Percutaneous ethanol (PEIT) and calcitrol (PCIT) injection therapy are ineffective in treating severe secondary hyperparathyroidism

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Abstract

Background. Secondary hyperparathyroidism (2HPT) is a frequent complication of long-term dialysis treatment and, despite recent advances in medical therapy, surgical parathyroidectomy (PTX) is required in a considerable number of uremic patients. Recently, other modalities of therapy, such as ultrasound-guided percutaneous parathyroid injection of ethanol (PEIT) or of calcitriol (PCIT), have been used to treat refractory 2HPT. Our objectives were to evaluate the efficacy of these therapeutic modalities and to analyse their effects on parathyroid cell proliferation.

Methods. Nineteen haemodialysis patients with severe 2HPT were studied. Ten underwent PEIT (Group I) and nine underwent PCIT (Group II). After treatment, five patients in each group were submitted to PTX. Parathyroid cell proliferation was appraised at the beginning and at the end of the study by fine-needle aspiration biopsy, making use of immunocytochemical testing for Ki-67. The surgically removed glands were submitted to histopathological analysis and cellular proliferation was evaluated.

Results. Both PEIT and PCIT proved inefficient in controlling 2HPT. Comparing study onset with day 60, both groups showed a significant decrease in serum-ionized calcium: 5.3±0.3 vs 5.1±0.5 mg/dl (P=0.03) in Group I and 5.5±0.4 vs 5.4±0.3 mg/dl (P=0.03) in Group II. Other laboratory parameters were unchanged. There was a significant, although transitory, enlargement in glandular volume in Group II at day 30 when compared with study onset (1.5±0.6 vs 1.7±0.7 cm³, P=0.02). When comparing the two groups, Group I showed a glandular volume smaller than that of Group II at days 30 (1±0.5 vs 1.7±0.7 cm³, P=0.003), 60 (0.8±0.4 vs 1.5±0.9 cm³, P=0.006) and 90 (0.8±0.5 vs 1±0.7 cm³, P=0.02). Cellular proliferation, which was equally elevated in both groups at the beginning of the study, could not be evaluated at the end due to lack of material. The majority of glands obtained through PTX presented intensive cellular proliferation and contained areas of nodular hyperplasia, even those glands with a volume of <0.5 cm³.

Conclusion. In our experience, both PCIT and PEIT were unable to control severe 2HPT in chronic hemodialysis patients. We believe that the severity of the 2HPT in the study patients, in conjunction with the fact that we excluded from treatment parathyroid glands with a volume of <0.5 cm³, were the most important causes of this failure.

Keywords: calcitriol injection; ethanol injection; Ki-67; parathyroid cellular proliferation; secondary hyperparathyroidism; sonographic guidance

Introduction

Secondary hyperparathyroidism (2HPT) remains a significant cause of morbidity in patients with chronic renal failure, mainly due to bone complications and cardiovascular disease [1]. Calcitriol is currently used to reduce parathyroid hormone levels in such patients. However, a significant number of patients fail to respond to calcitriol therapy and may have to undergo parathyroidectomy (PTX). It has been reported that PTX becomes necessary in 10–30% of patients who have undergone haemodialysis for >10 years [2]. The abnormal parathyroid response to calcitriol may be attributed to the development of nodular hyperplasias composed of monoclonal proliferating cells with lower-density calcitriol receptors [3]. Therefore, other modalities of therapy have been advocated for treatment of refractory 2HPT: parathyroid gland percutaneous injection of either ethanol (PEIT) or calcitriol (PCIT). PEIT was first reported by Solbiati et al. [4] as an
alternative to PTX in patients with high surgical risk and its success has been documented by other authors. Kitaoaka et al. [5] injected calcitriol directly into enlarged parathyroid glands using Doppler ultrasonography (Doppler US) guidance. Their results show that PCIT may be an effective alternative for 2HPT treatment.

Our present study aimed to evaluate these new modalities in the treatment of patients with severe 2HPT and to determine their effects on parathyroid cell proliferation by means of immunocytochemical testing for Ki-67.

