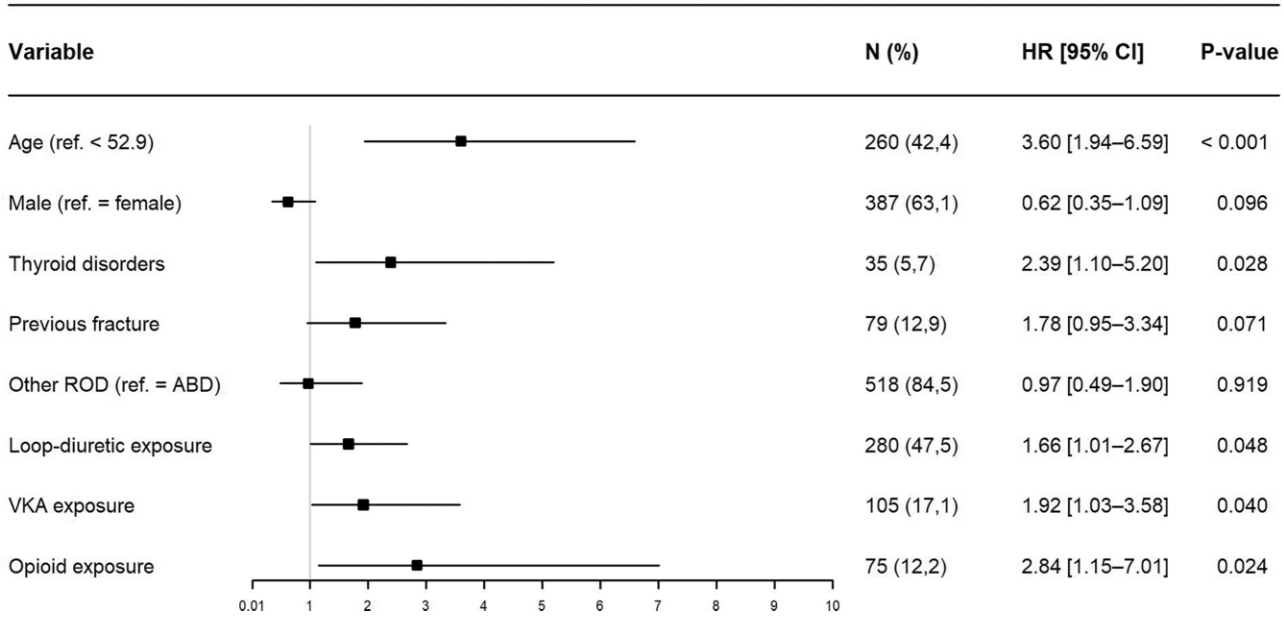


Figure 3: Multivariable Cox proportional hazards model with time-dependent covariates including the baseline variables associated with incident fractures ($P < .05$). To avoid overfitting the model, phosphate-calcium parameters and bone remodeling markers were replaced by the "renal osteodystrophy" variable (ABD or other). Serum creatinine was excluded from the analysis because of collinearity with renal osteodystrophy. ROD, renal osteodystrophy.

all cross-sectional studies that included patients with opioid dependence [37]. The present study did not evidence an association between opioid exposure and changes over time in BMD. Taken as a whole, these data suggest that the most likely facture mechanism in our study population was an elevated risk of falls.

A very recent retrospective, population-based study in Korea focused on risk factors for incident fractures in hemodialysis patients, peritoneal dialysis patients and kidney transplant recipients. The researchers also assessed the association between fractures and the use of certain medications (steroids, vitamin D and its analogs, phosphate binders, anti-osteoporotic medications, anti-depressants, opioids, and gabapentinoids) but (in contrast to our study) only at baseline (defined by at least 30 days of prescriptions filled in the year preceding the index date) and not over time. For the study population as a whole (and not specifically the subset of kidney transplant recipients) Kim *et al.* observed (i) a significant positive association between the incidence of incident fractures and the use of steroids, anti-osteoporotic medications, anti-depressants, opioids and gabapentinoids at baseline, and (ii) a significant negative (protective) association between the incidence of incident fractures and the use of vitamin D and phosphate binders at baseline [38]. Furthermore, the researchers did not exhaustively collect data on fracture risk factors, such as a previous fracture; indeed, large cohort studies [7, 39] and (to some extent) our present results show that a previous fracture is a risk factor for incident fractures in kidney transplant recipients.

