Universitat Autònoma de Barcelona

PREDICTIVE BIOMARKERS OF HYPERCALCIURIA IN PATIENTS WITH PERMANENT POSTSURGICAL HYPOPARATHYROIDISM. CLINICAL IMPLICATIONS

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DEDICATORIA

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Dedicado a los pacientes que un día perdieron la función paratiroidea como daño colateral de una intervención quirúrgica de tiroidectomía que yo mismo pude haber indicado.

AGRADECIMIENTOS

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ABBREVIATIONS

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- **BID:** twice a day
- **CaSR**: calcium-sensing receptor
- **CI**: confidence interval
- **DCT**: distal convoluted tubule
- **EMA**: European Medicines Agency
- **eGFR**: estimated glomerular filtration rate
- **HIAA**: 5-hydroxyindole acetic acid
- **HR**: hazard ratio
- **IV**: intravenous
- **MELAS**: mitocondrial encephalopathy lactic acidosis stroke
- **MgSO4**: magnesium sulphate
- **N/A**: not applicable
- **OPG**: osteoprotegerin
- **PABA**: para-aminobenzoic acid
- **PTH**: parathyroid hormone
- **QoL**: quality of life
- **RANKL**: receptor activator of nuclear factor κB ligand
- **rhPTH**: recombinant human PTH
- **ROC**: receiver operating characteristics
- **SD:** standard deviation
- **TAL**: thick ascending loop
- **TID:** ter in die (3 times/day)

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1.- SUMMARY

1.1 Summary

Hypercalciuria is an adverse event of postsurgical hypoparathyroidism treatment which can lead to nephrolithiasis, nephrocalcinosis and renal insufficiency. The collection of 24-hour urine is often considered an unreliable method to detect hypercalciuria.

The objective of the study was to find useful predictive biomarkers of hypercalciuria in patients with permanent postsurgical hypoparathyroidism receiving treatment with oral calcium and calcitriol supplements.

We performed a prospective cross-sectional study in an outpatient hospital clinical setting. A total of 42 consecutive stable patients with permanent postsurgical hypoparathyroidism taking oral calcium and calcitriol supplements, and 17 adult controls without hypoparathyroidism, were included. The main outcome measure was hypercalciuria, defined as 24-hour urine calcium above 300 mg.

Results: As expected, of the variables compared between controls and patients, only those that were related to the hypoparathyroid condition showed significant differences (calcium supplements, calcitriol dosage, serum parathyroid hormone, and urinary calcium excretion). Patients without hypercalciuria (n=26) *vs.* those with hypercalciuria (n=16) had lower levels of serum 1,25-dihydroxyvitamin D (32.4$\pm$11.7 pg/ml *vs*. 45.8$\pm$9.3 pg/ml; p<0.001), lower albumin-corrected serum calcium (8.42$\pm$0.46 mg/dl *vs*. 8.73$\pm$0.48 mg/dl; p=0.047), and similar serum parathyroid hormone levels (12.7$\pm$5.4 pg/ml *vs.* 11.2$\pm$7.4 pg/ml; p:ns). Multiple linear regression analysis showed a significant independent relationship between 1,25-dihydroxyvitamin D and urinary calcium excretion (B= 7.566$\pm$1.679; p<0.001). A cut-off value of 33.5 pg/ml for serum 1,25-dihydroxyvitamin D to predict the absence of hypercalciuria had 100% sensitivity and 65.4% specificity, and the area under the ROC curve was 0.829. No patients with serum 1,25-dihydroxyvitamin D under 33.5 pg/ml presented hypercalciuria, regardless of the level of albumin-corrected serum calcium.

Five patients with hypercalciuria, albumin-corrected serum calcium level above 8.5 mg/dl, and serum levels of 1,25-dihydroxyvitamin D above 33.5 pg/ml, were

treated by having their vitamin D3 supplements and calcitriol doses reduced, without thiazide diuretics being added. They normalized their urinary calcium excretion, and their serum 1,25-dihydroxyvitamin D levels were simultaneously lowered to less than 33.5 pg/ml.

Conclusions: Serum 1,25-dihydroxyvitamin D is a useful biomarker to predict the absence of hypercalciuria in patients with permanent postsurgical hypoparathyroidism who are receiving treatment with oral calcium and calcitriol supplements. The measurement of serum 1,25-dihydroxyvitamin D will permit us to limit the measurement of 24-hour urine calcium to those patients with serum levels of 1,25-dihydroxyvitamin D above 33.5 pg/ml.

We propose an algorithm based on the monitorization of serum 1,25-dihydroxyvitamin D that will improve the clinical management of patients with permanent postsurgical hypoparathyrodism that are receiving supplementary treatment.

1.2 Resumen

La hipercalciuria es un efecto adverso del tratamiento de los pacientes con hipoparatiroidismo postquirúrgico que puede provocar litiasis renal, nefrocalcinosis e insuficiencia renal. La recogida de orina de 24 horas para detectar hipercalciuria suele considerarse un método poco fiable.

El objetivo del estudio fue buscar biomarcadores predictores de hipercalciuria en pacientes con hipoparatiroidismo postquirúrgico que recibían tratamiento con suplementos de calcio y calcitriol.

Realizamos un estudio prospectivo transversal en un entorno clínico hospitalario ambulatorio. Se incluyeron 42 pacientes consecutivos estables afectos de hipoparatiroidismo postquirúrgico permanente que eran tratados con suplementos orales de calcio y calcitriol y 17 controles adultos sin hipoparatiroidismo. La variable principal fue la hipercalciuria, definida como una excreción urinaria de calcio superior a 300 mg/24 horas.

Resultados: como era de esperar, de las variables comparadas entre los controles y los pacientes, sólo aquellas relacionadas con el estado de hipoparatiroidismo mostraron diferencias estadísticamente significativas (suplementos de calcio, dosis de calcitriol, hormona paratiroidea sérica y excreción urinaria de calcio). Los pacientes sin hipercalciuria (n=26) *vs.* aquéllos con hipercalciuria (n=16) tuvieron niveles inferiores de 1,25-dihidroxivitamina D sérica (32,4$\pm$11,7 pg/ml *vs*. 45,8$\pm$9,3 pg/ml; p<0,001), así como de calcio sérico corregido por la albúmina (8,42$\pm$0,46 mg/dl *vs*. 8,73$\pm$0,48 mg/dl; p:0,047), y una concentración similar de hormona paratiroidea sérica (12,5$\pm$5,4 pg/ml *vs.* 11,2$\pm$7,4 pg/ml; p:ns). En el análisis de regresión lineal múltiple se apreció una relación independiente y estadísticamente significativa entre la 1,25-dihidroxivitamina D y la excreción urinaria de calcio (B= 7,566$\pm$1,679; p<0,001). Un valor de punto de corte en 33,5 pg/ml de la 1,25-dihidroxivitamina D sérica para predecir la ausencia de hipercalciuria tuvo un 100% de sensibilidad y un 65,4% de especificidad, con un área bajo la curva ROC de 0,829. Ningún paciente con un nivel sérico de 1,25-dihidroxivitamina D inferior a 33,5 pg/ml tuvo hipercalciuria, cualquiera que fuese el nivel de calcio sérico corregido por la albúmina.

Cinco pacientes con hipercalciuria, con un calcio sérico corregido por la albúmina superior a 8,5 mg/dl y con un nivel de 1,25-dihidroxivitamina D superior a 33,5 pg/ml fueron tratados mediante reducción en las dosis de los suplementos de vitamina D3 y calcitriol, sin añadirles diuréticos tiazídicos. En todos ellos se normalizó la excreción urinaria de calcio al tiempo en que los niveles séricos de 1,25-dihidroxivitamina D descendieron por debajo de 33,5 pg/ml.

Conclusiones: la concentración sérica de 1,25-dihidroxivitamina D es un biomarcador útil para predecir la ausencia de hipercalciuria en los pacientes con hipoparatiroidismo postquirúrgico permanente que reciben tratamiento con suplementos orales de calcio y calcitriol. La determinación del 1,25-dihidroxivitamina D permite restringir la medición de la excreción urinaria de calcio en 24 horas a los pacientes con valores séricos de 1,25-dihidroxivitamina D sérico por encima de 33,5 pg/ml.

Proponemos un algoritmo basado en la monitorización de los niveles séricos de 1,25-dihidroxivitamina D que puede mejorar el manejo clínico de los pacientes con hipoparatiroidismo postquirúrgico en tratamiento con suplementos orales de calcio y calcitriol.

2.- INTRODUCTION

2.- INTRODUCTION

Postsurgical hypoparathyroidism is a rare condition and it is the most frequent form of hypoparathyroidism. Usually, after a surgical thyroidectomy, some patients become hypoparathyroid, for a few weeks in most cases, and permanently in less so. The treatment of hypoparathyroidism requires careful attention to ensure a good quality of life for patients, and to prevent the appearance of adverse events such as hypocalcemic episodes, seizures, cataracts, nephrolithiasis, nephrocalcinosis, renal insufficiency, etc.

Research on hypoparathyroidism is scarce. In recent years, most of the papers refer to a new modality of treatment with human-recombinant parathyroid hormone that is still lacking in wide indication. Meanwhile, clinical studies on hypoparathyroidism may contribute to validating, extending or modifying the classical recommendations that were established many years ago about the best control for patients with hypoparathyroidism.

A thorough review of the basic and clinical issues is necessary to better understand the physiopathological alterations in hypoparathyroidism, and to appreciate the importance of hypercalciuria in these patients. In this thesis, I carry out a review of the postsurgical hypoparathyroidism, and hypercalciuria in patients with hypoparathyroidism, before presenting a prospective cross-sectional clinical study addressed to searching for ways to improve the detection and control of hypercalciuria. The results of this study offer an interesting new biomarker that makes the detection of hypercalciuria more reliable and contributes to its resolution by means of therapeutic adjustements. Finally, the conclusions and the lines of future research are reported.

I am aware of some redundancies in the text, written for the best understanding of each chapter without having to read the previous chapters. I apologize for any inconvenience this may cause.

3.- POSTSURGICAL HYPOPARATHYROIDISM

3.- POSTSURGICAL HYPOPARATHYROIDISM

Postsurgical hypoparathyroidism is the most common form of hypoparathyroidism [1-8]. Between 65% and 90% of chronic hypoparathyroidism cases are due to postsurgical etiology [4,9-12].

Surgery on the thyroid or parathyroid glands or adjacent neck structures and neck dissection surgery for malignancy may lead to acute and chronic hypoparathyroidism. Postoperative hypoparathyroidism is more likely to occur in patients who have undergone more than one neck operation and/or when extensive thyroid resection is required. Surgery for substernal goiter, and head or neck malignancies involving the anterior neck structures or Graves' disease, have all been shown to increase the risk of postoperative hypoparathyroidism. Apart from hypothyroidism, chronic hypoparathyrodiism is the most common long-term complication after total thyroidectomy [3,5,13-15].

3.1.- Epidemiology

A broad range of criteria have been used to define the incidence of postsurgical hypoparathyroidism: (1) clinical criteria, symptomatic *versus* asymptomatic; (2) biochemical parameters, serum calcium and/or intact PTH levels below specified levels; (3) therapeutic criteria, requirement for calcium and or vitamin D treatment; and (4) duration of calcemic support, time interval of the above therapy [14,16] . Besides different definitions of postsurgical hypoparathyroidism, Edafe, *et al.* [17] observed that the studies used different methods of assessment of outcomes and predictors, so clearly, robust definitions are warranted. In addition, most studies were single-arm cohort studies in which intergroup comparisons were made within subgroups of the same cohort. Few studies used multivariable analysis to adjust for potential confounders, and factors included in individual multivariable analyses varied across studies [17]. The review of Lorente-Poch, *et al.* [16] summarizes the problems in estimating prevalence of postsurgical hypoparathyroidism (Table 1).

Lack of clear definitions
Conflict of interest
Variety of laboratory ranges for normocalcemia and reference values
Timing of blood sampling in the postoperative period
Wide range in thyroid procedures included in the analysis
Different case mix
Small series
Missing data innational audits
Different policies for calcium and vitamin D supplements
Short/incomplete follow-up
Follow-up not performed by the surgical team but by referring physicians

Table 1. Problems in estimating prevalence of postsurgical hypoparathyroidism [16].

The rates of postsurgical hypoparathyroidism vary across centers and with different procedures and surgical expertise. In addition, attempting to compare data from surgical series is difficult because of the diversity of postoperative electrolyte supplementation protocols and discharge criteria utilized by different surgeons. Regimens vary from no supplementation (unless the patient exhibits symptoms of hypocalcemia) to empiric calcium, magnesium, and vitamin D derivative supplementation. Some protocols utilize intra- and/or post-operative serum calcium or intact PTH levels to guide degree of supplementation and discharge status [18].

Estimation of the incidence of postsurgical hypoparathyroidism reveals that despite the goal of preserving parathyroid glands, transient hypoparathyroidism that occurs days to weeks after surgical manipulation of neck structures is very common, ranging from 6.9% to 50% [5,8,15,17,19,20-35]. Usually, hypocalcemia will occur after 10% to 20% of thyroidectomies [36] and this increases to 50% or more after thyroidectomy for Graves' disease or thyroid cancer, particularly when combined with a bilateral central compartment lymph node dissection [29,37,38].

Anywhere from 3% to 30% of patients with postoperative hypocalcaemia will develop chronic or permanent hypoparathyroidism after 6 to 12 months on from a total thyroidectomy [33]. Permanent hypoparathyroidism can occur with a frequency as high

as 6.6% of patients undergoing thyroid surgery [2,7,8,15,17,24,26,30-33,35]. Surgical centers with experienced endocrine surgeons and a high case volume report rates of post-thyroid surgical permanent hypoparathyroidism of 0.9-1.6% [5,22,23,25,27,36,39,40] which could be underestimates [16,26,30,41]. The addition of a central compartment lymphadenectomy increases this risk to between 1% and 15% [5,26,28,29]. These studies emphasize the importance of expertise and experience.

3.2.- Etiopathogenesis

Postoperative hypoparathyroidism is a multifactorial phenomenon. It is worth noting that the extent of resection and surgical technique may have a greater impact on permanent postoperative hypoparathyroidism than thyroid pathologic condition [22].

Etiologies of postsurgical hypoparathyroidism include injury to the parathyroid glands or their blood supply and inadvertent resection of parathyroid tissue [35,42]. The extent of cervical dissection determines the risk of development of hypoparathyroidism [43]. Transient hypocalcemia is the consequence of prolonged manipulation and transient hypoxia of one or more parathyroid glands, whereas the loss of more than one parathyroid may result in mild to severe definitive hypocalcemia [5]. Risk factors for postsurgical hypocalcemia are listed in Table 2.

The manipulation of the parathyroid glands, even without their removal, can lead to transient disruption of PTH production and/or release. This is the reason for most iatrogenic hypoparathyroidism occurrences. Bilateral central neck operations, including total thyroidectomy, bilateral central neck dissection, and total laryngectomy can result in hypoparathyroidism even in circumstances in which the parathyroid glands themselves are identified and preserved [14,32,44,48,52-54]. The risk for postoperative hypocalcaemia is between 2.0 and 2.7 times higher when central neck dissection is performed [32,53] because the inferior parathyroid glands are located in the central neck compartment [44,48,53]. The increased morbidity with central lymph node dissection may be attributed in part to operator expertise [44,53,55,56].

Manipulation or removal of 1 or more unilateral parathyroid glands (e.g., in focused parathyroid exploration or thyroid lobectomy), whether normal or hyperfunctioning, generally does not result in transient hypoparathyroidism as long as

there are other normal undisturbed ipsi- or contra-lateral parathyroid glands [57]. Hypocalcemia can occur in patients at risk for hungry bone syndrome including elderly patients with osteoporosis and those with very large parathyroid adenomas or longstanding parathyroid disease, but this is not true hypoparathyroidism [58].

Fewer parathyroid glands identified during total thyroidectomy may result in gland injury and accidental parathyroidectomy [22,26]. A few parathyroid glands kept *in situ* due to incidental parathyroidectomy or autotransplantation has repeatedly been reported to be a crucial factor leading to acute parathyroid insufficiency [15,16,30,35,40,44,56].

Risk factors for postsurgical hypocalcemia and hypoparathyroidism
Bilateral central neck surgery
Surgery for thyroid malignancy with or without central neck dissection
Surgery for parathyroid hyperplasia
Bilateral neck exploration for primary hyperparathyroidism
Autoimmune thyroid disease
Hungry bone syndrome
Subesternal goiter
Accidental or inadvertent parathyroidectomy
Parathyroid auto-transplantation
Number of functioning parathyroid glands remaining *in situ*
Prior central neck surgery
Age younger than 45 years and age older than 50 years
Female sex
Vitamin D deficiency
Pregnancy
Lactation
Prior gastric bypass surgery
Use of drains after thyroid surgery
Prolonged duration of surgery
Low-volume thyroid surgeon

Table 2. Risk factors for postsurgical hypoparathyroidism [5,6,14,15,17,22,25,26,30,31,33-35,42,44-51].

In cases of total parathyroidectomy for multigland parathyroid disease with autotransplantation, a period of postoperative hypoparathyroidism can be anticipated [26]. For this reason, some surgeons prefer a subtotal parathyroid resection. Such patients will require medical support during the immediate postoperative period [14].

Autoimmune and inflammatory thyroid disease, whether Hashimoto thyroiditis or Graves' disease, increases the risk of postsurgical hypoparathyroidism with total thyroidectomy [59]. Graves' disease has been associated with both transient and permanent hypocalcemia [22]. This could be due to increased bone turnover, and difficult operations owing to increased vascularity of the thyroid gland [60].

Classic hungry bone syndrome refers to the rapid, profound, and prolonged hypocalcaemia associated with hypophosphataemia and hypomagnesaemia, and this is exacerbated by suppressed PTH levels, which follows parathyroidectomy in patients with severe primary hyperparathyroidism and preoperative high bone turnover. It is a relatively uncommon but serious adverse effect of parathyroidectomy. The severe hypocalcaemia is believed to be due to increased influx of calcium into bone, due to the sudden removal of the effect of high circulating levels of PTH on osteoclastic resorption, leading to a decrease in the activation frequency of new remodelling sites and to a decrease in remodelling space. Various risk factors have been suggested for the development of hungry bone syndrome, including older age, weight/volume of the resected parathyroid glands, radiological evidence of bone disease, and vitamin D deficiency. The syndrome is reported in 25–90% of patients with radiological evidence of hyperparathyroid bone disease *versus* only 0–6% of patients without skeletal involvement. Normalization of bone turnover is required for resolution of the hypocalcaemia which may last for a number of months after successful surgery. Preoperative treatment with bisphosphonates has been suggested to reduce post-operative hypocalcaemia [61].

A similar but less severe form of the hungry bone syndrome may also be observed following medical or surgical treatment of hyperthyroidism associated with high bone turnover, in which hypocalcaemia may occur in up to 46% of patients and may last for up to 12 weeks after initiation of treatment. In contrast to the case with the hungry bone syndrome of severe hyperparathyroidism, in treated hyperthyroidism, hypocalcaemia is associated with appropriately significant increases in PTH levels [21,61].

The results of previous studies searching for an association between vitamin D deficiency and post-thyroidectomy hypocalcaemia have been somewhat contradictory. Although vitamin D deficiency has been proposed a risk factor for postsurgical hypoparathyroidism in some studies [41,47,49,62], others authors found no correlation between preoperative vitamin D levels and the low post-thyroidectomy calcium level [45,63,64]. To the best of our knowledge, there have been no intervention studies with vitamin D metabolites other than calcitriol or alphacalcidol (these metabolites are not intended to replace a state of vitamin D deficiency) that have been designed to clarify this issue. There are several possible explanations for the contradictory findings of these studies: (1) some studies are retrospective with relatively low numbers of patients, (2) the metabolite of vitamin D evaluated (25-dihydroxyvitamin D or 1,25-dihydroxyvitamin D) and the definitions of vitamin D deficiency and hypocalcaemia vary between studies, (3) the use of selective and/or routine calcium/vitamin D supplementation is variable, and (4) variables related to type of intervention taken into account such as the number of functioning glands left behind, the extent of surgery, the experience of the surgeon, hyperthyroidism, retrosternal goiter, concomitant neck dissection, and thyroid carcinoma.

The cause of hypocalcemia after thyroidectomy is mainly secondary to trauma to the parathyroids resulting in transient or permanent hypoparathyroidism. Intact parathyroids will maintain normal serum calcium as long as there is calcium available in bone that can be mobilized. Moreover, the accepted levels for vitamin D deficiency identify people who are completely asymptomatic. Parathyroid hormone regulates the 1-α-hydroxylase in the kidney, which is the enzyme that converts 25-hydroxyvitamin D to its biologically active form, 1,25-dihydroxyvitamin D. Thus, a possible explanation for the lack of an association between vitamin D deficiency and post-thyroidectomy hypocalcaemia is that hypoparathyroidism results in decreased conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D regardless of the amount of 25-hydroxyvitamin D available [64]. This theory is supported by studies that have shown that the routine postoperative administration of calcitriol or alphacalcidol does have some benefit in reducing the risk of hypocalcemia after thyroidectomy [28].

However, vitamin D deficiency in patients with autoimmune hyperthyroidism may be a risk factor for postsurgical hypocalcaemia. Increased bone turnover and increased calcium influx from bone may prevent development of secondary

hyperparathyroidism of vitamin D–deficiency states in Graves disease. Postoperative transient hypoparathyroidism has been found to be the most significant parameter to determine the development of postoperative hypocalcaemia in vitamin D-deficient hyperthyroid patients [49].

Preoperative disorders of calcium and vitamin D absorption and metabolism such as those seen in patients who have undergone bariatric surgery (especially Roux-en-Y gastric bypass) also increase the risk of postoperative hypocalcemia following bilateral central neck surgery [65]. Pregnancy and lactation may place a patient at increased risk of postoperative hypocalcemia [66, 67] probably due to vitamin D deficiency [68]. Older age and female sex are also associated with vitamin D deficiency [69,70].

