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“Impact of Cholecalciferol supplementation on Skeletal and non-Skeletal manifestations in patients with Primary Hyperparathyroidism submitted to Parathyroidectomy or followed-up without surgery: Cardiovascular Outcomes.”

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Abstract

Context: Cardiovascular (CV) complications are a still debated issue in patients with biochemically mild primary hyperparathyroidism (PHPT) and may be related to both the PHPT condition itself and the hypovitaminosis D.

Objective: To evaluate the prevalence, nature and reversibility of CV disease and associated risk factors in a large cohort of postmenopausal mild PHPT patients surgically cured (PTx Group) or observed for two years without surgical intervention (no-PTx Group). To preliminarily assess, in both group, if the administration of two different doses (800 UI and 2000 UI) of cholecalciferol (VitD) versus no supplementation could affect the CV disease evolution.

Design: Randomized longitudinal prospective open label study.

Settings: The study was conducted in the Endocrinology Unit of two university hospitals.

Participants: 91 post-menopausal women with PHPT (38 in the PTx Group and 53 in the no-PTx Group) participated in the study.

Outcome Measures: Cardiac and vascular damage (blood pressure, transthoracic echocardiography and carotid ultrasonography), lipids and glucose metabolism, renin-angiotensin system (RAAS) activity.

Results: Arterial hypertension (AH) was found in 50.5% of patients and was not associated with PHPT after adjusting data for major CV risk factor. Diastolic dysfunction, LV hypertrophy and valve calcifications were diagnosed in 54.9%, 13.2% and 12.1% of patients and were respectively predicted by body mass index (BMI) and advancing age, by the presence of AH and by advancing age and the presence of AH, respectively. Similarly, advancing age was the only significant predictor of the presence of carotid plaque and AH was the only significant predictor of carotid intima-media thickness. We did not find any association between calcium, PTH or 25OHD and all glycemic parameters. No activation of RAAS was found in normotensive mild PHPT patients. All CV complications and risk factors were neither reversed nor significantly improved by surgery and/or VitD administration up to 24 months' follow-up.

Conclusions: The high incidence of CV disease and metabolic derangements reported in mild PHPT may be primarily related to the coexistence of AH, advanced age or increased BMI. Moreover, the administration of VitD supplements would seem to have a neutral effect at least as regards CV complications and CV risk factors in mild PHPT patients.

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1. INTRODUCTION

1.1 Primary Hyperparathyroidism

1.1.1 Definition and Epidemiology

Primary Hyperparathyroidism (PHPT) is a common endocrine disorder due to an abnormal secretion of parathyroid hormone (PTH) from one or more of the four parathyroid glands (1-5). PHPT is the most common cause of hypercalcaemia in outpatients and is established biochemically by the concurrent finding of hypercalcaemia and levels of PTH that are either clearly elevated or inappropriately normal for the hypercalcaemic state (1-5). Actually, a normocalcaemic variant of PHPT is recognized and diagnosed by elevated PTH levels with persistently normal concentrations of albumin-adjusted total and Ca2+ (1-6).

In the past, PHPT was a rare and generally symptomatic disorder (1-5). Over the last decades, the incidence of PHPT has increased due to the routine serum calcium evaluation, especially in industrialized countries where cases of asymptomatic and/or normocalcaemic PHPT are diagnosed regularly (1-5).

PHPT occurs more frequently in women than in men (F/M 4:1 in United States, F/M 4.9:1 in Italy) with a peak of incidence in the early postmenopausal years (7, 8). A 21/1000 PHPT prevalence was found in women aged 55-75 years, which is equivalent to 3/1000 prevalence in general population (7, 8). However, there is wide variability in prevalence estimates of PHPT, in particular for the normocalcaemic form due to the limited published experience as well as to how vitamin D deficiency is defined (3).

1.1.2 Aetiology and Risk Factors

PHPT is caused by a solitary parathyroid adenoma (PA) in 80% of cases, whereas four-gland hyperplasia (PH) accounts for 10–15%, multiple adenomas for 5% and parathyroid cancer (PC) for <1% of cases (1-5). As opposed to the female predominance in benign causes of PHPT, PC has an

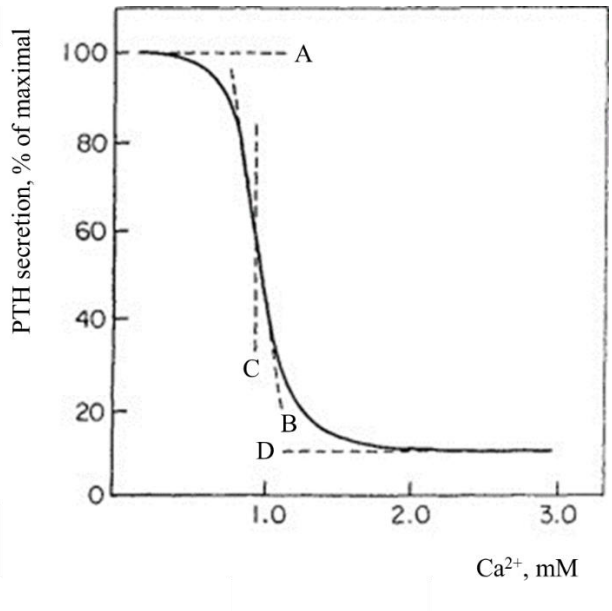
equal frequency of occurrence in both sexes and is usually diagnosed in the fifth decade of life (9-13). Without disease-specific serum and radiological markers, PC is typically diagnosed intraoperatively and/or by postoperative histologic examination of the resected tissue (9-13). Patients with PC typically have markedly elevated serum calcium (>14 mg/dl) and PTH levels (at least twice the upper limit of normal) along with evidence for target organ involvement. Fewer than 10% of PC are hormonally non-functional and characterized by the involvement of surrounding structures by the tumour mass (palpable solid neck mass associated with hoarseness and dysphagia) (9-14).

In all forms of PHPT, there is loss of normal feedback suppression of serum levels of calcium upon the synthesis and secretion of PTH, due to increased parathyroid cell mass and/or a reduction in the number of calcium sensing receptors (CASR) on parathyroid cells (15). PTH secretion is not completely autonomous and can usually be partially inhibited by increased levels of serum calcium (15). Considering the inverse sigmoidal relationship between PTH secretion and serum ionized calcium levels (Ca2+) (Figure 1), the increase in PTH secretion in PHPT is due to:

1. an elevation in the set-point of the calcium-PTH curve (i.e. the major determinant of the severity of the hypercalcemia) (15),
2. a variable change in the slope of the calcium-PTH curve due to relative non-suppressibility of PTH secretion (16).

The degrees of hypersecretion and non-suppressibility are a function of tumour mass and can range from none in patients with very small adenomas to considerable in patients with large ones.

Figure 1. PTH-calcium response curve.



The four parameters that describe the relationship between PTH secretion and Ca2+: A) maximal secretory rate at low Ca2+, B) Slope at the midpoint of the curve, C) Midpoint (or set point): the level of Ca2+ producing half of the maximal inhibition of PTH release, D) minimal secretory rate at high Ca2+.
Abbreviations: Ca2+, ionized calcium.
Modified from Brown, E.M. The Parathyroids, Second Edition.

PHPT is generally a sporadic disease (1-5). However, in 2-5% of cases it can occur in hereditary forms (1-5, 17). The aetiology of PHPT remains elusive in the majority of patients with sporadic disease. Risk factors associated with the development of PHPT include external radiation in childhood (18-19), exposure to a nuclear incident in adults (19), long-term lithium therapy (20), thiazide diuretics (21) and chronic low-calcium diet (22). Radioactive iodine (RAI) therapy (for the treatment of either benign or malignant thyroid disease) has been suggested to cause PHPT but data are conflicting (23, 24). The genetic pathogenesis of sporadic PHPT is often unclear. Most sporadic PA and PC are monoclonal whereas PH possibly originate from a stimulus for generalized (polyclonal) parathyroid cell proliferation, even though monoclonal tumours may also occur (25, 26). Two genes, *CCND1* (which encodes cyclin D1) and *MEN1* (which encodes menin), were involved in the development of sporadic PHPT are (27, 28). Somatic mutations in *MEN1* occur in 12–35% of

sporadic PA, whereas rearrangement or overexpression of *CCND1* can occur in 20–40% (27, 28). Recent studies have also implicated *CDC73*, *CTNNB1*, *CDKN1B* and *AIP* (which encodes the aryl hydrocarbon receptor-interacting protein) in a small percentage of PA (5, 29-31). *CDC73* is the most common mutated gene in sporadic PC (32). Mutations in *PRUNE2* (which encodes protein prune homologue 2) have also been associated with the development of PC (33). Other work suggests a potential role of microRNA-296 as a novel tumour-suppressor gene in PC (34).

The genetic syndromes associated with PHPT include *Multiple Endocrine Neoplasia type 1* (MEN1), *Multiple Endocrine Neoplasia type 2A* (MEN2A), *Multiple Endocrine Neoplasia type 4* (MEN4), *Hereditary Hyperparathyroidism-jaw Tumour syndrome* (HPT-JT), *Familial Isolated Hyperparathyroidism* (FIHP), *Familial Hypercalcaemic Hypocalciuria* (FHH) and *Neonatal Severe Hyperparathyroidism* (NSHPT) (1-5, 17) (Table 1). General features, common to almost all the forms of hereditary PHPT, mainly consist of: 1) multiglandular involvement, and 2) earlier age of onset than sporadic form of PHPT (1-5, 17).

Table 1. Genetic syndromes associated with PHPT.

Hereditary disorder	Inheritance	Gene/Protein (Chromosomal locus)	Phenotype
MEN1	AD	*MEN1*/menin (11q13.1)	PHPT (95%); pancreatic neuroendocrine tumours (40%); anterior pituitary adenomas (30%); additional features: adrenal adenomas, carcinoid tumours, lipomas, cutaneous angiofibromas and collagenomas
MEN2A	AD	*RET*/proto-oncogene c-Ret (10q11.21)	PHPT (20%); Medullary thyroid cancer (90%); pheochromocytomas (50%); Cutaneous lichen amyloidosis*, Hirschsprung disease*
MEN4	AD	*CDKN1B*/p27 (12p13.1)	PHPT (~80%), anterior pituitary tumors (~40%), pancreatic neuroendocrine tumors; other features: carcinoid, adrenocorticoid tumours, thyroid tumours, reproductive organ tumours and renal angiomyolipomas
HPT-JT	AD	*CDC73 also known as HRPT2*/parafibromin (1q31.2)	PHPT (80%) with a high prevalence of parathyroid carcinomas (>15%); ossifying fibromas of the mandible and maxilla (>30%); other features: renal and uterine tumors, pancreatic adenocarcinoma, testicular mixed germ cells and Hürthle cell thyroid adenomas
FIHP	AD	*MEN1*/menin (11q13.1) *CDC73*/parafibromin (1q31.2) *CASR*/CASR (3q13.3–q21.1) *GCM2*/GCM motif protein 2, also known as hGCMb (6p24.2) *CDKN1B*/p27 (12p13.1)	Isolated PHPT
FHH	AD	*CASR*/CASR (3q13.3–q21.1) *GNA11*/GNA11 (19p13.3) *AP2S1*/AP-2 complex subunit sigma (19q13.32)	Mild PTH-dependent hypercalcemia, associated with low concentration of urinary calcium
NSHPT	AD/AR	*CASR*/CASR (3q13.3–q21.1)	Severe neonatal PHPT

Abbreviations: MEN1, Multiple Endocrine Neoplasia type 1; MEN2A, Multiple Endocrine Neoplasia type 2A; MEN4, Multiple Endocrine Neoplasia type 4; HPT-JT, Hereditary Hyperparathyroidism-jaw Tumour syndrome; FIHP, Familial Isolated Hyperparathyroidism; FHH, Familial Hypercalcaemic Hypocalciuria; NSHPT, Neonatal Severe Hyperparathyroidism; AD, Autosomal Dominant; AR, Autosomal Recessive; PHPT, Primary Hyperparathyroidism; PTH: Parathyroid Hormone.
* depending upon the specific *RET* mutation.

1.1.3 Clinical Presentation, Diagnosis and Differential Diagnosis

To date, the clinical presentation of PHPT includes three phenotypes:

1- Classical PHPT with target organ involvement (i.e. with renal and skeletal complications);

2- Asymptomatic PHPT (i.e. without no overt signs of PHPT or target organ manifestations other than hypercalcaemia);

3- Normocalcaemic PHPT (NC-PHPT) (i.e. persistently normal serum albumin-corrected and ionized serum calcium concentrations with elevated PTH level and all known secondary causes of a high PTH ruled out) (1-6, 35).

Recently a fourth phenotype has been added to the previous ones, namely PHPT with normal calcium and PTH values but with histological alteration of the parathyroid glands (36, 37).

The clinical presentation of PHPT depends on several factors including the frequency of biochemical screening, the use of targeted testing in the differential diagnosis of osteoporosis and the prevalence of vitamin D deficiency in a given population. It is important to note that individuals who are asymptomatic or normocalcaemic can still show evidence of complications and target organ involvement (1-5, 35, 39-41).