Treatment with immunosuppressive drugs (and particularly steroids) is reportedly a major cause of bone loss in the months following kidney transplantation. We have already highlighted the positive effect on bone (in terms of BMD gain) of early steroid withdrawal 1 year after kidney transplantation [8]. Nikkel *et al.* reported that the long-term fracture risk was lower in patients with early steroid withdrawal than in patients on corticosteroid-based immunosuppression [7]. In contrast, Evenepoel *et al.* did not evidence an association between the cumulative steroid dose and incident fractures [41]. In the present study, steroid exposure was not associated with incident fractures; this was probably due to a lack of statistical power because very few study participants were not on long-term steroid therapy.

With regard to anti-osteoporotic drugs, we observed a positive effect of bisphosphonates on changes over time in the T-score for the wrist but did not evidence protective effects of calcium, vitamin D or bisphosphonates on incident fractures. Our results are in line with the literature data. With regard to bisphosphonates, most of the relevant studies (including meta-analyses) have found that the use of bisphosphonates by kidney transplant recipients is associated with a BMD gain [42–44] but not with a lower risk of incident fractures [42–44]. Concerning vitamin D, a recent study did not find an increase in BMD after 2 years of vitamin D supplementation [45]; this is also consistent with the literature data [46]. On the same lines, there is no evidence to suggest that vitamin D supplementation reduces the fracture risk [46]. Moreover, a recent publication by the American College of Nephrology emphasized the lack of studies of the effectiveness of vitamin D supplementation on fracture prevention [46]. In the present study, however, a lack of statistical power probably prevented us from seeing an effect of vitamin D supplementation on incident fractures; very few study participants were not exposed to this drug class.

Of the 63 patients with an incident fracture in the present study, the 42 patients who had performed a DXA in the year before or after the fracture were more likely to have osteopenia than osteoporosis. This observation might be explained by renal osteodystrophy (a component of CKD-MBD and therefore present before transplantation), which very often affects recipients and progresses after transplantation [47]. Since DXA assesses bone mass only, it cannot detect the bone

Table 2. Effects of loop diuretic, VKA, opioid, steroid and bisphosphonate exposure times and baseline osteoporosis risk factors on T-scores, as found in a multivariable linear mixed model.

