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ABSTRACT

Context. Nephrolithiasis (NL) and primary hyperparathyroidism (HPTH) are metabolic complications of Paget's Disease of Bone (PDB), but recent data regarding their prevalence in PDB patients are lacking.

Objectives. Study 1: To compare the prevalence of primary HPTH and NL in 708 PDB patients and in 1803 controls. Study 2: To evaluate the prevalence of NL-metabolic risk factors in 97 PDB patients with NL, 219 PDB patients without NL, 364 NL patients without PDB and 219 controls, all of them without HPTH

Design. Cross-sectional multicentric study

Setting. Italian referral Centers for metabolic bone disorders

Participants. PDB patients from the AIP (Associazione Italiana malati di osteodistrofia di Paget) registry. Participants to the Olivetti Heart and the Siena Osteoporosis Studies

Main Outcome Measures. HPTH; NL; NL-metabolic risk factors

Results. PDB patients showed higher prevalence of primary HPTH and NL compared to controls ($p<0.01$). The NL recurrence occurs more frequently in patients with polyostotic PDB. About half of PDB patients without NL showed one or more NL-related metabolic risk factors. The hyperoxaluria (HyperOx) prevalence was higher in PDB patients with NL compared to NL patients without PDB and in PDB patients without NL compared to controls ($p=0.01$). PDB patients with HyperOx showed a longer lapse of time from the last aminobisphosphonate treatment.

Conclusions. NL and HPTH are frequent metabolic complication of PDB. The NL occurrence should be evaluated in PDB patients, particularly in those with polyostotic disease and/or after aminobisphosphonate treatment, in order to apply an adequate prevention strategy.

Keywords: Paget's disease of bone. Nephrolithiasis. Hyperparathyroidism. Hyperoxaluria.

INTRODUCTION

Nephrolithiasis (NL) is a highly prevalent disease worldwide with rates ranging from 7%–13% in North America, 5%–9% in Europe, and 1%–5% in Asia (1). Despite several types of kidney stones have been described, the large majority of them are composed by calcium-oxalate and calcium-phosphate salts (altogether up to 85%), and by uric acid salts (5%–10%) (2). Other than primary hyperparathyroidism (HPTH) (3), other disorders of bone and mineral metabolism are considered as risk factors for NL (4), including Paget's disease of bone (PDB) (5). PDB is a chronic and focal metabolic bone disorder characterized by increased and disorganized bone turnover, affecting one or more skeletal sites, i.e. monostotic and polyostotic PDB respectively. The skeletal sites involved by PDB show pathognomonic radiological and scintigraphic changes (6). Epidemiological surveys indicate that PDB is the second most common metabolic bone disease after osteoporosis worldwide, although marked ethnic and geographical variations in its prevalence has been observed (7-10). PDB affects both genders with a slight predominance in males and is primarily observed in middle-aged or older adults (6, 11, 12). Aminobisphosphonates are considered the first-choice treatment for PDB (6, 11, 12). Of interest, both NL and primary HPTH were described as possible non-malignant, metabolic complications of PDB (5, 13, 14), although recent data regarding their prevalence and clinical relevance in PDB patients are not available in the international literature. Furthermore, to our best knowledge, no data are available regarding the prevalence of metabolic risk factors for NL in PDB patients. Starting from these considerations, we performed two cross-sectional studies (figure 1). The aim of the first study (Study 1) was to evaluate the prevalence of NL and primary HPTH in PDB patients included in the registry of the Italian Association of PDB patients (AIP, Associazione Italiana malati di osteodistrofia di Paget) (15) compared with non-pagetic subjects enrolled in two epidemiological cohort studies, the Olivetti Heart Study (OHS) (16) and the Siena Osteoporosis Study (SiOp) (17,18). The aim of the second study (Study 2) was to evaluate the prevalence of more common metabolic risk factors for NL [i.e. hypercalciuria (HyperCa), hypocitraturia (HypoCit), hyperoxaluria (HyperOx), hyperuricuria (HyperUr) and hypomagnesuria (HypoMg)] (2) in the follwing groups of patients: a) PDB patients with NL and without primary HPTH; b) PDB patients with neither NL nor primary HPTH; c) Controls with neither PDB, primary HPTH, nor NL; and d) Non-pagetic patients with NL and normal parathyroid function.

METHODS

NL- and primary HPTH- prevalence study (Study 1). Data regarding the self-reported history of NL were extracted from medical records of all consecutive Italian PDB patients referring from January 1st, 2012 to December 31st, 2015 at the Units of Bone and Mineral Disorders of Turin, Siena, and Naples, three main national centres for the diagnosis and treatment of PDB (15) which used an unique approach for clinical classification and management of PDB and its co-morbidities, including assessment of NL and primary HPTH history by a fixed sequence questionnaire and PDB treatment (18, 19). The PDB diagnosis was based on biochemical evaluation, bone scintigraphy, and subsequent X-ray examination of areas of increased isotope uptake in all subjects (6, 11, 12). Self-reported history of NL was also evaluated in 1803 subjects enrolled in the OHS and in SiOP using the same questionnaire (20, 21). The OHS and SiOP are two prospective epidemiological cohort-studies planned and carried out by the same University Departments involved in this study to evaluate the metabolic risk factors for cardiovascular and mineral disorders in healthy controls (18). For the present study, were identified as OHS-SiOp controls, the subjects without clinical or historical evidence of PDB enrolled in both studies (18). All subjects reporting bilateral NL and/or the excretion or surgical removal of two or more kidney stones were considered recurrent stone formers. Fasting venous blood samples were taken for the determination of total calcium (sCa), magnesium (sMg), phosphate (sPO4), intact parathyroid hormone (PTH), 25-hydroxyvitamin D (25OHD), total alkaline phosphatase (ALP), thyroid stimulating hormone, and creatinine levels in all study participants. According to Fillée and colleagues, PTH levels >7.6 pmol/l or > 5.8 pmol/l were considered indicative of primary HPTH in subjects with 25OHD < 50 nmol/l or > 50 nmol/l, respectively (22). The subjects with an estimated glomerular filtration rate (eGFR) ≤60 mL/min/1.73m^2 (23), and/ or six months or less treatment with bisphosphonates or calcitonin and/or incomplete data were excluded from the estimation of primary HPTH prevalence in study cohorts