The main laboratory findings of PHPT and its differential diagnosis are shown in Table 2.

The diagnosis of PHPT is established biochemically by documenting hypercalcaemia with simultaneously elevated PTH levels. By contrast, in almost all other aetiologies of non-parathyroid hypercalcaemia (i.e. malignancy, vitamin D intoxication, granulomatous diseases, lymphomas, etc) the PTH is clearly suppressed (42). One exception of this basic rule is the ectopic PTH secretion from a non-parathyroid tumour, a rare condition occasionally documented in late-stage malignancies (43,44).

Actually, even inappropriately normal PTH levels (i.e. > 20 - 25 pg/ml given a normal range of ~10 to 65 pg/mL) in the presence of hypercalcaemia are consistent with a diagnosis of PHPT (1-5, 45).

Before proceeding to the diagnostic evaluation, it is of paramount importance to check and correct a possible 25-hydroxyvitamin D (25OHD) deficiency (35, 40) (Table 2).

When the PTH is only minimally elevated or within the normal range, the benign inherited FHH must be excluded. The major feature that distinguishes FHH from PHPT, is a low urine calcium excretion and a calcium/creatinine (Ca/Cr) clearance ratio below 1% (formula: (24-hour urine calcium x serum creatinine) / (serum calcium x 24-hour urine creatinine), Table 2) (38). A family history of hypercalcaemia, especially in young children, and the absence of sign and symptoms of hypercalcemia are characteristic of this disorder and support the diagnosis together with the mutational analysis of CASR, GNA11 and AP2S1 (35).

In mild forms of PHPT, serum calcium levels can intermittently fall into the normal range. This finding is compatible with the diagnosis of PHPT as long as a recurrent pattern of hypercalcaemia is evident as opposed to NC-PHPT in which total and Ca2+ levels are persistently normal in the presence of elevated PTH levels and all secondary causes for hyperparathyroidism ruled out (Table 3) (1-5, 35, 46-47).

Table 2. Differential Diagnosis of PHPT, Laboratory Findings.

Disease	**Laboratory test**			
	Serum Calcium	**Intact PTH**	**Urinary Calcium (mg/24hours)**	**Ca/Cr clearance**
PHPT	Elevated	Normal or elevated	Normal or elevated	> 0.02
Non-parathyroid Hypercalcaemia	Elevated	Low (< 20 pg/mL)	Generally high	
PHPT with vitamin D deficiency	Normal or elevated	Elevated	Low-normal or low (<200)	
FHH	Elevated	Normal or mildly elevated	Low (< 100)	< 0.01
Normocalcaemic PHPT	Normal	Elevated	Normal	
Secondary hyperparathyroidism due to vitamin D deficiency	Normal or low	Elevated	Low	

Abbreviations: PTH, parathyroid hormone; PHPT, primary hyperparathyroidism; FHH, familial hypocalciuric hypercalcemia; Ca/Cr clearance, calcium to creatinine clearance ratio.

Table 3. Causes of secondary hyperparathyroidism.

Chronic kidney Disease
Decreased calcium intake Food Intolerance (Milk/Lactose) Phytates Strict vegan
Calcium Malabsorption Vitamin D Deficiency (Sunlight Deprivation, Pigmented Skin in Northern Latitudes, Liver Disease, Anticonvulsant Treatment) Bariatric Surgery Celiac Disease Pancreatic Disease Inflammatory Bowel Disease Cystic Fibrosis Corticosteroid Treatment Ageing
Renal Calcium Loss Idiopathic Hypercalciuria Loop Diuretics
Inhibition of Bone Resorption Bisphosphonates Denosumab Hungry Bone Syndrome
Lactation/Post Lactation
Rhabdomyolysis
Sepsis
Burns
Acute Pancreatitis
Metastatic Prostate Cancer
Vitamin D Dependent or Resistant Rickets or Osteomalacia
Pseudohypoparathyroidism

1.1.4 Overview of Classical Complications and management

PHPT targets the kidney and the skeleton (1-5). The classical symptoms and signs of PHPT reflect the combined effects of increased PTH secretion and hypercalcemia (1-5).

Skeletal involvement. Clinical parathyroid bone disease (or osteitis fibrosa cystica) is rarely seen today in developed countries where more subtle forms of skeletal involvement are observed (1-5, 48-50). Radiographic signs of osteitis fibrosa cystica include demineralized skeleton, “salt-and-pepper” skull appearance, tapering of the distal clavicles, subperiosteal bone resorption of the phalanges, bone cysts and brown tumors of the long bones (49). Low bone mineral density (BMD), in particular at

cortical sites (forearm and hip) as compared with trabecular sites (spine), is frequently observed even in asymptomatic PHPT patients (51-53). However, imaging technologies, such as high-resolution peripheral quantitative computed tomography (HRpQCT) and trabecular bone score (TBS) of lumbar spine dual-energy x-ray absorptiometry (DEXA) images, showed that in PHPT both cortical and trabecular bone compartments are adversely affected (54-56). Indeed, an increased risk of all fractures including vertebral fractures (VFx) is observed in PHPT patients and is associated with HRpQCT and TBS values independently from BMD (57-59).

DEXA is a standard of care for the evaluation of PHPT. BMD measured at three skeletal sites (lumbar spine, hip, and forearm) is used to determine the advisability of surgery in asymptomatic patients and to monitor patients over time, whether or not they have PTx (50). Moreover, a screening for silent VFx (by x-ray CT, MRI) is recommended in all patients and if present, PTx is recommended independently from DEXA values (50)

Successful parathyroidectomy (PTx) is associated with increases in BMD and TBS (54, 60) but data on fracture risk reduction are still inconclusive (61). In mild PHPT, in the short term (two years), PTx and antiresorptive therapy increase BMD to a similar degree and each represents a reasonable option in a patient with mild PHPT and low BMD (62-64). However, no data are available on fracture risk and long-term efficacy and safety of these therapies. Thus, PTx remains the treatment of choice for osteoporotic patients with PHPT (50).

Renal involvement. The most common clinical manifestations of PHPT are hypercalciuria and nephrolithiasis (65). Nephrolithiasis occurs in approximately 15 to 20% of PHPT patients (65-67). However, renal imaging (abdominal ultrasound, x-ray or spiral CT scan of the kidneys) indicates that the prevalence of subclinical disease is much higher reaching 55% of patients (68). Inversely, nephrocalcinosis, polyuria and polydipsia are much less common in modern PHPT, as are renal dysfunctions (i.e. estimated glomerular filtration rate, eGFR, < 60 ml/min) (39, 65). A reduced eGFR is present in 15 to 17% of PHPT patients and is prevalently related to the presence of traditional risk

factors, such as age, hypertension, use of antihypertensive medication and fasting glucose levels rather than clinical or biochemical indices of PHPT (50, 69, 70). Kidney imaging is recommended in the clinical assessment of any PHPT patients because positive imaging constitute an indication for surgery (50). However, PTx, unlike the benefit shown in reducing the risk of kidney stones, seems to not improve renal function (71, 72).

Parathyroidectomy is the only cure for PHPT and is recommended in all symptomatic patients (5). In asymptomatic PHPT patients, surgery is suggested if evidence of skeletal or renal subclinical complications of the disease exists (50, Table 4).

Table 4. Guidelines for recommending surgery in asymptomatic PHPT patients.

Serum calcium (>upper limit of normal)	1.0 mg/dL
Skeletal	BMD by DEXA: T-score < - 2.5 at lumbar spine, total hip, femoral neck, or distal 1/3 radius or Vertebral fracture by x-ray, CT, MRI, or VFA
Renal	Creatinine clearance < 60 ml/min or 24-h urine for calcium > 400 mg/day and increased stone risk by biochemical stone risk analysis* or presence of nephrolithiasis or nephrocalcinosis by x-ray, ultrasound, or CT
Age, years	<50

Patients need to meet only one of these criteria to be advised to have parathyroid surgery.
*analysis of 24-h urine composition (volume, pH, calcium, sodium, oxalate, citrate, uric acid, urea, creatinine) available through most commercial laboratories.
Abbreviations: BMD, bone mineral density; DEXA, dual-energy x-ray absorptiometry, CT, computer tomography; MRI, magnetic resonance imaging; VFA, vertebral fracture assessment.
Modified form Bilezikian JP et al. (50)

Regular monitoring is recommended (Table 5) for those who do not meet any criteria for PTx or choose to be observed or are poor surgical candidates for significant comorbidities (50). However, even in subjects who do not meet any criteria for PTx, surgery is always an option because it is the only definitive therapy for PHPT (50) and almost 40% of patients developed one or more indications

for PTx over 15 years of follow-up (73). Moreover, surgery is also indicated in patients for whom medical surveillance is not possible (50).

Table 5. Recommendations for monitoring PHPT patients who do not undergo parathyroid surgery.

Serum calcium	Annually
Skeletal	BMD by DEXA every 1–2 y (3 sites), x-ray or VFA of spine if clinically indicated (i.e., height loss, back pain)
Renal	eGFR, annually; serum creatinine, annually. If renal stones suspected, 24-h biochemical stone profile, renal imaging by x-ray, ultrasound, or CT

Abbreviations: BMD, bone mineral density; DEXA, dual-energy x-ray absorptiometry; VFA, vertebral fracture assessment; eGFR, estimated glomerular filtration rate; CT, computer tomography; MRI, magnetic resonance imaging.
Modified form Bilezikian JP et al. (50)

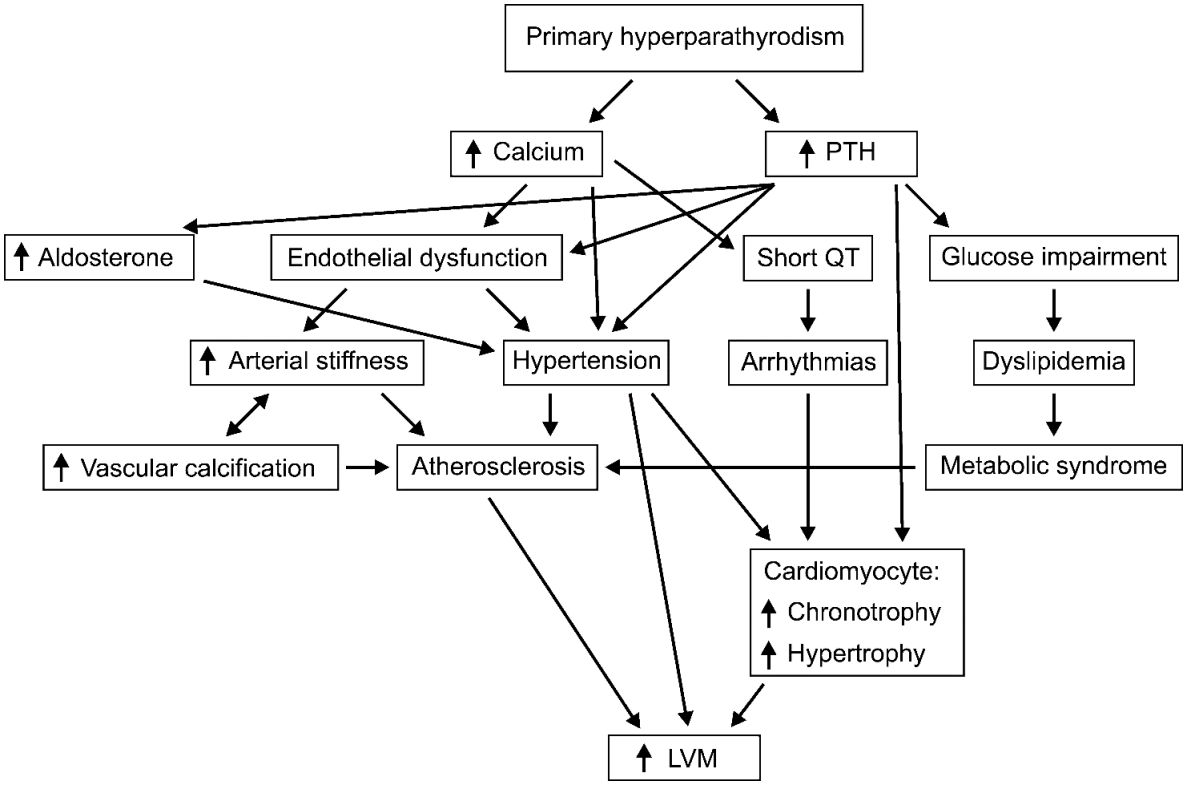
Medical therapy for those who cannot undergo PTx is used only if there is the need to lower the serum calcium concentration or to improve the BMD. To date, no single drug is able to satisfy both needs and long-term data are insufficient regarding benefit and safety of single and/or combined medical therapy in PHPT patients (50). Calcimimetics reduce serum levels of calcium and, although they are not primarily indicated for this purpose, estrogens, bisphosphonates and denosumab can increase BMD and possibly lower serum calcium concentrations by inhibition of bone resorption (40, 50, 74). Low dose thiazides (i.e. 12.5 mg daily) can been used for the treatment of hypercalciuria but close monitoring of serum calcium is required (75). In all PHPT patients, vitamin D supplementation (25OHD target 20 or 30 ng/mL) and a moderate calcium intake (800 - 1000 mg/day) are recommended (50, 76, 77).