	Lumbar spine, N = 411			Total hip, N = 359			Wrist, N = 387		
Fixed effects	Estimate ± SEE	t-value	P-value	Estimate ± SEE	t-value	P-value	Estimate ± SEE	t-value	P-value
Time	0.02859 ± 0.02 559	1.117	.264	−0.0319 ± 0.01686	−1.892	.058	−0.14886 ± 0.0289	−5.150	<.001
Age	−0.03446 ± 0.14222	−0.242	.809	−0.27406 ± 0.10196	−2.688	.007	−0.17976 ± 0.166	−1.083	.279
Male (ref. = female)	−0.18405 ± 0.14595	−1.261	.207	0.08893 ± 0.10 539	0.844	.399	−0.66461 ± 0.17113	−3.884	<.001
BMI	0.73113 ± 0.13 789	5.302	<.001	0.80224 ± 0.09 974	8.043	<.001	0.51639 ± 0.16 073	3.213	.001
Smoking	−0.10471 ± 0.1362	−0.769	.442	−0.03304 ± 0.09779	−0.338	.735	0.22357 ± 0.1598	1.399	.162
Alcohol consumption	−0.47046 ± 0.25939	−1.814	.070	−0.27767 ± 0.17915	−1.550	.121	−0.32826 ± 0.29266	−1.122	.262
Thyroid disorders	−0.40427 ± 0.28787	−1.404	.160	−0.58548 ± 0.20108	−2.912	.004	−0.17573 ± 0.3417	−0.514	.607
Previous fracture	−0.31167 ± 0.20789	−1.499	.134	−0.53382 ± 0.15567	−3.429	.001	−0.44573 ± 0.24504	−1.819	.069
Rheumatoid arthritis	−0.53973 ± 0.62402	−0.865	.387	−0.77585 ± 0.42403	−1.830	.067	−0.32842 ± 0.80357	−0.409	.683
Other autoimmune diseases	−0.12346 ± 0.28397	−0.435	.664	0.03044 ± 0.20 476	0.149	.882	−0.05687 ± 0.34095	−0.167	.868
Diabetes mellitus	0.38109 ± 0.18 453	2.065	.039	−0.11482 ± 0.13535	−0.848	.396	0.28076 ± 0.2165	1.297	.195
Sedentary lifestyle	−0.08966 ± 0.16611	−0.540	.589	−0.22967 ± 0.12296	−1.868	.062	−0.32354 ± 0.19357	−1.671	.095
ABD (ref. = other ROD)	−0.25402 ± 0.19781	−1.284	.199	−0.27635 ± 0.14481	−1.908	.056	−0.46572 ± 0.2321	−2.007	.045
Loop diuretics	−0.00046 ± 0.0002	−2.288	.022	−0.0004 ± 0.00026	−1.546	.122	−0.00055 ± 0.00025	−2.195	.028
VKAs	0.00025 ± 0.00 042	0.603	.546	−0.00006 ± 0.00017	−0.356	.722	0.00017 ± 0.00 033	0.515	.607
Opioids	0.00082 ± 0.00 079	1.034	.301	0.00007 ± 0.00 033	0.201	.840	−0.00003 ± 0.00058	−0.044	.965
Bisphosphonates	0.00035 ± 0.00 026	1.331	.183	0.00016 ± 0.00 021	0.776	.438	−0.00055 ± 0.00032	−1.709	.087
Steroids	−0.00028 ± 0.00011	−2.604	.009	−0.00011 ± 0.00006	−1.819	.069	−0.00026 ± 0.00011	−2.388	.017
Time × loop diuretics	0.00009 ± 0.00 004	2.056	.040	0.00005 ± 0.00 007	0.728	.467	0.00009 ± 0.00 005	1.992	.047
Time × VKAs	0.00005 ± 0.00 012	0.369	.712	−0.00005 ± 0.00004	−1.323	.186	−0.00007 ± 0.00007	−1.101	.271
Time × opioids	−0.00004 ± 0.00021	−0.204	.838	0.00006 ± 0.00 007	0.893	.372	0.00003 ± 0.00 013	0.233	.815
Time × bisphosphonates	0.00004 ± 0.00 005	0.814	.415	0.00004 ± 0.00 005	0.849	.396	0.00016 ± 0.00 007	2.414	.016
Time × steroids	−0.00002 ± 0.00003	−0.565	.572	0.00004 ± 0.00 002	1.562	.118	0.00011 ± 0.00 004	2.940	.003
Random effects	Variance ± SEE			Variance ± SEE			Variance ± SEE		
Subject	1.700 ± 1.304			0.773 ± 0.879			2.182 ± 1.477		
Slope	0.010 ± 0.101			0.004 ± 0.061			0.015 ± 0.125		

ROD, renal osteodystrophy; SEE, standard error of estimate.

microarchitecture disorder that results from renal osteodystrophy. Hence, along with bone demineralization, renal osteodystrophy is a risk factor for fracture [47]. Our present univariate analyses showed that baseline serum PTH and osteocalcin levels (which may reflect ABD) were lower in the fracture group than in the no-fracture group. However, we did not analyze serum PTH and osteocalcin levels at time points close to the fracture. Furthermore, there are no data to suggest that ABD is more of a risk factor for fractures than renal osteodystrophy with a high level of bone turnover.