Estimation of metabolic risk factors for NL (Study 2)- The metabolic risk factors for NL were estimated in the following groups: a) PDB with NL and without primary HPTH (PDB NL), b) PDB patients with neither NL nor primary HPTH (PDB not NL), c) controls from the OHS-SiOp cohorts, with neither NL, PDB, nor primary HPTH, which are age-, sex-, and BMI- matched to PDB not NL (CT), and d) non-pagetic stone formers over the age of 40 years without primary HPTH, enrolled in the same lapse of time by the same Centres (NL not PDB). To determine the metabolic risk factors for NL, on free diet, 24-hour urine samples were collected and analyzed for calcium (uCa), magnesium (uMg), phosphate (uPO_4), sodium (uNa), potassium (uK), chloride (uCl), citrate (uCit), creatinine (uCrea), oxalate (uOx) and urate (uUr) concentrations. Total urinary volume (V) was also

determined (24, 25). HypoCit was defined as uCit <1.7 mmol/24h ; hyperCa as uCa >7.5 mmol/24h in men, >6 mmol/24h in women or >0.1 mmol/Kg/24h; hyperOx as uOx >444 mmol/24h; hyperUr as a uUr >4.8 mmol/24h in men and >4.5 mmol/24h in women; and hypoMg as uMg <2 mmol/24h (26). The subjects with active PDB (ALP higher than the normal range), primary HPTH, hyper- or hypo-thyroidism (27), eGFR ≤60 mL/min/1.73m^2, chronic diarrhea, dietary or medical treatment for NL (24), six months or less treatment with bisphosphonate or calcitonin, incomplete data collection, and current treatment with calcitriol, ergocalciferol, diuretics, angiotensin-converting enzyme inhibitors, glucocorticoids or estrogens were not considered for the analyses of metabolic risk factors for NL.

Statistical analysis. All statistical analyses were performed using the IBM SPSS Statistics software, version 23 (International Business Machines Corporation, Armonk, New York). Data were expressed as means ± standard deviation for continuous variables and as absolute; percentage values for discrete variables. Analysis of variance (ANOVA) or chi-squared test were used to assess differences in main characteristics. Cox proportional hazard models were used to estimate odds ratios (ODs), with 95% confidence intervals (CIs). The multivariate model was adjusted for age, sex, BMI, and eGFR. A p value < 0.05 was considered as statistically significant. To evaluate the prevalence of NL-metabolic risk factors in PDB patients with neither NL nor primary HPTH and in controls with neither NL nor primary HPTH, we selected a representative sample of the study populations, based on a sample size calculation (28), using an electronically generated randomization schedule. PDB patients and controls were assigned a unique and sequential identification number based on the chronological order of entry into the study. Written informed consent has been obtained from each study participant after full explanation of the purpose and nature of all used procedures. The study protocol was approved by the Federico II University Ethical Committee.

RESULTS

Prevalence of primary HPTH in PDB patients and in OHS-SiOp controls (Study 1).

From January 1st, 2012 to December 31st, 2015, 708 PDB patients (PDB polyostotic: PDB monostotic 398:310) were examined in the three AIP Centres. Among them, 401 (PDB polyostotic: PDB monostotic 226: 175) showed 25OHD levels < 50 nmol/l and 307 (PDB polyostotic: PDB monostotic 172:135) showed 25OHD levels > 50 nmol/l. In the same lapse of time, among OHS-SiOp controls, 979 showed 25OHD levels < 50 nmol/l and 824 showed 25OHD levels > 50 nmol/l. The clinical characteristics of PDB patients and OHS-SiOp controls,

classified according to 25OHD levels, were reported in table 1. Overall, 106 PDB patients (15.0%) and 74 controls (4.1 %) showed PTH levels indicative of primary HPTH according to vitamin D status (22) (figure 1). At univariate analysis, the prevalence of primary HPTH observed in PDB patients was significantly higher compared to OHS-SiOp controls [OR 4.11 95% C.I.:3.01-5.61, p<0.01]. This association remained significant also in a model adjusted for age, sex, BMI, and eGFR [OR 4.24 95% C.I.:3.07-5.68, p<0.01]. Thirty-four PDB patients with primary HPTH (32.1%) and 24 OHS-SiOp controls with primary HPTH (32.4%) reported a personal history of NL. No significant differences in levels of sCa, sMg, sPO4, intact PTH and 25OHD were observed between HPTH-PDB patients with or without NL as well as between HPTH-OHS-SiOp controls with or without NL (d.n.s.). Analyzing the medical records of enrolled patients, 22 PDB patients and 18 controls had had the clinical diagnosis of primary HPTH before the enrolment for this study.

Prevalence of NL in PDB patients and in OHS-SiOp controls without primary HPTH (Study 1).