1.1.5 Cardiovascular Complications

PHPT has been associated with increased cardiovascular (CV) morbidity and mortality but data are still controversial and PTx resulted in conflicting outcomes in particular in those with mild forms of disease (39, 49, 78-83). Indeed, the latest endocrine guidelines on PHPT management do not consider CV disease as an indicator for PTx and a CV risk assessment is currently not suggested in PHPT patients (50). Actually, some experts support the consideration of PTx for possible mitigation of CV risk factors on a case-by-case basis (84).

Both hypercalcemia and elevated PTH levels could affect the CV system through different mechanisms (Figure 2). PTH receptors were identified in cardiomyocytes, vascular smooth muscle, and endothelial cells (80, 85). Specifically, in the heart, PTH exerts a direct hypertrophic action upon cardiomyocytes through protein kinase C activation (80, 86-87). Other potential mechanisms include PTH's ionotropic effects on the heart via its ability to increase heart rate and coronary blood flow (80, 88). In vessels, PTH may induce endothelial dysfunction increasing pro-atherosclerotic and pro-inflammatory factors like endothelin-1, interleukin-6, beta-1 integrin (89, 90). Also, PTH may increase renin release and activate the renin-angiotensin system (RAAS, see next chapter). On the other hand, calcium influences the cardiomyocytes and vascular smooth muscle cell contractility and regulates the endothelial permeability (80, 91-93) .

Figure 2. Proposed mechanisms behind the observed increase CV risk in PHPT.



Abbreviations: PTH, Parathyroid Hormone; LVM, Left Ventricular Mass
Modified from Pepe J et al., EJE 2017 (80).

CV complications associated with PHPT include arterial hypertension (AH), arrhythmia, left ventricular hypertrophy (LVH), atherosclerosis and vascular and valvular calcification (80, 81, 94-96). Moreover, an increased frequency of impaired glucose tolerance (IGT), type 2 diabetes mellitus (T2DM), insulin resistance (IR), dyslipidaemia and metabolic syndrome was reported in both normocalcaemic and hypercalcaemic PHPT patients, contributing in part to increase CV risk in these population (97-102).

The difficulty to clearly define the relationship between CV complications and PHPT is linked to several factors: 1) differences in enrolled patient varying from NC-PHPT to symptomatic PHPT; 2) differences in the methods utilized for excluding confounding pre-existing CV disease; 3) differences in the choice of CV endpoints and timepoint; 4) study design and sample size (the outcomes are mainly derived from observational, cross-sectional and single centre studies, only a few longitudinal randomized controlled trials (RCT) are available).

Arterial hypertension is frequently seen in association with PHPT (prevalence 40-60% of patients), even among patients with mild disease (80, 81, 101, 103). In a recent National Inpatient Sample Study, the presence of PHPT remained strongly correlated with AH (OR 1.3, P<0.001) in patients with PHPT (n=37,922) as compared to the general population (n=33,094,451) after a multivariate regression analysis adjusted for age, gender, and other CV risk factors including DM, hyperlipidemia, obesity, tobacco use, and chronic kidney disease (104). However, the effect of PTx on AH remain controversial. Several studies demonstrated that following PTx there was a reduction in blood pressure after six months (105, 106), and up to 5 years (107) while others did not report significant reduction (108). The only RCT of PTx versus observation in 116 patients with asymptomatic PHPT did not demonstrate between-group differences in change in blood pressure in a two-year analysis (109).

In addition to AH, PHPT appears to be associated with an increased risk of developing LVH and impaired diastolic filling even in the mild form of the disease (83, 110, 111). In contrast, this association was not found in other studies (112-114). Likewise, results from studies assessing changes in cardiac structure and function after PTx are also conflicting with evidence of a significant benefit of PTx only in observational studies including severe and symptomatic PHPT patients (111, 115, 116). RCTs of PTx vs observation did not reveal a statistically significant benefit of PTx but tended to include patients with lower levels of calcium and PTH than those included in observational studies. In the RCT published by Persson et al (n = 49, mean calcium 10.6 mg/dL, PTx n= 26) baseline left ventricular (LV) dimension correlated with PTH levels but there was no echocardiographic difference in cardiac structure or function between PTx vs observation group after 2 years (117). A 2015 meta-analysis, which included 15 studies (4 RCTs) for a total of 457 patients enrolled, indicated that PTx was associated with a decline in LV mass and that higher levels of PTH predict a greater CV improvement (118). However, the benefit of PTx, was observed only in studies with durations of 6 months or less and the clinical significance of the observed decline in LV mass was uncertain because mean LV mass was normal preoperatively in most of the studies included in the meta-analysis (118).

Myocardial and valvular calcifications have clearly been described in PHPT patients with severe hypercalcemia (80, 81). Studies in mild PHPT are limited but an association with subclinical aortic valve calcification was reported in mild PHPT patients (94). PTH, but not serum calcium concentration, predicted aortic valve calcification (94), but there was no improvement after PTx (119).

Mild PHPT was associated with subclinical carotid and coronary vascular manifestations. The mean carotid intima-media thickness (IMT), a strong predictor of systemic atherosclerosis and cerebrovascular events, was significantly higher in patients with PHPT compared with controls (120, 121). In addition, increased aortic stiffness (96, 122) and carotid vascular stiffness (120) were associated with PTH levels. Even in NC-PHPT, mean carotid IMT values were significantly higher compared to controls, but PTx led to an improvement in IMT only in hypercalcemic patients and not in mild and NC-PHPT (119, 123). The coronary flow reserve (CFR) seems to be reduced in PHPT patients compared to controls (116, 124) and to improve after PTx (116) but an increased risk in coronary artery disease is not suggested (125).

Untreated classical and/or severe PHPT was associated with an increased risk of CV mortality (78, 81, 83, 126). Survival benefit after PTx was demonstrated in some studies whereas others reported persistent increased CV risk even after successful PTx (83, 127-129). Moreover, several studies suggest that the increased risk of CV death may not be applicable to modern asymptomatic PHPT patients. Indeed when patients were stratified by calcium levels, only patients in the highest quartile of serum calcium (11.2-16 mg/dl) had reduced survival supporting a relationship between degree of hypercalcemia and CV mortality (126). Moreover in the study by Nilsson et al., when patients were stratified by year of surgery, those operated on after 1985 had no excess mortality suggesting that modern patients may differ in risk (83).

Further, serum PTH rather than serum calcium appeared to be associated with an increased risk of fatal and non-fatal CV disease (130).

1.1.6 The interplay between Aldosterone, Vitamin D and Parathyroid Hormone in Cardiovascular Risk

Compelling evidences support a bidirectional interaction between the RAAS and PTH that might explain the increased CV risk observed in PHPT patients (131-134). Both plasma aldosterone concentration (PAC) and PTH were independently associated with CV mortality, with evidence for a synergistic interaction (135). Similarly, an important role in the pathogenesis of cardiac remodelling in primary aldosteronism (PA) was attributed to PTH (136). In humans, PTH infusion caused significant increase of urinary tetrahydroaldosterone excretion without any changes of plasma renin and potassium levels (137). Moreover, several small sample size studies documented increased PAC in patients with PHPT and a significant decrease of PAC, plasma renin activity, angiotensin II and blood pressure after PTx (138, 139). Conversely, other studies observed no RAAS activation in PHPT raising doubts about a significant interaction between the PTH and the RAAS (140, 141).

On the other hand, patients with PA had higher PTH levels when compared to matched individuals with essential hypertension (EH) (142-144). In these patients, normalization of serum calcium and PTH levels were reported after treatment of PA with either adrenal surgery or mineralocorticoid receptor antagonists (143-147). PA patients frequently had secondary hyperparathyroidism but case reports of coincident PA and PHPT have been reported (148-152). Moreover, even in individuals without PA, higher serum aldosterone levels were independently associated with higher PTH levels (153). In PA patients, increased levels of PTH may be triggered through aldosterone-mediated tubular (and potentially intestinal) calcium and magnesium losses with secondary hyperparathyroidism (144, 146, 147, 154, 155) and prolonged secondary hyperparathyroidism might eventually lead to tertiary hyperparathyroidism. On the basis of gene expression and immunohistochemistry studies, both the angiotensin II type 1 receptor (ATR1) and the mineralocorticoid receptor (MR) were expressed in parathyroid tissue (152, 156). In primary culture of human parathyroid cells, both aldosterone and angiotensin II increased PTH secretion, by acting through MR and ATR1 rspectively and these effects were abolished by canrenone and irbesartan (157). Vice versa, the PTH receptor type 1 was expressed

in aldosterone producing adenoma and hyperplasia cells at both the mRNA and the protein level (152).

Vitamin D deficiency appears to have an independent role as a risk factor in CV disease (158-162). Vitamin D deficiency and insufficiency are common in PHPT patients and lower serum 25OHD levels were associated with higher serum PTH levels in PHPT making either or both plausible mediators of deleterious CV effects in PHPT (66, 163, 164). Vitamin D receptors (VDR) have a broad tissue distribution that includes cardiomyocytes, vascular endothelial, smooth muscle cells and adrenal gland (165-169) suggesting additional physiological functions beyond calcium homeostasis. In VDR knockout mice, renin, angiotensin II and PAC levels were drastically increased (170). These mouse developed AH and cardiac hypertrophy that were corrected by treatment with captopril (an ACE inhibitor) or losartan (an angiotensin II AT1 receptor antagonist) confirming that over-stimulation of the RAAS was indeed responsible for these abnormalities (170). Moreover 1,25-dihydroxyvitamin D (1,25(OH)2D3) suppressed renin gene expression independently from calcium, PTH and angiotensin II feedback regulation (170-173). In humans, numerous epidemiological and clinical studies confirmed an inverse relationship between vitamin D and blood pressure and/or plasma renin activity and/or concentration of angiotensin II in both normotensive and hypertensive patients (174-178). A linear correlation between the rise in blood pressure or the prevalence of AH and the latitudes north or south of the equator was found (179) and ultraviolet light has been reported to lower blood pressure in patients with mild EH (180). In clinical trials, vitamin D treatment was reported to reduce blood pressure in hypertensive or elderly patients (181, 182). Treatment with vitamin D in hypertensive and hypovitaminotic patients has been shown to reduce the plasma renin activity, angiotensin II levels, blood pressure, and myocardial hypertrophy (183, 184) but data are still controversial (185, 186). Specifically in PHPT patients, an inverse association between LV hypertrophy and 25OHD was seen (112) but studies assessing vitamin D treatment in patients with PHPT and concomitant vitamin D deficiency are necessary to assess whether vitamin D deficiency and its repletion affect CV health in these population.

2. AIMS OF THE STUDY

Cardiovascular complications are a still debated issue in patients with biochemically mild PHPT. Although CV outcomes and mortality have been consistently reported to be increased in classical PHPT, the effects of mild PHPT on the CV system have been less deeply studied. Moreover, 25OHD deficiency and insufficiency are common in PHPT patients. Given the postulated role of vitamin D deficiency on CV disease (158-162), the CV complications in PHPT may be related to both the PHPT condition itself and the hypovitaminosis D.

The overall goals of this study were:

1) to evaluate the prevalence, nature and reversibility of CV disease and associated risk factors in two different groups of postmenopausal mild PHPT patients:

   a) subjects not fulfilling the surgical criteria for PTx apart from osteoporosis (i.e. no-PTx Group);

   b) subjects candidates for PTx (i.e. PTx Group);

2) to preliminarily assess if two different doses of cholecalciferol (VitD) supplementation (800 UI and 2000 UI) vs no supplementation (0 UI) can affect the evolution of these complications in the two groups of patients.

This study was part of a larger project named "Impact of cholecalciferol supplementation on skeletal and non-skeletal manifestations in patients with Primary Hyperparathyroidism submitted to parathyroidectomy or followed up without surgery" (the "*VitD-PHPT project*") with the following end points:

1) Bone Mineral Density measured at lumbar spine (LS), femoral neck (FN) , total hip (TH) and distal third of radius (TR);

2) Clinical and morphometric VFx;

3) Renal function and nephrolithiasis;

4) Lipids and glucose metabolism;

5) Cardiac and vascular damage: AH, diastolic dysfunction, left ventricular (LV) hypertrophy, ejection fraction (%), valve calcification, posterior LV wall thickness, ventricular septum thickness, peak transaortic pressure gradient, LV end-diastolic dimension, estimated systolic pulmonary artery pressure (sPAP), carotid intima-media thickness (IMT), carotid plaque;

6) RAAS activity;

7) Balance, gait and risk of fall.

Here we report data on the CV complications of mild PHPT.