Our study had several strengths and several limitations. The use of Cox proportional hazards models with time-dependent covariates and LMMs enabled us to take periods of drug exposure in account; simply dichotomizing the participants as "exposed" or "not exposed" during the study period would have led to a loss of information and would not have reflected real-life clinical practice. However, a lack of statistical power prevented us from building a multivariable Cox proportional hazards models that included all known osteoporosis risk factors, thus, residual confounding cannot be ruled out. Another strength was our comprehensive collection of data in general and data on drug exposure periods in particular, since all the patients' prescriptions were available for analysis. Although we cannot be sure that all the study participants were fully compliant, poor compliance would be unlikely for loop diuretics and VKAs, notably. Moreover, we did not have data on the drugs' dose levels and regimen; this preventing us from analyzing cumulative doses. Another strength was the very low proportion of missing data, for which we nevertheless performed multiple imputations using a validated method. Despite our consideration of the vast majority of osteoporosis risk factors, we found that menopausal status and the presence or absence of a family history of osteoporosis were poorly documented in the participants' medical records and so were not included in our analyses. Modifiable osteoporosis risk factors (such as BMI, smoking, alcohol consumption and a sedentary lifestyle) were considered at baseline only; any subsequent changes over time were not considered. Furthermore, the lack of literature data dissuaded us from considering possible persistent drug effects (with the exception of bisphosphonates) after discontinuation; this lack of consideration might have led to falsely positive (protective) results for certain drug classes. For example, a fracture that occurred after drug discontinuation (but while the drug's toxic effects on bone were still present), was not recorded as in the exposure period of this drug. Although we did not observe these events, it is still possible that the toxic effect of certain drug classes was underestimated for this reason. Furthermore, we might not have comprehensively identified all incident fractures and particularly asymptomatic high or middle thoracic vertebral fractures. Indeed, some lumbar or low thoracic vertebral fractures were documented incidentally on the CT scans of the abdomen and pelvis performed every 2 years. On these scans, however, the vertebrae were only visible up to Th7 or Th8. It is much less likely that all non-vertebral fractures were not identified, since they would have been symptomatic fractures documented at all annual check-ups. Furthermore, it would have been more appropriate to use Z-scores than T-scores in the LMMs. However, this was not possible because Z-scores were poorly reported in the medical records, and we did not have direct access to DXA data. Moreover, renal osteodystrophy (defined from biological parameters) was only considered at baseline but not over time. Other study limitations included loss-to-follow-up bias and competition bias in the Cox models, since we right-censored deceased patients. Other limitations were related to the study's observational and retrospective design, which prevented us from forming comparable groups. In contrast, the study's single-center design ensured a homogeneous population in terms of management and assessment.

CONCLUSION

In a study of a cohort of kidney transplant recipients with long-term follow-up, we found that exposures to loop diuretics, VKAs and opioids were associated with incident fragility fractures. Further clinical studies are necessary to assess the fracture risk of other drug classes (e.g. thiazide diuretics and direct oral anticoagulants) that may be less harmful for bone.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests. The results presented in this paper have not been published previously in whole or part, except in abstract format.

AUTHORS' CONTRIBUTIONS

B.B. contributed to the conception/design of the work, acquisition, analysis and interpretation of data for the work, and drafting the manuscript. A.N. contributed to the acquisition of data for the work, and drafting the manuscript. A.S. contributed to the acquisition of data for the work and revised the work critically for important intellectual content. C.P. contributed to the acquisition of data for the work and revised the work critically for important intellectual content. K.M. revised the work critically for important intellectual content. F.B. revised the work critically for important intellectual content. S.M.L. revised the work critically for important intellectual content. Y.B. revised the work critically for important intellectual content. V.G.-C. contributed to interpretation of data for the work, revised it critically for important intellectual content. G.C. contributed to the conception/design of the work, revised the work critically for important intellectual content. S.L. contributed to the conception/design of the work, the interpretation of data for the work, and the drafting of the manuscript. All authors approved the version to be published, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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ETHICS APPROVAL AND PATIENT CONSENT STATEMENT

In line with the French legislation on retrospective analyses of routine clinical practice, patients were not required to give their informed consent. On admission to hospital, however, patients could refuse the use of their medical data for research purposes. This protocol was approved by an institutional committee (with competency for studies not requiring approval by an investigational review board) and was registered with the French National Data Protection Commission (Commission Nationale de l'Informatique et des Libertés, Paris, France; reference: PI2019_843_0055).

DATA AVAILABILITY STATEMENT

Data available on request. The data underlying this article will be shared on reasonable request to the corresponding author.

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