Among 602 PDB patients without primary HPTH (mean age 67.6 ± 11.7 years, M:F 324:278, PDB polyostotic: PDB monostotic 341: 261), 97 (mean age 63.9 ± 10.3 years, M:F 61:36, PDB polyostotic: PDB monostotic 50: 47) reported a personal history of NL. Among the 1729 OHS-SiOp controls without primary HPTH, 138 (mean age 63.1 ± 9.1 years, M:F 83:55) declared a personal history of NL. At univariate analysis, the prevalence of NL observed in PDB patients was significantly higher compared to controls (16.1% vs. 8.0%, OR 2.21 95% C.I.:1.67-2.92, p<0.01,). This association remained significant also in a model adjusted for age, sex, BMI, and eGFR [OR 2.26 95% C.I.:1.72-2.98, p<0.01]. Thirty-nine PDB patients (M: F 23:16) and 46 controls (M: F 27:19) reported a recurrent NL. The rate of NL recurrence was not significant different between pagetic and non pagetic subjects (40.2% vs. 33.3%; p=0.33). Interestingly, among PDB cases with recurrent NL, 27 (M:F 16:11) had polyostotic disease and 12 (M:F 7:5) had monostotic disease, consistent with a higher prevalence of polyostotic disease in the presence of recurrent NL (69.2% vs. 30.8%, p<0.01, OR 3.42, 95% C.I.: 1.45-8.09).

Metabolic risk factors for NL in patients and controls without primary HPTH (Study 2).

Clinical characteristics of PDB NL, PDB not NL (PDB polyostotic: PDB monostotic 120:99), NL not PDB, and CT were reported in table 2. The large majority of NL cases with or without PDB showed at least one metabolic risk factor for NL. The percentage of PDB not NL cases showing at least one metabolic risk factor for NL was significantly higher compared to CTs (p<0.05). The percentage prevalence of metabolic risk factors for NL in PDB NL, PDB not NL, CT, and NL not PDB was depicted in figure 2. The following between groups comparisons were assessed:

1) PDB NL *vs.* PDB not NL: In a multivariate model adjusted for age, sex, BMI and eGFR, PDB NL cases showed a higher prevalence of HyperCa (34/97 *vs.* 43/219, p<0.05; OR: 1.73, 95%C.I.: 1.20-2.58), HypoCit (38/97 *vs.* 48/219, p<0.05; OR: 1.81, 95%C.I.: 1.28-2.56), HyperOx (29/97 *vs.* 38/219, p<0.05; OR: 1.75, 95%C.I.: 1.16-2.68) and HyperUr (14/97 *vs.* 11/219; p<0.05; OR: 2.67, 95%C.I.: 1.15-5.69) compared to PDB not NL group.

2) PDB not NL *vs.* CT: In a multivariate model adjusted for age, sex, BMI and eGFR, an increased prevalence of HyperOx was observed in PDB not NL group compared to CT group (38/219 *vs.* 19/219; p<0.05; OR: 2.11, 95% C.I.: 1.29-3.46). No additional differences in the prevalence of the remaining metabolic risk factors for NL were observed between the two groups.

3) PDB NL vs. NL not PDB. Among PDB NL group, HypoCit was the most frequently observed metabolic risk factor for NL (38/97). In a multivariate model adjusted for age, sex, BMI and eGFR, the prevalence of HyperOx was significantly higher in PDB NL group compared to NL not PDB group (29/97 *vs.* 59/364; p<0.05; OR: 1.85, 95% C.I.: 1.19-2.58). No additional differences in the prevalence of the remaining metabolic risk factors for NL were observed between the two groups.

Clinical characteristics of PDB patients with HyperOx.

Considering that HyperOx was the only metabolic risk factor for NL significantly higher in PDB NL group vs. NL not PDB group and that a similar difference was observed between PDB not NL group and CT group, we evaluated the clinical characteristics of all PDB patients with (n=67, 21.2%, mean age 63.6 ± 10.8 years, M:F 34:33, polyostotic: monostotic 31:36) or without HyperOx. No differences in mean age, BMI, male to female ratio and extension of disease were observed between PDB patients with or without HyperOx (d.n.s). Interestingly, PDB patients with HyperOx showed a longer lapse of time, expressed in months, from the last aminobisphosphonate treatment (Zoledronic acid or Neridronic acid) compared to PDB patients without HyperOx (28.2 ± 9.3 vs. 16.1 ± 8.7 months for PDB patients with and without HyperOx, respectively, p<0.05). This associations remained significant after adjustment for age, sex, BMI, eGFR, and PDB extension (monostotic vs. poliostotic)