Unrecognized prior reduction in parathyroid function, such as that following prior central neck surgery, where 1 or more parathyroid glands might have been unknowingly removed or compromised, increases the risk of hypoparathyroidism after additional central neck surgery. Such increased risk may not be detectable with preoperative testing [14].

3.3.- Actions to minimize postsurgical hypoparathyroidism

Strategies directed to minimizing postsurgical hypocalcemia include preoperative evaluation of vitamin D status and prevention of hypocalcaemia with glucocorticoid treatment, and intraoperative parathyroid preservation and autotransplantation.

3.3.a Preoperative strategies: evaluation of vitamin D status and glucocorticoid treatment

Some authorities have proposed measuring 25-hydroxyvitamin D blood levels preoperatively in order to mitigate postoperative hypoparathyroidism. If the patient is identified as being severely vitamin D-deficient (25-hydroxyvitamin D lower than 20 ng/mL), then aggressive treatment with high-dose vitamin D (a daily ergocalciferol dosage of 50,000 IU can safely be given for 5 days) should be considered [52]. If 25-hydroxyvitamin D is between 20 and 30 ng/mL, less aggressive replacement is sufficient; typically, 50,000 IU vitamin D3 (cholecalciferol) is given by mouth weekly to

correct the vitamin D deficiency and hopefully reduce the risk of postoperative hypocalcaemia. Ergocalciferol or vitamin D2 is also an option for replacement. Care should be taken in cases of hyperparathyroidism not to cause a further significant elevation of serum calcium with vitamin D therapy [14,71].

In addition to increasing the predictive value of serum phosphate in postsurgical hypocalcaemia (see chapter 3.4.c), vitamin D replacement also reduces the risk of hungry bone syndrome [63].

Dexamethasone (8 mg intravenously) given 90 minutes before skin incision is not yet a standard but has been shown to reduce both the rate of transient hypoparathyroidism (12.8% *versus* 37% in the placebo group) and laryngeal nerve palsy (4.9% *versus* 8.4% in the placebo group) in a single study [72].

3.3.b Intraoperative parathyroid preservation and autotransplantation

Intraoperative preservation of the parathyroid glands with their blood supply intact, typically from the inferior thyroid artery, is paramount for preventing hypoparathyroidism. The question of how many parathyroids must be preserved to maintain a normal serum calcium level remains unresolved. Most authors believe that a single functioning gland is enough to restore normal parathyroid activity [2,73], but others believe that the integrity of at least 3 glands is necessary [47]. Thomusch, *et al.* [22] performed a large study and demonstrated that at least 2 parathyroid glands should be identified and preserved during bilateral thyroid surgery to avoid permanent postoperative hypoparathyroidism. However, during thyroid surgery, the identification of the parathyroid glands is not always possible [74]. Lorente-Poch, *et al.* [30] confirmed an inverse relationship between the number of parathyroid glands remaining *in situ* and the incidence of postoperative hypoparathyroidism. In contrast, Puzziello, *et al.* [5] in a prospective study of 2,361 thyroidectomies reported a higher risk of transient hypocalcaemia associated with intraoperative identification and preservation of parathyroid glands (29.2% *versus* 18.7% in patients in whom the identification of parathyroid glands was not achieved; $p<0.01$); however, the risk of permanent hypocalcaemia was five times lower.

There is considerable controversy as to whether autotransplantation may help to preserve parathyroid function [33,75]. Autotransplantation of a parathyroid gland is less effective than a careful preservation of the parathyroid with its vascular supply to avoid postsurgical hypoparathyroidism [15,16,30,31,76]. Parathyroid autotransplants may be placed in neck or forearm muscle. The latter may be preferred to avoid revision neck surgery and recurrent laryngeal nerve injury. Recovery from hypoparathyroidism in patients undergoing parathyroid autotransplantation is known to generally to take from 7 days to 1 month postoperatively [74]. There is some literature suggesting that autotransplantation is not always functionally successful [15,26]. The problem with many studies that have investigated autotransplantation following thyroid cancer surgery is that it is hard to know if residual parathyroid function is related to preserved glands or the transplanted gland(s) [14].

A large retrospective study comparing the occurrence of hypoparathyroidism after autotransplantation of 0, 1, 2, or 3 parathyroid glands following total thyroidectomy found 9.8%, 11.9%, 15.1%, and 31.4% ($p<0.05$) incidence rates of temporary hypoparathyroidism, respectively; the corresponding rates for permanent hypoparathyroidism were 0.98%, 0.77%, 0.97%, and 0% (p: ns) [37]. The number of parathyroid glands autotransplanted seems to be inverse to the reduction of temporary hypoparathyroidism, and not related to the prevention of permanent hypoparathyroidism. Routine autotransplantation *versus* selective autotransplantation of a parathyroid gland in a study resulted in an increased incidence of transient hypocalcemia (25% *vs.* 15%; $p=0.014$) without a decreased incidence of permanent hypocalcemia (1.7% *vs.* 1.8%), negating the value of routine parathyroid gland autotransplantation [77]. In another study the rate of permanent hypoparathyroidism was three-fold higher among patients who received autotransplantation (9.8% *vs.* 3.1%; $p=0.002$) [30].

3.4.- Intraoperative or immediate postoperative prediction of hypoparathyroidism

There is no consensus regarding how to predict postoperative hypocalcemia after total thyroidectomy [52]. However, PTH levels during the first 24 hours after thyroidectomy seem to be more accurate for prediction of hypoparathyroidism than serum calcium concentrations [33].

3.4.a Parathyroid hormone measurement

Intact PTH levels alone or combined with serum calcium levels can guide the decision to begin prophylactic oral calcium. Several studies have demonstrated the effectiveness of PTH levels in the early postoperative period from various time points: in the postanesthesia care unit immediately after surgery, to 1, 2, 4, 6, or 24 hours later. The normal short half-life of PTH (3-5 minutes) supports relying on early postoperative PTH levels. Typically, a PTH level <10 to 15 pg/mL is predictive of later hypocalcemia [14]. However, in patients with vitamin D deficiency, serum PTH is not an accurate predictor of post-thyroidectomy hypocalcaemia because this is a condition associated with increased PTH levels [78]. A large prospective study showed that a 3-hour postoperative decline in PTH levels could predict the risk of permanent hypoparathyroidism. The findings also indicated that a stable or increased postoperative PTH concentration rules out the possibility of persistent hypoparathyroidism [27].

The American Association of Clinical Endocrinologist and the American College of Endocrinology [14] propose that patients with a PTH value above 15 ng/mL measured 20 minutes or later after surgery can be discharged on prophylactic oral calcium supplements. These patients are at extremely low risk of developing postoperative hypocalcemia and can be considered for early discharge from the hospital if they are otherwise stable [45]. Patients with PTH less than 15 ng/mL are at risk of developing hypocalcemia, but with a high false-positive rate [27,45], and it has been recommended starting them on calcitriol (0.5 mcg BID) in addition to oral calcium supplements, and keeping them under observation overnight [14].

3.4.b Serum calcium measurement

Postoperative calcium testing has also been used as a means for assessing postoperative hypoparathyroid risk and stratifying patients for observation and/or discharge. Unfortunately, the lag time for calcium changes is greater than that for PTH, and a calcium nadir may not occur for 24 to 72 hours following surgery [27]. Absolute numbers and trends of calcium as well as total *versus* ionized calcium measurements have been used to establish clinical guidance [24]. If calcium levels are stable or increase over an observation period, discharge is generally considered safe. If calcium levels

continue to decline despite medical treatment, the patient requires optimization of calcium replacement therapy and ongoing observation until calcium stability or increase is observed [52]. In the study of Järhult, *et al.* [31] of 640 thyroidectomized patients, the absolute postoperative level of serum calcium was better predictor for postsurgical hypoparathyrodism than the decrease in serum calcium concentration. A normal postoperative serum calcium level could effectively rule out symptoms and later permanent hypoparathyroidism, but patients with subnormal calcium levels were not necessarily symptomatic. In this study, only with serum calcium levels lower than 1.85 mmol/l (7.41 mg/dl) on postoperative day 1 was the positive likelihood ratio high enough to predict hypocalcaemic symptoms and permanent hypoparathyroidism with reasonable accuracy. Calcium levels, when used, are recommended to be drawn the evening after surgery, the following morning, and every 6 to 12 hours thereafter [14,52].

Other authors found that a PTH level of ≤15 pg/mL on postoperative day 1, and serum calcium level of ≤7.6 mg/dl on postoperative day 2, are the best parameters for predicting postsurgical hypoparathyrodiism. This approach may be dangerous given the low level of calcium. Other combinations such as PTH levels at a different cut-off (10 or 12 pg/mL) on different postoperatorive days (1 or 4) combined with serum calcium levels (≤7.6 mg/dl with or without clinical symptoms or <8.4 mg/d with neuromuscular symptoms) on postoperative day 1, 2, 3, or 4 have less positive predictive value for postsurgical hypoparathyrodism [40].

A rational approach was proposed by Khan, *et al.* [52]. Serum calcium, albumin, magnesium, phosphorus, and PTH levels are measured 6 hours after surgery; if the corrected serum calcium concentration is less than 8 mg/dl or if PTH is undetectable, calcium and calcitriol replacement therapy is initiated. Patients are re-evaluated on the morning following surgery. If the corrected calcium level is increasing or the PTH concentration is greater than 5 pg/mL, calcium and calcitriol supplementation is titrated down and patients are discharged with subsequent close outpatient follow-up. This includes obtaining calcium and PTH values in 5 to 7 days. All patients are educated on signs and symptoms of hypocalcemia and given explicit instructions about taking calcium supplementation if necessary.

3.4.c Serum phosphate measurement

Immediate access to PTH measurement is expensive and not widely available. Serum phosphate responds rapidly to changes in circulating PTH levels, and its measurement is readily available in all hospitals. In humans, the change in the serum phosphate concentration in response to a change in PTH concentration is almost immediately detected whereas the alteration in serum calcium level may be delayed [27]. Sam, *et al.* [63] reported that serum phosphate could be a reliable biochemical predictor of post-thyroidectomy hypocalcaemia in patients without vitamin D deficiency. Patients with a vitamin D level > 25 nmol/l (10 ng/ml) that developed hypocalcaemia requiring treatment from day 1 onwards had an overnight rise in serum phosphate to >1.44 mmol/l (4.46 mg/dl) (100% sensitivity and specificity for predicting hypocalcaemia). Patients who had a vitamin D level < 25 nmol/l that also developed hypocalcaemia had an attenuated rise in serum phosphate. This may be explained by the concomitant development of hungry bone syndrome. In addition, patients with vitamin D deficiency typically have hypophosphataemia. Hungry bone syndrome is characterized by deposition of calcium and phosphate in bone following an abrupt fall in PTH release in patients with preexisting hyperparathyroidism (primary or secondary). However, a number of caveats must be considered in using serum phosphate to predict hypocalcaemia following thyroidectomy, so this approach has not been widely implemented [63].

3.5.- Diagnosis of postsurgical hypoparathyroidism

Hypocalcemia commonly occurs within 1 day after total thyroidectomy. Calcium levels reach a nadir 3 days after surgical intervention, and the kinetics of PTH after thyroid resection suggest that PTH levels are lowest 3 hours after total thyroidectomy [27].

A diagnosis of postsurgical hypoparathyroidism should be considered in a postsurgery patient with hypocalcemia and low PTH levels [2,6,51,52]. Relative hypoparathyroidism or parathyroid insufficiency may exist postoperatively defined as clinical symptoms of hypoparathyroidism requiring medical treatment, even though measured laboratory values may be within normal ranges [14]. Some patients with manifest clinical symptoms of hypocalcemia despite eucalcemia are usually seen

following surgery for primary or tertiary hyperparathyroidism or aggressive thyroid surgery and probably represent acute calcium lowering, which precedes a lagging reset of the calcium-sensing receptor system acclimatized to prior hypercalcemia [14].

Table 3 shows the criteria to diagnose postsurgical hypoparathyroidism, proposed by The American Association of Clinical Endocrinologist and the American College of Endocrinology [14]. However, most endocrinologists define postsurgical hypoparathyroidism as the combined presence of hypocalcemia (serum calcium < 2.0 mM or < 8.0 mg/dl) with an inadequate PTH concentration (either frankly low or inappropriately normal -below 15 ng/L- intact PTH levels) [33].

Clinical
Subjective hyperesthesias of the distal extremities
Perioral numbness/tingling
Nocturnal leg cramps
Chvostek/Trousseau signs
Biochemical
Hypocalcemia: total calcium, corrected to a serum albumin of 4.0 g/L, <8.6 mg/dl (2.15 mmol/L).
Ionized calcium < 4.6 mg/dl (1.15 mmol/L)
Hypoparathormonemia: PTH less than 12 or 15 pg/mL (varies from lab to lab based on assay used)

Table 3. Diagnostic criteria for hypoparathyroidism proposed by the American Association of Clinical Endocrinologist and the American College of Endocrinology, 2015 [14].

The cut-off value and timing of blood sampling used to define postoperative hypocalcemia differ. Some authors have considered that an early postoperative decrease (3 to 24 hours after thyroidectomy) in serum PTH concentrations associated with an albumin corrected serum calcium <8 mg/dl, is consistent with acute parathyroid insufficiency after total thyroidectomy. Total serum calcium is cheap to measure and easy to interpret and is preferable to ionized calcium concentrations which are highly dependent on blood sampling, transport, and pH. A cut-off of 8 mg/dl (2 mmol/L) corrects for recumbency and mild hemodilution, and only exceptionally are symptoms of hypocalcemia observed above this value. As the timing of calcium

measurement is critical, these authors propose that postoperative hypocalcemia be defined as albumin-corrected serum calcium <8 mg/dl (2 mmol/L) 24 hours after total thyroidectomy, and recommend that oral treatment with calcium and calcitriol be started if serum calcium drops below this value [16,25,30,48].

3.6.- Classification of postsurgical hypoparathyroidism

Hypoparathyroidism following surgery is commonly classified as temporary or transient, and chronic or permanent. The most common time marker used to delineate between these two conditions is 12 months following surgery [5,14,16,50,74,79] although some authors consider only 6 months to be appropriate [2,3,7,51]. While transient hypoparathyroidism after neck surgery is relatively common, often called 'stunning' of the glands, permanent hypoparathyroidism is less common. There is no difference in postoperative calcium levels between patients with transient hypocalcaemia and those who develop permanent hypoparathyroidism [31].

About 60-70% of patients with postsurgical hypoparathyroidism recover parathyroid gland function within 4-6 weeks after surgery and thus do not develop permanent disease [3,16,48]. The rest will progress to protracted hypoparathyroidism characterized by low serum PTH levels and the need for continued treatment. The most relevant factor leading to protracted hypoparathyroidism is the number of functioning parathyroid glands remaining *in situ;* clinical and disease-related variables lose significance at this stage [16,30].

Finally, about 15–25% of patients with protracted hypoparathyroidism will develop chronic hypoparathyroidism (Figure 1). The significant variables influencing negatively the long-term parathyroid function are fewer parathyroid glands remaining *in situ*, undetectable PTH level at 1 month and a low serum calcium concentration during the first weeks after thyroidectomy. Other clinical and disease-related variables do not seem to influence parathyroid function recovery [80].

A normal to high calcium level at 1 month after thyroidectomy under treatment with calcium and vitamin D appears to increase the chance of parathyroid gland recovery (Figure 2). The risk of chronic hypoparathyroidism is closely related to the number of parathyroid glands remaining *in situ* at operation: 16% for cases with one

to two preserved glands, 6% for three glands, and 2.5% for four glands [30]. A nomogram combining serum calcium and PTH concentrations at 1 month after thyroidectomy has been constructed to predict the likelihood of recovery of the parathyroid function [81].

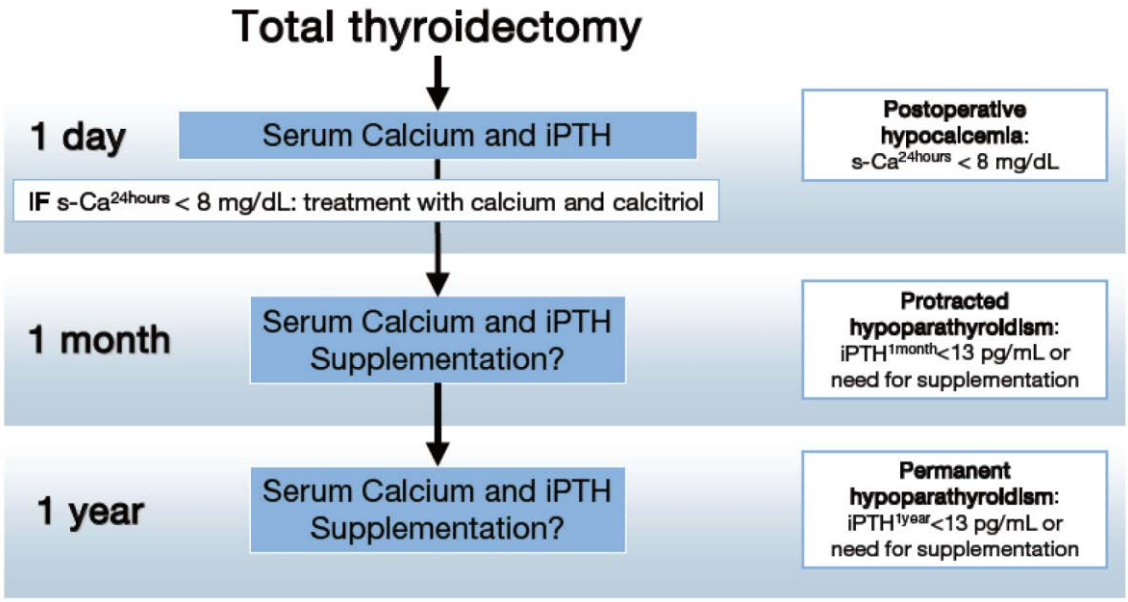

Figure 1. Classification of postsurgical hypoparathyroidism [16].

Recently, Kim, *et al.* [74] performed a singular prospective study of patients in whom recovery of parathyroid function occurred more than a year after diagnosis of postsurgical hypoparathyroidism. Of 1,467 patients who underwent total thyroidectomy with central compartment dissection without parathyroid autotransplantation, 22 (1.49%) presented with permanent postoperative hypoparathyroidism. In 5 of these 22 patients (22.7%), the PTH levels increased steadily and returned to normal in 27.6 $\pm$ 2.9 months, after which supplementation of calcium and vitamin D could be discontinued. This study shows that recovery from permanent hypoparathyroidism is a rare condition that deserves to be referred. The authors hypothesize that the delayed recovery of parathyroid function observed in these 5 patients may have been due to the slow but steady recovery of blood flow via neovascularization occurring over the small surface area of the remaining parathyroid, and/or neovascularization occurring slowly in devascularized parathyroid glands after central compartment node dissection, thereby delaying the recovery of parathyroid function. Previously, Page, *et al.* [25],

discussing the best time to diagnose permanent postsurgical hypoparathyroidism, reported the recovery of parathyroid function in 5 patients 18 months after surgery.

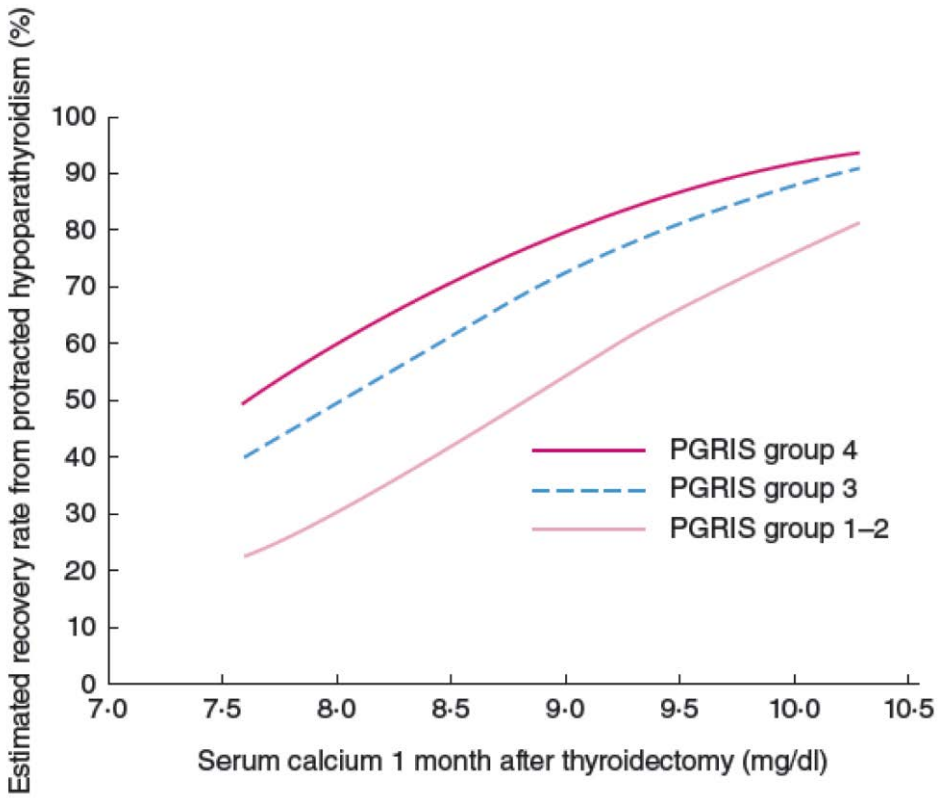

Figure 2. Evolution of protracted hypoparathyroidism [30]. Estimated percentage of recovery from protracted hypoparathyroidism according to number of parathyroid glands remaining *in situ* (PGRIS) and the serum calcium concentration (mg/dl) 1 month after total thyroidectomy. Group 1–2 (one or two PGRIS), group 3 (three PGRIS) and group 4 (all four glands remaining *in situ*).