3. SUBJECTS AND METHODS

Study subjects

This randomized longitudinal prospective open label study was conducted at the Endocrine Clinic Unit, IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo (FG), Italy and at the Endocrinology Unit, IRCCS, Fondazione Ca' Granda, Ospedale Maggiore Policlinico, Milano (MI), Italy. Patients recruitment was carried out by each Health Centre, with a centralized randomization.
Cases were eligible if they were postmenopausal female PHPT patients aged 45-80 years.
The diagnosis of PHPT was based on increased ionized (> 1.32 mmol/l) or albumin-corrected serum calcium (> 10.2 mg/dl), with increased or inappropriately normal intact PTH (normal values 10 - 65 pg/ml) after excluding FHH as previously explained. The diagnosis of NC-PHPT was made after careful exclusion of all secondary causes for hyperparathyroidism (Table 3).

Exclusion criteria were:

1) familial forms of PHPT (Table 1);
2) serum calcium > 13 mg/dL;
3) malignancy other than non-melanoma skin cancer;
4) other diseases or conditions, beside PHPT, known to affect bone metabolism (i.e. menopause before 45 years, thyrotoxicosis, gastrointestinal disorders, chronic renal failure, chronic hepatic disease, depression, alcoholism, eating disorders, rheumatological or hematological diseases, hypercortisolism, etc.);
5) concurrent (or taken within the last two years) therapy with drugs known to affect mineral metabolism or PTH and calcium levels (i.e. bisphosphonates, cinacalcet, glucocorticoids, teriparatide, thiazide diuretics, hormonal adjuvant therapy, lithium).

In patients already on VitD therapy at the time of recruitment or in whom VitD was administered to rule out secondary hyperparathyroidism, VitD therapy was stopped for at least 4 months prior to study entry.

All patients gave written, informed consent. This study was approved by local committee of both involved centers.

Study design

In all patients, at baseline (T0), the following parameters were evaluated:

1) familiar and personal history of myocardial infarction, angina, angioplasty, coronary artery bypass surgery, deep vein thrombosis, T2DM, hypercholesterolemia, cigarette smoking (categorized as smokers and non-smokers);
2) personal history of falls and incident fractures;
3) weight, height, body mass index (BMI), blood pressure, heart rate, anamnestic collection of type and number of medications with particular attention to hypertensive, lipid-lowering and hypoglycemic medications;

4) fasting sample for serum calcium, albumin, Ca2+, phosphorus, intact PTH, 25OHD, creatinine, alkaline phosphatase total activity (ALP), C-terminal telopeptide (CTX);

5) 3 h and 24 h urine collection for calcium and creatinine;

6) fasting sample for total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, APOB/APOA1 ratio, PAC and plasma direct renin concentration (DRC), glucose, insulin and glycated hemoglobin (HbA1c);

7) in non-diabetic patients, glucose and insulin 120 min after the administration of an oral glucose tolerance test (OGTT) with 75 g of glucose;

8) BMD at LS, FN, TH and TR and anteroposterior and lateral spinal radiographs (Appendix A);

9) transthoracic echocardiography and carotid ultrasonography;

10) upper abdomen ultrasonography;

11) risk of fall (Tinetti and Conley scale, Appendix A).

Subjects were then divided in PTx Group and no-PTx Group and then randomized to treatment with 800 UI or 2000 UI of VitD or no treatment (0 UI) for the next 24 months.

Subsequent follow-up evaluations were at 6- (T1), 12- (T2), 18- (T3) and 24- (T4) months.

Patients in the PTx Group were operated after baseline evaluation. In this group, the examinations scheduled at time T1 were performed at the suspension of the post-surgery calcium and calcitriol therapy and from there the subsequent times (T2, T3 and T4) were calculated.

In both groups at T1, T2, T3 and T4 follow-up evaluations, the parameters in points 1), 2), 3), 4) and 5) were re-evaluated.

In both groups at T2 and T4 follow-up evaluations, the parameters in points 6), 7), 8), 9), 10) and 11) were also re-evaluated.

Compliance with vitD therapy was assessed at each follow-up visit and patients with a compliance < 80% were excluded from the study.

Assays

Fasting samples for serum calcium, phosphorus, albumin, creatinine, ALP, total cholesterol, triglycerides, HDL, glycemia, HbA1c and insulin were measured by standard technique. Total calcium was corrected for serum albumin according to the formula: (total calcium + (4.4 – albumin mg/dl) x 0.8) (reference interval 8.2 - 10.2 mg/dL) (187). Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula: total cholesterol minus HDL-cholesterol minus triglycerides/5 in mg/dl (188). Serum Ca2+ was measured by ion-selective electrode (AVL LIST GmbH Medizintechnik, Graz, Austria). Urinary calcium and creatinine were measured in 24h urine collections by standard technique and calcium clearance [formula: (24-hour urine calcium x serum creatinine) / (serum calcium x 24-hour urine creatinine)] was calculated in order to exclude the presence of benign FHH. Serum intact PTH was measured by an ICMA (LIAISON N-TACT PTH 2 Assay, Diasorin, Stillwater, MN, USA, reference interval 10 - 65 pg/mL). 25OHD concentration was measured by a commercial RIA (DiaSorin, Stillwater, MN, USA). CTX were measured by the serum CrossLaps ELISA (Immunodiagnostic System Ltd) (normal values 140 -1350 pg/mL). APOA1 and APOB were measured by quantitative automated immunoturbidimetric assay (Intermedical, S.r.l., Villaricca, Italy). Insulin resistance was estimated by HOMA-index calculated from fasting blood glucose and insulin, normal values < 2.5 (189). Type 2 diabetes mellitus was diagnosed if fasting blood glucose levels were equal to or greater than 126 mg/dl or equal to or greater than 200 mg/dl after an oral load of 75 gr of glucose or if HbA1c was 48 mmol/mol or higher (190). Two tests are used to confirm the diagnosis of diabetes (190). For clinical convenience, plasma PAC and DRC were measured only in supine position by CLIA technology (LIAISON, DiaSorin, Stillwater, MN, USA) in patients not on medications known to alter the RAAS (191). Hypertension was defined as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg (192)

Transthoracic echocardiography, carotid ultrasonography and upper abdomen ultrasonography

Cardiac indexes were evaluated with high-resolution B-mode cardiac ultrasound according to a standardized protocol (193). Carotid vascular abnormalities were evaluated with high-resolution B-mode carotid ultrasound using standardized protocols (120). Values of IMT of 0.9 mm or higher were considered abnormal (194). Mean IMT was calculated by averaging the right and left IMT values while maximum IMT was the highest value of IMT found between the left and right measurements. Abdomen ultrasonography was performed using standardized protocols.

Statistical analysis

Statistical analysis was performed using SPSS version 26 statistical package (SPSS Inc, Chicago, IL). The Kolmogorov-Smirnov test was used to assess if variables were normally distributed. At baseline, differences between groups were assessed with the $\chi 2$ test or Fisher Exact test, as appropriate, for categorical variables. Comparison of continuous variables was performed using unpaired T-test or Mann-Whitney U-test, as appropriate. Categorical variables were described by number and percentages while continuous variables were expressed as mean value ± standard deviation (SD). Relationships between albumin-corrected total calcium, Ca2+, 25OHD and PTH, and glycemic and metabolic indexes and cardiac and carotid variables were assessed with Pearson or Spearman correlation, as appropriate. Partial correlation were used to measure the degree of association between two variables by controlling for confounding variables. In the whole study project population, a multiple regression analysis was carried out to determine whether PTH was an independent predictor of AH, the ventricular septum thickness, the posterior LV wall thickness and the peak transaortic pressure gradient after controlling for age, BMI, hypertension status and 25OHD levels. Moreover, a multiple regression analysis including PTH, age, AH and T2DM was used to assess whether 25OHD was an independent predictor of carotid plaque and maximum IMT values. Standardized β-coefficients were calculated to determine which predictor had the greatest effect on the outcome.

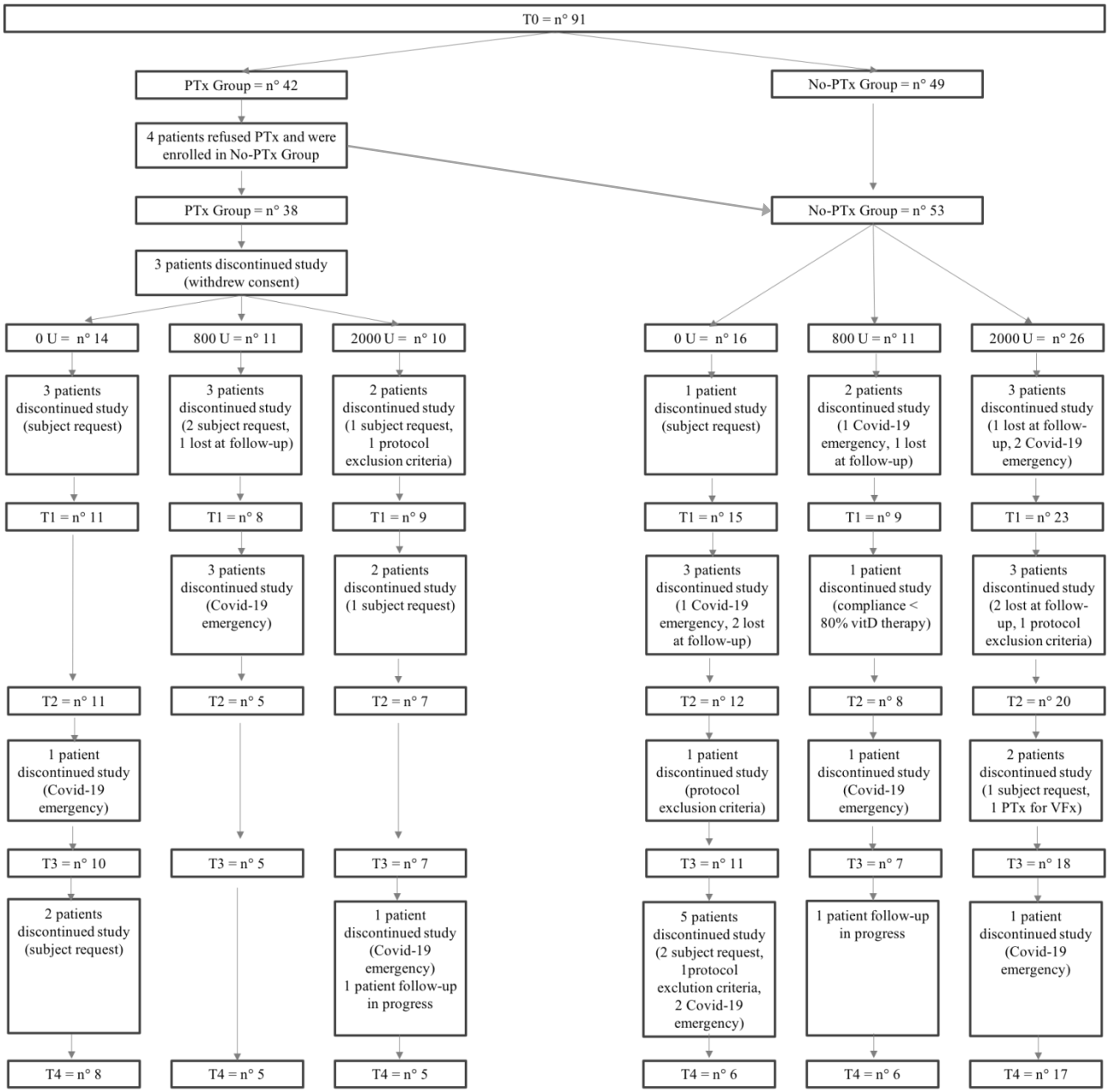
For the follow-up evaluation in PTx Group and no-PTx Group separately, a paired sample T-test was used to determine the mean differences between baseline and follow-up measures (specified for each variable). One-factor ANCOVA was used to compare means change in two time points in the whole group and in different randomization groups (0 UI, 800 UI and 2000 UI) accounting for baseline values and others covariates (specified for each variable). A repeated-measures ANOVA was used to compare within-subjects (time) and between-subjects (treatment group) mean changes in outcomes over time. The McNemar test was used to analyze differences over time on dichotomous dependent variables in different randomization groups (0 UI, 800 UI and 2000 UI). A two-tailed *P* value <0.05 was considered statistically significant.

4. RESULTS

Between November 2016 and January 2021, 91 consecutive PHPT post-menopausal woman meeting the previously listed inclusion and exclusion criteria and who agreed to participate in the VitD-PHPT project were enrolled in the study. Among them, 42 patients were enrolled in the PTx Group and 49 in the no-PTx Group. 4 patients in the PTx Group refused surgery and then were included in the no-PTx Group. Finally, 38 patients were enrolled in the PTx Group and 53 patients in the no-PTx Group. Of the 91 patients enrolled, 75 patients (28 in the PTx Group) completed the follow-up at 6 months (T1), 63 patients (23 in the PTx Group) completed the follow-up at 12 months (T2), 58 patients (22 in the PTx Group) completed the follow-up at 18 months (T3) and 47 patients (18 in the PTx Group) completed the follow-up at 24 months (Figure 3).