DISCUSSION

Previous studies suggested that PDB patients may have an increased risk of primary HPTH and NL (5,13,14,29). The latter was mainly related to an increase in serum and urinary calcium due to active, polyostotic PDB and/or to the occurrence of primary HPTH. Our data demonstrate that PDB patients are at increased risk of NL independently of disease activity or the presence of primary HPTH. Moreover, the disease extension significantly influences the recurrence rate of NL in PDB patients, since it occurs more frequently in the presence of polyostotic disease. As expected, PDB patients with primary HPTH are also at increased risk of NL, with similar prevalence rates of non-pagetic primary HPT patients, as reported in the literature (3, 30). Altogether these data confirm and extend the available information, further supporting the relationship between metabolic bone disorders and NL. Analyzing NL-related metabolic risk factors in patients and controls without primary HPTH, a higher prevalence of HyperOx was observed in PDB cases with NL compared to PDB patients without NL, controls and even non-pagetic NL cases. Of interest, PDB patients with HyperOx overall considered showed a longer lapse of time from the last aminobisphosphonate treatment compared to PDB patients without HyperOx. Before, during and after aminobisphosphonate treatment an adequate supplementation of vitamin D and calcium is generally recommended to avoid the occurrence of life-threatening electrolytes disorders, in particular hypocalcemia and hypophosphatemia (31-33). In this respect, dietary calcium intake is a key factor for oxalate absorption and excretion (34): a reduction in calcium intake reduces the calcium concentration in the intestinal lumen preventing the formation of insoluble and non-absorbable calcium-oxalate salts in this anatomical site. In this way, a higher quantity of soluble oxalate salts remains free in the intestinal lumen and can be easily absorbed and excreted by kidney, thus increasing uOx (35). Accordingly, a normal calcium diet (1 gr/die) is the only dietary intervention that may significantly reduce uOx (24, 35, 36). Likewise, a long-term adherence to a diet featuring normal calcium, low protein and low salt intakes may significantly decreases uCa, uOx and the calcium-oxalate relative supersaturation indexes in people with recurrent NL, thus reducing the numbers of stone recurrences (24, 36, 37). Unfortunately, the adherence to recommendation for guarantee an adequate dietary calcium intake decreases gradually and significantly over time, in particular when these interventions are prescribed as single treatment (37-40). In addition, experimental studies suggest a possible effect of aminobisphosphonate treatment on gut microbioma (41), which in turn can significantly influence oxalate intestinal absorption and excretion (42). We thus speculate that a reduced adherence to prescribed vitamin D and calcium supplementation after aminobisphosphonate treatment over time (43), particularly in the absence of a normocalcic and hyposodic diet,

alone or in association to possible effects of aminobisphosphonate treatment on gut microbioma, might explain the occurrence of HyperOx in PDB patients long-time after the aminobisphosphonate treatment. The study 1 results indicate that PDB patients have an increased prevalence of primary HPTH compared to controls. Despite this association has been described for several years, the mechanisms that underlies the development of primary HPTH in pagetic patients is still unknown. In PDB patients, the treatment with antiresorptive drugs may induce a secondary HPTH. (44). The possibility that tertiary HPTH could evolve from the secondary form should be considered, particularly when calcium and vitamin D requirements cannot be chronically met by dietary sources and/or by supplements (13). The knowledge about the molecular biology of both PDB and primary HPTH suggest another possible pathogenic link between the two disorders. In effect, both the *sequestosome 1* (*SQSTM1*) and the *multiple endocrine neoplasia type 1 (MEN1)* genes, which cause a significant percentage of PDB and primary HPTH cases respectively (45,46) share NF-κB as a common interacting transcription factor (45, 47), even if through different ways. Furthermore, mutations of these genes may influence the NF-κB functional properties and, consequently, the transcription of several other genes relevant to the biological activities of bone and parathyroid cells (13). In this scenario, the role of different mutation of *SQSTM1* gene showing distinct effects on protein structure and function may be an intriguing working hypothesis (48).

Study strengths and limits: The major strength of our study is represented by the large and clinically well characterized study populations examined. In addition, we have performed the analysis of NL-related metabolic risk factors in PDB patients during aminobisphosphonate-induced disease remission (i.e. total ALP serum levels within the normal range). This approach allows to consider PDB *per se* as a significant risk factor for NL independently from metabolic activity. Our study had also some limits. The major limitation relates to the cross-sectional nature, which prevents from establishing cause-effect relationships. A carry over effect of PDB diagnosis on studies results should be also considered. In study designs different from crossover trials, which can be routinely checked for this bias, common sense and alertness for unusual patterns in the data are the only defenses against it (50). Data regarding self-reported NL diagnosis was obtained from a fixed sequence questionnaire used from several years by study authors also in studies not involving PDB patients. On the other hand, the low number of PDB patients with diagnosis of primary HPTH before the enrolment and the lack of recent data regarding parathyroid function in PDB patients indicates that parathyroid function is not systematically evaluated in PDB patients, in particular when their clinical management is performed by physicians lacking a training in bone and mineral metabolism (13). This probably follows the early findings of normal circulating concentrations of PTH and calcitonin in PDB patients (50,51).

A critical point is also the diagnostic criteria used to define the primary HPTH in study cohorts, considering that they have not been validated in PBD patients. The Fillée criteria (22) also does not completely exclude the probability of a secondary HPTH due to antiresorptive treatment. To minimize this phenomenon however, PTH levels were determined at least six months after the last treatment and in PDB patients with disease remission. In addition, the prevalence of primary HPTH in our control study cohorts is higher than those reported in the literature between the end of the last Century and the beginning of the present one (52). However, our data are in agreement to those available in the more recent literature showing a significant increase in the incidence of primary HPTH in the United States and in Europe in the last two decades, because of an increase in the use of screening methods. (53).

Although further *ad hoc* studies are needed to better clarify these data, the study results may contribute to significantly improve the clinical management of PDB patients, indicating the necessity to investigate the risk of NL and its metabolic risk factors in this setting. This approach is particularly important in PDB patients with polyostotic disease which are at increased risk of NL recurrence, also considering the NL related morbidity (54).

Data Availability

All data generated or analyzed during this study are included in this published article or in the data repositories listed in References. The dataset generated and analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Figure legends

Figure 1. Studies flow-chart.

The study 1 was performed to evaluate the prevalence of primary hyperparathyroidism (HPTH) and nephrolithiasis (NL) in patients with Paget's disease of bone (PDB) and in subjects enrolled in the Olivetti Heart Study (OHS) and in the Siena Osteoporosis Study (SiOp) without Paget's disease of bone (OHS-SiOp controls). Primary HPTH was defined according to criteria proposed by Fillée et al. (22).