Subjects and methods

In the period from May 2000 to June 2002, 19 patients with refractory 2HPT were assessed for PEIT or PCIT feasibility. Patients were considered for intervention if they met the following criteria: serum intact parathormone concentration (iPTH) ≥ 450 pg/ml and one or more parathyroid glands with ≥ 0.5 cm³ volume, according to neck ultrasound. Patients with ectopic glands determined by 99m-tc-sestamibi parathyroid scintigraphy and those submitted previously to surgical PTX were excluded. Ten patients were submitted to PEIT (Group I) and nine to PCIT (Group II). Patients who were non-responders to PEIT or PCIT were submitted to PTX. Informed consent from all patients was obtained in advance and protocols were approved by the ethical committee of the institution.

All patients had been treated by haemodialysis for 93.2 ± 51.5 (Group I) or 73.6 ± 37.6 months (Group II). Dialysis was performed three times weekly using 3.5 mEq/l dialysate calcium. Duration of each dialysis was 4 h and dialysis protocols were not changed during the study. In both groups, the main cause of end-stage renal failure was chronic glomerulonephritis.

Due to hypercalcaemia, calcium supplements or calcium-containing phosphate binders (calcium carbonate) were used by very few patients in either group. In those patients, there were no statistical differences in dosage between the groups. During the study, the dosages of oral phosphate binders remained unchanged. No patient received calcitriol at the onset of the study.

Ethanol injection (Group I)

Neck ultrasound examination was performed using a colour Doppler ultrasonogram (GE Logik 500, WI, USA) with a 10-MHz Scanner. Glandular volumes were estimated by measurement of three dimensions of the glands, and glands with a volume of ≥ 0.5 cm³ were treated. We chose the largest gland for the first ethanol injection and, when indicated, the next largest gland(s) underwent ethanol injections as well. Ethanol injection was performed using a 22-gauge needle, and the tip of the needle was guided to the centre of the gland. The amount of absolute ethanol injected was calculated according to gland volume. If gland volume was between 0.5 and 1 cm³, the ethanol injected was 80% of gland volume, but if the gland volume was > 1 cm³ the ethanol injected was 50% of gland volume. Treatment control was performed at 2 weeks. An assessment of tissue destruction was made using colour Doppler blood mapping to assess the blood supply, and the serum iPTH was checked. Our objective was a serum iPTH between 200 and 300 pg/ml. If the iPTH did not decrease to < 300 pg/ml and the colour Doppler was still positive, we applied a new injection in the same gland until the blood flow subsided. For those patients with multiple enlarged glands, we first injected ethanol into the largest gland. If serum iPTH failed to decrease to < 300 pg/ml, despite the effective parathyroid tissue destruction verified by the Doppler US, we injected ethanol into the next largest gland. We repeated this process until serum iPTH decreased to < 300 pg/ml or all glands > 0.5 cm³ had been injected. As a co-adjuvant, we also used calcitriol pulse therapy (1–4 µg/three times per week) when ionized calcium was < 5.4 mg/dl and phosphorus was below 6 mg/dl. The follow-up period was 90 days.

Calcitriol injection (Group II)

Neck ultrasound examination was performed using the same method as was used in the ethanol injection, and the same criteria was used for gland selection. The calcitriol injection (calcitriol solution 1 µg/ml, Abbott) was performed using a 22-gauge needle, and the tip of the needle was guided to the centre of the gland. Calcitriol injections were performed three times per week for 2 weeks (total injections = six per gland). The injected volume of calcitriol was 80% of the calculated gland volume. In those patients with multiple enlarged glands, we injected all at the same time. Over the course of the protocol, no additional injections were given. Our objective was a serum iPTH between 200 and 300 pg/ml. We also used the same criteria for initiating calcitriol pulse therapy, and the follow-up period was also 90 days.

Parathyroidectomy

Five patients in either group considered non-responders were submitted to PTX. The elapsed time between the end of the protocol and PTX was 6.2 ± 0.8 and 7.8 ± 0.8 months for Groups I and II, respectively. The technique used was total PTX—with or without forearm autograft. The routine procedure was total PTX with forearm autograft, but in those cases where local dissection was difficult, total PTX without autograft was performed. Some of the parathyroid tissue removed during the operation was routinely cryopreserved.