Unfortunately, due to the Covid-19 health emergency, 13 patients discontinued the study while 9 patients, were not able to complete all the examinations scheduled for the specific follow-up times due to unavailability of the medical/nursing staff or personal will.

Figure 3. VitD-PHPT project participants.



Footnotes. PTx Group: subjects who are candidates for parathyroidectomy, non PTx Group: subjects not fulfilling the surgical criteria for parathyroidectomy apart from osteoporosis

Baseline evaluation, characteristics of whole population.

Consistent with the known demographics of PHPT in Italy (8), the whole study group had biochemical evidence typical of mild PHPT [serum calcium = 10.2 ± 0.8 mg/dl (normal range = 8.2 - 10.2 mg/dl)]. NC-PHPT was diagnosed in 3 patients.

The prevalence of hypovitaminosis D (i.e. 25OHD < 30 pg/ml) in the whole population of VitD-PHPT study was 84.1%. The prevalence of classical complications of PHPT were: osteoporosis 75.8% (69 patients) and nephrolithiasis 31.9% (29 patients). T2DM was diagnosed in 12 (13.2%) and insulin resistance in 17 (21.5%, excluding T2DM patients) patients. Dyslipidemia was diagnosed in 64 (70%) patients, an abnormal IMT in 16 (22.9%, excluding patients on lipid-lowering therapy) patients and a carotid plaque was present in 25 (27.4%) patients. Concerning CV comorbidity, AH was diagnosed in 46 (50.5%) patients, a diastolic dysfunction in 50 (54.9%) patients [23 (54.8%) excluding hypertensive patients], valve calcifications in 11 (12.1%) patients and LV hypertrophy in 12 (13.2%) patients [1 (2.2%) excluding hypertensive patients].

In Table 6 baseline characteristics of the study participants are shown according to vitamin D status (i.e. 25OHD < 30 ng/ml vs 25OHD ≥ 30 ng/ml). There was no statistically significant difference in age, BMI, creatinine, calcium-phosphoric parameters, PAC, DRC, glycemic metabolism and lipid profile between the two groups. The prevalence of osteoporosis, fragility Fx, nephrolithiasis, AH, T2DM and dyslipidemia was not different between the two groups. Regarding the echocardiographic and carotid ultrasonography data, there were no between-group differences in all evaluated parameters (Table 7).

Table 6. Baseline characteristics of the study participants according to vitamin D status.

	25OHD < 30 (n 74)	25 OHD ≥ 30 (n 14)	p-value	Normal Range
Age (years)	62.4 ± 7.7	62.3 ± 9.2	0.97	
BMI (kg/m^2)	28.1 ± 6.6	26.1 ± 3.9	0.28	18 - 25
Creatinine (mg/dl)	0.70 ± 0.2	0.70 ± 0.1	1.00	0.4 -1.2
Calcium (mg/dl)	10.2 ± 0.8	9.9 ± 0.6	0.13	8.2 - 10.2
Ca2+ (mmol/l)	1.45 ± 0.1	1.41 ± 0.1	0.10	1.13 - 1.32
PTH (pg/ml)	157.4 ± 80.7	130.2 ± 50.7	0.23	10 - 65
Phosphorous (mg/dl)	2.8 ± 0.5	2.7 ± 0.4	0.66	2.7 - 4.5
Urinary Calcium (mg/day)	298.0 ± 128.8	281.0 ± 97.5	0.65	< 400
ALP (U/L)	95.2 ± 33.3	107.6 ± 45.0	0.23	35 - 140
CTX (pg/ml)	860 ± 400	750 ± 400	0.32	140 -1350
Glycemia (mg/dl)*	95.4 ± 19.1	87.2 ± 12.8	0.13	70 - 100
HbA1c (mmol/mol)*	39.1 ± 8.0	36.4 ± 4.3	0.27	20 - 42
HOMA index*	2.4 ± 2.0	3.2 ± 2.8	0.86	< 2.5
Total Cholesterol (mg/dl)**	211.7 ± 35.2	202.4 ± 32.8	0.47	< 200
HDL (mg/dl)**	62.6 ± 15.2	67.9 ± 17.8	0.35	> 50
LDL (mg/dl)**	129.5 ± 32.3	114.1 ± 33.0	0.19	< 129
Triglycerides (mg/dl)**	100.8 ± 39.4	101.6 ± 31.2	0.95	< 150
ApoB/A **	0.61 ± 0.2	0.60 ± 0.1	0.81	< 0.8
PAC (pg/ml)	82.9 ± 45.3	63.2 ± 36.2	0.47	37 - 310
DRC (mIU/L)	10.3 ± 5.7	9.5 ± 7.6	0.79	4.2 – 59.7
Osteoporosis, %	55 (75.3)	12 (85.7)	0.50	
Prevalent fragility fx, %***	5 (6.8)	1 (7.1)	1.00	
Nephrolithiasis, %	25 (34.2)	3 (21.4)	0.53	
Cigarette smokers, %	8 (10.8)	0 (0)	0.35	
Hypertension, %	36 (48.6)	7 (50)	1.00	
T2DM, %	11 (14.9)	1 (7.1)	0.68	
Dyslipidaemia, %	53 (71.6)	9 (64.3)	0.75	

Abbreviations: 25OHD, 25-hydroxyvitamin D; BMI, body mass index; Ca2+, ionized calcium; ALP, alkaline phosphatase total activity; CTX, C-terminal telopeptide; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; PAC, plasma aldosterone concentration; DRC, plasma direct renin concentration; Fx, fractures.

Data are expressed as mean (SD).

***** Excluding T2DM patients from the analysis.

** Excluding patients on lipid-lowering therapy from the analysis.

***Vertebral or non-vertebral fragility Fx.

Table 7. Baseline echocardiographic and carotid measures according to vitamin D status.

	25OHD < 30 (n 74)	25 OHD ≥ 30 (n 14)	p-value	Normal Range
Ejection fraction (%)	64.3 ± 4.5	64.3 ± 4.3	0.99	> 55%
Posterior LV wall, (mm)	9.8 ± 1.4	9.3 ± 1.8	0.29	6 - 10
Ventricular septum, (mm)	10.0 ± 1.5	9.3 ± 1.8	0.15	6 - 10
Peak transaortic pressure gradient, (mm Hg)	9.4 ± 5.9	6.5 ± 0.7	0.49	< 25
LV end-diastolic dimension, (mm)	45.4 ± 4.7	44.6 ± 4.9	0.31	39 - 54
sPAP (mmHg)	27.4 ± 4.7	26.3 ± 6.1	0.41	< 35
LV hypertrophy (Yes/No)	10 (13.7)	1 (8.3)	1.00	
Diastolic dysfunction (Yes/No)	41 (64.1)	8 (66.7)	1.00	
Valve calcification (Yes/No)	9 (12.5)	2 (16.7)	0.65	
Mean carotid IMT (mm)	0.83 ± 0.2	0.93 ± 0.3	0.35	0.7 - 0.9
Maximum carotid IMT (mm)	0.88 ± 0.2	0.99 ± 0.3	0.19	0.7 - 0.9
Carotid plaque (Yes/No)	23 (32.4)	1 (9.1)	0.16	

Abbreviations: PTx, parathyroidectomy; LV, Left ventricle; sPAP, estimated systolic pulmonary artery pressure; IMT, carotid intima-media thickness.
Data are expressed as mean (SD), or as number (percentages).

Baseline evaluation, PTx Group vs no-PTx Group.

As shown in Table 8, there was no statistically significant difference in age, BMI, creatinine, 25OHD levels, PAC, DRC, glycemia, HbA1c, HOMA index and lipid profile between PTx and no-PTx patients. As expected, in PTx Group serum total and ionized calcium, PTH, ALP, CTX and urinary calcium levels were higher, while serum phosphorous levels was lower. The prevalence of osteoporosis and fragility Fx was not different between the two groups while, as expected, nephrolithiasis was more frequent in PTx Group patients. No differences were found in the prevalence of T2DM and dyslipidemia between the two groups. With the same CV risk factors (including personal and family history of CV events, data not shown), patients in the PTx Group were found to be more hypertensive than patients in the no-PTx Group.

The echocardiographic and carotid ultrasonography data in PTx and no-PTx Group are shown in Table 9. Ventricular septal thickness, posterior LV wall thickness, peak transaortic pressure gradient were significantly higher in PTx Group. Similarly, the presence of LV hypertrophy was also greater in the PTx Group. There were no between-group differences in the ejection fraction, LV end-diastolic

dimension, PAPs, presence of diastolic dysfunction and valve calcification. Patients in the PTx Group had higher values of maximum carotid IMT than patients in no-PTx Group.

Table 8 . Comparison of baseline clinical and biochemical data of PTx Group vs no-PTx Group.

	No-PTx Group (n 53)	PTx Group (n 38)	p-value	Normal Range
Age (years)	61.3 ± 8.1	64.2 ± 7.9	0.10	
BMI (kg/m^2)	27.0 ± 5.4	28.8 ± 7.1	0.18	18 - 25
Creatinine (mg/dl)	0.69 ± 0.1	0.71 ± 0.2	0.50	0.4 -1.2
Calcium (mg/dl)	9.9 ± 0.5	10.6 ± 0.9	**0.00**	8.2 - 10.2
Ca2+ (mmol/l)	1.39 ± 0.1	1.52 ± 0.1	**0.00**	1.13 - 1.32
PTH (pg/ml)	121.9 ± 55.7	197.1 ± 81.2	**0.00**	10 - 65
Phosphorous (mg/dl)	2.9 ± 0.5	2.6 ± 0.4	**0.00**	2.7 - 4.5
Urinary Calcium (mg/day)	262.8 ± 90.7	343.4 ± 146.1	**0.00**	< 400
25OHD (ng/ml)	22.3 ± 11.1	19.1 ± 8.1	0.14	> 30
ALP (U/L)	91.5 ± 28.8	106.8 ± 42.6	**0.05**	35 - 140
CTX (pg/ml)	760 ± 300	960 ± 400	**0.02**	140 -1350
Glycemia (mg/dl)*	89.1 ± 9.7	88.3 ± 7.4	0.71	70 - 100
HbA1c (mmol/mol)*	36.9 ± 4.2	36.3 ± 3.7	0.50	20 - 42
HOMA index*	1.9 ± 1.0	2.5 ± 1.6	0.12	< 2.5
Total Cholesterol (mg/dl)**	213.7 ± 32.7	206.7 ± 34.6	0.40	< 200
HDL (mg/dl)**	63.9 ± 16.1	60.1 ± 16.0	0.36	> 50
LDL (mg/dl)**	130.5 ± 33.6	124.4 ± 32.3	0.46	< 129
Triglycerides (mg/dl)**	100.6 ± 34.7	106.0 ± 50.2	0.60	< 150
ApoB/A**	0.64 ± 0.2	0.57 ± 0.1	0.16	< 0.8
PAC (pg/ml)	86.2 ± 41.6	68.4 ± 50.7	0.32	37 - 310
DRC (mIU/L)	10.3 ± 5.9	9.8 ± 6.1	0.81	4.2 - 59.7
Hypovitaminosis D, %	41 (77.3)	33 (86.8)	0.38	
Osteoporosis, %	37 (69.8)	32 (84.2)	0.07	
Prevalent fragility fx, %***	2 (3.7)	4 (10.5)	0.20	
Nephrolithiasis, %	4 (7.5)[#]	25 (65.8)	**0.00**	
Cigarette smokers, %	2 (3.7)	6 (15.7)	0.05	
Hypertension, %	20 (37.7)	26 (68.4)	**0.01**	
T2DM, %	7 (13.2)	5 (13.1)	0.60	
Dyslipidaemia, %	40 (75.5)	24 (63.2)	0.15	

Abbreviations: PTx, parathyroidectomy; BMI, body mass index; Ca2+, ionized calcium; 25OHD, 25-hydroxyvitamin D; ALP, alkaline phosphatase total activity; CTX, C-terminal telopeptide; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; PAC, plasma aldosterone concentration; DRC, plasma direct renin concentration; Fx, fractures; T2DM, type 2 diabetes mellitus. Data are expressed as mean (SD), or as number (percentages).

***** Excluding T2DM patients from the analysis.

** Excluding patients on lipid-lowering therapy from the analysis.

***Vertebral or non-vertebral fragility Fx.

[#]Patients who refused surgery and were included in the no-PTx Group.

Table 9. Comparison of baseline echocardiographic and carotid measures of PTx Group vs no-PTx Group.