The study 2 was performed to evaluate the prevalence of metabolic risk factors for NL in PDB patients with NL and without primary HPTH (PDB NL), in PDB patients with neither NL nor primary HPTH (PDB not NL), in OHS-SiOp controls with neither NL, PDB, nor primary HPTH (CT), and in patients with NL over the age of 40 with neither PDB nor primary HPTH (NL not PDB). PDB not NL patients were a representative sample of PDB patients with neither NL nor primary HPTH examined in the first study. CTs were selected from the OHS-SiOp cohorts and were age-, sex- and body mass index- matched to PDB not NL. All examined subjects were enrolled by the Bone and Mineral Disorders Centres of Turin, Siena, and Naples, Italy, from January 1st, 2012 to December 31st, 2015.

Figure 2. Prevalence of metabolic risk factors for NL in patients and controls without primary hyperparathyroidism enrolled for the study 2.

PDB: Paget's disease of bone. NL: nephrolithiasis. PDB NL: PDB patients with NL. PDB not NL: PDB patients without NL. NL not PDB: patients with NL over the age of 40 without PDB. CT: controls with neither NL or PDB patients. CTs were selected from the Olivetti Hearth and Siena osteoporosis studies and were age-, sex- and body mass index- matched to PDB not NL. HypoCit = hypocitraturia (urinary citrate excretion <1.7 mmol/24h) (26); HyperCa = hypercalciuria (urinary total calcium excretion >7.5 mmol/24h in men, >6 mmol/24h in women or >0.1 mmol/kg/24h) (26); HyperOx = hyperoxaluria (urinary oxalate excretion >444 mmol/24h) (26); HyperUr = hyperuricuria (urinary urate excretion >4.8 mmol/24h in men and >4.5 mmol/24h in women) (26); HypoMg = hypomagnesiuria (urinary magnesium excretion <2 mmol/24h) (26).

References

1. Ziemba JB, Matlaga BR. Epidemiology and economics of nephrolithiasis. Investig Clin Urol. 2017;58(5):299-306.
2. Coe FL, Evan A, Worcester E. Kidney stone disease. J Clin Invest. 2005(10);115:2598–2608.
3. Bilezikian JP, Bandeira L, Khan A, Cusano NE. Hyperparathyroidism. Lancet. 2018;391(10116):168-178.
4. Lucato P, Trevisan C, Stubbs B, Zanforlini BM, Solmi M, Luchini C, Girotti G, Pizzato S, Manzato E, Sergi G, Giannini S, Fusaro M, Veronese N. Nephrolithiasis, bone mineral density, osteoporosis, and fractures: a systematic review and comparative meta-analysis. Osteoporos Int. 2016;27 (11):3155-3164.
5. Siris ES. Epidemiological aspects of Paget's disease: family history and relationship to other medical conditions. Semin Arthritis Rheum. 1994;23(4):222-225
6. Gennari L, Rendina D, Falchetti A, Merlotti D. Paget's Disease of Bone. Calcif Tissue Int. 2019;104(5):483-500.
7. Seitz S, Priemel M, von Domarus C, Beil FT, Barvencik F, Amling M, Rueger JM. The second most common bone disease: a review on Paget's disease of bone. Eur J Trauma Emerg Surg. 2008;34(6):549–553
8. Corral-Gudino L, Borao-Cengotita-Bengoa M, Del Pino-Montes J, Ralston S. Epidemiology of Paget's disease of bone: a systematic review and meta-analysis of secular changes. Bone. 2013;55(2):347–352
9. van Staa TP, Selby P, Leufkens HG, Lyles K, Sprafka JM, Cooper C. Incidence and natural history of Paget's disease of bone in England and Wales. J Bone Miner Res. 2002;17 (3):465–471
10. Rendina D, Gennari L, De Filippo G, Merlotti D, de Campora E, Fazioli F, Scarano G, Nuti R, Strazzullo P, Mossetti G. Evidence for increased clinical severity of familial and sporadic Paget's disease of bone in Campania, southern Italy. J Bone Miner Res. 2006;21(12):1828–1835
11. Ralston SH, Corral-Gudino L, Cooper C, Francis RM, Fraser WD, Gennari L, Guañabens N, Javaid MK, Layfield R, O'Neill TW, Russell RGG, Stone MD, Simpson K, Wilkinson D, Wills R, Zillikens MC, Tuck SP. Diagnosis and Management of Paget's Disease of Bone in Adults: A Clinical Guideline. J Bone Miner Res. 2019;34 (4):579-604.
12. Ralston SH. Clinical practice. Paget's disease of bone. N Engl J Med. 2013 (7);368:644–650
13. Brandi ML, Falchetti A. What is the relationship between Paget's disease of bone and hyperparathyroidism? J Bone Miner Res. 2006;21(Suppl 2):P69-P74