4.- JUSTIFICATION: HYPERCALCIURIA IN PATIENTS WITH HYPOPARATHYROIDSM

4.- JUSTIFICATION: HYPERCALCIURIA IN PATIENTS WITH HYPOPARATHYROIDISM

4.1.- Epidemiology

Treatment of patients with hypoparathyroidism by means of calcium and vitamin D analogs increases the risk of hypercalciuria, nephrocalcinosis, nephrolithiasis, and impaired renal function. The rates of these complications, however, have been difficult to estimate given the lack of large natural history studies. In one cross sectional study of 25 patients with postsurgical hypoparathyroidism, 23% had 24-hour urine calcium excretion higher than 300 mg and 8% had asymptomatic nephrolithiasis on renal ultrasound. Renal function was normal in all patients [82]. In another cross-sectional study of 33 patients with hypoparathyroidism of diverse etiologies, 15% reported a history of nephrolithiasis [83]. In one National Institutes of Health cohort (n=27), 41% had nephrocalcinosis and 33% had an estimated glomerular filtration rate below 60 mL/min/1.73 m^2 [84]. In a large longitudinal cohort of 120 hypoparathyroid patients (66% of postsurgical etiology) with a follow-up for 7.4 $\pm$ 5.1 yr to assess serum and urine laboratory results and renal and brain imaging, only 44% of patients had at least one 24-hour urine calcium collection [9]. Of these, 38% had at least one 24-hour urine calcium over 300 mg. Higher serum calcium was associated with higher urine calcium values, but there was wide variation because some patients with concomitant measured serum and urine calcium had urine calcium levels above 300 mg/24-hour associated with low or normal serum calcium levels, and some patients with normal or high levels of serum calcium had no hypercalcuria (Figure 3). In this series, two patients (with idiopathic hypoparathyroidism) required renal transplant due to nephrocalcinosis; 31% of patients with renal imaging had either renal stones or nephrocalcinosis, and 41% of patients had chronic kidney disease stage 3 or higher (estimated glomerular filtration rate below 60 mL/min/1.73 m^2). Rates of chronic kidney disease stage 3 or higher were 2- to 17-fold greater than age-matched healthy controls. In a Danish National Patient Registry, 688 patients with permanent postsurgical hypoparathyroidism were identified. Compared with a group of age- and gender-matched population-based controls, patients had an almost five times increased risk of renal stones (HR: 4.82; 95% CI: 2.00–11.64) and kidney failure (HR: 4.95; 95% CI: 2.88–8.50). Moreover, although not statistically significant, more patients than controls received treatment with dialysis [10]. In a recent cohort of 32

patients with postsurgical hypoparathyrodism with a follow-up of 78±68 months, a 15.6% had renal failure and a 10% had nephrolithiasis [85].

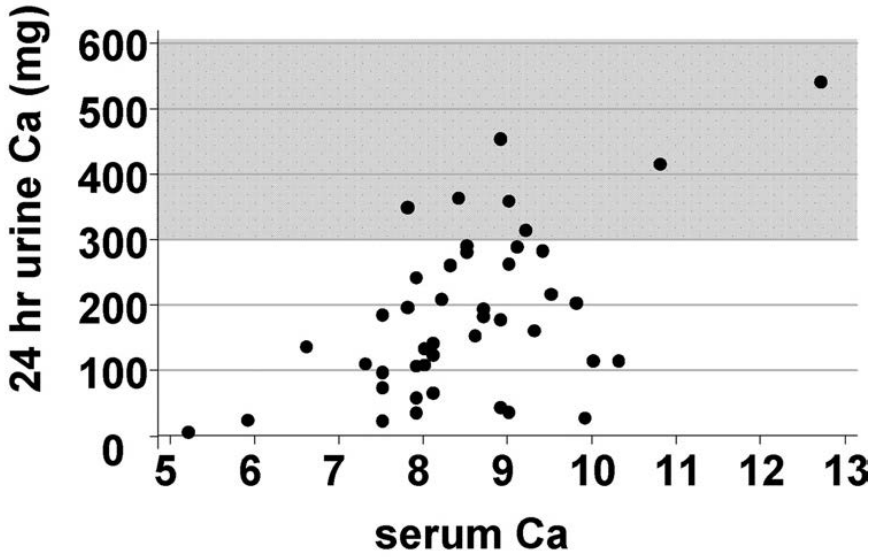

Figure 3. Scatterplot of all 24-hour urine calcium levels with serum calcium levels recorded the same day [9]. Unshaded areas represent target range (<300 mg).

Overall, the higher proportion of patients with renal disease in these studies suggests that monitoring and optimizing therapy to preserve renal function is of critical importance for patients with hypoparathyroidism. The association of hypercalciuria with renal calcification and impaired renal function reinforces the existing recommendations to maintain serum calcium in the low-normal range and 24-hour urinary calcium excretion below 300 mg. Careful monitoring and adjustment of treatment to reduce urinary calcium excretion is a key part of the medical management of these patients [9].

4.2.- Pathophysiology

PTH is necessary for the conservation of calcium in the proximal and distal renal tubules, and many patients with hypoparathyroidism have hypercalciuria which can lead to nephrocalcinosis, nephrolithiasis, and renal failure [1,3,68]. The kidney is especially vulnerable in patients with hypoparathyroidism because the filtered load of calcium increases directly with increases in the serum calcium level. In the absence of PTH to promote renal calcium reabsorption, the additional calcium absorbed must be excreted through the kidneys [2]. Long-term treatment with calcium and vitamin D does not restore physiologic calcium homeostasis and often results in hypercalciuria even in the face of normocalcemia, thereby increasing the risk of renal sequellae [82].

Hypoparathyroidism has traditionally been associated with calcium-containing urolithiasis and renal impairment because of an increased calcium–phosphate product. Loss of renal PTH action decreases renal tubular reabsorption of calcium and excretion of phosphate causing hypercalciuria and hyperphosphataemia respectively [2]. Thus, patients with chronic hypoparathyroidism have been found to have an increased risk of renal complications [10]. Impaired renal function has been associated with the age of the patient, duration of the disease and relative time with hypercalcaemia (Figure 4) [9,82]. However, despite keen interest in improving the management of hypoparathyroidism, large cohort studies describing typical treatment patterns, optimized target laboratory parameters and rates of complications are scarce [6].

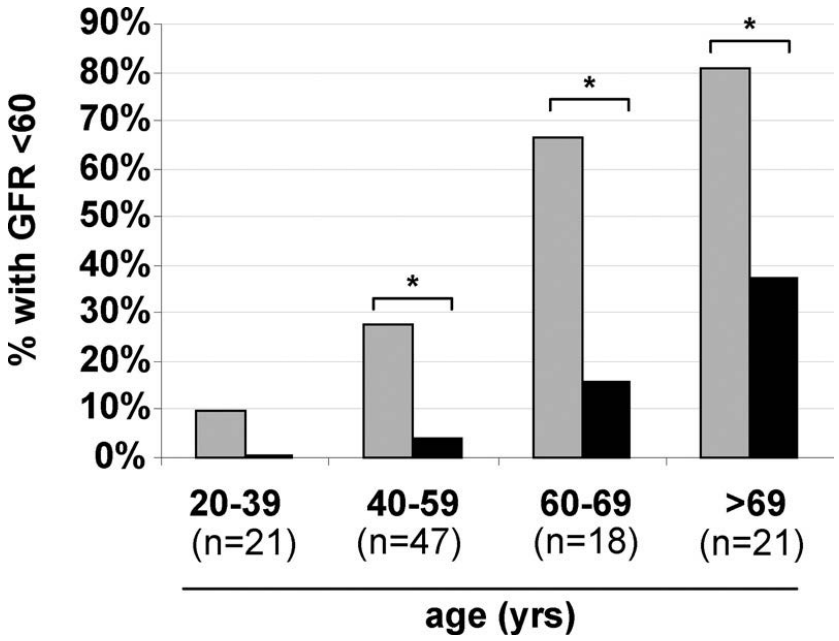

Figure 4. Proportion of patients with eGFR below 60 ml/min/1.73 m2 by age group [9].
Gray columns, hypoparathyroid cohort; black columns, population norms.
*, $p < 0.001$ for comparison by one-sample t test .

A major therapeutic challenge in patients with hypoparathyroidism is achieving consistently effective management of the hypocalcemia while avoiding hypercalciuria and other complications. In the absence of PTH the tubular reabsorption of calcium is lowered and much of the absorbed calcium in the intestine is lost in the urine [86]. It is therefore advisable to maintain serum calcium at the low end of the normal range. Although undertreatment can cause symptomatic hypocalcemia, overtreatment and associated hypercalciuria can cause nephrolithiasis, nephrocalcinosis, and renal insufficiency. Not surprisingly, hypercalciuria is a common complication of therapy and patients frequently have increased urinary calcium excretion [1-3,68,86-88]. Hypercalciuria

will occur typically before the serum calcium increases [2,3,89-92]. Close monitoring of the laboratory profile has been warranted in all patients being treated with large amounts of calcium and vitamin D preparations [2,3,68].

Therapy with rhPTH has so far not been documented to cause a significant sustained reduction in 24-hour urinary calcium. Plasma levels of rhPTH peak after 1-2h with a plasma half-life of approximately 1.5 h. Accordingly, rhPTH does reduce urinary calcium as long as PTH is present in the circulation. However, once- or twice-a-day injection does not provide a sustained exposure of PTH to the renal tubules throughout the day and no marked net effect on 24-hour urinary calcium is therefore present. Moreover, the rise in plasma calcium levels in the hours following an injection of PTH increases the filtered load of calcium and may thereby in part antagonize the net effect on urinary calcium as induced by PTH-mediated increased renal tubular calcium reabsorption. In addition, rhPTH causes an increase in the synthesis of 1,25-dihydroxyvitamin D with a peak concentration approximately 10 h after an injection. This may contribute to the lack of an effect of rhPTH injections on the 24-hour urinary calcium excretion, as 1,25-dihydroxyvitamin D might maintain plasma calcium during the second half of the day, while causing an increase in urinary calcium excretion [11].

To prevent renal damage, historically urinary calclium excretion lower than 300 mg/24 hour has been recommended [2,52]. More recently some authors empirically keep this value for men and recommended a cut-off of 250 mg/24 hour for women or less than 4 mg/kg/24 hour for both sexes [6].

In summary, because patients with permanent hypoparathyroidism lose the ability to reabsorb calcium through the renal tubular system, they are prone to developing hypercalciuria, renal stones, nephrocalcinosis and renal insufficiency. In these patients, hypercalciuria can be minimized by maintaining a low-normal serum calcium level and treating with thiazide diuretics. Urinary calcium excretion of less than 300 mg/24 hour should be maintained with periodic evaluations of 24-hour urinary calcium [52].

4.3.- Pitfalls in the diagnosis of hypercalciuria

Measurement of urinary calcium excretion was first conducted as an indicator test of the presence or absence of hypercalcemia or hypocalcemia (urinary Sulkowitch test). As results were confusing, this test was discontinued for this purpose [93].

However, in the following years and until today, periodic measurement of 24-hour urinary calcium excretion has been recommended in patients with hypoparathyroidism even if they are stable [2,6,94]. This approach is based on avoiding renal complications if hypercalciuria appears as a consequence of the treatment with calcium and vitamin D supplements. This is clearly illustrated by Quack, *et al.* [95]. They reported a series of five postsurgical patients with hypoparathyroidism admitted because impairment of renal function during dihydrotachysterol therapy continued for 4 to 50 years. In all cases calcium levels were determined, ranging from 3.08 to 4.97 mmol/l (12.3 to 19.88 mg/dl) after inappropiately long intervals. Creatinine levels ranged from 277 to 365 µmol/l. All patients suffered symptoms of severe hypercalcaemia, three of them needing intensive care unit treatment. As confirmed by renal biopsy, the impairment of renal function was due to calcifications. Monitoring has been inadequate in all cases regarding the pharmacokinetics of dihydrotachysterol. Urinary calcium excretion as an early marker of vitamin D overdose was never determined. Serum creatinine and calcium levels had not been checked on a regular basis. Hence renal function and the capacity to eliminate calcium declined unperceived, a vicious circle which finally led to severe hypercalcaemia. This study is a clear example from reality that patients with hypoparathyroidism live. Any pharmacological treatment for hypoparathyroidism can only be safely conducted as long as the necessary monitoring is performed.

In the absence of other reliable alternative indicators of hypercalciuria, this procedure has been implemented despite volume omissions in 24-hour urine collections that can produce false negative results [96-98]. Complete 24-hour urine may be not properly collected [97-100] because it may be a nuisance to the patient, leading to an inaccurate collection [101]. Complete urine collection may be difficult to obtain, due to forgetfulness. Urinations after bedtime occur frequently and it is probably these specimens that most often are 'forgotten', giving rise to erroneously low solute excretions in supposedly 24-hour urines [102]. Also contributing to incomplete urine collections are misplacement of samples, lack of container capacity, erroneous

inclusion of urine from the first void, and loss of urine during defecation. This is particularly so when studying individuals performing their routine lifestyles, as opposed to patients in, for example, a metabolic ward. Furthermore, it may be inconvenient to carry containers of urine around for a full day [97].

Twenty-four-hour urine collections are unpopular with both patients and laboratory staff, even more so if they need to be done twice yearly over a lifetime [96,100]. Consequently, it is not uncommon to have reports of compliance with this approach by means of 24-hour urine collections in a minority (44%) of patients. Nonetheless, among patients that perform this procedure, 38% had at least one 24-hour urine calcium excretion over 300 mg [9].

Thus, all these inconveniences imply that the necessary diagnosis of hypercalciuria by means of 24-hour urine collections may often be unreliable [97,99,100]. Thus, reliable indicators of 24-hour urine collections were investigated. Measurements of urinary creatinine and para-aminobenzoic acid have been used to check the completeness of 24-hour urine specimens.

In studies where missed volumes were controlled for and outliers were not excluded on a statistical basis, the range of within-subject variation of urine creatinine excretion rate during 24 hours was 9–24% [97,103]. Estimates of the between individual variation in the creatinine excretion rate, controlling for incomplete samples, are around 18% to 21% for men, and 17% to 25% for women [97]. The creatinine excretion rate is higher for men than for women, and it decreases with age and increases with exercise, muscle mass and intake of meat. Furthermore, the excretion rate varies due to the diurnal, seasonal and, for women, menstrual cycles [97,103]. A further source of variation in urine creatinine is the completeness of the collection itself. Furthermore, the normal range for urine creatinine excretion is so wide that only about a 30% of incomplete collections have low creatinine excretion [103].

			Urinary creatinine			Urinary creatinine
Age (years)	n	Body weight (Kg)	mg/24h	mg/kg/24h	n	mg/kg/24h
Males						
20-29	12	68.4	1,625 ± 137	23.8 ± 2.3	0	
30-39	10	70.9	1,520 ± 130	21.9 ± 1.5	4	21.7 ± 3.7
40-49	32	77.5	1,544 ± 421	19.7 ± 3.2	7	21.9 ± 2.4
50-59	37	75.7	1,445 ± 252	19.3 ± 2.9	14	19.8 ± 2.3
60-69	23	73.4	1,252 ± 364	16.9 ± 2.9	13	19.1 ± 3.8
70-79	18	69.5	919 ± 132	14.2 ± 3.0	10	13.2 ± 2.7
80-89	12	56.3	651 ± 238	11.7 ± 4.0	1	9.0
90-99	5	67.6	612 ± 188	9.4 ± 3.2	2	10.7 ± 2.1
Total	149				51	
Females						
20-29	32	58.1	1,135 ± 224	19.7 ± 3.9	0	
30-39	14	60.9	1,218 ± 191	20.4 ± 3.9	4	19.4 ± 4.7
40-49	48	60.1	1,056 ± 256	17.6 ± 3.9	10	16.5 ± 3.6
50-59	34	67.8	989 ± 246	14.9 ± 3.6	11	16.7 ± 2.8
60-69	23	65.4	871 ± 283	12.9 ± 2.6	13	15.4 ± 3.4
70-79	27	58.3	685 ± 184	11.8 ± 2.2	12	10.1 ± 3.9
80-89	32	55.3	578 ± 154	10.7 ± 2.5	4	11.3 ± 1.0
90-99	9	52.4	433 ± 113	8.4 ± 1.4	1	12.9
Total	219				55	

Table 4. Normal values for urine creatinine according sex and age [104]. Results from 149 males and 219 females with serum creatinine ≤ 1.4 mg/dl and from 51 males and 55 females with elevated serum creatinine values between 1.5 and 5.0 mg/dl.

Kampmann, *et al.* [104] published what became a reference study to date. They studied 368 hospitalized patients to measure endogenous creatinine clearance and serum and urine creatinine. Their values of urine creatinine grouped by sex, age and renal disease have been recommended to assist in the completeness of 24-hour urine collections [105,106] (Table 4 and Figure 5). Of note, in the group of 106 patients with renal disease and serum creatinine values between 1.4 and 5 mg/dl, the urinary creatinine expressed as mg/kg/24 hours was not significantly different from that of patients without renal disease. Urinary creatinine was determined in three consecutive 24-hour samples and the mean value was used. Only variations in 24-hour creatinine excretion less than 25% were accepted. Many of the elderly patients had a catheter which ensured that the urine was collected quantitatively.

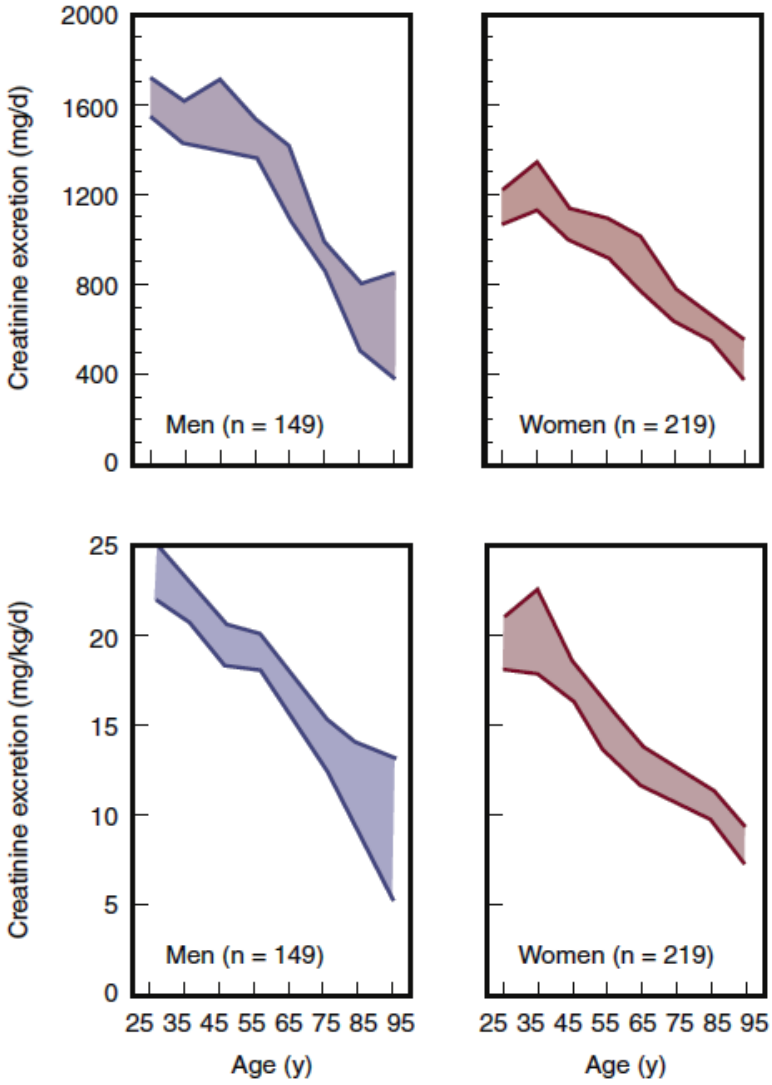

Figure 5. Age related differences in urinary creatinine excretion in normal men (left) and woman (right) [106]. Shaded areas represent 95% confidence intervals calculated from the data of Kampmann and co-workers (1974) [104]. Values in the upper panels are milligrams per day; values in the lower panels are milligrams per kilogram of body weight per day.

Completeness of 24-hour urine collection can be verified using PABA (para-aminobenzoic acid). After rapid absorption from the gut, PABA is extensively metabolized and conjugated in the liver, before being completely and rapidly cleared by the kidneys and excreted in the urine [98,102]. Bingham & Cummings [107] introduced PABA as an objective marker to validate the completeness of 24-hour urine collection. Normally, three 80 mg PABA tablets are taken orally in conjunction with main meals on the day of urine collection, and PABA recovery in the urine above 85% of total ingested dose indicates urine has been collected for 24 hours.

PABA has been reported to be more sensitive and reliable for verification of the completeness of 24-hour urine collections than urine creatinine [103]. However, PABA is not free of drawbacks. Overcollection cannot be detected using PABA, and it appears that people sometimes forget or refuse to take the capsules. It is also suggested that differences in the meal-time patterns may interfere with the PABA recovery test [108]. In other studies, PABA excretion over 24 hours decreases with the age of the subject by about 1% per ten years of age from age 30 [102,109]. Additionally, some question the analytical method used for PABA determination because the colorimetric method co-determines aromatic amines, compounds that can originate from the intake of a number of commonly used drugs, notably paracetamol and sulphonamides [110].

The number of incomplete 24-hour samples has been estimated in a few studies, where the self-reported number of incomplete samples ranged from 3 to 22%. Based on 24-hour urine samples from participants who reported having ingested the full dose of PABA, an estimate of 17% incomplete samples was obtained. Based on the available information, Garde, *et al.* [97] concluded that it seemed reasonable to assume that the typical percentage of incomplete 24-hour urine samples in non-hospitalized individuals is 15–20%. The recovery of PABA in 24-hour urine samples judged as incomplete corresponds to missed volumes from around 30% [97].

To overcome the problems associated with 24-hour urine collections, including inconvenience and accuracy of collection, and not always accurately detecting urine creatinine or PABA, alternative markers in plasma or serum have been investigated successfully in other settings. As an example, Carling, *et al.* [100] found that fasting plasma 5-HIAA concentration provides a more convenient screening test for carcinoid syndrome and overcomes the problems associated with 24-hour urine collections, without any loss of diagnostic precision.