	No-PTx Group (n 53)	PTx Group (n 38)	p-value	Normal Range
Ejection fraction (%)	63.5 ± 4.2	65.2 ± 4.6	0.08	> 55%
Posterior LV wall, (mm)	9.4 ± 1.5	10.2 ± 1.4	**0.02**	6 - 10
Ventricular septum, (mm)	9.6 ± 1.4	10.3 ± 1.6	**0.05**	6 - 10
Peak transaortic pressure gradient, (mm Hg)	6.9 ± 2.5	12 ± 7.3	**0.01**	< 25
LV end-diastolic dimension, (mm)	44.9 ± 4.4	45.6 ± 5.3	0.50	39 - 54
sPAP (mmHg)	27.6 ± 4.2	26.8 ± 5.7	0.51	< 35
LV hypertrophy (Yes/No)	3 (5.7)	9 (25)	**0.01**	
Diastolic dysfunction (Yes/No)	30 (61.2)	20 (68.9)	0.33	
Valve calcification (Yes/No)	4 (7.7)	7 (20)	0.11	
Mean carotid IMT (mm)	0.80 ± 0.2	0.89 ± 0.2	0.12	0.7 - 0.9
Maximum carotid IMT (mm)	0.85 ± 0.3	0.95 ± 0.2	**0.01**	0.7 - 0.9
Carotid plaque (Yes/No)	12 (24.5)	13 (37.1)	0.23	

Abbreviations: PTx, parathyroidectomy; LV, Left ventricle; sPAP, estimated systolic pulmonary artery pressure; IMT, carotid intima-media thickness.
Data are expressed as mean (SD), or as number (percentages).

Baseline evaluation, relationship between 25OHD, PTH, calcium and glycemic and metabolic indexes.

Excluding patients on lipid lowering therapy from the analysis, a significant negative correlation was found between 25OHD levels and LDL cholesterol (r = - 0.323; p = 0.01). However, 25OHD was not associated with total cholesterol, HDL cholesterol, triglycerides and Apo B/A levels. The correlation between 25OHD and LDL cholesterol (r = -0.453; p = 0.00) remained significant after controlling for the effects of age, BMI, and T2DM by a third-order partial correlation.

A significant negative correlation was found between 25OHD levels and glycemia (r_s= -0.29; p = 0.01) but not with HbA1c, or HOMA index. This correlation was no longer maintained by excluding T2DM patients from the analysis (data not shown).

A significant positive correlation was found between PTH levels and BMI (r = 0.35; p = 0.00). There was no association between PTH levels and lipid profile, Apo B/A and all glycemic indexes. Total calcium level, but not Ca2+, correlated negatively with total cholesterol levels (r = -0.21; p = 0.04) . This correlation was no longer maintained by excluding from the analysis patients on lipid-lowering

therapy (data not shown). There was no association between total calcium levels and Ca2+ and HDL, LDL, triglycerides, Apo B/A levels and all glycemic indexes (data not shown).

Baseline evaluation, relationship between 25OHD, PTH, calcium and echocardiographic and carotid measures in PHPT and regression analysis.

25OHD was negatively associated with the ventricular septum thickness (r_s = -0.23; p = 0.04). There was no association between 25OHD levels and the presence of AH, diastolic disfunction, arterial pressure values (both systolic and diastolic) and with others echocardiographic parameters.

The correlation between 25OHD and the ventricular septum thickness loses statistical significance after controlling for the effects of PTH, BMI, age and systolic arterial pressure by a fourth-order partial correlation (increasing PTH, BMI, age and systolic arterial pressure were all associated to increasing ventricular septum thickness). 25OHD was negatively associated with the presence of a carotid plaque (r = -0.22, p = 0.05) and positively associated with maximum IMT values (r_s = 0.25, p = 0.03) but not with carotid medium IMT values and plaque thickness (data not shown).

Higher levels of PTH were associated with the presence of AH (r = 0.26, p = 0.02), systolic arterial pressure (r_s 0.21, p = 0.04 and r_s 0.29, p = 0.05 excluding patients on antihypertensive therapy), ventricular septum thickness (r_s= 0.34; p = 0.00), the posterior LV wall thickness (r_s = 0.34; p = 0.02), the peak transaortic pressure gradient (r_s = 0.40; p = 0.01), the presence of LV hypertrophy (r = 0.25; p = 0.04). There was no association between PTH levels and diastolic arterial pressure and with others echocardiographic parameters (data not shown). PTH levels were positively associated to maximum carotid IMT values (r_s = 0.27; p = 0.02) but not with all others carotid parameters. Excluding all hypertensive patients from the analysis, PTH remained associated with ventricular septum thickness (r_s= 0.33; p = 0.03), the posterior LV wall thickness (r_s = 0.33; p = 0.03), the peak transaortic pressure gradient (r_s = 0.60; p = 0.00) but not with the presence of LV hypertrophy (r = 0.28, p = 0.06). The correlation between PTH and AH was no longer maintained after controlling for the effects of BMI, age, presence of dyslipidemia, T2DM and smoking by a fifth-order partial correlation (r = 0.05, p =

0.63). The correlation between PTH and the ventricular septum thickness and the posterior LV wall thickness remained significant ($r = 0.57$; $p = 0.00$ and $r = 0.58$; $p = 0.00$, respectively) after controlling for the effects of 25OHD, BMI, age and systolic arterial pressure by a fourth-order partial correlation even excluding from the analysis all patients on antihypertensive therapy, ($r = 0.64$; $p = 0.01$ and $r = 0.59$; $p = 0.02$, respectively). At variance, the correlation between PTH and the peak transaortic pressure gradient after controlling for the same variables was not significant.

Total calcium and Ca2+ were positively associated with the presence of AH ($r = 0.22$, $p = 0.03$; $r = 0.21$, $p = 0.05$, respectively). There was no association between albumin-corrected calcium level and Ca2+ levels and all echocardiographic and carotid measures (data not shown).

The correlation between total calcium and Ca2+ with AH lost its significance after controlling for the effects of BMI and age (both associated with rising calcium levels and AH) by a second-order partial correlation (data not shown).

Because increasing BMI and age were significantly associated with rising PTH ($r = 0.33$, $p = 0.00$; $r = 0.29$, $p = 0.01$, respectively), increasing ventricular septum thickness ($r_s = 0.44$, $p = 0.00$; $r_s = 0.24$, $p = 0.03$, respectively), increasing peak transaortic pressure gradient ($r_s = 0.40$, $p = 0.01$; $r_s = 0.46$, $p = 0.00$, respectively), and diastolic disfunction ($r = 0.33$, $p = 0.00$; $r = 0.33$, $p = 0.00$, respectively) and BMI was associated with increasing posterior LV wall thickness ($r_s = 0.41$, $p = 0.00$) a multiple regression model including PTH, BMI and age was used to assess these relationships (Table 10). Hypertension status, known to affect cardiac structure and function in general population, was included in the regression analysis (Table 10).

Table 10: Multiple Logistic Regression models of LVH, ventricular septum thickness, posterior LV wall thickness and peak transaortic pressure gradient in the whole group of PHPT patients.

Variable	B	SE	β	p-value
LVH				
PTH	0.001	0.000	0.157	0.15
BMI	0.011	0.006	0.192	0.08
Age	-0.003	0.005	-0.070	0.51
AH	0.205	0.073	0.297	**0.01**
Ventricular septum thickness				
PTH	0.002	0.002	0.112	0.28
BMI	0.076	0.025	0.314	**0.00**
Age	0.014	0.019	0.076	0.45
AH	0.871	0.295	0.291	**0.00**
Posterior LV wall thickness				
PTH	0.003	0.002	0.159	0.14
BMI	0.070	0.025	0.292	**0.01**
Age	0.002	0.019	0.011	0.91
Hypertension	0.758	0.301	0.255	**0.01**
Peak transaortic pressure gradient				
PTH	0.011	0.012	0.148	0.34
BMI	0.387	0.158	0.384	**0.02**
Age	0.297	0.114	0.397	**0.01**
AH	-0.257	1.619	-0.023	0.86

Abbreviations: BMI, Body Mass Index; LV, left ventricle; LVH, left ventricular hypertrophy; AH, arterial hypertension.

BMI and hypertension were both significant predictors of the ventricular septum thickness and the posterior LV wall thickness regardless of PTH values and age (model coefficient of determinations R^2 = 0. 306 and R^2 = 0.262, respectively, Table 10). Using the same multiple regression model, BMI and age were both significant predictors of peak transaortic pressure gradient regardless of PTH values and age (model coefficient of determination R^2 = 0. 379).

Because increasing age was associated with the presence of a carotid plaque (r = 0.39, p = 0.00) and increasing age and IA were both significantly associated with maximum carotid IMT values (r_s = 0.25, p = 0.03 and r_s = 0.39, p = 0.00) a multiple regression model including 25OHD, PTH, age and IA was used to assess these relationships (Table 11). T2DM was included in the model because it tended to be associated with the presence of carotid plaque (r = 0.21, p = 0.06).

Table 11: Multiple Logistic Regression models of carotid plaque and maximum carotid IMT in the whole group of PHPT patients.

Variable	B	SE	β	p-value
Carotid plaque				
25OHD	-0.004	0.005	-0.090	0.42
PTH	0.000	0.001	-0.052	0.63
Age	0.024	0.007	0.404	**0.00**
AH	0.020	0.098	0.022	0.84
T2DM	0.155	0.147	0.116	0.30
Maximum carotid IMT				
25OHD	0.005	0.003	0.198	0.10
PTH	0.000	0.000	0.129	0.28
Age	0.004	0.004	0.121	0.33
AH	0.150	0.057	0.311	**0.01**
T2DM	-0.019	0.086	-0.026	0.83

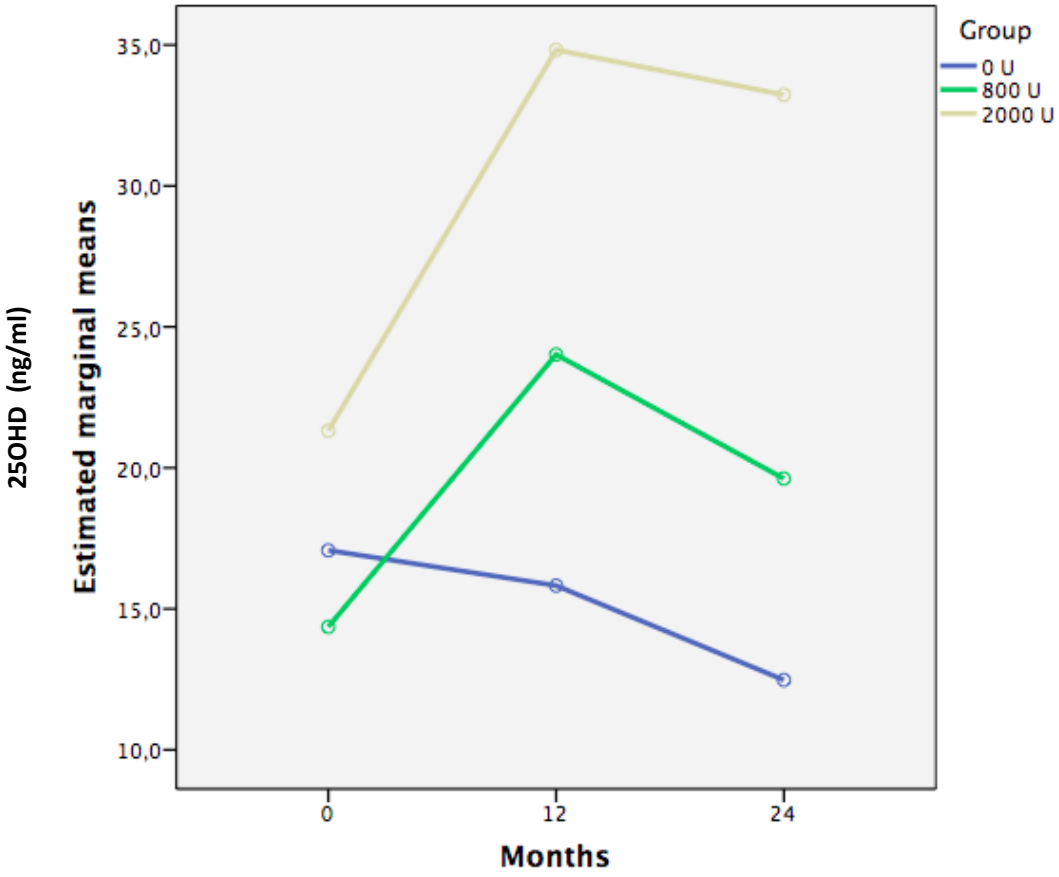
Abbreviations: 25OHD, 25-hydroxyvitamin D; AH, arterial hypertension; T2DM, Type 2 Diabetes Mellitus; IMT, carotid intima-media thickness.

Advancing age was the only significant predictor of the presence of carotid plaque regardless of 25OHD and PTH levels, AH and T2DM (model coefficient of determinations R^2 = 0. 222, Table 11). Using the same multiple regression model, AH was the only significant predictor of maximum carotid IMT regardless of 25OHD and PTH levels, age and T2DM (model coefficient of determination R^2 = 0. 193).

No-PTx group: follow-up evaluation according to the group of randomization.