Biochemical measurements

Serum iPTH levels (normal range, 8–76 pg/ml) were measured by using a radioimmunoassay (Cis-Bio International, SELSA PTH, Gif-sur-Yvette, France). Serum-ionized calcium (normal range, 4.8–5.4 mg/dl), serum phosphorus (normal range, 2.3–4.6 mg/dl), and serum alkaline phosphatase (normal range, 60–170 IU/l) were determined with an automated analyser (Auto analyser Covas-Integra, Roche). Each parameter was determined at the onset of the study and at 30, 60 and 90 days after the first injection. Serum 1,25(OH)₂ vitamin D (normal range, 15.9–55.6 pg/ml) was measured by using radioimmunoassay (Diasorin, MN, USA) at study onset and at day 14.

Parathyroid scintigraphy

In order to identify patients with ectopic glands before the treatment, a parathyroid study using i.v. 99m-Tc-MIBI
(25 mCi) was performed and static images were taken using a scintillation camera (ECAM, Siemens, IL, USA) at 15 and 180 min after injection of the radiotracer.

**Parathyroid cellular proliferation study**

In order to study the parathyroid cellular proliferation, all patients were submitted to Doppler US-guided puncture/fine-needle aspiration (PFNA) of the largest parathyroid gland before and after the follow-up period. All specimens were fixed in 10% formalin and were routinely processed in paraffin. Serial sections were cut and mounted on glass slides. These sections were deparaffinized in xylene and rehydrated through an ethanol series. After blocking endogenous peroxidase activity with 3% hydrogen peroxide, the sections were then placed in jars containing citric acid and heated in a pressure cooker for 5 min. Those sections were incubated in a humidified chamber with the primary antibody overnight at 4°C. The following primary antibodies were used: Ki-67 (MIB 1, diluted 1:1200, Dako, Denmark); thyroglobulin (diluted 1:40 000, Dako, Denmark) and PTH (diluted 1:4000 Dako, Denmark). After rinsing, the slides were incubated with biotinylated antiserum (Dako, Denmark); thyroglobulin (diluted 1:40 000, Dako, Denmark) and then with a streptavidin–biotin complex/HRP (Dako, Denmark). Slides were then developed with a freshly prepared diaminobenzedine chromogen (Sigma Chemical Co.) in the presence of 3% H2O2. Sections were counterstained with haematoxylin. Tonsil, thyroid and parathyroid tissues were used as positive controls and normal serum was substituted for the primary antibodies as a negative control. The immunochemistry for PTH and thyroglobulin provides a method for confirming parathyroid origin of samples. Immunohistochemical testing for Ki-67 permits evaluation of parathyroid cellular proliferation. The quantification of Ki-67 was carried out under 1000× magnification. Immunoreactive cells were counted in selected areas of each specimen and the label index was calculated as the number of positive immunoreactive nuclei per 1000 cells.

The parathyroids removed at PTX were submitted to histopathological study and their cellular proliferation was also analysed by immunohistochemical testing for Ki-67. The quantification of Ki-67 was performed under 400× magnification and expressed as cells/0.2 mm². For each section, 20 microscopic fields, each corresponding to an area of 0.2 mm², were evaluated.

**Statistical analysis**

All results are expressed as means±SD. Statistical significance was determined by unpaired or paired Student’s t-test in case of a single comparison. Multivariate analysis (Wald’s test) was used for multiple comparisons. Pearson’s correlation was employed to analyse a possible overall correlation. P values of <0.05 were considered significant.

**Results**

**Clinical and biochemical data**

As can be seen in Table 1, there were no differences in demographic and clinical characteristics between the groups other than age; the patients in Group II were older than those in Group I (33.8±5.9 and 45.4±9 years, P= 0.02). In Group I, 13 glands were treated by 33 injections (2.5 injections/gland, 3.3 injection/patient). In Group II, 13 glands were treated with 54 injections (6 injections/gland, 6 injections/patient) according to the study protocol.