5.- OBJECTIVE

5.- OBJECTIVE

Postsurgical hypoparathyroidism is the most common form of hypoparathyroidism [6,9,14,22,25,39,55,94]. The normalization of serum calcium levels in patients with hypoparathyroidism is usually achieved by using calcium and vitamin D supplements, commonly in the form of calcitriol [6,9,14,55,84,95,111]. However, the optimal management of hypocalcemia has not been investigated in clinical trials, and treatment is based largely on accepted conventional practice [68]. In addition to controlling symptoms, the goals of therapy in patients with hypoparathyroidism are to maintain an albumin-corrected serum calcium level in the low-normal range or slightly below the lower limit of the reference range (approximately 8.0 to 8.5 mg/dl), a calcium-phosptate product below 55 mg^2/dl^2, and a 24-hour urinary calcium level below 300 mg [2,6,7,9,14,55,111].

Excessive urinary calcium excretion is a drawback of treatment with calcium and vitamin D supplements in patients with hypoparathyroidism [6,7,9,55,111,112]. Chronic hypercalciuria may lead to calcium-containing urolithiasis, nephrocalcinosis, and impaired renal function [6,9,84,95,111,113]. Monitoring and optimization of therapy to preserve renal function has been considered to be of critical importance for patients with hypoparathyroidism [9,111]. Traditionally, to monitor hypercalciuria, twice-yearly measurement of 24-hour urinary calcium is recommended in patients with hypoparathyroidism, once the treatment has been stabilized [2,6,7,55].

However, 24-hour urine collection is often unreliable because of volume omissions. In this regard, it is well known that a 24-hour urine may be not properly collected [98] because it may be a nuisance for the patient that leads to an inaccurate collection [101]. The typical percentage of incomplete 24-hour urine samples in non-hospitalized individuals is 15-20% with missed volumes from around 30% [97]. Moreover, 24-hour urine collections are unpopular with both patients and laboratory staff, even more if it needs to be done twice yearly over a lifetime [96]. It has been reported that the compliance rate for 24-hour urine collection may be as low as 44% for patients with hypoparathyroidism with a follow-up of 7.4 $\pm$ 5.1 years [9].

To our knowledge, no clinical studies in recent years have been specifically aimed at evaluating which factors are related to the presence of hypercalciuria in

patients with hypoparathyroidism who are receiving supplementary treatment. At the same time, it has become common for patients with hypoparathyroidism to be treated with both calcitriol (1,25-dihydroxyvitamin D) and cholecalciferol (vitamin D3) supplements when pills containing fixed doses of calcium and cholecalciferol are prescribed as treatment. However, at the present time, we can routinely measure serum metabolites such as calcidiol, calcitriol and PTH to analyze their influence on hypercalciuria. Thus, it is possible that new considerations about the best control of patients with hypoparathyroidism may be taken into account, and new insights may be gained into the best means of managing supplemental treatment in order to avoid hypercalciuria and its potentially deleterious effects on the kidneys.

The objective of this study was to find biomarkers that could be useful for predicting hypercalciuria in patients with permanent postsurgical hypoparathyroidism whose condition had been stabilized with oral calcium and calcitriol, with or without other vitamin D supplements.

6.- PATIENTS AND METHODS

6.- PATIENTS AND METHODS

6.1.- Patients

Over a period of 24 months (November 2014 to November 2016), 42 consecutive stable outpatients with permanent postsurgical hypoparathyroidism after a total thyroidectomy performed because of benign (n=18) or malignant (n=24) thyroid conditions were enrolled in this study. All patients with malignancy were in complete remission at the time of the study. The control group consisted of 17 adults with postsurgical hypothyroidism, without hypoparathyroidism, who were enrolled more than 1 year after undergoing thyroidectomy. The study was reviewed and approved by the ethics committee of the Hospital Universitari Mútua de Terrassa. Written informed consent was obtained from all patients and controls.

6.2.- Definition of postsurgical hypoparathyroidism

Hypoparathyroidism was confirmed in all patients by low serum levels (usually below 15 pg/ml) of intact PTH during hypocalcemia (albumin-corrected serum calcium levels below 8 mg/dl) 24 to 48 hours after total thyroidectomy. Hypoparathyroidism was permanent, given that all patients showed undetectable or subnormal PTH concentrations (usually below 15 pg/ml in the absence of raised serum calcium levels) and required calcium supplements with calcitriol to achieve albumin-corrected serum calcium levels above 8 mg/dl for more than 1 year after thyroidectomy was performed [14,16,40,48,55].

6.3.- Protocol of treatment

The patients took oral calcium and oral vitamin D supplements. They were all receiving calcitriol treatment, and in addition, 18 patients were also treated with vitamin D3 (cholecalciferol) supplements that were added in fixed doses to the tablets of calcium supplements. In a food survey, we quantified the level of calcium intake on 3 different days per week, using a food composition table [114].

Therapy and clinical conditions were stable in at least the three months prior to data collection. No patient had renal insufficiency or was being treated with other

drugs that could have interfered with calcium homeostasis, such as parathyroid hormone, thiazide or loop diuretics, systemic glucocorticoids, antiresorptive drugs, antiepileptic drugs, or lithium [6].

In our clinical procedure, patients with hypercalciuria and albumin-corrected serum calcium above 8.5 mg/dl are advised to reduce oral calcium supplements to a maximun of 500 mg of calcium element tid and/or to reduce aditionally 0.25 mcg/day the dosage of calcitriol; patients with hypercalciuria and albumin-corrected serum calcium below 8.5 mg/dl are treated with added thiazide diuretics.

6.4.- Primary endpoint

The primary endpoint was 24-hour urine calcium excretion, which was measured in a single urine sample. Emphasis was placed on the importance of patients providing complete 24-hour urine specimens. The reliability of urine specimens collected was tested by measuring urine creatinine. For each group of age and sex, the values were considered suitable if they were within the mean ± 2 standard deviations of values for subjects with normal renal function as previously reported [104,106] and shown in the following table:

MALES		FEMALES	
Age (years)	Urine creatinine mg/24h (mean ± 2 SD)	Age (years)	Urine creatinine mg/24h (mean ± 2 SD)
20-29	1,625 ± 274	20-29	1,135 ± 448
30-39	1,520 ± 260	30-39	1,218 ± 382
40-49	1,544 ± 842	40-49	1,056 ± 512
50-59	1,445 ± 504	50-59	989 ± 492
60-69	1,252 ± 728	60-69	871 ± 566
70-79	919 ± 264	70-79	685 ± 368
80-89	651 ± 476	80-89	578 ± 308
90-99	612 ± 376	90-99	433 ± 226

Using this criterion, four urine collections were rejected because of low levels of creatinine, and the patients were asked to provide more reliable 24-hour urine specimens in the following weeks which, on this occasion, were reliable.

In considering whether a patient had hypercalciuria, a threshold of 300 mg for 24-hour urine calcium excretion was applied [2,14,52,55].

6.5.- Variables collected to analize

The variables recorded to analyze their influence on 24-hour urine calcium excretion included the following:

- Patients: age and sex, time from hypoparathyroidism ocurrence, current weight, size, body mass index (BMI), and dietary calcium intake.
- Treatment conditioning factors: daily doses of calcium supplements and vitamin D3 supplements, daily dose of calcitriol, last dose of calcitriol before the blood draw, time interval between the last intake of calcitriol and the blood draw, and doses of thyroxine treatment.
- Serum analytic variables: calcium, albumin, phosphorus, magnesium, creatinine, thyrotropin, free thyroxine, parathyroid hormone, 25-hydroxivitamin D, and 1,25-dihydroxyvitamin D. Blood was drawn after an 8-hour fasting period.
- Urine analytic variables: 24-hour creatinine excretion.

Creatinine clearance was estimated as [urine creatinine (mg/dl) * urine volume (ml)] / [serum creatinine (mg/dl) * 1440]. Albumin-corrected serum calcium was calculated as follows: serum calcium (mg/dl) + [(4 - albumin (gr/dl)) * 0.8].

6.6.- Laboratory methods

Serum calcium was measured by the 5-nitro-5'-methyl-BAPTA (NM-BAPTA) method. Urine calcium was measured by the ortho-cresolphthalein complexone method. Serum albumin was measured by the bromocresol green (BCG) method and serum 25-hydroxivitamin D and intact PTH were determined using electrochemiluminescence immunoassay. Serum 1,25-dihydroxyvitamin D was determined by radioimmunoassay (125I RIA Kit, DiaSorin®, Stillwater, Minnesota). The

reference values were 8.6 to 10.0 mg/dl (coefficient of variation 0.61%) for serum calcium, 15.2 to 65.7 pg/ml (coefficient of variation 3.05%) for PTH, and 25 to 66 pg/ml (coefficient of variation 10%) for serum 1,25-dihydroxyvitamin D.

6.7.- Statistical methods

The normality of the continuous variables was determined by the Kolmogorov-Smirnov test. Continuous variables that could influence the presence of hypercalciuria were investigated with Student *t* test, and categorical variables with Chi-Square test. Differences in the means of variables after modifications in treatment were explored with the Wilcoxon test for paired data. Statistical relationships between quantitative variables were investigated with Pearson's correlation coefficient and linear multiple regression analysis (stepwise method). Receiver operating characteristic (ROC) curves were generated to examine the power of variables to discriminate between patients with and without hypercalciuria. Areas under ROC curves were compared using the Hanley and McNeil statistic method [115]. Statistical significance was set at $p<0.05$ (2-tailed). Statistical analysis was performed with Epidat software, version 3.1 (Servizo Galego de Saúde, Galicia, Spain).

7.- RESULTS

7.- RESULTS

7.1.- Comparison of patients and controls

A summary of control and patient data, treatment, and analytic results is displayed in Table 5. No patient had hypercalcemia, and all patients had a calcium-phosphorus product below 55 mg^2/dl^2. As expected, of the variables compared

	Controls	Patients	p value
n	17	42	
Age (years)	57.2±13.2	52.8±13.9	ns
Sex (female/ male)	16 / 1	31 / 11	ns
Weight (kg)	73.8±19.1	75.2±14.4	ns
BMI (kg/m^2)	30.13±9.67	28.48±7	ns
Duration of hypoparathyroidism (months)	N/A	90.5±90	---
Dietary calcium intake per day (mg)	597±204	650±246	ns
Treatment:			
- Calcium supplements (mg/day)	705±750	1,438±785	0.002
- Vitamin D3 supplements (UI/day)	470±640	716±746	ns
- Calcitriol (mcg/day)	0	0.47±0.20	<0.001
- Last dose of calcitriol (mcg)	N/A	0.29±0.11	---
- Time interval between last dose of calcitriol and the blood draw (h)	N/A	14.9±6.0	---
- Thyroxine (mcg/day)	118±38	125±25	ns
Serum analytic variables:			
- Calcium (mg/dl)	9.12±0.6	8.95±0.47	ns
- Albumin (g/dl)	4.4±0.39	4.51±0.29	ns
- Alb-corrected serum calcium (*)	8.79±0.51	8.54±0.48	0.092
- Phosphorus (mg/dl)	3.7±0.7	4±0.63	ns
- Product calcium-phosphorus (mg^2/dl^2)	33.7±5.1	35.9±6	ns
- Magnesium (mg/dl)	2.05±0.18	1.98±0.18	ns
- Creatinine (mg/dl)	0.74±0.18	0.77±0.12	ns
- Thyrotropin (mUI/l)	1.8±3.2	3.0±4.8	ns
- Free thyroxine (ng/ml)	1.61±0.33	1.54±0.32	ns
- Parathyroid hormone (pg/ml)	37.8±22.7	12.1±6.2	<0.001
- 25-hydroxyvitamin D (ng/ml)	27.7±9.1	31.3±12.9	ns
- 1,25-dihydroxyvitamin D (pg/ml)	39.7±13.3	37.5±12.5	ns
Urine analytic variables:			
- Calcium (mg/24h)	160±91	292±170	0.004
- Creatinine (mg/24h)	1,035±308	1,184±418	ns
Clearance of creatinine (ml/min)	99.5±28.5	107.5±37	ns

Table 5. Baseline characteristics of controls and patients, their treatment, and analytic parameters. Values of variables are expressed as mean ± SD or number when appropriate. (*): Albumin-corrected serum calcium (mg/dl). N/A: not applicable.

between controls and patients, only those that were related to the hypoparathyroid condition showed significant differences (calcium supplements, calcitriol dosage, serum parathyroid hormone, and urinary calcium excretion).

7.2.- Comparison of patients with and without hypercalciuria

	Patients with hypercalciuria	Patients without hypercalciuria	p value
n	16	26	
Age (years)	48.6±14.7	55.4±13.1	ns
Sex (female/ male)	11 / 5	20 / 6	ns
Weight (kg)	72.4±9.1	76.8±16.8	ns
BMI (kg/m^2)	25.75±3.22	30.17±8.15	<0.05
Duration of hypoparathyroidism (months)	59.9±53.3	119±101	0.018
Dietary calcium intake per day (mg)	698±156	621±287	ns
Treatment:			
- Calcium supplements (mg/day)	1,462±737	1,423±827	ns
- Vitamin D3 supplements (UI/day)	940±750	578±723	ns
- Calcitriol (mcg/day)	0.54±0.22	0.43±0.18	0.08
- Last dose of calcitriol (mcg)	0.28±0.12	0.31±0.11	ns
- Time interval between last dose of calcitriol and the blood draw (h)	14.2±5.8	15.4±6.1	ns
- Thyroxine (mcg/day)	129±25	123±25	ns
Serum analytic variables:			
- Calcium (mg/dl)	9.14±0.50	8.83±0.43	0.041
- Albumin (g/dl)	4.51±0.34	4.51±0.27	ns
- Alb-corrected serum calcium (*)	8.73±0.48	8.42±0.46	0.047
- Phosphorus (mg/dl)	3.76±0.79	4.15±0.48	0.089
- Product calcium-phosphorus (mg^2/dl^2)	34.6±8.2	36.6±4.1	ns
- Magnesium (mg/dl)	1.92±0.15	2.02±0.19	0.06
- Creatinine (mg/dl)	0.77±0.12	0.77±0.12	ns
- Thyrotropin (mUI/l)	2.7±3.4	3.2±5.5	ns
- Free thyroxine (ng/ml)	1.56±0.39	1.52±0.28	ns
- Parathyroid hormone (pg/ml)	11.2±7.4	12.7±5.4	ns
- 25-hydroxyvitamin D (ng/ml)	34.1±10.0	29.6±14.4	ns
- 1,25-dihydroxyvitamin D (pg/ml)	45.8±9.3	32.4±11.7	<0.001
Urine analytic variables:			
- Calcium (mg/24h)	440±187	201±63	<0.001
- Creatinine (mg/24h)	1,338±393	1,086±410	0.059
Clearance of creatinine (ml/min)	120.4±29.6	99.3±39.3	0.074

Table 6. Baseline characteristics of patients with and without hypercalciuria, their treatment, and analytic parameters.
Values of variables are expressed as mean ± SD or number when appropriate. (*): Albumin-corrected serum calcium (mg/dl).

Otherwise, comparing patients with and without hypercalciuria (Table 6), the latter had a higher BMI, longer evolution of hypoparathyroidism, and lower levels of serum calcium and 1,25-dihydroxyvitamin D. Interestingly, the serum PTH levels of patients with and without hypercalciuria were similar.

7.3.- Analysis of urinary calcium excretion

In the patient group, urinary calcium excretion correlated significantly with the daily dose of calcitriol (r= 0.443; p=0.003) (Table 7), and with the serum level of 1,25-dihydroxyvitamin D (r= 0.539; p<0.001) (Table 7, Figure 6). A clear tendency was detected between urinary calcium excretion and the serum level of PTH (r= -0.303; p=0.051) (Table 7, Figure 7), and albumin-corrected serum calcium levels (r=0.275, p=0,078), but the correlations did not reach statistical significance (Table 7, Figure 8). In the multiple linear regression analysis only the serum levels of 1,25-dihydroxyvitamin D (B= 7.566 $\pm$ 1.679; 95% CI 4.170 to 10.961; p<0.001), and the serum levels of PTH (B= -9.211 $\pm$ 3.397; 95% CI -16.083 to -2.339; p=0.01) remained significantly associated with urinary calcium excretion (Table 9).

Table 7. Correlation matrix.

		URINE CALCIUM (mg/24h)	AGE (years)	WEIGHT (Kg)	BMI (Kg/m^2)	CALCIUM ORAL TOTAL (mg/day)	VIT. D3 DOSE (UI/day)	CALCI-TRIOL DOSE (µg/day)	THYRO-XINE DOSE (µg (µg/day)
URINE CALCIUM (mg/24h)	r	1	-.241	-.198	-.282	.166	.068	.443	-.068
	p		.123	.209	.070	.293	.669	.003	.670
	n	42	42	42	42	42	42	42	42
AGE (years)	r	-.241	1	.093	.232	.045	-.091	-.239	-.273
	p	.123		.557	.139	.778	.567	.127	.081
	n	42	42	42	42	42	42	42	42
WEIGHT (Kg)	r	-.198	.093	1	.819	-.164	-.255	.024	.633
	p	.209	.557		.000	.299	.103	.878	.000
	n	42	42	42	42	42	42	42	42
BMI (Kg/m^2)	r	-.282	.232	.819	1	-.238	-.078	-.165	.315
	p	.070	.139	.000		.129	.622	.296	.042
	n	42	42	42	42	42	42	42	42

		URINE CALCIUM (mg/24h)	AGE (years)	WEIGHT (Kg)	BMI (Kg/m^2)	CALCIUM ORAL TOTAL (mg/day)	VIT. D3 DOSE (UI/day)	CALCI-TRIOL DOSE (µg/day)	THYRO-XINE DOSE (µg/day)
CALCIUM ORAL TOTAL (mg/day)	r	.166	.045	-.164	-.238	1	.207	.275	-.097
	p	.293	.778	.299	.129		.188	.078	.542
	n	42	42	42	42	42	42	42	42
VIT. D3 DOSE (UI/day)	r	.068	-.091	-.255	-.078	.207	1	-.130	-.130
	p	.669	.567	.103	.622	.188	.000	.413	.411
	n	42	42	42	42	42	42	42	42
CALCITRIOL DOSE (µg/day)	r	.443	-.239	.024	-.165	.275	-.130	1	-.008
	p	.003	.127	.878	.296	.078	.413		.959
	n	42	42	42	42	42	42	42	42
THYROXINE DOSE (µg/day)	r	-.068	-.273	.633	.315	-.097	-.130	-.008	1
	p	.670	.081	.000	.042	.542	.411	.959	
	n	42	42	42	42	42	42	42	42
ALBUMIN-CORRECTED SERUM CALCIUM (mg/dl)	r	.275	-.099	-.455	-.364	-.002	.069	-.011	-.243
	p	.078	.531	.002	.018	.988	.666	.944	.121
	n	42	42	42	42	42	42	42	42
SERUM TSH (mU/ml)	r	.113	-.167	.238	.137	-.009	-.107	.085	.102
	p	.477	.289	.129	.388	.954	.499	.591	.521
	n	42	42	42	42	42	42	42	42
SERUM FREE THYROXINE (ng/ml)	r	-.214	.194	-.132	-.070	.087	.080	-.315	.127
	p	.175	.219	.405	.662	.582	.612	.042	.425
	n	42	42	42	42	42	42	42	42
SERUM PTH (pg/ml)	r	-.303	.142	.108	.246	-.256	-.025	-.323	.104
	p	.051	.371	.498	.116	.101	.876	.037	.512
	n	42	42	42	42	42	42	42	42
SERUM 25-OH-VIT-D (ng/ml)	r	.123	.018	-.349	-.220	-.160	.417	-.137	-.360
	p	.436	.912	.023	.161	.311	.006	.389	.019
	n	42	42	42	42	42	42	42	42
SERUM 1,25-dOH-VIT-D (pg/ml)	r	.539	-.286	-.143	-.220	-.067	.136	.292	.121
	p	.000	.066	.367	.162	.672	.392	.061	.445
	n	42	42	42	42	42	42	42	42

		ALBUMIN-CORRECTED SERUM CALCIUM (mg/dl)	SERUM TSH (mU/mL)	SERUM FREE THYROXINE (ng/ml)	SERUM PTH (pg/ml)	SERUM 25-OH-VIT-D (ng/ml)	SERUM 1,25-dOH-VIT-D (pg/ml)
URINE CALCIUM (mg/24h)	r	.275	.113	-.214	-.303	.123	.539
	p	.078	.477	.175	.051	.436	.000
	n	42	42	42	42	42	42
AGE (years)	r	-.099	-.167	.194	.142	.018	-.286
	p	.531	.289	.219	.371	.912	.066
	n	42	42	42	42	42	42
WEIGHT (Kg)	r	-.455	.238	-.132	.108	-.349	-.143
	p	.002	.129	.405	.498	.023	.367
	n	42	42	42	42	42	42
BMI (Kg/m2)	r	-.364	.137	-.070	.246	-.220	-.220
	p	.018	.388	.662	.116	.161	.162
	n	42	42	42	42	42	42
CALCIUM ORAL TOTAL (mg/day)	r	-.002	-.009	.087	-.256	-.160	-.067
	p	.988	.954	.582	.101	.311	.672
	n	42	42	42	42	42	42
VIT. D3 DOSE (UI/day)	r	.069	-.107	.080	-.025	.417	.136
	p	.666	.499	.612	.876	.006	.392
	n	42	42	42	42	42	42
CALCITRIOL DOSE (µg/day)	r	-.011	.085	-.315	-.323	-.137	.292
	p	,944	.591	.042	.037	.389	.061
	n	42	42	42	42	42	42
THYROXINE DOSE (µg/day)	r	-.243	.102	.127	.104	-.360	.121
	p	.121	.521	.425	.512	.019	.445
	n	42	42	42	42	42	42
ALBUMIN-CORRECTED SERUM CALCIUM (mg/dl)	r	1	-.072	.207	-.124	.294	.259
	p		.651	.189	.435	.059	.097
	n	42	42	42	42	42	42
SERUM TSH (mU/ml)	r	-.072	1	-.352	-.158	-.090	.260
	p	.651		.022	.319	.569	.097
	n	42	42	42	42	42	42

		ALBUMIN-CORRECTED SERUM CALCIUM (mg/dl)	SERUM TSH (mU/mL)	SERUM FREE THYROXINE (ng/ml)	SERUM PTH (pg/ml)	SERUM 25-OH-VIT-D (ng/ml)	SERUM 1,25-dOH-VIT-D (pg/ml)
SERUM FREE THYROXINE (ng/ml)	r	.207	-.352	1	.180	-.045	-.205
	p	.189	.022		.254	,777	.192
	n	42	42	42	42	42	42
SERUM PTH (pg/ml)	r	-.124	-.158	.180	1	.004	.059
	p	.435	.319	.254		.978	.710
	n	42	42	42	42	42	42
SERUM 25-OH-VIT-D (ng/ml)	r	.294	-.090	-.045	.004	1	.099
	p	.059	.569	.777	.978		.533
	n	42	42	42	42	42	42
SERUM 1,25-dOH-VIT-D (pg/ml)	r	.259	.260	-.205	.059	.099	1
	p	.097	.097	.192	.710	.533	
	n	42	42	42	42	42	42

7.4.- Analysis of calcitriol treatment and serum levels of 1,25-dihydroxyvitamin D

A detailed analysis of serum levels of 1,25-dihydroxyvitamin D and calcitriol treatment was performed and is shown in Table 8.