As expected, there was a significant interaction between group of randomization and 25OHD levels at follow-up (F(4,44) = 2.65, p = 0.05 25OHD*group interaction), Figure 4. In particular 25OHD values at 0, 12 and 24 months, respectively were 0 U group, 17.1 (± 7.14), 15.8 (± 5.41), and 12.5 (± 2.6) ng/ml (p = 0.51 within subjects, p = 0.80 vs 800 U group, p = 0.00 vs 2000 U group); 800 U group, 14.4 (± 6.41), 24.0 (± 8.30), and 19.6 (± 2.60) ng/ml (p = 0.07 within subjects, p = 0.80 vs 0 UI group, p = 0.00 vs 2000 UI group); and 2000 UI group, 21.3 (± 8.73), 34.8 (± 10.6), and 33.2 (± 7.00) ng/ml (p = 0.00 within subjects, p = 0.00 vs 0 UI group and 800 UI group).

Figure 4. 25-hydroxyvitamin D levels in no-PTx Group patients over time in the three randomization groups.



In no-PTx group, VitD supplementation both at 800 UI and 2000 UI is not associated with an increase in serum or urinary calcium. In particular Ca2+ values at 0, 12 and 24 months, respectively, were: 0 UI group, 1.45 (± 0.08), 1.42 (± 0.09), and 1.45 (± 0.10) mmol/l (p = 0.36); 800 UI group, 1.36 (± 0.02), 1.35 (± 0.03), and 1.36 (± 0.02) mmol/l (p = 0.56); and 2000 UI group, 1.37 (± 0.04), 1.36 (± 0.05), and 1.38 (± 0.04) mmol/l (p = 0.18) [F(4,50) = 0.19, p = 0.95 ionized calcium*group interaction]. Total calcium values at 0, 12 and 24 months, respectively, were: 0 UI group, 10.2 (± 0.65), 10.0 (± 0.57), and 10.0 (± 0.70) mg/dl (p = 0.41); 800 UI group, 9.7 (± 0.39), 9.5 (± 0.42), and 9.6 (± 0.33) mg/dl (p = 0.43); and 2000 UI group, 9.8 (± 0.51), 9.7 (± 0.47), and 9.8 (± 0.40) mg/dl (p = 0.36) [F(4,52) = 0.37, p = 0.83 total calcium*group interaction]. Urinary calcium values at 0, 12,

and 24 months, respectively, were: 0 UI group, 313.4 (± 70.7), 296.7 (± 48.5), and 297.3 (± 79.9) mg/day (p = 0.81); 800 UI group, 290.9 (± 107.6), 280.0 (± 56.3), and 252.5 (± 113.8) mg/day (p = 0.78); and 2000 UI, 261.6 (± 87.0), 269.3 (± 90.4), and 277.5 (± 86.1) mg/day (p = 0.70), [F(4,44) = 0.49, p = 0.75 urinary calcium*group interaction]. No differences were seen in PTH levels within and between randomization groups (data not shown).

Ionized calcium levels, total calcium levels, PTH levels and urinary calcium excretion remained constant over time and not differ between group even when grouping patients to VitD supplementation yes (800 UI plus 2000UI group) or no (0 UI group), regardless of dose.

No differences were seen in creatinine levels and renal function (evaluated with The Cockcroft and Gault formula) within and between randomization groups (data not shown).

No differences were seen in PAC and DRC at 0, 12 and 24 months within and between randomization groups (data not shown). 2 patients developed hypertension at 12 months follow-up (both in 0 UI group, p = 0.50 vs baseline) and 2 patients developed hypertension at 24 months follow-up (1 in 0 UI group and 1 in 2000 UI group, p = 0.50 vs baseline). Excluding patients on antihypertensive therapy from the analysis, diastolic pressure, but not systolic pressure, significantly increase at 24 months follow-up (71.8 ± 8.2 vs 76.1 ± 6.5, p = 0.05) independently of randomization group (data not shown).

No differences were seen in BMI, total cholesterol, HDL, triglycerides, LDL and APO B/A levels at 0, 12 and 24 months within and between randomization group even excluding patients assuming lowering-lipids therapy (data not shown). 2 patients developed dyslipidemia at 12 months follow-up (both in 2000 UI group, p = 1.00 vs baseline) and 3 patient developed dyslipidemia at 24 months follow-up (1 in 800 UI group and 2 in 2000 UI, p = 1.00 vs baseline).

Concerning glucose metabolism, no differences were seen in glycemia, HbA1c levels and HOMA index over time within and between randomization groups excluding T2DM patients from the analysis (data not shown). No patient developed T2DM during follow-up.

No differences were seen within and between groups in all cardiac parameters during follow-up. In particular, at 12 and 24 months follow-up no differences were seen in ventricular septum thickness

(p = 0.71 and p = 0.36, respectively) and in posterior LV wall thickness (p = 0.63 and p = 0.12, respectively) between randomization groups adjusting for baseline data and the presence of hypertension and BMI values. 3 patients developed AH during the follow-up (2 in 0 UI group and 1 in 2000 UI group, p = 0.50) and in 2 patients, blood pressure control worsened with the need to increase antihypertensive therapy (both in 2000 UI group). No patients developed CV events during the follow-up.

No differences were seen within and between groups in all evaluated carotid parameters excluding patients in lipids-lowering therapy and adjusting for baseline data, age and the presence of AH (data not shown).

PTx Group: follow-up evaluation according to the group of randomization.

All patients in PTx group resulted surgically cured from PHPT. In Table 12 are reported baseline and post-PTx biochemical values. Normal calcium values were maintained in all patients until the end of follow-up (data not shown).

Table 12. Comparison of baseline and post-PTx biochemical data in PTx-Group patients.

	Pre-PTx (T0)	**Post-PTx (T1)**	**p-value**
Calcium (mg/dl)	10.5 ± 0.8	8.9 ± 0.3	**0.00**
Ca2+ (mmol/l)	1.51 ± 0.1	1.21 ± 0.0	**0.00**
PTH (pg/ml)	195.3 ± 79.3	68.6 ± 34.5	**0.00**
Phosphorous (mg/dl)	2.6 ± 0.4	3.2 ± 0.5	**0.00**
Urinary Calcium (mg/day)	340.7 ± 148.5	173.2 ± 77.1	**0.00**

Abbreviations: PTx, parathyroidectomy; Ca2+, ionized calcium.
Data are expressed as mean (SD).

As expected, there was a significant interaction between group of randomization and 25OHD levels at follow-up (F(4,22) = 3.90, p = 0.02 25OHD*group interaction). The post-hoc analysis revealed that this interaction was statistically significant only for the 2000 UI group. In particular, 25OHD values at 0, 12 and 24 months, respectively were 0 UI group, 15.3 (± 6.54), 21.4 (± 10.83), and 16.0 (± 4.87) ng/ml (p = 0.17 within subjects, p = 0.81 vs 800 UI group, p = 0.24 vs 2000 UI group); 800

UI group, 13.8 (± 5.80), 28.5 (± 13.67), and 27.9 (± 15.77) ng/ml (p = 0.14 within subjects, p = 0.81 vs 0 UI group, p = 1.0 vs 2000 UI group); and 2000 UI group, 12.5 (± 3.70), 31.1 (± 9.42), and 38.2 (± 12.35) ng/ml (p = 0.01 within subjects, p = 0.24 vs 0 UI group, p = 1.00 vs 800 UI group).

After surgery, no differences were seen in Ca2+, total calcium and PTH levels between randomization groups (data not shown). Similarly no differences were seen in urinary calcium excretion between randomization groups (data not shown).

No differences were seen in creatinine levels and renal function (evaluated with The Cockcroft and Gault formula) within and between randomization groups (data not shown).

2 patients developed AH at 12 months follow-up (both in 0 UI group, p = 0.50), 2 other patients developed AH at 24 months follow-up (1 in 0 UI group and 1 in 2000 UI group, p = 0.13 vs baseline). In 2 patients blood pressure control worsened with the need to increase antihypertensive therapy (1 in 0 UI group and 1 in 800 UI group) while in 1 patient blood pressure control improved (2000 UI group). Excluding patients on antihypertensive therapy from the analysis, both systolic and diastolic pressure significantly increase at 24 months follow-up (120.4 ± 9.5 vs 131.1 ± 13.4, p = 0.01 and 72.4 ± 4.8 vs 80.1 ± 8.5, p = 0.04, respectively) independently of randomization group (data not shown). Unfortunately, the dosages of PAC and DRC available in the PTx group were too few to allow the statistical analysis to be performed. No patients developed CV events during the follow-up.

Excluding from the analysis patients assuming lowering-lipids therapy, no differences were seen in total cholesterol, HDL, triglycerides, LDL and APO B/A levels at 0, 12 and 24 months within and between randomization group (data not shown).

4 patients developed dyslipidemia at 12 months follow-up (2 in 0 UI group, 1 in 800 UI group and 1 in 2000 UI group, p = 0.38) and 2 patients developed dyslipidemia at 24 months follow-up (both in = UI group, p = 0.25 vs baseline). A significant increment in BMI values was seen over time (p = 0.02) independently from randomization group (data not shown).

Excluding T2DM patients from the analysis, a significant increment in HbA1c levels was seen over time (p = 0.01) but this was independent from randomization group (F(4,14) = 0.425, p = 0.78

HbA1c*group interaction). No differences were seen over time in other glycemic parameters within and between randomization groups (data not shown). No patient developed T2DM during follow-up. No differences were seen within and between groups in all cardiac parameters during follow-up, however a non-significant reduction in ventricular septum thickness at 12 months (10.6 ± 1.3 vs 10.2 ± 1.5, p = 0.08) and in posterior LV wall thickness at 24 months (10.6 ± 1.2 vs 9.7 ± 2.1, p = 0.09) was seen. However, at 12 and 24 months follow-up, no differences were seen in ventricular septum thickness (p = 0.34 and p = 0.63, respectively) and in posterior LV wall thickness (p = 0.87 and p = 0.71, respectively) between randomization groups adjusting for baseline data and the presence of AH and BMI values.

No differences were seen within and between groups in all evaluated carotid parameters excluding patients in lipids-lowering therapy and adjusting for baseline data, age and the presence of AH (data not shown).

5. DISCUSSION

This study was aimed to evaluate the prevalence, nature and reversibility of CV disease and associated risk factors in a large cohort of mild PHPT patients surgically cured or observed for two years without surgical intervention. Moreover we preliminarily assessed in both group if the administration of VitD supplements could affect the evolution of these complications.

Our data showed that AH is a frequent finding even in mild PHPT patients (50.5% of our study group). Parathyroid hormone and calcium values, but not 25OHD, were associated with AH but this association was lost when data were adjusted for major CV risk factors. Moreover, AH was neither reversed nor significantly improved by PTx, and VitD administration had no effect on this outcome.

With regard to cardiac structure and function, we found a high prevalence of diastolic dysfunction even in normotensive patients (54.9%) which, however, was not associated with the presence of PHPT but rather with BMI and age. Left ventricular hypertrophy was diagnosed in 13.2% of PHPT patients. However, only 2.2% of PHPT patients without AH showed LVH and, in keeping, LVH was

predicted only by the presence of AH. Similarly, the ventricular septum thickness and posterior LV wall thickness were predicted by AH and BMI. However, at variance with LVH, PTH levels remained associated with the ventricular septum thickness and posterior LV wall thickness even after excluding hypertensive patients from the analysis and after adjusting for independent predictors of these comorbidity (i.e. age, BMI and systolic pressure values). In both PTx and no-PTx Groups, neither surgery or observation nor VitD therapy resulted in a significant modification of the echocardiographic parameters up to 24 months follow-up. Valve calcifications were present in 12.1% of patients and were related to age and to the presence of AH rather than to calcium, PTH and 25OHD levels. No improvement of valve calcifications was appreciated after PTx.

With regard to lipid metabolism and carotid pathology, we found that patients with the worst biochemical picture had significantly higher values of maximum carotid IMT. Parathyroid hormone and 25OHD levels were positively associated to maximum carotid IMT values. Moreover, a significant negative association between 25OHD levels and LDL cholesterol values and with the presence of a carotid plaque was found at baseline but no effects of VitD supplements over time on these parameters were found. However, advancing age was the only significant predictor of the presence of carotid plaque and AH was the only significant predictor of maximum carotid IMT regardless of 25OHD and PTH levels, age and T2DM. Parathyroidectomy had no effect on either the lipid profile or the carotid parameters. Finally, we did not find any association of calcium, PTH or 25OHD with all glycemic parameters nor an improvement after PTx.