14. Gutteridge DH, Gruber HE, Kermode DG, Worth GK. Thirty cases of concurrent Paget's disease and primary hyperparathyroidism: sex distribution, histomorphometry, and prediction of the skeletal response to parathyroidectomy. Calcif Tissue Int. 1999;65(6):427-435
15. http://www.pagetitalia.com Accessed on 11/04/2020
16. http://www.olivettiheartstudy.org Accessed on 11/04/2020
17. Rendina D, Gianfrancesco F, De Filippo G, Merlotti D, Esposito T, Mingione A, Nuti R, Strazzullo P, Mossetti G, Gennari L. FSHR gene polymorphisms influence bone mineral density and bone turnover in postmenopausal women. Eur J Endocrinol. 2010;163(1):165-72.
18. Rendina D, De Filippo G, Merlotti D, Di Stefano M, Succoio M, Muggianu SM, Bianciardi S, D'Elia L, Coppo E, Faraonio R, Nuti R, Strazzullo P, Gennari L. Vitamin D status in Paget disease of bone and efficacy-safety profile of cholecalciferol treatment in pagetic patients with hypovitaminosis D. Calcif Tissue Int. 2019;105(4):412-422
19. Gennari L, Gianfrancesco F, Di Stefano M, Rendina D, Merlotti D, Esposito T, Gallone S, Fusco P, Rainero I, Fenoglio P, Mancini M, Martini G, Bergui S, De Filippo G, Isaia G, Strazzullo P, Nuti R, Mossetti G. SQSTM1 gene analysis and gene environment interaction in Paget's disease of bone. J Bone Miner Res. 2010;25 (6):1375–1384
20. Rendina D, Mossetti G, De Filippo G, Benvenuto D, Vivona CL, Imbroinise A, Zampa G, Ricchio S, Strazzullo P. Association between metabolic syndrome and nephrolithiasis in an inpatient population in southern Italy: role of gender, hypertension and abdominal obesity. Nephrol Dial Transplant. 2009;24 (3):900-906.
21. Strazzullo P, Barba G, Vuotto P, Farinaro E, Siani A, Nunziata V, Galletti F, Mancini M, Cappuccio FP. Past history of nephrolithiasis and incidence of hypertension in men: a reappraisal based on the results of the Olivetti Prospective Heart Study. Nephrol Dial Transplant. 2001;16(11): 2232–2235
22. Fillée C, Keller T, Mourad M, Brinkmann T, Ketelslegers JM. Impact of vitamin D-related serum PTH reference values on the diagnosis of mild primary hyperparathyroidism, using bivariate calcium/PTH reference regions. Clin Endocrinol. 2012;76 (6):785–789
23. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-612.

24. Gambaro G, Croppi E, Coe F, Lingeman J, Moe O, Worcester E, Buchholz N, Bushinsky D, Curhan GC, Ferraro PM, Fuster D, Goldfarb DS, Heilberg IP, Hess B, Lieske J, Marangella M, Milliner D, Preminger GM, Reis Santos JM, Sakhaee K, Sarica K, Siener R, Strazzullo P, Williams JC; Consensus Conference Group.. Metabolic diagnosis and medical prevention of calcium nephrolithiasis and its systemic manifestations: a consensus statement. J Nephrol. 2016;29 (6):715–734
25. https://uroweb.org/wp-content/uploads/Urolithiasis-2010.pdf Accessed on 11/04/2020
26. Mossetti G, Vuotto P, Rendina D, Numis FG, Viceconti R, Giordano F, Cioffi M, Scopacasa F, Nunziata V. Association between vitamin D receptor gene polymorphisms and tubular citrate handling in calcium nephrolithiasis. J Intern Med. 2003;253 (2):194-200
27. Cooper DS, Biondi B. Subclinical thyroid disease. Lancet. 2012;379 (9821):1142–1154
28. http://www.epicentro.iss.it/stumenti/SampleSize. asp Accessed on 11/04/2020
29. Kanis JA. Pathophysiology and treatment of Paget's disease of bone. 2nd ed. London: Martin Dunitz, Inc; 1998
30. Castellano E, Attanasio R, Boriano A, Borretta G. Clinical presentation of primary hyperparathyroidism in older adults. J Endocr Soc. 2019;3 (12):2305-2312.
31. Kreutle V, Blum C, Meier C, Past M, Müller B, Schütz P, Borm K. Bisphosphonate induced hypocalcaemia—report of six cases and review of the literature. Swiss Med Wkly. 2014;144:w13979
32. Kaur U, Chakrabarti SS, Gambhir IS. Zoledronate induced hypocalcemia and hypophosphatemia in osteoporosis: a cause of concern. Curr Drug Saf. 2016;11(3):267–269
33. Tufano A, Rendina D, Conca P, Matani B, Di Minno G. Hypocalcemia and hypophosphatemia after treatment with zoledronic acid in a patient with AL amyloidosis. Intern Emerg Med. 2019;14 (3):447-449.
34. Whittamore JM, Hatch M. The role of intestinal oxalate transport in hyperoxaluria and the formation of kidney stones in animals and man. Urolithiasis. 2017;45 (1):89-108.
35. Borghi L, Schianchi T, Meschi T, Guerra A, Allegri F, Maggiore U, Novarini A. Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. N Engl J Med. 2002;346 (2):77-84
36. Prezioso D, Strazzullo P, Lotti T, Bianchi G, Borghi L, Caione P, Carini M, Caudarella R, Ferraro M, Gambaro G, Gelosa M, Guttilla A, Illiano E, Martino M, Meschi T, Messa P, Miano R, Napodano G, Nouvenne A, Rendina D, Rocco F, Rosa M, Sanseverino R, Salerno A, Spatafora S, Tasca A, Ticinesi

A, Travaglini F, Trinchieri A, Vespasiani G, Zattoni F; CLU Working Group. Dietary treatment of urinary risk factors for renal stone formation. A review of CLU Working Group. Arch Ital Urol Androl. 2015;87(2):105-20.