	Value (mean ± SD)	Correlation with the level of serum 1,25-dihydroxyvitamin D (pg/ml)	p value for correlation
Daily dose of calcitriol (µg/day):			
-All patients (n=42)	0.47 ± 0.20	r =0.292	0.061
-Patients with hypercalciuria (n=16)	0.54 ± 0.22	r= 0.475	0.063
-Patients without hypercalciuria (n=26)	0.43 ± 0.18	r= 0.012	ns
Last dose of calcitriol taken (µg):			
-All patients (n=42)	0.29 ± 0.11	r = 0.150	ns
-Patients with hypercalciuria (n=16)	0.28 ± 0.12	r = 0.291	ns
-Patients without hypercalciuria (n=26)	0.31 ± 0.11	r = 0.229	ns
Time interval between the last intake of calcitriol and the blood draw (hours):			
-All patients (n=42)	14.9 ± 5.8	r = - 0.073	ns
-Patients with hypercalciuria (n=16)	14.2 ± 5.8	r = - 0.208	ns
-Patients without hypercalciuria (n=26)	15.4 ± 6.1	r = 0.055	ns

Table 8. Results and correlations of calcitriol treatment and serum levels of 1,25-dihydroxyvitamin D.

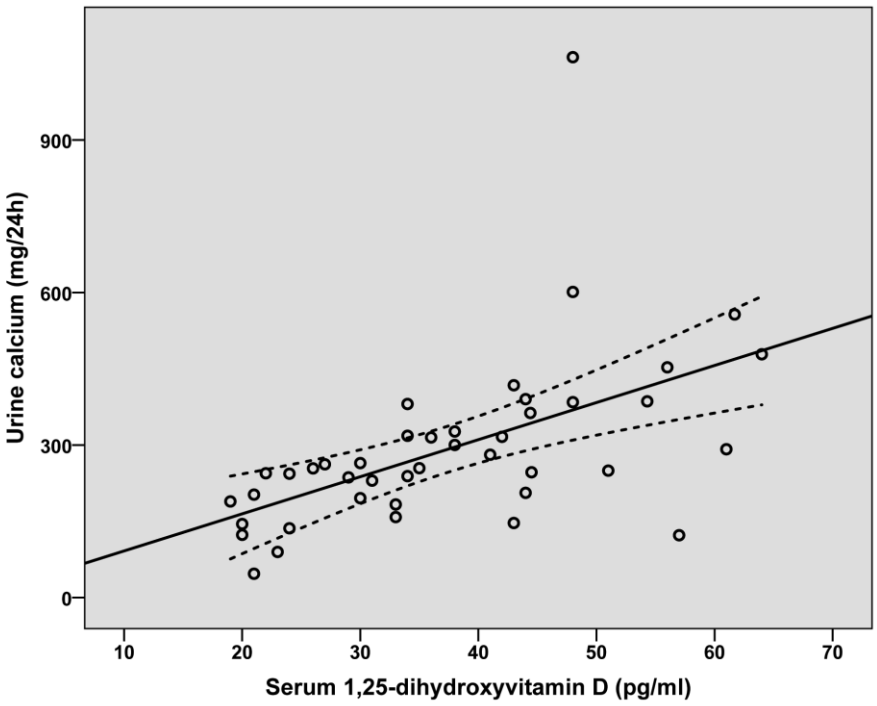

Figure 6. Correlation and linear regression between urinary calcium excretion and serum 1,25-dihydroxyvitamin D.
(r=0.539, p<0.001; Urine calcium (mg/24h) = 7.29 * serum 1,25-dihydroxyvitamin D (pg/ml) + 18.76; dashed lines represent 95% CI of regression line).

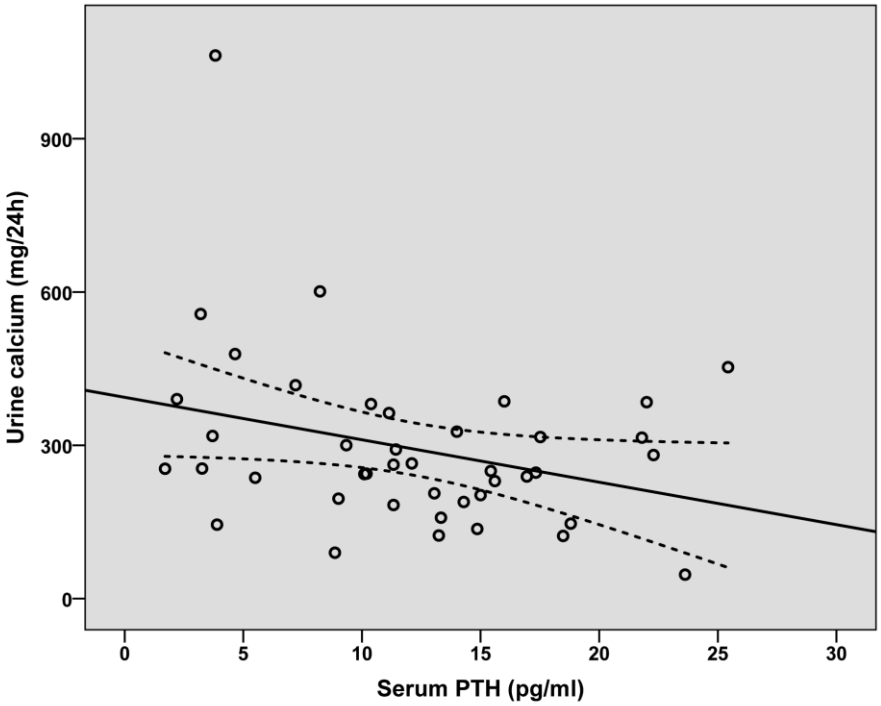

Figure 7. Correlation and linear regression between urinary calcium excretion and serum PTH.
(r=-0.303, p=0.051; Urine calcium (mg/24h) = -8.30 * serum PTH (pg/ml) + 393.86; dashed lines represent 95% CI of regression line).

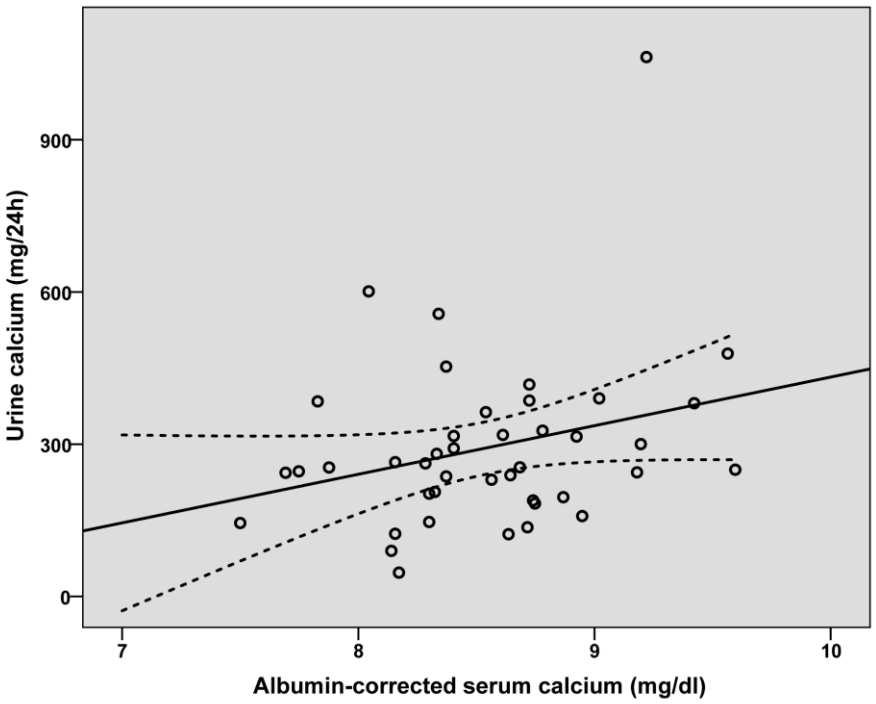

Figure 8. Correlation and linear regression between urinary calcium excretion and albumin-corrected serum calcium.
(r=0.275, p=0.078; Urine calcium (mg/24h) = 95.78 * serum albumin-corrected calcium (mg/dl) – 525.53; dashed lines represent 95% CI of regression line).

Table 9. Multiple linear regression analysis (stepwise method) of urine calcium excretion with daily dose of calcitriol, serum level of PTH, serum level of 1,25-dihydroxyvitamin D, and albumin-corrected serum calcium levels.

Summary of the model

			Statistics				
Model	R	R squared	Evolution in R squared	Evolution in F	gl1	gl2	Significance of the evolution in F
1	.539[a]	.290	.290	16.359	1	40	.000
2	.635[b]	.403	.113	7.351	1	39	.010

(a): Model 1: predictive variables: constant and serum 1,25-dihydroxyvitamin D.
(b): Model 2: predictive variables: constant, serum 1,25-dihydroxyvitamin D and serum PTH.

ANOVA[c]

Model		Sum of squares	gl	Mean squared	F	Sig.
1	Regression	346,134.509	1	346,134.509	16.359	.000[a]
	Residual	846,366.940	40	21,159.173		
	Total	1,192,501.449	41			
2	Regression	459,792.090	2	240,181.322	13.153	.000[b]
	Residual	854,143.117	51	18,259.969		
	Total	1,313,935.207	53			

Coefficients[c]

Model		Unstandarized coefficients		Standarized coefficients	t	Sig.	95% CI for B	
		B	Tipical Error	Beta			Lower limit	Upper limit
1	(Constant)	18.768	71.353		.263	.794	-125.44	162.977
	Serum 1,25-dihy-droxyvitamin D	7.296	1.804	.539	4.04	.000	3.650	10.94
2	(Constant)	120.84	76.231		1.58	.121	-33.344	275.039
	Serum 1,25-dihy-droxyvitamin D	7.566	1.679	.559	4.50	.000	4.170	10.961
	Serum PTH	-9.211	3.397	-.336	-2.71	.010	-16.083	-2.339

(c): dependent variable: urine calcium excretion.

Excluded variables[c]

Model		Beta in	t	Significance	Partial Correlation
1	Serum PTH	-.336[a]	-2.711	.010	-.398
	Albumin-corrected serum calcium	.145[a]	1.050	.300	.166
	Daily dose calcitriol	.312[a]	2.368	.023	.355
2	Albumin-corrected serum calcium	.096[b]	.741	.119	.119
	Daily dose calcitriol	.215[b]	1.580	.248	.248

(c): dependent variable: urine calcium excretion.

7.5.- The ROC curves

The ROC curves for the serum level of 1,25-dihydroxyvitamin D, the serum level of PTH, and the level of albumin-corrected serum calcium, used to discriminate between patients with and without hypercalciuria, are shown in Figure 9. Areas under ROC curves were 0.829 (95% CI, 0.706 to 0.953) for serum 1,25-dihydroxyvitamin D, 0.591 (95% CI, 0.397 to 0.786) for serum PTH, and 0.692 (95% CI, 0.525 to 0.859) for albumin-corrected serum calcium. When these areas were compared, a nearly significant difference was found (Chi-squared=3.7678; df: 2; p=0.0522).

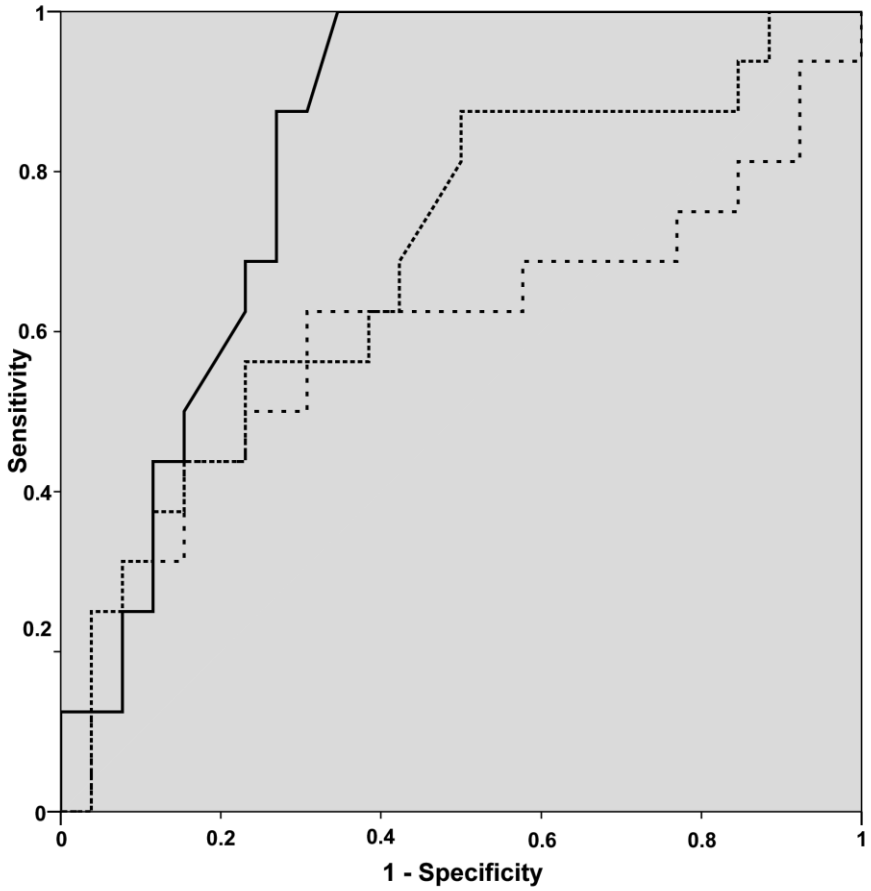

Figure 9. ROC curves for serum 1,25-dihydroxyvitamin D (——), albumin-corrected serum calcium (-----), and serum level of PTH (▪ ▪ ▪), to discriminate between patients with and without hypercalciuria.

Proposed cut-off values for these variables to predict absence of hypercalciuria with 100% sensitivity are shown in Table 10.

Variable	*Cut-off value*	*Sensitivity*	*Specificity (95% CI for specificity)*
Albumin-corrected serum calcium (mg/dl)	≤ 7.78	100%	11.5% (0 to 23.7)
Serum PTH (pg/ml)	≤ 26.4	100%	0% (0)
Serum 1,25-dihydroxyvitamin D (pg/ml)	≤ 33.5	100%	65.4% (47.1 to 83.7)

Table 10. Cut-off values for each variable selected from ROC curves to detect cases with absence of hypercalciuria by their higher specificity for 100% sensitivity.

7.6.- Relationship between serum 1,25-dihydroxyvitamin D, serum and urine calcium

Figure 10 shows the distribution of patients on the basis of their serum levels of 1,25-dihydroxyvitamin D and 24-hour urine calcium excretion. Patients have been grouped according to their levels of albumin-corrected serum calcium. No patient with a serum 1,25-dihydroxyvitamin D level equal to or less than 33.5 pg/ml had hypercalciuria, regardless of the level of albumin-corrected serum calcium (lower than 8.5 mg/dl, 8.5 to 9.5 mg/dl or higher than 9.5 mg/dl). In the group of patients with serum 1,25-dihydroxyvitamin D level higher than 33.5 pg/ml, a 64% (16/25) had hypercalciuria.

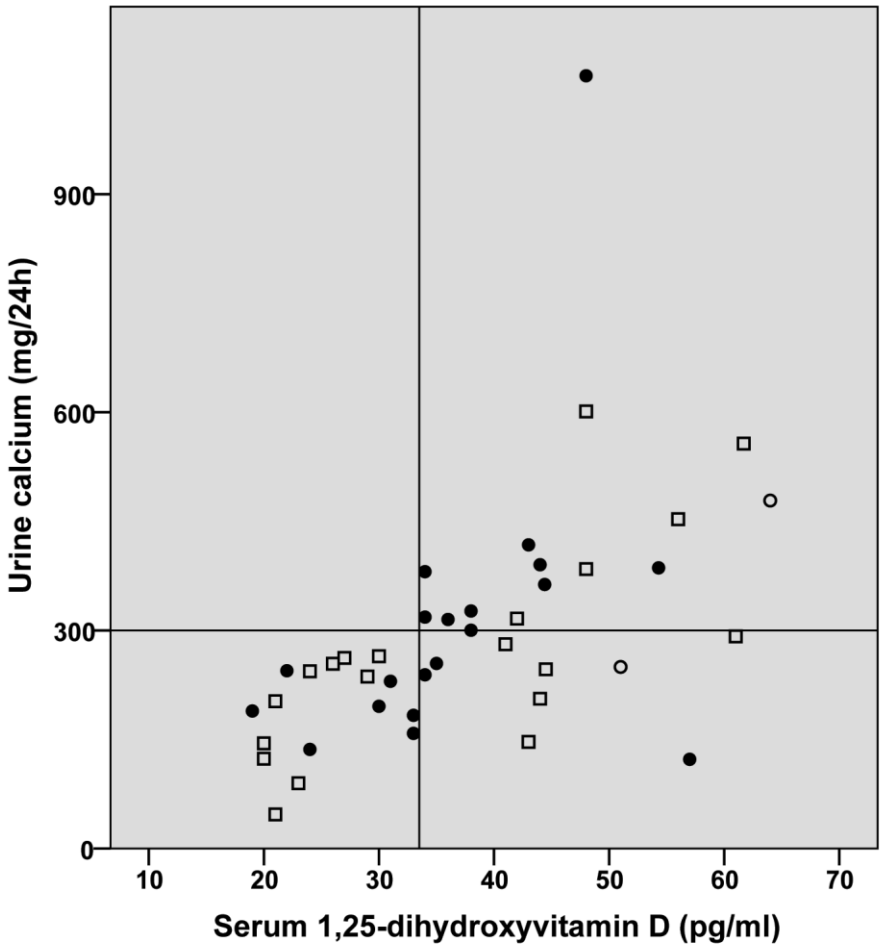

Figure 10. Distribution of patients grouped by their albumin-corrected serum calcium (□ lower than 8.5 mg/dl, ● between 8.5 and 9.5 mg/dl, ○ higher than 9.5 mg/dl) depending on their level of serum 1,25-dihydroxyvitamin D and urine calcium. The vertical line intersects the X axis at 33.5 pg/ml.

7.7.- Analysis of the serum levels of 1,25-dihydroxyvitamin D

When comparing patients with serum 1,25-dihydroxyvitamin D levels equal to or less than (n=17) and greater than (n=25) 33.5 pg/ml, serum PTH was similar in the groups (11.4 ± 5.0 pg/ml *vs.* 12.7 ± 6.9 pg/ml; p: ns) (Figure 11), as was albumin-corrected serum calcium (8.37 ± 0.45 mg/dl *vs.* 8.65 ± 0.48 mg/dl; p= 0.07) (Figure 12), but not 24-hour urine calcium excretion (188.5 ± 64.0 mg/24h *vs.* 363.5 ± 184.5 mg/24h; p<0.001) (Figure 13). A correlation between serum 1,25-dihydroxyvitamin D and creatinine clearance (r=0.381, p=0.014) (Figure 14) was found. A clear tendency was detected between serum levels of 1,25-dihydroxyvitamin D and daily dose of calcitriol (r= 0.292, p=0.061) but the correlation did not reach statistical significance. However, we did not find any significant relationship between serum levels of 1,25-dihydroxyvitamin D and the last dose of calcitriol before blood draw, the time interval between the last intake of calcitriol and the blood draw, serum PTH, age, weight, or sex.

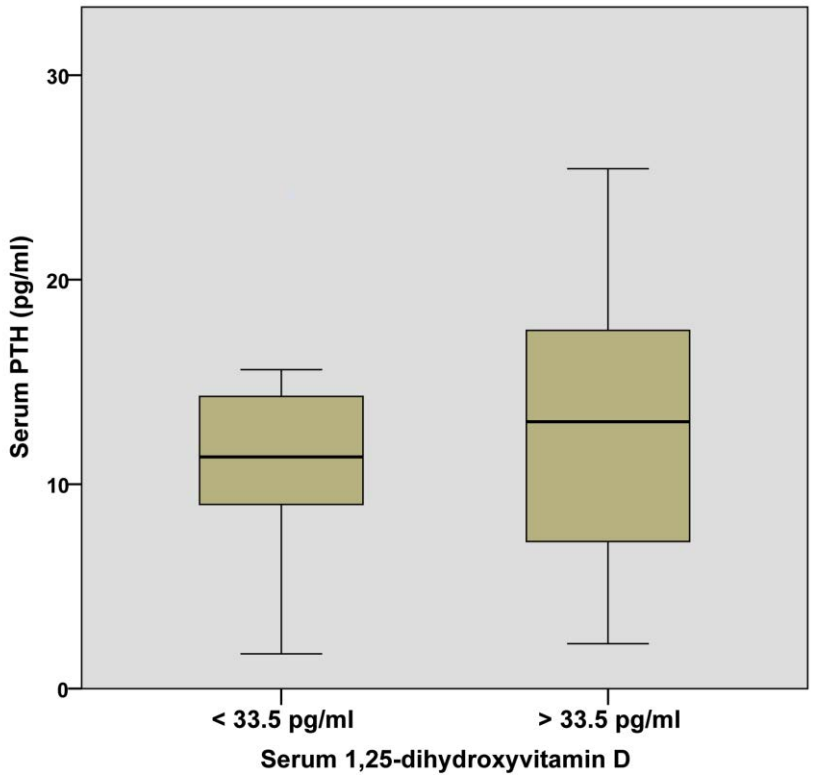

Figure 11. Serum levels of PTH in patients grouped by their serum 1,25-dihydroxyvitamin D level (11.4 ± 5.0 pg/ml *vs.* 12.7 ± 6.9 pg/ml; p:ns). Boxes represent percentile 25 to percentile 75. Horizontal line inside the box represents median value. Vertical lines up and down represent percentile 95 and percentile 5.