Comparing study onset and day 60, the mean value of ionized calcium decreased from 5.3±0.3 to 5.1±0.5 mg/dl (P = 0.03) in Group I and from 5.5±0.4 to 5.4±0.3 mg/dl (P = 0.03) in Group II. The mean value of serum 1,25(OH)₂ vitamin D was within normal range at study onset range and there was no change in serum level at day 14 in Group I (18.7±14.7 vs 16.6±6.8 pg/ml, NS) or Group II (32.6±18.7 vs 22.4±8.5 pg/ml, NS). There was also no change in the levels of iPTH during the course of the study in either groups. None of the patients had sufficient reduction in iPTH to reach the target range (200–300 pg/ml). Therefore, none of the patients was considered a responder. Regarding phosphorus and alkaline phosphatase, there were no changes during the course of the study in either group. There were no differences in any biochemical parameters between the groups during the course of the study.

**Parathyroid scintigraphy and sonographic results**

Parathyroid scintigraphy was negative for ectopic glands in all patients. As for sonographic results, at the beginning of treatment, the mean of gland volumes in Group I was similar to that observed in Group II (1.2±0.9 vs 1.5±0.6 cm³). In Group I, gland volumes did not change during the course of the study. On the other hand, in Group II, the gland volumes increased at day 30 (1.5±0.6 vs 1.7±0.7 cm³, P = 0.02). When we compared the two groups, we found gland volumes in Group I to be smaller than in Group II at days 30 (1±0.5 vs 1.7±0.7 cm³, P = 0.003), 60 (0.8±0.4 vs 1.5±0.9 cm³, P = 0.006) and 90 (0.8±0.5 vs 1±0.7 cm³, P = 0.02). We did not find correlations between gland volumes and biochemical and immunocytochemical parameters. Blood supply evaluated by Doppler flow

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**Table 1. Basal clinical and biochemical data**

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Normal values</th>
</tr>
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<tbody>
<tr>
<td>Male:female</td>
<td>3:7</td>
<td>1:8</td>
<td>–</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.8±5.9</td>
<td>45.4±9a</td>
<td>–</td>
</tr>
<tr>
<td>Months on dialysis</td>
<td>93.2±5.1</td>
<td>73.6±37.6</td>
<td>–</td>
</tr>
<tr>
<td>Ionized calcium (mg/dl)</td>
<td>5.3±0.3</td>
<td>5.5±0.4</td>
<td>4.8–5.4</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>6.1±1</td>
<td>6.4±1.1</td>
<td>2.3–4.6</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>513.9±364.5</td>
<td>436.9±392.9</td>
<td>60–170</td>
</tr>
<tr>
<td>Intact PTH (pg/ml)</td>
<td>1136.8±678.7</td>
<td>1277.1±566.9</td>
<td>8–76</td>
</tr>
<tr>
<td>1,25(OH)₂ vitamin D (pg/ml)</td>
<td>18.7±14.7</td>
<td>32.6±18.7</td>
<td>15.9–55.6</td>
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</table>

Values are expressed as mean±standard deviation. a vs Group I (P < 0.05).
mapping disappeared after ethanol and calcitriol injections.

Calcitriol pulse therapy

Improvement in the biochemical profile (control of hypercalcaemia and of hyperphosphataemia) in some patients permitted the use of calcitriol pulse therapy. Five patients from Group I were treated with i.v. calcitriol administered at a mean dosage of 4.5 μg/week for a mean period of 45 days. In Group II, two patients were also treated with i.v. calcitriol administered at a mean dosage of 6 μg/week for a mean period of 67 days. The calcitriol therapy was stopped due to development of hyperphosphataemia in all patients.

Side effects

PEIT and PCIT were safe procedures. The side effects were limited, with the exception of the almost universal incidence of spontaneously remitting local pain or burning sensation at time of injection. Mild dysphonia was observed in only one patient (Group I); however, it subsided in 48 h. We also observed haematoma around the injected glands in two cases (one in each group) that retrograded completely with clinical treatment.