Our data confirmed the frequent association of PHPT and AH even among patients with mild disease as previously reported (80, 81, 101, 103, 104). Both hypercalcemia and elevated PTH levels could affect the CV system through direct or indirect mechanisms (i.e. hypertrophic and ionotropic effect on the heart, cardiomyocytes and vascular contractility, regulation of endothelial permeability, activation of RAAS, release of endothelial pro-atherosclerotic and pro-inflammatory factors) (86-93) and in a recent population study from a national database, the presence of PHPT independently predicted the risk of AH after a multivariate regression analysis adjusted for the main CV risk factors

(age, sex, T2DM, hyperlipidemia, obesity, tobacco use and renal function) (104). In our series neither calcium nor PTH values were significantly associated with the presence of AH after correction for the same CV risk factors. Moreover, the relationship between PHPT and AH remain unclear due to the controversial effects of PTx on AH in particular in mild forms. Several studies demonstrated that following PTx there was a reduction in blood pressure after six months (105, 106), and up to 5 years (107), while others did not report significant reduction (108). The only RCT of PTx versus observation in 116 patients with asymptomatic PHPT did not demonstrate between-group differences in change in blood pressure in a 2 year analysis (109). In our study there was no improvement in blood pressure values in PTx Group patients up to 2 years of follow-up. In fact, a significant worsening of both systolic and diastolic pressures was observed at 24 months. It must be considered that in patients older than 50 years PHPT, but also AH and other CV risk factors, are frequent and may be not linked each other (195). In agreement, in Italy, the prevalence of AH in women aged 35-79 years (therefore on average younger than our study group) is high, exceeding 40% (196). Therefore, the fact that PTx did not ameliorate AH suggests that this latter is independent from PHPT. However, it also possible that structural vessel changes caused by chronically elevated PTH or longstanding EH (or a combination of both) may become irreversible with time and therefore an earlier PTx (in the first years of the disease) could probably have more beneficial effect on CV outcomes (197). Unfortunately, in most patients of our study we could not establish the onset of PHPT and the disease duration before study protocol entry and whether or not AH was already present before the diagnosis of PHPT. Moreover, a role of RAAS could be hypothesized since RAAS activity was found to be higher in PHPT patients (138, 139). Unfortunately, in our study we assessed the RAAS activity only in patients not on drug therapy known to affect the RAAS (191) and, in this subgroup, we found no activation of the RAAS.

Consistently with the lack of a causal link between mild PHPT and CV complications, the high incidence of diastolic dysfunction and LVH in our PHPT patients appeared to be linked to BMI and age and to BMI and AH, respectively, rather than to calcium, PTH or 25OHD values. Likewise,

we did not find any significant beneficial effect of PTx on CV echocardiographic parameters nor a worsening in non-PTx Group patients. Several (83, 110, 111), but not all (112-114) observational studies have reported an association between PHPT and LVH and diastolic dysfunction even in the mild form of the disease but many of the available studies are limited by the absence of a control group or lack of consideration of other coexisting CV risk factors. Results from studies assessing the changes in the cardiac structure and function after PTx are also conflicting. Indeed, only in observational studies including severe and symptomatic PHPT patients evidence of a significant benefit of PTx is reported (111, 115, 116). Differently, RCTs evaluating PTx vs observation, including patients with milder PHPT than those included in observational studies, did not reveal CV benefits of PTx (114, 117). A 2015 meta-analysis, which included 15 studies (4 RCTs) for a total of 457 patients enrolled, confirmed that CV benefits of PTx were limited to short-term studies (6 months or less in duration), with the best results obtained in observational studies that included patients with more severe disease (118). Therefore, the result of the present study reinforce the idea that in PHPT the CV complications are mainly typical of the more severe forms of the disease.

Myocardial and valvular calcifications have been consistently described in PHPT patients with severe hypercalcemia (80, 81) and studies in mild PHPT are limited. In our series valve calcifications were not associated with calcium, PTH or 25OHD levels but only with age and the AH presence and no improvement after PTx was found, in keeping with previous studies (119).

Although we have seen an association between PTH levels and maximum IMT values and between 25OHD levels and the presence of carotid plaque, our data suggest that, in mild PHPT, AH and advancing age are the major determinant of IMT and carotid plaque. Both VitD supplementation and PTx proved ineffective in these area after adjusting data for age and the presence of AH. Previous data showed that IMT was significantly higher in patients with mild PHPT as compared to a control group and that PTH was an independent predictor of carotid stiffness (120). However one study suggested that IMT was increased only in PHPT patients with associated CV risk factors (198). Moreover, as in our study population, a lack of favorable change in serum lipoproteins after PTx has

been already reported (199-201) and neither IMT nor carotid stiffness improved 1 or 2 years after PTx (119, 123).

We did not find any association of calcium, PTH or 25OHD with all glycemic parameters nor an improvement after PTx. Indeed, in our case series, a significant increase in HbA1c and BMI values was observed in PTx-Group patients during follow-up. However, previous large–scale observational and epidemiological data suggest that increased PTH concentrations are positively associated with abnormal glucose metabolism (97-99, 199) and in contrast to our results, PTx was proven to significantly decrease fasting blood glucose and insulin levels and HOMA-index even in mild PHPT and NC-PHPT patients (102, 106, 199). The reasons for the reduction in glycemic parameters after PTx and the clinical impact of these finding still remain to be clarified. Indeed, no significant correlation between beta cell function or insulin sensitivity and serum levels of calcium, phosphate, 1,25-dihydroxyvitamin D or PTH have been ever found (202).

It has been reported that 25OHD deficiency may have an independent role as a risk factor in CV disease (158-162). The proposed mechanisms whereby 25OHD contributes to CV disease include its inhibitory effects on inflammatory cytokines (203), improved glycemic control and insulin sensitivity (204), and inhibition of the RAAS (170). In particular, in PHPT patients there is a high prevalence of hypovitaminosis D (164) and lower serum 25OHD levels are associated with higher serum PTH levels potentially enhancing its deleterious CV effects (163). However, in clinical trials, VitD treatment has yielded inconclusive results on blood pressure control and CV disease risk in general population (176, 182, 185, 186). To our knowledge, this is the first study that provided data on the relationship between 25OHD, calcium and PTH levels and CV status in a non-replete mild PHPT population and that investigated changes in CV risk factors, cardiac and carotid structures without and with different doses of VitD supplements over time. At baseline, we did not find any differences in calcium and PTH levels and all CV endpoints in patients with low vitamin D values as compared to patients with normal vitamin D values. Furthermore, in our follow-up data, VitD supplementation in mild PHPT patients showed no clinical significance in this area. Moreover, it is known that 25OHD levels are

highly heritable and variants near genes involved in cholesterol synthesis, hydroxylation, and vitamin D transport affect vitamin D status (205). Genetic factors like vitamin D receptor (VDR) polymorphism, may be also associated with some CV comorbidity in PHPT patient, as shown in patients with secondary hyperparathyroidism due to renal failure (206). Unfortunately, due to the small number of patients enrolled in the different randomization groups who fully completed the follow-up at 24 months (largely due to the COVID-19 health emergency), our data on the contribution of vitamin D supplementation to CV and metabolic complications must be considered preliminary and subject to statistical bias. Despite these limitations, we confirmed that VitD supplementation is safe in patients with mild PHPT (207, 208). Indeed in no-PTx Group patients, supplementation both at 800 U and 2000 U day is not associated with an increase in serum or urinary calcium.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

Our results suggested that the high incidence of CV disease and metabolic derangements reported in mild PHPT are not tied to a simple cause-and-effect relationship but may be primarily related to the coexistence of AH, advanced age or increased BMI. In support of this hypothesis, the fact that after PTx, CV structural and functional parameters and CV risk factors did not resolve or significantly improve.

Despite the statistical limitations due to the small number of patients that completed 24-months follow-up evaluation and the lack of a complete evaluation of the RAAS in hypertensive PHPT patients, we carefully evaluated multiple aspects of the CV system in a homogeneous group of patients with mild PHPT, and we were being able to investigate the relative roles of PTH, calcium and 25OHD as distinct from the main CV risk factors. An age- and sex-matched control group could also have provided more comprehensive information on the prevalence and evolution of CV risk factors and disease and on RAAS activity compared to our mild PHPT population and this is a limitation of the present study.

In conclusion, our data support the need of a routine CV evaluation in mild PHPT patients in order to identify patients at greatest CV risk. However, the possible improvement of CV complications should not be listed among the indications for PTx. Moreover the administration of VitD supplements would seem to have a neutral effect at least as regards CV complications and risk factors in mild PHPT patients.

More prospective data on CV outcomes in mild PHPT are needed to further elucidate these points. It is probable that a follow-up of more than 24 months is necessary to highlight the effects of both PTx and VitD supplementation on the CV system. Additionally, the analysis of the polymorphic variants of the VDR gene could further clarify the relationship between PHPT, 25OHD and CV disease.

7. APPENDIX A. Supplementary material on methods.

BMD and fractures

BMD was measured by dual X-ray absorptiometry (DEXA, Hologic Discovery, Software version 13.3:3, Bedford MA, USA), at the lumbar spine (L1–L4, LS in vivo precision 1.0%), femoral neck (FN, in vivo precision 1.8%), total hip (TH, in vivo precision 1.7%) and non-dominant forearm (distal third of radius, TR, in vivo precision 1.4%). The technologist performing DEXA was blinded to the patient treatment category. BMD was expressed as T-scores (difference from the mean BMD value of healthy young people in SD units) or Z-scores (age-matched comparison in SD units) .

At the same intervals, a conventional spinal radiograph in lateral and anteroposterior projection (T4–L4) was obtained with standardized technique and VFx diagnosed on visual inspection using the semiquantitative visual assessment (209).

The presence of other incident, non-vertebral, fractures was investigated with a detailed anamnestic collection at each visit.

Osteoporosis was diagnosed if LS or FN or TH or TR T-score was ≤ -2.5 and/or in the presence of one or more fragility fractures (210).

Risk of fall

The balance, gait and risk of fall were assessed by Conley Scale and Tinetti Scale (211, 212). The Conley Scale consists of six dichotomous items (Figure 5). Each item's lowest score is always 0, whereas the highest score range from 1 to 3. The total score of the scale may range from 0 to 10, where 0 indicates the absence of risk of falling and scores > 2 indicate higher risk of falling (211). Scoring of the Tinetti Scale (Figure 6) is done on a three point ordinal scale with a range of 0 to 2. A score of 0 represents the most impairment, while a score of 2 represents independence. The individual scores are then combined to form three measures; an overall gait assessment score, and overall balance assessment score, ad a combined gait and balance score. The maximum score for the gait

component is 12 points. The maximum score for the balance component is 16 points. The maximum total score is 28 points. In general, patients who score ≤ 24 points are at risk for fall with the higher risk for < 19 points (212).

Figure 5. Conley Scale.

	YES	NO
History (patient report)		
1- Have you ever fallen in the last 3 months?	2	0
2- Have you ever experienced dizziness or vertigo in the last 3 months?	1	0
3- Have you ever wet or soiled yourself on the way to the bathroom in the last 3 months?	1	0
Observations (nursing assessment and observation)		
4- Impaired gait/shuffle/wide base, unsteady walk	1	0
5- Agitation	2	0
6- Impaired judgments/lack of safety awareness	3	0
TOTAL		

Figure 6. Tinetti Scale.

NAME:_____ DATE:_____

BALANCE

Instructions: Person is seated in a hard armless chair. The following maneuvers are tested.

1. Sitting balance
Leans or slides in chair 0
Steady, safe 1 ____

2. Rising
Unable without help 0
Uses arms to help 1
Able without use of arms 2 ____

3. Attempts to rise
Unable without help 0
Requires more than one attempt 1
Able to rise with one attempt 2 ____

4. Immediate standing balance (first 5 sec)
Unsteady (staggers, moves feet, trunk sways) 0
Steady but uses walker or cane or grabs other objects for support 1
Steady without any support 2 ____

5. Standing balance
Unsteady 0
Steady but has wide stance (medial heels more than 4 inches apart) or uses cane or walker or other support 1
Narrow stance without support 2 ____

6. Nudged (person stands with feet as close as possible; examiner pushes light on person's sternum with palm of hand three times)
Begins to fall 0
Staggers, grabs, but catches self 1
Steady 2 ____

7. Eyes closed
Unsteady 0
Steady 1 ____

8. Turning 360 degrees
Discontinuous steps 0
Continuous steps 1
Unsteady (grabs, staggers) 0
Steady 1 ____

9. Sitting down
Unsafe (misjudges distance, falls into chair) 0
Uses arms or not a smooth motion 1
Safe, smooth motion 2

Balance score _____/16

GAIT

Instructions: Person stands with examiner, walks down Hallway or across room, first at usual pace, then back at rapid but safe pace (using usual walking aid such as cane or walker).

10. Initiation of gait
Any hesitancy or multiple attempts to start 0
No hesitancy 1 ____

11. Step length and height
(a) Right swing foot
Does not pass left stance foot with step 0
Passes left stance foot 1
Right foot does not completely clear floor 0
Right foot completely clears floor 1 ____
(b) Left swing foot
Does not pass right stance foot with step 0
Passes right stance foot 1
Left foot does not clear floor completely 0
Left foot completely clears floor 1 ____

12. Step symmetry
Right & left step length do not appear equal 0
Right & left step appear equal 1 ____

13. Step continuity
Stopping or discontinuity between steps 0
Steps appear continuous 1 ____

14. Path (estimate in relation to 12-inch floor tiles; observe excursion of 1 ft over about 10 ft of the course.
Marked deviation 0
Mild or moderate deviation or uses walking aid 1
Straight without walking aid 2 ____

15. Trunk
Has marked sway or uses walking aid 0
No sway but has flexion of knees or back or spreads arms out while walking 1
No sway, no flexion, no use of arms, and no use of walking aid 2 ____

16. Walking stance
Heels apart 0
Heels almost touch while walking 1 ____

Gait Score _____

Total _____/28

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