37. Escribano J, Balaguer A, Roqué i Figuls M, Feliu A, Ferre N. Dietary interventions for preventing complications in idiopathic hypercalciuria. Cochrane Database Syst Rev. 2014;2:CD006022
38. Penniston KL, Wertheim ML, Nakada SY, Jhagroo RA. Factors associated with patient recall of individualized dietary recommendations for kidney stone prevention. Eur J Clin Nutr. 2016;70 (9):1062-1067
39. Hess B. Renal stone clinic survey: calcium stone formers' self-declared understanding of and adherence to physician's recommendations. Urolithiasis. 2017;45 (4):363-370.
40. Barrett-Connor E, Wade SW, Downs RW, Ganiats T, Hochberg M, Recker RR, Stolshek BS. Self-reported calcium use in a cohort of postmenopausal women receiving osteoporosis therapy: results from POSSIBLE US™. Osteoporos Int. 2015;26 (8):2175-2184
41. Ermer MA, Kottmann SC, Otten JE, Wittmer A, Poxleitner P, Pelz K. In vitro investigation of the antimicrobial effect of three bisphosphonates against different bacterial strains. J Oral Maxillofac Surg. 2018;76 (3):553-560
42. Ticinesi A, Nouvenne A, Chiussi G, Castaldo G, Guerra A, Meschi T. Calcium Oxalate nephrolithiasis and gut microbiota: not just a gut-kidney axis. a nutritional perspective. Nutrients. 2020;20;12(2):548.
43. http://www.agenziafarmaco.gov.it/sites/default/files/Determinazione_446-2017_agg_nota79.pdf accessed on 11/04/2020
44. Fenton AJ, Gutteridge DH, Kent GN, Price RI, Retallack RW, Bhagat CI, Worth GK, Thompson RI, Watson IG, Barry-Walsh C, Matz RL. Intravenous aminobisphosphonate in Paget's disease: clinical, biochemical, histomorphometric and radiological responses. Clin Endocrinol (Oxf). 1991;34(3):197-204.
45. Gennari L, Rendina D,Picchioni T, Bianciardi S, Materozzi M, Nuti R, Merlotti D. Paget's disease of bone: an update on epidemiology, pathogenesis and pharmacotherapy Expert Opin Orphan Drugs. 2018, 6(8):485-496
46. Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, Debelenko LV, Zhuang Z, Lubensky IA, Liotta LA, Crabtree JS, Wang Y, Roe BA, Weisemann J,

Bogusky MS, Agarwal SK, Kester MB, Kym YS, Heppner C, Dong Q, Spiegel AM, Burns AL, Marx SJ Positional cloning of the gene for multiple endocrine neoplasia type 1. Science 1997 276:404–407

47. Heppner C, Bilimoria KY, Agarwal SK, Kester MB, Whitty LJ, Guru SC, Chandrasekharappa C, Collins FS, Spiegel AM, Marx SJ, Burns AL The tumor suppressor protein menin interacts with NB-kB proteins and inhibits NF-κB-mediated transactivation. Oncogene 2001 20:4917–4925
48. Goode A, Long JE, Shaw B, Ralston SH, Visconti MR, Gianfrancesco F, Esposito T, Gennari L, Merlotti D, Rendina D, Rea SL, Sultana M, Searle MS, Layfield R. Paget disease of bone-associated UBA domain mutations of SQSTM1 exert distinct effects on protein structure and function. Biochim Biophys Acta. 2014;1842(7):992-1000.
49. Cleophas T.J. (1999) Carryover Effects in Clinical Research. In: Human Experimentation. Springer, Dordrecht
50. Burckhardt PM, Singer FR, Potts JT Jr Parathyroid function in patients with Paget's disease treated with salmon calcitonin. Clin Endocrinol (Oxf) 1973 2:15–22
51. Kanis JA, Heynen G, Walton RJ Plasma calcitonin in Paget's disease of bone. Clin Sci Mol Med 1977 52:329–332.
52. Adami S, Marcocci C, Gatti D. Epidemiology of primary hyperparathyroidism in Europe. J Bone Miner Res. 200;17(Suppl 2):N18-N23
53. Bilzekian, Yeh MW, Ituarte PH, Zhou HC, Nishimoto S, Liu IL, Harari A, Haigh PI, Adams AL. Incidence and prevalence of primary hyperparathyroidism in a racially mixed population. J Clin Endocrinol Metab. 2013;98(3):1122-1129.
54. Zhe M, Hang Z. Nephrolithiasis as a risk factor of chronic kidney disease: a meta-analysis of cohort studies with 4,770,691 participants. Urolithiasis. 2017;45 (5):441-448.

Table 1. Clinical characteristics of PDB patients and OHS-SiOp controls, according to 25OHD levels

	PDB patients (n= 708)		OHS-SiOp Controls (n= 1803)	
25OHD	≤ 50 nmol/L	>50 nmol/L	≤ 50 nmol/L	>50 nmol/L
N (n; %)	401; 56.6	307; 43.4	979; 54.3	824; 45.7
M : F (n; %)	219; 54.6 : 182; 45.4	181; 58.9 : 126; 41.1	554; 56.6 : 425; 43.4	424; 51.5 : 400; 48.5
Age (years)	69.6±11.3	66.2±10.8	62.8±7.0	62.8±6.8
BMI (Kg/m^2)	27.9±4.5	26.9±4.2	27.7±3.6	27.0±3.1
25OHD (nmol/L)	33.2±10.2	80.2±10.4	32.7±10.5	80.5±13.2
Vit D Suppl (n, %)	216; 53.8	240; 78.2	234; 23.9	330; 40.0
iPTH (pmol/L)	4.65±1.81	4.35±1.54	4.19±1.64	3.34±1.22
HPTH (n, %)	77; 19.2	29; 9.44	47; 5.8	27; 3.3
Calcium (mmol/L)	2.31±0.11	2.33±0.11	2.31±0.10	2.33±0.12

Data are expressed as mean±standard deviation for continuous variables and absolute; percentage for categorical variables. PDB: Paget's disease of bone. OHS: Olivetti Heart Study. SiOp: Siena Osteoporosis Study. M: males. F: females. BMI: Body mass index. Vit D Suppl: number of patients and controls taking vitamin D (cholecalciferol) supplementation. iPTH: intact parathormone. HPTH: Primary hyperparathyroidism. According to Fillèe and colleagues (22), PTH levels > 7.6 pmol/L or > 5.8 pmol/L were considered indicative of hyperparathyroidism in PDB patients with 25OHD < 50 nmol/L or > 50 nmol/L, respectively.