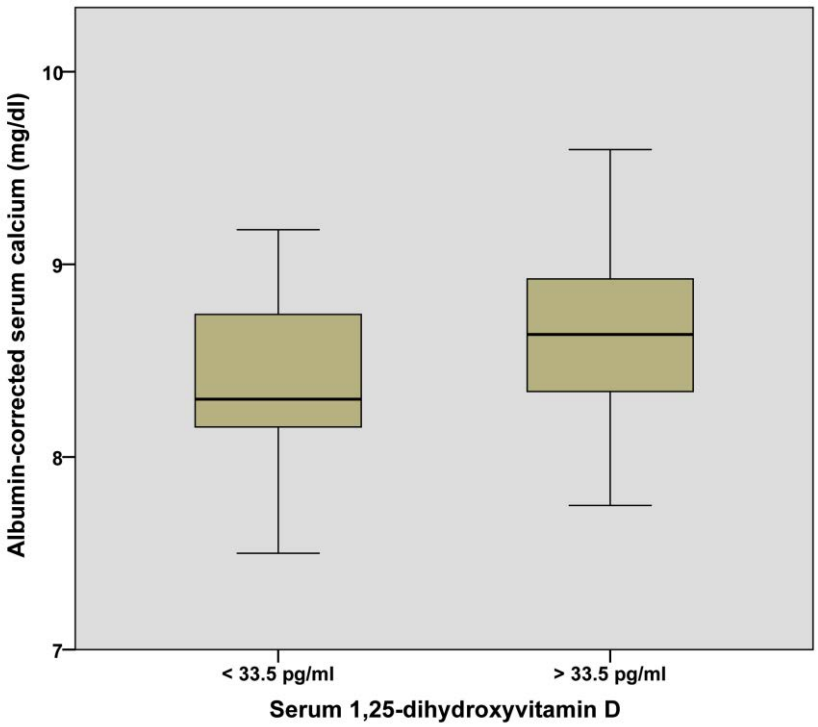

Figure 12. Albumin-corrected serum calcium levels in patients grouped by their serum 1,25-dihydroxyvitamin D level (8.37 ± 0.45 mg/dl *vs.* 8.65 ± 0.48 mg/dl; p= 0.07). Boxes represent percentile 25 to percentil 75. Horizontal line inside the box represents median value. Vertical lines up and down represent percentile 95 and percentile 5.

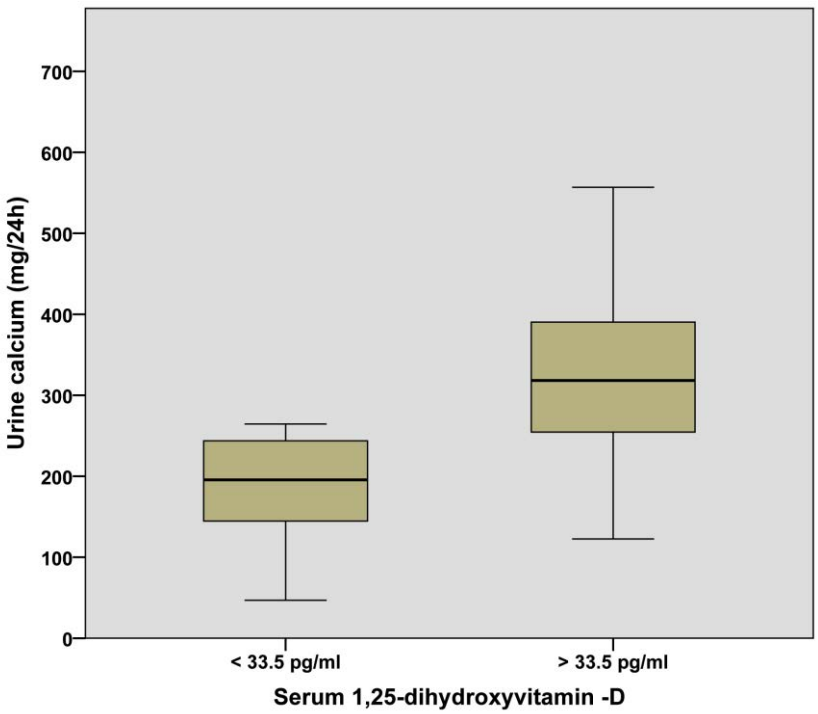

Figure 13. Urine calcium levels in patients grouped by their serum 1,25-dihydroxyvitamin D level (188.5 ± 64.0 mg/24h *vs.* 363.5 ± 184.5 mg/24h; p<0.001). Boxes represent percentile 25 to percentil 75. Horizontal line inside the box represents median value. Vertical lines up and down represent percentile 95 and percentile 5.

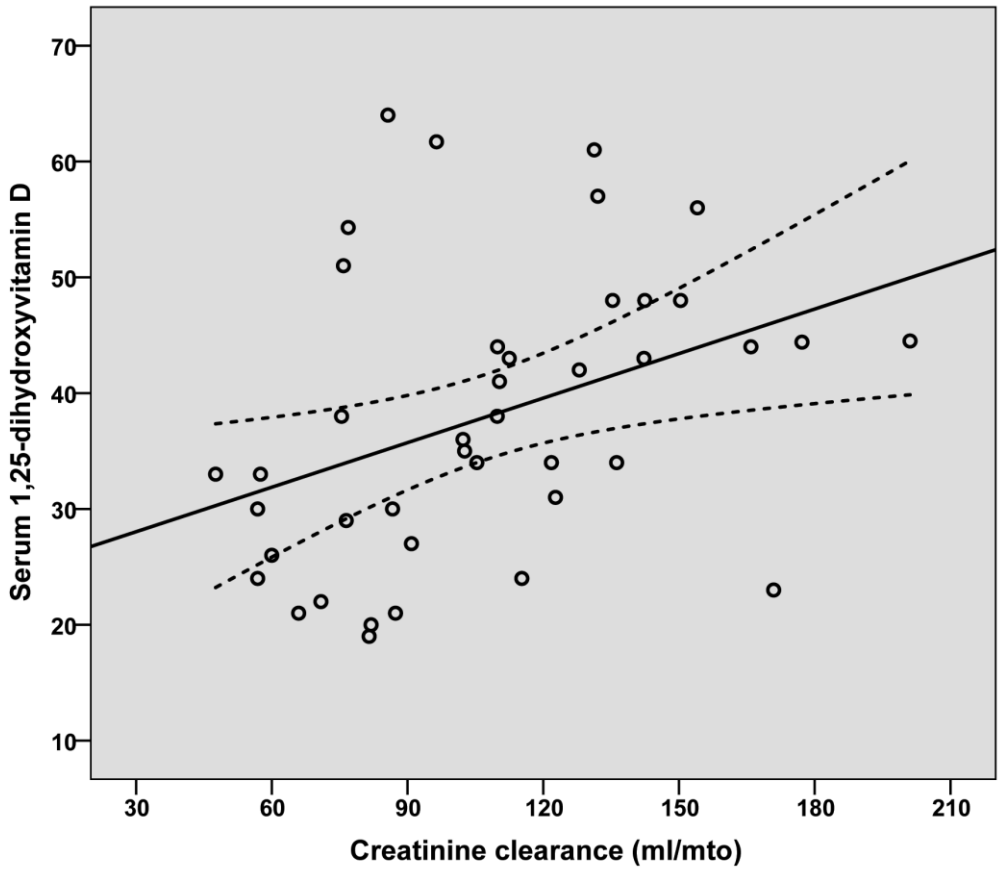

Figure 14. Correlation and linear regression between creatinine clearance and serum 1,25-dihydroxyvitamin D levels. (r=0.381, p=0.014; creatinine clearance (ml/min) = 1.135 * serum 1,25-dihydroxyvitamin (pg/ml) + 64.46; dashed lines represent 95% CI of regression line).

7.8.- Remisssion of hypercalciuria

Of 16 patients with hypercalciuria, 5 had an albumin-corrected serum calcium level below 8.5 mg/dl, and 11 had an albumin-corrected serum calcium level above 8.5 mg/dl. Among the latter, 5 patients who had vitamin D3 supplements and calcitriol doses reduced without the addition of thiazide diuretics had normalizaed urinary calcium excretion rates and, simultaneously, their serum 1,25-dihydroxyvitamin D levels decreased to less than 33.5 pg/ml (Table 11).

	Before hypercalciuria correction	After hypercalciuria correction	p value
Treatment:			
- Calcium supplements (mg/day)	1,400 ± 1,083	1,500 ± 1,118	ns
- Vitamin D3 supplements (UI/day)	896 ± 995	400 ± 565	ns
- Calcitriol (µg/day)	0.50 ± 0.17	0.35 ± 0.13	ns
- Thyroxine (µg/day)	147 ± 18	145 ± 21	ns
Serum analytic variables:			
- Albumin-corrected serum calcium (mg/dl)	8.78 ± 0.68	8.60 ± 0.32	ns
- Thyrotropin (mUI/l)	0.29 ± 0.25	0.71 ± 0.82	ns
- Free thyroxine (ng/ml)	1.83 ± 0.38	1.67 ± 0.45	ns
- Parathyroid hormone (pg/ml)	15.0 ± 7.7	16.3 ± 7.6	ns
- 25-hydroxyvitamin D (ng/ml)	30.4 ± 14.0	28.8 ± 10.3	ns
- 1,25-dihydroxyvitamin D (pg/ml)	45.6 ± 11.2	28.8 ± 10.3	<0.05
Urine analytic variables:			
- Calcium (mg/24h)	358 ± 74	232 ± 36	<0.05
- Creatinine (mg/24h)	1,098 ± 201	1,057 ± 218	ns
Clearance of creatinine (ml/min)	106.7 ± 28.1	102.2 ± 26.4	ns

Table 11. Changes in 5 patients with hypercalciuria and albumin-corrected serum calcium above 8.5 mg/dl, before and after correction of the hypercalciuria.

8.- DISCUSSION

8.- DISCUSSION

This is a prospective cross-sectional clinical study to identify useful biomarkers related to the presence of hypercalciuria in patients with permanent postsurgical hypoparathyroidism who were receiving treatment with oral calcium and calcitriol supplements, with or without vitamin D3 supplements. Serum levels of 1,25-dihydroxyvitamin D were independently related to 24-hour urinary calcium excretion, and we found a cut-off value for this variable that allowed us to predict the absence of hypercalciuria in all cases. To the best of our knowledge, this is the first evidence of a reliable serum biomarker for identifying patients who are at risk of hypercalciuria after permanent postsurgical hypoparathyroidism treated with calcium and calcitriol supplements.

8.1.- Measurement of 24-hour urine calcium excretion

A major therapeutic challenge in patients with hypoparathyroidism is the consistent effective management of the hypocalcemia while avoiding hypercalciuria and other complications. In the absence of PTH, the tubular reabsorption of calcium is reduced, and much of the absorbed calcium in the intestine is lost in the urine [86]. Measurement of urinary calcium excretion was first conducted as an indicator test of the presence or absence of hypercalcemia or hypocalcemia. As the results were confusing, this test was discontinued for this purpose [93]. However, in the following years up to now, periodic measurement of 24-hour urinary calcium excretion has been recommended for patients with hypoparathyroidism even if they are stable [2,6,94,111]. This approach is designed to avoid renal complications if hypercalciuria appears as a consequence of the treatment with calcium and vitamin D supplements. In the absence of other alternative reliable indicators of hypercalciuria, this procedure has been implemented despite the volume omissions in 24-hour urine collections that can produce false negative results (see Chapter 4.3) [96-98]. In this regard, Mitchell, *et al* [9] reported that compliance in 24-hour urine collections was only 44%, but of those patients who successfully completed this procedure, 38% had at least one excessive (over 300 mg) 24-hour urine calcium measurement. In our series, four urine specimens were rejected because of a low level of urine creatinine which made us suspect that the sample was incomplete. Of the 42 patients, 16 had hypercalciuria (38.1%), a figure equal to that of the Mitchell series.

8.2.- Serum calcium, serum levels of 1,25-dihydroxyvitamin D, and hypercalciuria

There is evidence to suggest that albumin-corrected serum calcium is of limited value to predict hypercalciuria in the setting of hypoparathyroidism [9] (see Chapter 4.1). Nevertheless, to minimize or prevent hypercalciuric episodes and their renal consequences, a policy of keeping the fasting serum calcium concentration around or below the normal limit has been advised [2,6,7,9,55,86,94,111,116,117]. However, to the best of our knowledge, serum levels of 1,25-dihydroxyvitamin D have not been previously reported as a useful predictive biomarker of hypercalciuria in patients with postsurgical hypoparathyroidism.

Serum 1,25-dihydroxyvitamin D is a powerful hypercalcemic metabolite [86,94,118]. Its serum levels depend on calcitriol dosage, the time interval between the last intake of calcitriol and the blood draw, PTH residual secretion, renal integrity, serum calcium levels, and calcidiol reserves [101]. Likewise, calcidiol is influenced by multiple factors [119]. Thus, different levels of serum 1,25-dihydroxyvitamin D can be achieved among patients taking the same dosage of calcitriol supplements. In our study, the absence of a relationship between serum levels of 1,25-dihydroxyvitamin D with the last dose of calcitriol, and the time interval between the last intake of calcitriol and the blood draw, could be explained by the fact that the time required for the calcitriol to reach its peak serum concentration after administration (3 to 6 hours) [111] is approximately one-third the interval of time between the last intake of calcitriol and the blood draw (14.9 ± 5.8 hours).

Our patients had serum 1,25-dihydroxyvitamin D levels of 38.3 $\pm$ 12.5 pg/ml, similar to those for the control group (39.7 $\pm$ 13.3 pg/ml). This may be attributed to the treatment with calcitriol. The administered treatment with calcitriol may have been responsible for restoring the low or low-normal levels of serum 1,25-dihydroxyvitamin D currently found in the patients with hypoparathyroidism before treatment is begun [2,7,55]. In the laboratory at our center, the normal serum levels of 1,25-dihydroxyvitamin D are 25 to 66 pg/ml; values between 20 and 60 pg/ml have been considered normal in healthy subjects by other authors [101]. Measurement of serum 1,25-dihydroxyvitamin D levels has been considered not to be necessary in the evaluation of patients with hypoparathyroidism [2,111].

Our data show that patients with permanent postsurgical hypoparathyroidism receiving treatment with oral calcium and oral calcitriol supplements are at risk of hypercalciuria above a specific level of serum 1,25-dihydroxyvitamin D (33.5 pg/ml), regardless of their albumin-corrected serum calcium. The presence of hypercalciuria with concomitant low, normal, or high levels of serum calcium was previously observed. In the largest reported longitudinal cohort of 120 patients with hypoparathyroidism (66% of postsurgical etiology), with follow-up for 7.4 $\pm$ 5.1 years, higher serum calcium levels were associated with higher urine calcium values. However, there was wide variability, and in 44 simultaneous urine and serum calcium measurements, 2 of 22 patients had urine calcium levels above 300 mg/24-hour, whereas they had serum calcium levels below 8.5 mg/dl; conversely 17 of the remaining 22 patients had serum calcium levels above 8.5 mg/dl without hypercalcuria [9].

In our study, the level of serum 1,25-dihydroxyvitamin D was shown to be a useful independent biomarker of the absence of hypercalciuria. Patients in whom serum levels of 1,25-dihydroxyvitamin D were equal to or below 33.5 pg/ml did not present hypercalciuria. In addition, serum levels of 1,25-dihydroxyvitamin D above 33.5 pg/ml suggested the presence of hypercalciuria. This test has a sensitivity of 100% with a specificity of 65.4% and an area under the ROC curve of 0.829. It is worth mentioning that a test with no discriminant ability will produce a ROC curve that follows the diagonal of the grid, and the area under the curve will be 0.5. If a test could discriminate perfectly, the ROC curve would pass throught the point (0,1) on the unit grid and the area under the curve would be 1. Values for the area under the ROC curve between 0.5 and 0.7 indicate low accuracy; values between 0.7 and 0.9 indicate that a test is useful for some purposes, and values above 0.9 indicate high accuracy [120]. Some diagnostic tests in medicine that are commonly used and recommended by expert panels have an area under the ROC curve between 0.7 and 0.8 [121].

8.3.- Idiopathic hypercalciuria *versus* elevated serum levels of 1,25-dihydroxyvitamin D

One could argue that patients with hypercalciuria may be afected by idiopathic hypercalciuria. It is our clinical practice that patients with hypercalciuria and albumin-corrected serum calcium above 8.5 mg/dl are advised to reduce oral calcium

supplements to a maximun of 500 mg of calcium element tid and/or to additionally reduce the dosage of calcitriol by 0.25 mcg/day; patients with hypercalciuria and albumin-corrected serum calcium below 8.5 mg/dl are treated by adding thiazide diuretics. In both cases, a new analytic control is performed 2-4 weeks later. In this series, of 16 patients with hypercalciuria, 5 had albumin-corrected serum calcium below 8.5 mg/dl, and 11 had albumin-corrected serum calcium above 8.5 mg/dl. Five of these latter patients were treated by reducing their oral doses of calcitriol and vitamin D3 supplements whereby they experienced remission of hypercalciuria. This decrease in the doses of vitamin D3 supplements (896 ± 995 to 400 ± 565 UI/day) and calcitriol (0.50 ± 0.17 to 0.35 ± 0.13 mcg/day) was not statistically significant, because the low number of patients confers a low power to detect differences. In the case of vitamin D3 supplements, their high standard deviation adds to the difficulty in finding statistically significant differences [122,123]. The combined reduction in vitamin D3 supplements and the dosage of calcitriol could have had an additive effect, resulting in a more intense decrease in 1,25-dihydroxyvitamin D serum levels (45.6 $\pm$ 11.2 to 28.8 $\pm$ 10.3 pg/ml; $p< 0.05$). Table 11 shows available data for these patients, before and after correcting hypercalciuria. Thus, it seems unlikely that such patients had idiopathic hypercalciuria. By contrast, it seems more plausible that the correction of hypercalciuria accounted for the changes in 1,25-dihydroxyvitamin D serum levels.

8.4.- Clinical implications

Results of our study suggest that monitoring of patients with permanent postsurgical hypoparathyroidism should include serum 1,25-dihydroxyvitamin D assessment. The measurement of 24-hour urine calcium should be limited to confirming hypercalciuria in those patients with serum levels of 1,25-dihydroxyvitamin D above 33.5 pg/ml. This approach seems more reliable and less cumbersome for patients than routine 24-hour urine collections.

We propose the following algorithm for the management of patients with hypoparathyroidism receiving supplementary treatment with calcium and calcitriol supplements:

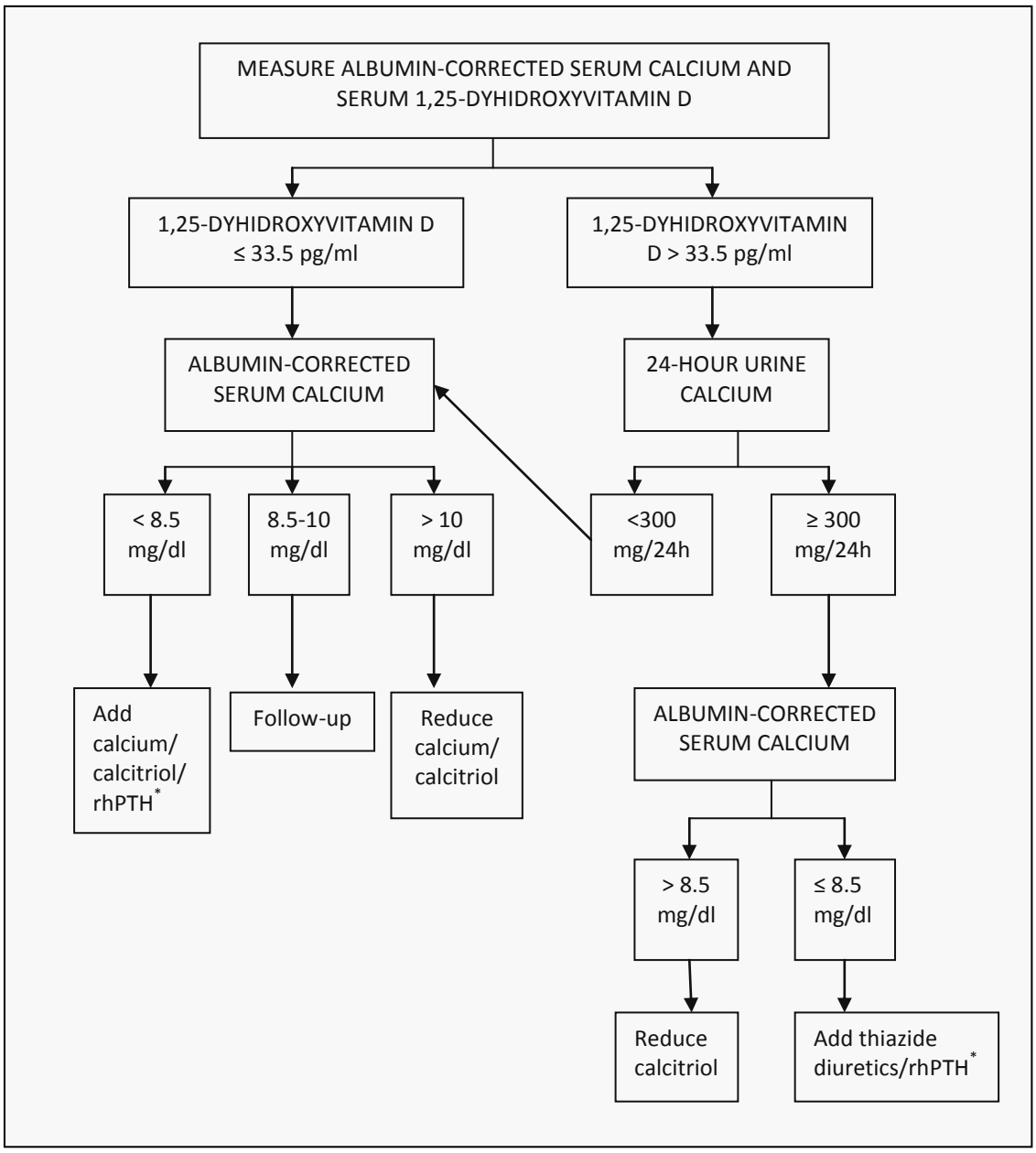

Figure 15. Proposed algorithm for managing patients with permanent postsurgical hypoparathyroidism receiving supplementary treatment.
(*): Recommended by an expert panel [7] and authorized by EMA in April 2017 but not yet available in Spain.