Parathyroidectomy: pathology of glands and cellular proliferation study

Five patients in each group were submitted to PTX. Two of those patients were submitted to renal transplant and the rest are still waiting for surgery. Our standard surgical procedure (performed in seven patients) was PTX with autograft. When local dissection was difficult (as it was with two patients in Group I and one patient in Group II) PTX without autograft was performed. All patients in both groups presented four glands. Mean gland volume in Group II was significantly greater than in Group I (1.3 ± 1.4 vs 0.6 ± 0.4 cm³, P = 0.04). Histopathological analysis was performed on 19 glands in Group I and 17 glands in Group II. Histopathology revealed nodular hyperplasia in a majority of glands, 73.6 and 81.2% in Groups I and II, respectively. In Group I, we found nodular hyperplasia in 70% (7/10) of glands with a volume of >0.5 cm³ and in 88.8% (8/9) of glands with a volume of <0.5 cm³. In Group II, we observed nodular hyperplasia in 83.3% (10/12) of glands with a volume of >0.5 cm³ and in 40% (2/5) of glands a volume of ≤0.5 cm³. When all glands were analysed together, nodular hyperplasia was found in 77% (17/22) of glands with a volume of >0.5 cm³ and in 71.4% (10/14) of glands with a volume of ≤0.5 cm³. The cellular proliferation in those glands evaluated through immunohistochemistry for Ki-67, showed a mean label index of 12.3 ± 11.2 and 18.1 ± 19.1 cells/mm² for Groups I and II, respectively. When we evaluated cellular proliferation of glands by volume, we observed that glands with a volume of >0.5 cm³ showed label indices, which were lower in Group I than in Group II (7.3 ± 5.3 vs 24.3 ± 20.1 cells/mm², P = 0.03). When glands had a volume of < 0.5 cm³, these indices were 15.4 ± 14.9 and 4.2 ± 1.6 cells/mm² for Groups I and II, respectively (NS). In Group II, glands with a volume of ≥0.5 cm³ showed a label index greater than those with a gland volume of < 0.5 cm³ (24.3 ± 20.1 vs 4.2 ± 1.6 cells/mm², P = 0.03).

When we analysed all glands, we found a positive correlation between gland volume and cellular proliferation (r = 0.51, P = 0.002) as can be seen in Figure 1. Glands with a volume of ≥0.5 cm³ had a label index of 17.2 ± 17.6 cells/mm² and glands with a volume of < 0.5 cm³ had a label index of 11.4 ± 12.4 cells/mm² (NS). The results of the histopathology and cellular proliferation count of the glands obtained through PTX are shown in Table 2.

![Fig. 1. Correlation between gland volume and cellular proliferation (Ki-67) in parathyroids obtained through PTX (n = 33, r = 0.51, P = 0.002).](https://academic.oup.com/ndt/article-abstract/19/3/657/1810702)

<table>
<thead>
<tr>
<th>Gland volume</th>
<th>Group I</th>
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<tr>
<td>NH Ki-67 (cells/mm²)</td>
<td>NH Ki-67 (cells/mm²)</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(≥0.5 cm³)</td>
<td>70% (7/10)</td>
<td>7.3 ± 5.34 (n = 8)</td>
<td>83.3% (10/12)</td>
<td>24.3 ± 20.1b (n = 11)</td>
<td>77% (17/22)</td>
<td>17.2 ± 17.6 (n = 19)</td>
<td></td>
</tr>
<tr>
<td>(&lt; 0.5 cm³)</td>
<td>88.8% (8/9)</td>
<td>15.4 ± 14.9 (n = 9)</td>
<td>40% (2/5)</td>
<td>4.2 ± 1.6 (n = 5)</td>
<td>71.4% (10/14)</td>
<td>11.4 ± 12.4 (n = 14)</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation. NH, nodular hyperplasia.

a vs Group I (P < 0.05).

b vs gland volume < 0.5 cm³ (P < 0.05).
Parathyroid glands submitted to PFNA: cellular proliferation study

The PFNA performed at the study onset provided sufficient material for analysis of seven patients in Group I and five patients in Group II. At the end of the study, the punctures were satisfactory in only one patient in Group I and two patients in Group II. The side effects were limited. One patient in Group I presented, around the gland, a haematoma, which disappeared completely with medical treatment. All glands showed positive immunochemistry for PTH and negative for thyroglobulin confirming parathyroid origin of samples. Cellular proliferation in the parathyroid at the study onset showed a label index of 19.6 ± 17.1 and 29 ± 9.5/1000 cells for Groups I and II, respectively. There was no difference in cellular proliferation between the two groups at the study onset. Cellular proliferation at the end of the study was impossible to evaluate due to lack of material.