Table 2

Clinical characteristics for nephrolithiasis in patients and controls without primary hyperparathyroidism enrolled for the study 2

	PDB NL	PDB not NL	NL not PDB	CT
Number (n)	97	219	364	219
Mean age (years)	63.9 ± 10.3	65.1 ± 10.3	61.2 ± 10.4	64.9 ± 10.2
Female (n;%)	36; 37.2	103; 47.0	149, 40.9	103; 47.0
Male (n;%)	61; 62.8	116; 53.0	215; 59.1	116; 53.0
BMI (Kg/m^2)	28.1 ± 4.3	27.1 ± 4.6	28.9 ± 4.7	27.1 ± 4.6
At least one (n;%)	93; 95.9 [a,b]	104; 47.5[b]	326; 89.6 [a,b]	65; 29.7

Data are expressed as mean ± standard deviation and absolute; percentage number for continuous and dichotomous variables respectively. PDB: Paget's disease of bone. NL: nephrolithiasis. BMI: body mass index. PDB NL = PDB patients with NL. PDB not NL = PDB patients without NL. CT = control subjects from the Olivetti Hearth Study and from the Siena Osteoporosis Study with neither PDB, nor NL. CT were age-, sex-, and BMI- matched to PDB not NL. NL not PDB = Patients over the age of 40 with NL and without PDB. a) $p < 0.05$ *vs.* PDB not NL. b) $p < 0.05$ *vs.* CT

Figure 1

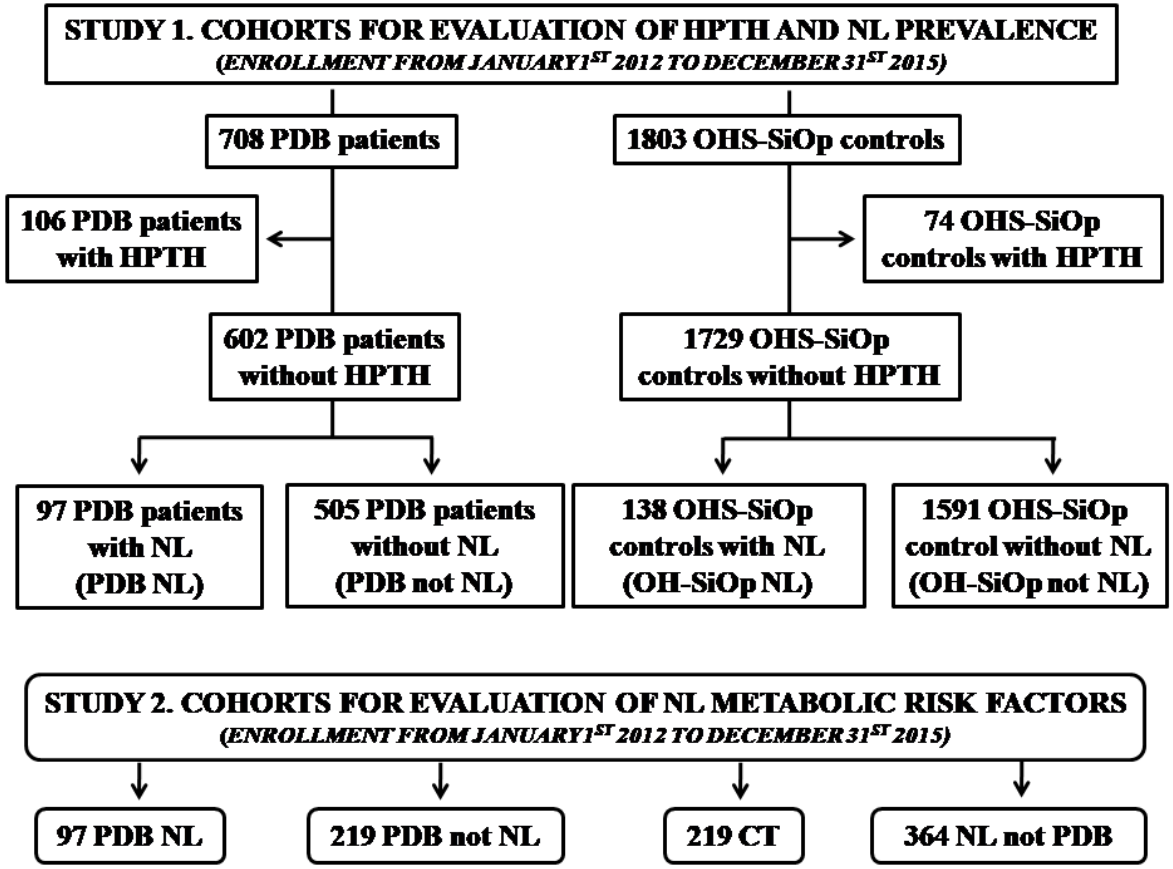

Figure 2