In clinical practice, if someone suspects hypercalciuria in a patient as a result of the level of 1,25-dihydroxyvitamin D, and hypercalciuria is confirmed by 24-hour urine collection, the serum level of 1,25-dihydroxyvitamin D may be an indicator that the dosage of oral calcitriol supplements should be reduced (if hypocalcemia is not present; if hypocalcemia is present, one should consider adding thiazide diuretics

before reducing the oral dose of calcitriol) in order to achieve a serum level of 1,25-dihydroxyvitamin D below 33.5 pg/ml. Likewise, this approach of reducing the dosage of oral calcitriol supplements in this situation (hypercalciuria with serum 1,25-dihydroxyvitamin D above 33.5 pg/ml without concomitant hypocalcemia) could also be an indicator of the recovery of parathyroid function when applied to patients with postsurgical hypoparathyroidism that is still considered nonpermanent, because it could advise about the recovery of parathyroid function.

Because serum 1,25-dihydroxyvitamin D is more closely related to the absence of hypercalciuria than is albumin-corrected serum calcium, it seems reasonable to suggest that when 1,25-dihydroxyvitamin D is lower than 33.5 pg/ml, the optimal goal of therapy in patients with permanent postsurgical hypoparathyroidism receiving treatment with oral calcium and calcitriol supplements should be to achieve normal serum calcium values instead of the currently recommended low-normal levels. In terms of clinical practice, looking at Figure 10, no patients receiving treatment with oral calcium and calcitriol supplements, without other treatments that could modify the urinary calcium excretion, had hypercalciuria when the serum 1,25-dihydroxyvitamin D level was equal to or lower than 33.5 pg/ml regardless of the level of albumin-corrected serum calcium.

Overall the proposed approach (Figure 15) reduces hypercalciuria and could reduce the high frequency of visits to emergency departments as well as hospital admissions for symptomatic hypocalcemia as a consequence of maintaining low-normal serum calcium.

8.5.- Mechanisms of hypercalciuria in patients with hypoparathyroidism

There are some mechanisms that explain why the presence of hypercalciuria may be influenced by the level of serum 1,25-dyhidroxyvitamin D in patients with hypoparathyroidism and normal or even low fasting serum calcium levels. Calcitriol enhances the intestinal absorption of calcium through an active transport system [124]. In our study, serum level of 1,25-hydroxyvitamin D was similar in patients and controls, but the intake of calcium was superior in patients. Thus, the intestinal absorption of calcium should have been greater in the patients, in particular those with higher levels

of 1,25-dyhidroxyvitamin D. Patients with low or undetectable levels of serum PTH had impaired renal reabsorption of calcium. Therefore, it follows that an increase in urinary calcium excretion occurs when larger amounts of calcium are filtered by the kidney and not reabsorbed by PTH [2,3].

On the other hand, the correlation between 1,25-dihydroxyvitamin D and creatinine clearance raises the possibility of a complementary mechanism of hypercalciuria mediated by 1,25-dihydroxyvitamin D. The reabsorption of calcium in the kidney is controlled by several factors, such as the filtered load of sodium, urine flow, and the activity of several hormones, most notably PTH [125]. A synthesis of the experimental results suggests that vitamin D3 metabolites affect distal tubular reabsorption of calcium via several mechanisms, and that these mechanisms are regulated directly or indirectly by 1,25-dihydroxyvitamin D and PTH [125]. The total amount of calcium filtered across the glomerulus under normal circumstances in a 24-hour period is about 8,000 mg [125,126]. Approximately 98% of the filtered load of calcium is reabsorbed in the proximal and distal tubules and the loop of Henle. In the proximal tubule, about 70% of the filtered load of calcium is reabsorbed predominantly through the paracellular pathway, independently of PTH action [127]. Volume expansion inhibits calcium reabsorption in the proximal tubule [125,126]. Likewise, shrinkage of extracellular volume has been suggested as a mechanism for reducing urinary calcium excretion by thiazide diuretics. Decreased extracellular volume leads to increased proximal calcium reabsorption [116]. Thus, our findings that levels of 1,25-dihydroxyvitamin D correlated positively with creatinine clearance could explain the lessened reabsorption of calcium in the proximal tubule; this may lead to the greater availability of calcium to the thick ascending loop of Henle and the distal tubule, where, in the absence of PTH, the mechanisms implicated in the reabsorption of calcium at this level are unable to assume this calcium overload, and consequently, hypercalciuria develops.

8.6.- Limitations of the study

One limitation of this study was the small number of patients recruited. Permanent postsurgical hypoparathyroidism is estimated to occur after approximately 0.5 to 6.6% of total thyroidectomies; the rates of this complication are even higher in some case series, whereas reported rates at endocrine surgical centers with high levels of expertise are 0.9 to 1.6% [2,6,14]. This low incidence of postsurgical

hypoparathyroidism is the main barrier to obtaining sufficient number of patients. In this regard, in most studies only 20 to 30 patients with hypoparathyroidism are included out of more than 1,000 thyroidectomized patients [4,46,48,74,84]. Furthermore, the demographic prevalence of permanent postsugical hypoparathyroidism has been estimated at between 6.4 and 22 cases/100,000 inhabitants [4,10,128]. The Hospital Universitari Mútua de Terrassa, where this study was carried out, is a public hospital with a population area of 200,000 inhabitants, so the 42 patients with permanent postsurgical hypoparathyroidism recruited in this study are almost all to be expected. So while the principal statistical problem associated with a small sample size is their low power for revealing effects that are genuinely true, our findings are not invalidated by this issue [122,123].

9.- CONCLUSIONS

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The main results of the study permit to conclude the following:

1.- Serum levels of 1,25-dihydroxyvitamin D are significantly and independently associated with 24-hour urinary calcium excretion.

2.- Serum levels of 1,25-dihydroxivitamin D are a useful biomarker for identifying patients at risk of hypercalciuria after permanent postsurgical hypoparathyroidism who are receiving treatment with calcium and calcitriol supplements. No patient with a serum 1,25-dihydroxyvitamin D level equal to or less than 33.5 pg/ml had hypercalciuria, regardless of the level of albumin-corrected serum calcium.

3.- The optimal initial goal of therapy in patients with permanent postsurgical hypoparathyroidism receiving treatment with oral calcium and calcitriol supplements should be to achieve normal serum calcium values, instead of the currently recommended low-normal levels to avoid hyperclaciuria, with a serum 1,25-dihydroxyvitamin D level lower than 33.5 pg/ml.

4.- Patients with hypoparathyrodisim who have hypercalciuria, serum 1,25-dihydroxivitamin D levels above 33.5 pg/ml, and albumin-corrected serum calcium above 8.5 mg/dl may be treated by having their calcitriol doses reduced. This approach allows for simultaneously decreasing the serum level of 1,25-dihydroxyvitamin D and normalizing the urinary calcium excretion.

5.- 1,25-dihydroxyvitamin D correlates with creatinine clearance and this association suggests a renal influence of 1,25-dihydroxyvitamin D on the tubular reabsortion of calcium.

6.- The monitoring of patients with permanent postsurgical hypoparathyroidism by including the measurement of serum levels of 1,25-dihydroxyvitamin D will permit us to limit the measurement of 24-hour urine calcium to those patients with serum levels of 1,25-dihydroxyvitamin D above 33.5 pg/ml. Overall, this new clinical approach will improve the management of patients with permanent postsurgical hypoparathyroidism.

10.- FUTURE LINES OF WORK

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As consequence of the results and conclusions of this thesis, future lines of research may be developed in the following areas:

- Economic appraisal on the routine implementation of the serum levels of 1,25-dihydroxivitamin D in the follow-up of the patients with hypoparathyroidism.

- Follow-up of the renal complications in hypoparathyroid patients with serum calcitriol lower than 33.5 pg/ml *vs* a control population or a historical cohort.

- To consider salt intake and renal excretion of sodium as a cofactor of the renal excretion of calcium in hypoparathyroid patients.

- To evaluate the usefulness of serum levels of 1,25-dihydroxivitamin D in other pathogenic models, as in patients with hiperparathyroidism: Has the replacement of low serum calcidiol levels an hypercalciuric effect in hyperparathyroid patients mediated by an increase in calcitriol serum levels?

- Comparision of the two classic treatment regimens in hypoparathyroid patients: high dose of calcium combined with a relative very low dose of activated vitamin D *vs* a lower dose of calcium supplements with a relatively high dose of activated vitamin D analogues.

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11.- REFERENCES

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12.- APPENDIX 1:

APPROVAL OF THE STUDY BY THE ETHICS COMMITTEE

12.- APPENDIX 1. Approval of the study by the ethics committee.

HOSPITAL UNIVERSITARI MÚTUA TERRASSA
CEIC

MútuaTerrassa

INFORME DEL COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA

Don Ramón Pla.
Presidente del Comité Etico de Investigación Clínica de:
HOSPITAL UNIVERSITARI MÚTUA DE TERRASSA

CERTIFICA:

Que este Comité ha evaluado en la reunión del día 25 de marzo de 2015 (Acta 03/15) el estudio titulado "Hypercalciuria and related factors in patients with postsurgical hypoparathyroidism".

y considera que,

Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto.
La capacidad del investigador y los medios disponibles son apropiados para llevar a cabo el estudio.

Y este Comité acepta que dicho protocolo sea realizado en el Centro HOSPITAL UNIVERSITARI MÚTUA TERRASSA por el Dr. Luis Garcia Pascual como investigador principal.

Lo que firmo en Terrassa a 25 de marzo de 2015.

Dr. Ramón Pla
Presidente del CEIC

13.- APPENDIX 2:
SCIENTIFIC PUBLICATION

13.- SCIENTIFIC PUBLICATION

A synthesis of this work has been published in one of the most relevant journals of endocrinology: The Journal of Clinical Endocrinology and Metabolism 2017; 102:259-62 (5.789 impact factor at 2017; in the first decile of the endocrinological journal ranking).

Serum 1,25-Dihydroxyvitamin D as a Biomarker of the Absence of Hypercalciuria in Postsurgical Hypoparathyroidism

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Context: Hypercalciuria is an adverse event of postsurgical hypoparathyroidism treatment that can lead to renal complications. The collection of 24-hour urine to detect hypercalciuria is often considered unreliable.

Objective: The purpose of this study was to find useful predictive biomarkers of hypercalciuria in patients with permanent postsurgical hypoparathyroidism receiving treatment with oral calcium and calcitriol supplements.

Design and Setting: The investigation was designed as a prospective cross-sectional study. An outpatient hospital clinic served as the study setting.

Patients: Fifty-four consecutive observations were made of 34 stable outpatients with postsurgical hypoparathyroidism taking oral calcium and calcitriol supplements, and 17 adult controls without hypoparathyroidism.

Intervention: There were no interventions.

Main Outcome Measure: Hypercalciuria was defined as 24-hour urine calcium >300 mg.

Results: Patients without hypercalciuria (n = 21) *vs* those with hypercalciuria (n = 33) had lower levels of serum 1,25-dihydroxyvitamin D (33.5 ± 11.9 pg/mL *vs* 45.8 ± 9.5 pg/mL; $P < 0.001$), similar albumin-corrected serum calcium (8.3 ± 0.5 *vs* 8.6 ± 0.5 mg/dL; *P* = nonsignificant), and serum parathyroid hormone (12.5 ± 5.7 *vs* 10.7 ± 6.8 pg/mL; *P* = nonsignificant). Multiple linear regression analysis showed an independent relationship between 1,25-dihydroxyvitamin D and urinary calcium excretion (B = 6.2 ± 1.423; $P < 0.001$). A cutoff value of 33.5 pg/mL for serum 1,25-dihydroxyvitamin D to predict the absence of hypercalciuria had 100% sensitivity and 63.6% specificity, and the area under the receiver operating characteristic curve was 0.797. No patients with serum 1,25-dihydroxyvitamin D levels of <33.5 pg/mL presented with hypercalciuria, regardless of the level of albumin-corrected serum calcium.

Conclusions: Routine measurement of serum 1,25-dihydroxyvitamin D may be useful as a biomarker to predict the absence of hypercalciuria in patients with permanent postsurgical hypoparathyroidism who are receiving treatment with oral calcium and calcitriol supplements. **(*J Clin Endocrinol Metab* 102: 259–266, 2017)**

Postsurgical hypoparathyroidism is the most common form of hypoparathyroidism (1–5). The normalization of serum calcium levels in patients with hypoparathyroidism is usually achieved by using calcium and vitamin D supplements, commonly in the form of calcitriol (2–7). However, the optimal management of hypocalcemia has

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Abbreviations: BMI, body mass index; CI, confidence interval; PTH, parathyroid hormone; ROC, receiver operating characteristic.

not been investigated in clinical trials, and treatment is based largely on accepted conventional practice (8). In addition to controlling symptoms, the goals of therapy in patients with hypoparathyroidism are to maintain an albumin-corrected serum calcium level in the low-normal range or slightly below the lower limit of the reference range (~8.0 to 8.5 mg/dL), a calcium-phosphate product of <55 mg^2/dL^2, and a 24-hour urinary calcium level of <300 mg (2–5, 7, 9, 10).

Excessive urinary calcium excretion is a drawback of treatment with calcium and vitamin D supplements in patients with hypoparathyroidism (2, 3, 5, 7, 10). Chronic hypercalciuria may lead to calcium-containing urolithiasis, nephrocalcinosis, and impaired renal function (3, 5–7). Monitoring and optimization of therapy to preserve renal function have been considered to be of critical importance for patients with hypoparathyroidism (3, 7). Traditionally, to monitor hypercalciuria, twice-yearly measurement of 24-hour urinary calcium is recommended in patients with hypoparathyroidism, once the treatment has been stabilized (2, 5, 9, 10). However, 24-hour urine collection is often unreliable because of volume omissions. As is well known, the 24-hour urine may not all be collected because the patient finds collection inconvenient (11, 12). Moreover, 24-hour urine collection is unpopular with both patients and laboratory staff, especially if it needs to be performed twice yearly on a regular basis (13). It has been reported that the compliance rate for 24-hour urine collection may be as low as 44% for patients with hypoparathyroidism with a follow-up of 7.4 ± 5.1 years (3).

To our knowledge, no clinical studies in recent years have been specifically aimed at evaluating which factors are related to the presence of hypercalciuria in patients with hypoparathyroidism who are receiving supplemental treatment. At the same time, it has become common for patients with hypoparathyroidism to be treated with both calcitriol (1,25-dihydroxyvitamin D) and cholecalciferol (vitamin D3) supplements when pills containing fixed doses of calcium and cholecalciferol are prescribed as treatment. However, at the present time, we can routinely measure serum metabolites such as calcidiol, calcitriol, and parathyroid hormone (PTH) to analyze their influence on hypercalciuria. Thus, it is possible to gain new insights into the best means of managing supplemental treatment to avoid hypercalciuria and its potentially deleterious effects on the kidneys.

The aim of this study was to find biomarkers that could be useful for predicting hypercalciuria in patients with permanent postsurgical hypoparathyroidism whose condition has been stabilized with oral calcium and calcitriol, with or without other vitamin D supplements.

Materials and Methods

Over a period of 18 months (November 2014 to May 2016), 34 consecutive stable outpatients with permanent postsurgical hypoparathyroidism after a total thyroidectomy that was performed because of benign (n = 15) or malignant (n = 19) thyroid conditions were enrolled in this study. Data on the variables included in the study were collected at 1 observation time point for all 34 patients. Data were collected at a second observation time point for 16 patients, and at a third observation time point for 4 patients; the doses of calcitriol and/or calcium supplements were modified at each observation point for patients who underwent second and third observations. All patients had achieved a stable condition for at least 3 months prior to data collection at every observation time point. Therefore, a total of 54 observations were included for analysis. All patients with malignancy were in complete remission at the time of the study. The control group consisted of 17 adults with postsurgical hypothyroidism, without hypoparathyroidism, who were enrolled more than 1 year after undergoing thyroidectomy. The study was reviewed and approved by the ethics committee of the Hospital Universitari Mútua de Terrassa. Written informed consent was obtained from all patients.

Hypoparathyroidism was confirmed in all patients by low serum levels (usually <15 pg/mL) of intact PTH during hypocalcemia (albumin-corrected serum calcium levels of <8 mg/dL) 24 to 48 hours after total thyroidectomy. Hypoparathyroidism was permanent, given that all patients showed undetectable or subnormal serum PTH concentrations (usually <15 pg/mL in the absence of raised serum calcium levels) and required calcium supplements with calcitriol to achieve albumin-corrected serum calcium levels >8 mg/dL for more than 1 year after thyroidectomy was performed (2, 4, 14–16).

The patients took oral calcium and oral vitamin D supplements. They were all receiving calcitriol treatment, and in addition, 18 patients were also treated with vitamin D3 supplements that were added in fixed doses to the calcium supplements. No patient was receiving parathyroid hormone treatment, had renal insufficiency, or was treated with other drugs that could have interfered with calcium homeostasis, such as thiazide or loop diuretics, glucocorticoids, antiresorptive drugs, antiepileptic drugs, or lithium (5). In a food survey, we quantified the level of calcium intake by taking an average of the calcium intake on 3 different days per week, using a food composition table (17).

The primary end point was 24-hour urine calcium excretion, which was measured in a single urine sample. In considering whether a patient had hypercalciuria, a threshold of 300 mg for 24-hour urine calcium excretion was applied (2, 4, 9, 18). Emphasis was placed on the importance of patients providing complete 24-hour urine specimens. The reliability of the urine specimens collected was tested by measuring urine creatinine. For each age and sex group, the values were considered suitable if they were within the mean $\pm$ 2 standard deviations of values for subjects with normal renal function, as previously reported (19). Using this criterion, 4 urine collections were rejected because of low levels of creatinine, and the patients were asked to provide more reliable 24-hour urine specimens in the following weeks.

The variables recorded to analyze their influence on 24-hour urine calcium excretion included the following: patient's age and sex, time from hypoparathyroidism occurrence, current

weight, size, body mass index (BMI), and dietary calcium intake; treatment-conditioning factors, including daily doses of calcium supplements and vitamin D3 supplements, daily dose of calcitriol, last dose of calcitriol before the blood draw, time interval between the last intake of calcitriol and the blood draw, and doses of thyroxine treatment; serum analytic variables, including calcium, albumin, phosphorus, magnesium, creatinine, thyrotropin, free thyroxine, PTH, 25-hydroxivitamin D, and 1,25-dihydroxyvitamin D (blood was drawn after an 8-hour fasting period); and urine analytic variables (24-hour creatinine excretion).

Creatinine clearance was estimated as [urine creatinine (mg/dL) × urine volume (mL)]/[serum creatinine (mg/dL) × 1440]. Albumin-corrected serum calcium was calculated as follows: serum calcium (mg/dL) + [(4 − albumin [g/dL]) × 0.8].

Serum calcium was measured by the 5-nitro-5′-methyl-BAPTA method. Urine calcium was measured by the orthocresolphthalein complexone method. Serum albumin was measured by the bromocresol green method, and serum 25-hydroxivitamin D and intact PTH were determined using electrochemiluminescence immunoassay. Serum 1,25-dihydroxyvitamin D was determined by radioimmunoassay (125I RIA Kit, DiaSorin, Stillwater, MN; coefficient of variation intra-assay, 7.2%; coefficient of variation interassay, 12.8%). The reference values were 8.6 to 10.0 mg/dL for serum calcium, 15.2 to 65.7 pg/mL for PTH, and 25 to 66 pg/mL for serum 1,25-dihydroxyvitamin D.

The normality of the continuous variables was determined by the Kolmogorov-Smirnov test. Continuous variables that could influence the presence of hypercalciuria were investigated with Student t test, and categorical variables with χ^2 test. Differences in the means of variables after modifications in treatment were explored with the Wilcoxon test for paired data. Statistical relationships between quantitative variables were investigated with Pearson's correlation coefficient and linear multiple regression analysis (stepwise method). Receiver operating characteristic (ROC) curves were generated to examine the power of variables to discriminate between patients with and without hypercalciuria. Areas under the ROC curves were compared using the Hanley and McNeil statistical method (20). Statistical significance was set at $P < 0.05$ (two-tailed). Statistical analysis was performed with EpiData software, version 3.1 (Epidat software, version 3.1, Servizo Galego de Saúde, Galicia, Spain).

Results

A summary of patient data, treatment, and analytic results is displayed in Table 1. No patient had hypercalcemia, and all patients had a calcium-phosphorus product below 55 mg^2/dL^2. As expected, of the variables compared between controls and patients, only those that were related to the hypoparathyroid condition showed significant differences (calcium supplements, calcitriol dosage, albumin-corrected serum calcium, serum PTH, and urinary calcium excretion). Otherwise, comparing patients with and without hypercalciuria, the latter were older and had a higher BMI, longer evolution of hypoparathyroidism, and lower levels of serum 1,25-dihydroxyvitamin D. Interestingly, the serum PTH levels of patients with and without hypercalciuria were similar.