Discussion

Despite improvements in clinical treatment for 2HPT, a considerable number of patients do not respond to calcitriol therapy. Consequently, surgery is indicated for these patients [2]. This non-response is related to enlarged parathyroid glands whose histopathology generally shows nodular hyperplasia [6,7]. PEIT and PCIT have been used as an alternative to PTX in controlling 2HPT.

PEIT has proved useful, reaching about a 50–80% success rate [8–10]. However, there is no agreement as to when it is indicated or exactly what is the most effective way to perform this procedure. In our study, we followed the protocol suggested by Fukagawa et al. [11]; nevertheless, we had no success in controlling 2HPT. Our patients presented severe 2HPT, which is characterized by hypercalcaemia, hyperphosphataemia, elevated serum alkaline phosphatase and iPTH. In fact, the average iPTH of our patients who underwent PEIT was 1136 pg/ml, which is extremely high compared with patients in other studies. Kitaoka et al. [12] were successful in controlling 2HPT in 77% of cases during the first week of treatment, but the average initial iPTH of their patients was 727 pg/ml. In another study, Kakuta et al. [10] were successful in 80% of treated patients showing initial iPTH average of 633 pg/ml. Rodriguez et al. [13] demonstrated how important the iPTH levels are as a predictor of response to clinical treatment and of the severity of 2HPT. These authors observed that, with an initial iPTH level of 400 pg/ml, there was an 80% probability of successful clinical treatment. The probability decreased to < 20% for an iPTH of 1200 pg/ml. Therefore, higher serum levels of iPTH increase the severity of the disease and lower the chance of therapeutic success. This being the case, we can say that our lack of success in PEIT could be due to the severity of 2HPT in our patients. Nodular hyperplastic parathyroid glands are characterized by having few calcitriol and calcium receptors. In addition, genetic abnormalities make them resistant to calcitriol therapy [3,14]. Tominaga et al. [7] found that glands with a volume ≥0.5 cm³ manifested nodular hyperplasia in 90% of cases. Based on this evidence, the objective of PEIT is to destroy these glands by means of glandular tissue reduction, so that parathyroid response to calcitriol is restored [12]. In the present study, an ephemeral control of hypercalcaemia and hyperphosphataemia allowed us to apply calcitriol pulse therapy in 50% of patients who underwent PEIT. Although all these therapeutic modalities were employed in the management strategy for these patients, we did not achieve the iPTH target level (200–300 pg/ml). The evaluation of the glands (after surgical excision) in five patients who underwent PEIT, demonstrated that 66.6% of injected glands showed fibrosis. This finding has been described by other authors and shows the harmful effect on ethanol on the parathyroid tissue. Furthermore, 88% of the pathological exams on glands < 0.5 cm³ showed nodular hyperplasia, contradicting Tominaga’s findings. Thus, we could conclude that, despite the parathyroid gland fibrosis, there was a small probability that we could control 2HPT through calcitriol pulse therapy once the remaining glands exhibited nodular hyperplasia.

Related to PCIT, even though this therapy was also inefficient in controlling 2HPT, two patients from this group showed improvement in their laboratory tests, which allowed us to apply i.v. calcitriol pulse therapy. In contrast to PEIT, few studies have evaluated PCIT as an alternative therapy in the management of 2HPT. Kitaoka et al. [5] used PCIT to treat seven patients with 2HPT and five of them became controllable. In their study, the average iPTH base level was 711 pg/ml and the mean gland volume was 0.6 cm³. These values are lower than those we observed, which were 1137 pg/ml and 1.5 cm³ for iPTH and gland volume, respectively. Once again, we believe that the degree of severity of 2HPT in our patients can explain the discrepancy of therapeutic results between studies. Recently, Shizaki et al. [15] evaluated the effect of direct injection of 22-oxacalcitriol to treat 2HPT. They demonstrated that there was a significant reduction in iPTH after a 12-week follow up (955 ± 433 vs 463 ± 285 pg/ml, P < 0.01). However, iPTH levels were above the target recommended for control of 2HPT [16,17]. In a case report, Chudek et al. [18] attempted treatment of a renal transplanted patient with severe 2HPT using PCIT. They were not successful, and as a result of unfavourable evolution of the disease, surgical treatment was indicated. In light of this, there is a lack of evidence concerning the efficiency of PCIT in controlling 2HPT.