In the patient group, urinary calcium excretion correlated significantly with the BMI ($r = -0.277$; $P = 0.043$), the serum level of PTH ($r = -0.323$; $P = 0.017$), and the serum level of 1,25-dihydroxyvitamin D ($r = 0.467$; $P < 0.001$). A clear tendency was detected between urinary calcium excretion and albumin-corrected serum calcium levels, but the differences did not reach statistical significance ($r = 0.265$; $P = 0.052$). In the multiple linear regression analysis, only the serum levels of 1,25-dihydroxyvitamin D (B = 6.248 ± 1.423; 95% confidence interval [CI], 3.391 to 9.106; $P < 0.001$) and the serum levels of PTH (B = −9.247 ± 2.879; 95% CI, −15.028 to −3.467; $P = 0.002$) remained significantly associated with urinary calcium excretion.

The ROC curves for the serum level of 1,25-dihydroxyvitamin D, the serum level of PTH, and the level of albumin-corrected serum calcium, used to discriminate between patients with and without hypercalciuria, are shown in Fig. 1. Areas under the ROC curves were 0.797 (95% CI, 0.679 to 0.916) for serum 1,25-dihydroxyvitamin D, 0.600 (95% CI, 0.437 to 0.762) for serum PTH, and 0.656 (95% CI, 0.503 to 0.808) for albumin-corrected serum calcium. When these areas were compared, a nonsignificant difference was found ($\chi^2 = 3.11$; df, 2; $P = 0.07$). Levels of serum 1,25-dihydroxyvitamin D of <33.5 pg/mL showed 100% sensitivity with 63.6% specificity in predicting the absence of hypercalciuria.

Figure 2 shows the distribution of patients on the basis of their 1,25-dihydroxyvitamin D and urine calcium serum levels. Patients have been grouped according to their levels of albumin-corrected serum calcium. No patient with a serum 1,25-dihydroxyvitamin D level of <33.5 pg/mL had hypercalciuria, regardless of the level of albumin-corrected serum calcium (<8.5 mg/dL, 8.5 to 9.5 mg/dL, or > 9.5 mg/dL).

When comparing patients with serum 1,25-dihydroxyvitamin D levels of less than and greater than 33.5 pg/mL, serum PTH was similar in the groups (10.9 ± 5.6 pg/mL *vs* 12.4 ± 6.5 pg/mL; P = nonsignificant), as was albumin-corrected serum calcium (8.37 ± 0.53 mg/dL *vs* 8.57 ± 0.52 mg/dL; P = nonsignificant), but not 24-hour urine calcium excretion (191.4 ± 68.4 mg/24h *vs* 343.7 ± 169.8 mg/24 h; $P < 0.001$). A correlation between serum 1,25-dihydroxyvitamin D and creatinine clearance (r = 0.34; $P = 0.013$) was found. However, we did not find any significant relationship between serum 1,25-dihydroxyvitamin D and the doses of oral calcitriol (daily dose and last dose before the blood draw), the time interval between the last intake of calcitriol and the blood draw, age, weight, or sex.

Of 21 patients with hypercalciuria, 7 had an albumin-corrected serum calcium level of <8.5 mg/dL, and 14 had

Table 1. Baseline Characteristics of Controls and Patients, Their Treatment, and Serum and Urine Analytic Parameters

	Controls	Patients	P^a	Patients With Hypercalciuria	Patients Without Hypercalciuria	P^b
Number	17	54		21	33	
Age (y)	57 ± 13.2	52 ± 15.3	ns	45 ± 15.9	56 ± 13.5	0.011
Sex (female/male)	16/1	39/15	ns	14/7	25/8	ns
Weight (kg)	73.8 ± 19.1	75.5 ± 13.7	ns	72.1 ± 9.1	77.7 ± 15.7	ns
BMI (kg/m^2)	30.13 ± 9.60	28.57 ± 6.96	ns	25.45 ± 3.60	30.55 ± 7.85	0.007
Duration of hypoparathyroidism (mo)	N/A	93.3 ± 89.5		63 ± 61.4	112.5 ± 99.7	0.028
Average dietary calcium intake (mg/d)	597 ± 204	613 ± 236	ns	636 ± 191	598 ± 262	ns
Treatment						
Calcium supplements (mg/d)	705 ± 751	1422 ± 752	< 0.001	1542 ± 754	1345 ± 751	ns
Vitamin D3 supplements (U/d)	470 ± 640	854 ± 899	ns	948 ± 715	794 ± 1005	ns
Calcitriol (μg/day)	0	0.54 ± 0.64	< 0.001	0.51 ± 0.21	0.56 ± 0.81	ns
Last dose of calcitriol (μg)	N/A	0.29 ± 0.11		0.27 ± 0.11	0.30 ± 0.10	ns
Time interval (h)[b]	N/A	14.9 ± 5.8		14.2 ± 5.7	15.3 ± 5.9	ns
Thyroxine (μg/d)	118 ± 38	126 ± 26	ns	127 ± 26	126 ± 26	ns
Serum analytic variables						
Calcium (mg/dL)	9.1 ± 0.6	8.9 ± 0.5	ns	9 ± 0.6	8.8 ± 0.4	0.07
Albumin (g/dL)	4.4 ± 0.4	4.5 ± 0.2	ns	4.5 ± 0.3	4.5 ± 0.2	ns
Albumin-corrected serum calcium (mg/dL)	8.7 ± 0.5	8.5 ± 0.5	< 0.05	8.6 ± 0.5	8.3 ± 0.5	0.06
Phosphorus (mg/dL)	3.7 ± 0.7	4 ± 0.6	0.065	3.9 ± 0.8	4.1 ± 0.5	ns
Calcium-phosphorus product (mg^2/dL^2)	33.7 ± 5.1	36.1 ± 5.9	ns	36.2 ± 8	36.1 ± 4.2	ns
Magnesium (mg/dL)	2.05 ± 0.18	1.98 ± 0.19	ns	1.92 ± 0.17	2.02 ± 0.2	0.06
Creatinine (mg/dL)	0.74 ± 0.18	0.77 ± 0.12	ns	0.78 ± 0.14	0.77 ± 0.12	ns
Thyrotropin (mU/L)	1.8 ± 3.2	2.9 ± 4.5	ns	2.4 ± 3.07	3.2 ± 5.3	ns
Free thyroxine (ng/mL)	1.61 ± 0.33	1.56 ± 0.29	ns	1.60 ± 0.35	1.54 ± 0.25	ns
Parathyroid hormone (pg/mL)	37.8 ± 22.7	11.8 ± 6.2	< 0.001	10.7 ± 6.8	12.5 ± 5.7	ns
25-hydroxyvitamin D (ng/mL)	27.7 ± 9.1	31.7 ± 13.1	ns	35.4 ± 10.6	29.3 ± 14.1	0.09
1,25-dihydroxyvitamin D (pg/mL)	39.7 ± 13.3	38.3 ± 12.5	ns	45.8 ± 9.5	33.5 ± 11.9	< 0.001
Urine analytic variables						
Calcium (mg/24 h)	160 ± 91	284 ± 157	0.003	417 ± 167	199 ± 66	< 0.001
Creatinine (mg/24 h)	1035 ± 308	1196 ± 393	ns	1324 ± 359	1113 ± 397	0.055
Other						
Creatinine clearance (mL/min)	99.53 ± 28.51	108.67 ± 34.93	ns	118.54 ± 27.50	102.19 ± 38.07	0.096

Values for variables are expressed as mean ± standard deviation or number when appropriate.

Abbreviations: N/A, not applicable; ns, nonsignificant.

[a]Comparisons were made (a) between controls and patients, and (b) between patients with hypercalciuria and patients without hypercalciuria.

[b]Between the last dose of calcitriol and the blood draw.

an albumin-corrected serum calcium level >8.5 mg/dL. Among the latter, 6 patients who had vitamin D3 supplements and calcitriol doses reduced without the addition of thiazide diuretics had normalized urinary calcium excretion rates and, simultaneously, their serum 1,25-dihydroxyvitamin D levels decreased to <33.5 pg/mL (Table 2).

Discussion

This clinical study was performed to identify useful biomarkers related to the presence of hypercalciuria in patients with permanent postsurgical hypoparathyroidism who were receiving treatment with oral calcium and calcitriol supplements, with or without other vitamin D supplements. Serum levels of 1,25-dihydroxyvitamin D were independently related to hypercalciuria, and we found a cutoff value for this variable that allowed us to predict the absence of hypercalciuria in all cases. To the best of our knowledge, this investigation provides evidence of a reliable serum biomarker for identifying patients who are at risk for hypercalciuria after permanent postsurgical hypoparathyroidism.

A major therapeutic challenge in patients with hypoparathyroidism is the consistent effective management of the hypocalcemia while avoiding hypercalciuria and other complications. In the absence of PTH, the tubular reabsorption of calcium is reduced, and much of the absorbed calcium in the intestine is lost in urine (21). Periodic measurements of 24-hour urinary calcium

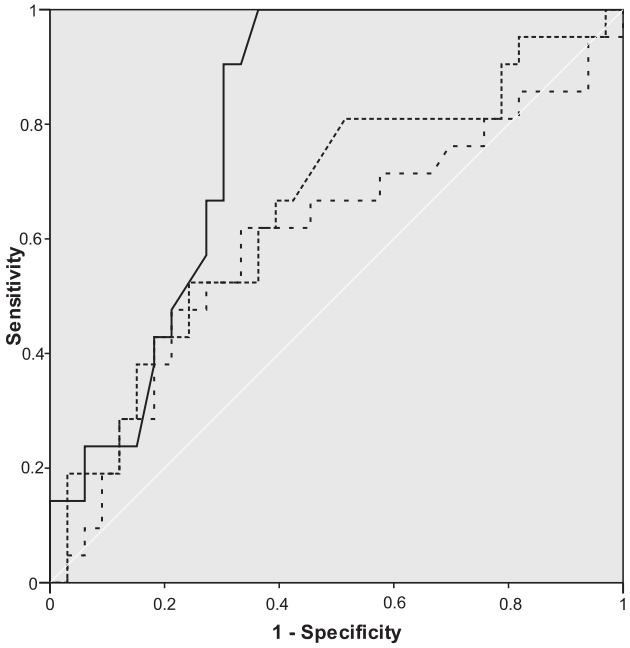

Figure 1. ROC curves for serum 1,25-dihydroxyvitamin D (—), albumin-corrected serum calcium (…) and serum PTH (– –) to discriminate between patients with and without hypercalciuria.

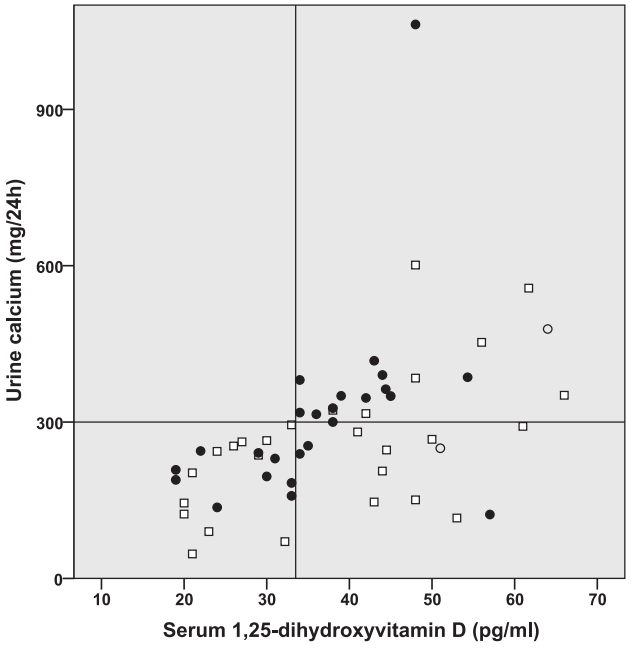

Figure 2. Distribution of patients grouped by their albumin-corrected serum calcium [<8.5 mg/dL (□); between 8.5 and 9.5 mg/dL (●); >9.5 mg/dL (○)], depending on their level of serum 1,25-dihydroxyvitamin D and urine calcium. The vertical line intersects with the *x*-axis at 33.5 pg/mL.

excretion have been recommended for patients with hypoparathyroidism (5, 7, 9) to avoid potential renal complications if hypercalciuria occurs as a consequence of the treatment with calcium and vitamin D supplements. In the absence of other alternative reliable indicators of hypercalciuria, this procedure has been implemented despite the volume omissions in 24-hour urine collections that can produce false-negative results (11, 13, 22). In this regard, Mitchell *et al.* (3) reported that compliance in 24-hour urine collections was only 44%, but of those patients who successfully completed the procedure, 38% had at least 1 excessive (>300 mg) 24-hour urine calcium measurement (3).

Serum 1,25-dihydroxyvitamin D is a powerful hypercalcemic metabolite (21, 23), and its levels depend on calcitriol dosage, the time interval between the last intake of calcitriol and the blood draw, PTH residual secretion, renal integrity, serum calcium levels, and calcidiol stores (7, 12). Likewise, calcidiol is influenced by multiple factors (24). Thus, different levels of serum 1,25-dihydroxyvitamin D can be achieved among patients taking the same dosage of calcitriol supplements. In our study, the absence of a relationship between serum levels of 1,25-dihydroxyvitamin D with the last dose of calcitriol, and the time interval between the last intake of calcitriol and the blood draw, could be explained by the fact that the time required for the calcitriol to reach its peak serum concentration after administration (3 to 6 hours) (7) is approximately one-third the interval of time between the last intake of calcitriol and the blood draw (14.9 ± 5.8 hours). Our patients had serum 1,25-dihydroxyvitamin D levels of 38.3 ± 12.5 pg/mL, similar to those for the control group (39.7 ± 13.3 pg/mL). The treatment with calcitriol may have been responsible for restoring the low or low-normal levels of serum 1,25-dihydroxyvitamin D currently found in the patient with hypoparathyroidism before treatment is begun (2, 9, 10).

Our data show that patients with permanent postsurgical hypoparathyroidism receiving treatment with oral calcium and oral calcitriol supplements are at risk for hypercalciuria above a specific level of serum 1,25-dihydroxyvitamin D (33.5 pg/mL), regardless of their albumin-corrected serum calcium level. The presence of hypercalciuria with concomitant low, normal, or high levels of serum calcium has previously been observed. In the largest reported longitudinal cohort of patients with hypoparathyroidism (66% of postsurgical etiology), higher serum calcium levels were associated with higher urine calcium values. However, there was wide variability, and in 44 simultaneous urine and serum calcium measurements, 2 of 22 patients had urine calcium levels >300 mg/24 h, whereas they had serum calcium levels of <8.5 mg/dL; conversely, 17 of the remaining 22 patients had serum calcium levels above 8.5 mg/dL without hypercalciuria (3).

In our study, the level of serum 1,25-dihydroxyvitamin D was shown to be a useful independent biomarker of the absence of hypercalciuria. This test had a sensitivity of 100% with a specificity of 63.6% and an area under the

Table 2. Changes in Patients (n = 6) With Hypercalciuria and Albumin-Corrected Serum Calcium Levels Greater Than 8.5 mg/dL, Before and After Hypercalciuria Correction by Reduction of Calcitriol Doses

	Before Hypercalciuria Correction	After Hypercalciuria Correction	*P*
Treatment			
Calcium supplements (mg/d)	1333 ± 983	1500 ± 1000	ns
Vitamin D3 supplements (U/d)	880 ± 890	333 ± 531	ns
Calcitriol (μg/d)	0.46 ± 0.19	0.33 ± 0.13	ns
Thyroxine (μg/d)	141.5 ± 22	139.5 ± 23	ns
Serum analytic variables			
Albumin-corrected serum calcium (mg/dL)	8.80 ± 0.61	8.62 ± 0.29	ns
Thyrotropin (mU/L)	0.31 ± 0.23	0.68 ± 0.73	ns
Free thyroxine (ng/mL)	1.82 ± 0.34	1.71 ± 0.42	ns
Parathyroid hormone (pg/mL)	14.6 ± 7.0	15.9 ± 6.9	ns
25-hydroxyvitamin D (ng/mL)	35.3 ± 17.4	25.4 ± 12.5	ns
1,25-dihydroxyvitamin D (pg/mL)	45.5 ± 10.1	29.1 ± 7.1	< 0.05
Urine analytic variables			
Calcium (mg/24 h)	357 ± 66	232 ± 36	< 0.01
Creatinine (mg/24 h)	1085 ± 182	1057 ± 218	ns
Creatinine clearance (mL/min)	103.74 ± 26.23	98.82 ± 25.08	ns

Abbreviation: ns, nonsignificant.

ROC curve of 0.797. Values for the area under the ROC curve between 0.5 and 0.7 indicate low accuracy, values between 0.7 and 0.9 indicate that a test is useful for some purposes, and values >0.9 indicate high accuracy (25). Some diagnostic tests in medicine that are commonly used and recommended by expert panels have an area under the ROC curve between 0.7 and 0.8 (26).

One could argue that patients with hypercalciuria could be affected by idiopathic hypercalciuria. However, in our series, the available data on 6 patients who experienced hypercalciuria remission after a reduction in their oral doses of calcitriol and vitamin D3 supplements suggest that this is very unlikely (Table 2). The decrease in the doses of vitamin D3 supplements (880 ± 890 to 333 ± 531 U/d) and calcitriol (0.46 ± 0.19 to 0.33 ± 0.13 μg/d) were not statistically significant, because the low number of patients conferred a low power for detecting differences. In the case of vitamin D3 supplements, their high standard deviation adds to the difficulty in finding the differences statistically significant (27, 28). The combined reduction in vitamin D3 supplements and the dosage of calcitriol could have had an additive effect, thus resulting in a more intense decrease in 1,25-dihydroxyvitamin D serum levels (45.5 ± 10.1 to 29.1 ± 7.1 pg/mL; $P < 0.05$) (12).

In clinical practice, if we suspect hypercalciuria in a patient as a result of the level of 1,25-dihydroxyvitamin D, and hypercalciuria is confirmed by 24-hour urine collection, the serum level may be an indicator that the dosage of oral calcitriol supplements should be reduced to achieve a 1,25-dihydroxyvitamin D level of <33.5 pg/mL (in the absence of hypocalcemia). This could also be an indicator of the recovery of parathyroid function when applied to patients with postsurgical hypoparathyroidism that is still considered nonpermanent.

Because serum 1,25-dihydroxyvitamin D is more closely related to hypercalciuria than is albumin-corrected serum calcium, it seems reasonable to suggest that when 1,25-dihydroxyvitamin D is lower than 33.5 pg/mL, the optimal goal of therapy in patients with permanent postsurgical hypoparathyroidism receiving treatment with oral calcium and calcitriol supplements should be to achieve normal serum calcium values instead of the currently recommended low-normal levels (2, 3, 5, 7, 9, 10, 21). In addition, this approach could reduce the high frequency of visits to emergency departments as well as hospital admissions for symptomatic hypocalcemia (3).

There are some mechanisms that explain why the presence of hypercalciuria may be influenced by the level of serum 1,25-dyhidroxyvitamin D in patients with hypoparathyroidism and normal or even low fasting serum calcium levels. Calcitriol enhances the intestinal absorption of calcium through an active transport system (29). In our study, the serum level of 1,25-hydroxyvitamin D was similar in patients and controls, but the intake of calcium was superior in patients. Thus, the intestinal absorption of calcium should have been greater in the patients, in particular those with higher levels of 1,25-dyhidroxyvitamin D. Patients with low or undetectable levels of serum PTH had impaired renal reabsorption of calcium. Therefore, it follows that an increase in urinary calcium excretion occurs when larger amounts of calcium are filtered by the kidney and not reabsorbed by PTH (9, 30).

The correlation between 1,25-dihydroxyvitamin D and creatinine clearance raises the possibility of a complementary mechanism of hypercalciuria mediated by 1,25-dihydroxyvitamin D. The reabsorption of calcium in the kidney is controlled by several factors, such as the filtered load of sodium, urine flow, and the activity of several hormones, most notably PTH (31). A synthesis of

the experimental results suggests that vitamin D3 metabolites affect distal tubular reabsorption of calcium via several mechanisms, and that the mechanisms are regulated directly or indirectly by 1,25-dihydroxyvitamin D and enhanced by PTH (31). The total amount of calcium filtered across the glomerulus under normal circumstances in a 24-hour period is about 8,000 mg (31, 32). Approximately 98% of the filtered load of calcium is reabsorbed in the proximal and distal tubules and the loop of Henle. In the proximal tubule, about 70% of the filtered load of calcium is reabsorbed, predominantly through the paracellular pathway, independent of PTH action (33). Volume expansion inhibits calcium reabsorption in the proximal tubule (31, 32). Likewise, shrinkage of extracellular volume has been suggested as a mechanism for reducing urinary calcium excretion by thiazide diuretics. Decreased extracellular volume leads to increased proximal calcium reabsorption (24). Thus, our finding that levels of 1,25-dihydroxyvitamin D correlated positively with creatinine clearance could explain the lessened reabsorption of calcium in the proximal tubule; this may lead to the greater availability of calcium to the thick ascending loop of Henle and the distal tubule, where, in the absence of PTH, the mechanisms implicated in the reabsorption of calcium at this level are unable to assume this calcium overload, and consequently, hypercalciuria develops.

One limitation of this study is the small number of patients recruited. The low incidence of postsurgical hypoparathyroidism (34) is the main barrier to obtaining a sizable series of patients; in most studies, even studies with more than 1000 patients who have undergone thyroidectomy, only 20 to 30 patients with hypoparathyroidism are included (4, 5, 9, 14, 15, 35, 36). Related to this, the principal statistical problem associated with a small sample size is the low power for revealing effects that are real, but this was not the case in the current study (27, 28).

In conclusion, our results suggest that measurement of serum 1,25-dihydroxyvitamin D should be included in the follow-up of patients with permanent postsurgical hypoparathyroidism. Twenty-four–hour urine calcium measurements could be limited to use for confirming hypercalciuria in patients with 1,25-dihydroxyvitamin D serum levels >33.5 pg/mL. This approach seems more reliable as well as more convenient for patients than do routine 24-hour urine collections. However, prospective studies to confirm these results are needed.

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