Histopathological analysis of surgically removed glands from Group II also showed glandular fibrosis in 75% of all glands that underwent PCIT. How PCIT functions is still unknown. It is believed that it may increase calcitriol concentration in the parathyroid directly, which would break the resistance of these glands to calcitriol. As a result, it would reduce the synthesis and the secretion of parathyroid hormone.
The appearance of fibrosis in our patients’ glands showed that calcitriol could cause glandular sclerosis and destruction in a fashion similar to that of ethanol. Even so, we found nodular hyperplasia in 40% of glands <0.5 cm³. As in Group I, this finding could be responsible for the lack of response to PCIT.

To date, there are no comparative studies concerning PEIT and PCIT effectiveness. We report that, although both therapeutic modalities were ineffective in treating 2HPT, we were able to apply calcitriol pulse therapy in a large number of patients in Group I, which could increase the chances of therapeutic response. Overall, patients in Group II developed glandular volumes greater than those in Group I. This fact is probably related to glandular edema stimulated by PCIT in Group II [5].

PFNA of parathyroid is performed as a diagnostic procedure and is useful in evaluating pre-surgical adenomas [19,20]. However, its application in evaluating parathyroid cell proliferation has not been described in the literature. In our research, PFNA of parathyroid performed prior to treatment provided sufficient material for analysis. This was not the case for post-treatment punctures. All were ultrasonically guided and performed without technical difficulties. The lack of material from these biopsies could be the result of glandular fibrosis caused by ethanol and calcitriol injections. This made evaluation of post-treatment cell proliferation impossible.

Parathyroid turnover is low [21], which changes in chronic renal failure. In cases of 2HPT, cell proliferation increases notably and is one of the important factors in resistant clinical treatment [3]. Abbona et al. [22] evaluated parathyroid cell proliferation in 2HPT patients using immunohistochemistry for Ki-67 and found a label index of 26/1000 cells, which is much higher than the index found in normal parathyroid (2/10 000 cells) [23]. In our study, pre-treatment biopsies confirmed intense cell proliferation for both groups, showing an index of 19.6±17.2 for Group I and 29.0±9.5/1000 cells for Group II. Post-surgical evaluation of cell proliferation confirmed the findings pointed out by PFNA. Furthermore, we observed a positive correlation between glandular volume and cell proliferation evaluated for Ki-67. It is worth emphasizing that, even in glands <0.5 cm³, we found high indices of cell proliferation, suggesting that these glands are resistant to clinical treatment.

In conclusion, in our experience direct calcitriol injection proved no more efficient in controlling 2HPT than did direct injection of ethanol. Our patients were characterized by an increase in biochemical markers and in cell proliferation indicative of severe 2HPT. Furthermore, parathyroids with a volume <0.5 cm³ were excluded from treatment. The majority of these glands presented nodular hyperplasia that is no doubt more resistant to oral or i.v. pulse therapy. We strongly believe that these events were crucial in the failure of the therapeutic techniques used in the present study. This being the case, care must be taken in selecting patients for these treatment modalities and local injection treatment of glands <0.5 cm³ should be considered. In addition, patients presenting with severe 2HPT (as documented by very high serum PTH levels, hypercalcaemia and hyperphosphataemia) should probably not be selected for either PEIT or PCIT.

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Conflict of interest statement. None declared.

References
13. Fukuda N, Tanaka H, Fukagawa M, Kurokawa K, Seino Y. Decreased 1,25-dihydroxyvitamin D3 receptor density in


17. Quarles LD, Lobaugh B, Murphy G. Intact parathyroid hormone overestimates the presence and severity of parathyroid-mediated osseous abnormalities in uremia. *J Clin Endocrinol Metab* 75: 145–150


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