SINGLE AND MULTIPLE GLAND DISEASE IN

PRIMARY HYPERPARATHYROIDISM

.

SINGLE AND MULTIPLE GLAND DISEASE IN

PRIMARY HYPERPARATHYROIDISM

UNI- EN MULTIGLANDULAIRE AFWIJKINGEN BIJ

PRIMAIRE HYPERPARATHYREOIDIE

PROEFSCHRIFT

Ter verkrijging van de graad van Doctor

aan de Erasmus Universiteit Rotterdam

op gezag van de Rector Magnificus

Prof. Dr. C. J. Rijnvos

en volgens besluit van het College van Dekanen.

De openbare verdediging zal plaatsvinden op

woensdag 9 september 1992 om 13.45 uur.

door

Hendrik Jacob Bonjer

geboren te Leiden

Promotiecommissie:

Promotores: Prof. Dr. H.A. Bruining

                         Prof. Dr. J.C. Birkenhäger

Overige leden: Prof. R.H. Nishiyama, M.D., Ph.D.

                         Prof. Dr. Th.J.M.V. van Vroonhoven

To Martha
and my parents

CONTENTS

Chapter 1

INTRODUCTION

One century after F. D. von Recklinghausen described "osteitis fibrosa cystica generalisata" the etiology and pathogenesis of primary hyperparathyroidism remain poorly understood.[1] The clinical picture and pathology of primary hyperparathyroidism have changed considerably during the past 70 years. The first patients operated upon for primary hyperparathyroidism had severe bone disease which was caused by enlargement of a single parathyroid gland ("adenoma"). In the 1930's Albright and Bauer reported the relationship between renal concrements, nefrocalcinosis and primary hyperparathyroidism.[2] One decade later peptic ulcers and acute pancreatitis were associated with primary hyperparathyroidism.[3,4] At present, half of the patients with primary hyperparathyroidism is asymptomatic or has vague symptoms as muscular weakness, fatigue and mental disturbances.[5] This changed clinical picture of primary hyperparathyroidism is probably due to the early detection of primary hyperparathyroidism after the introduction of automated routine assessment of serum calcium.

The first pathological entity reported in primary hyperparathyroidism was a parathyroid "adenoma", removed by Mandl in 1925.[6] Churchill described in 1933 three patients with enlargement of all four parathyroid glands.[7] Microscopical examination of these glands showed acinar-like arrangement of large water clear cells. This entity was called "water clear cell hyperplasia". For unknown reasons "water clear cell hyperplasia" has become rare. In 1958 Cope et al. reported another form of hyperplasia which was referred to as "chief cell hyperplasia".[8] Parathyroid "adenomas" and "chief cell hyperplasia" were difficult to differentiate microscopically. No single differentiating morphological criterion has been reported. Separating parathyroid "adenomas" and "chief cell hyperplasia" grossly was also difficult since asymmetrical enlargement of the parathyroid glands in "chief cell hyperplasia" was frequently seen.[9]

Although at first the incidence of "chief cell hyperplasia" was considered to be low, Paloyan suggested in the 70's that most cases of primary hyperparathyroidism were caused by hyperplasia of the parathyroid glands. Therefore, Paloyan recommended subtotal parathyroidectomies in all patients with primary hyperparathyroidism.[10] However, permanent hypoparathyroidism is a common complication of subtotal parathyroidectomies, and evidence was lacking that most patients with primary hyperparathyroidism had hyperplasia. Therefore, subtotal parathyroidectomy was not accepted as a routine treatment of primary hyperparathyroidism.

In 1971 Bruining hypothesized that all cases of primary

hyperparathyroidism showed hyperplasia in the early stage of the disease.[11] Hyperplasia would transite into nodular hyperplasia and finally an adenoma would evolve with involution of the remaining three parathyroid glands. An increase of the incidence of hyperplasia was attributed to the earlier diagnosis of primary hyperparathyroidism.

In 1984 Ghandur and Kimura redefined the pathology of primary hyperparathyroidism and reclassified 172 cases with primary hyperparathyroidism.[12] In 75 % of cases with single gland enlargement hyperplasia was found at microscopical examination of all parathyroid glands what induced the term "focal hyperplasia". Almost half of the patients with focal hyperplasia developed post-operatively evidence of parathyroid hyperfunction after removal of the enlarged parathyroid gland.

The unresolved pathology of primary hyperparathyroidism has resulted in a continuous debate on the optimal surgical treatment of primary hyperparathyroidism. Bilateral and unilateral approaches are advocated. Some surgeons use the histological appearance of parathyroid tissue at frozen section to determine the extent of parathyroidectomies.[10,13-15] When a hyperplastic parathyroid gland is reported by the pathologist, 3 or 3 1/2 parathyroids are removed. An alternative approach to parathyroid surgery is to rely solely on the gross characteristics of parathyroid glands and remove only enlarged glands.[16-18]

The scope of this thesis is:
- To review diagnostic procedures in primary hyperparathyroidism
- To review localization studies of parathyroid glands in primary hyperparathyroidism
- To assess the optimal surgical treatment of primary hyperparathyroidism by studying the rates of persistent or recurrent hyperparathyroidism
- To attempt to classify primary hyperparathyroidism by histopathology
- To determine DNA patterns in parathyroid glands in primary hyperparathyroidism

4

References

1.   Von Recklinghausen F. Die fibrose oder deformirende Ostitis, die Osteomalacie und die osteoplastische Carcinose, in ihren gegenseitigen Beziehungen. Festschr. Rudolf Virchow (Berlin), 1-89, 1891.

2.   Albright F, Aub JC and Bauer W. Hyperparathyroidism: common and polymorphic condition as illustrated by seventeen proved cases from one clinic. JAMA, 102, 1276, 1934.

3.   Rogers HM, Keating FR Jr, Morlock CG and Barker NW. Primary hypertrophy and hyperplasia of parathyroid glands associated with duodenal ulcer: report of additional case, with special reference to metabolic, gastrointestinal and vascular manifestations. Arch Int Med, 79, 307, 1947.

4.   Cope O, Culver PJ, Mixter CG Jr and Nardi GL. Pancreatitis, diagnostic clue to hyperparathyroidism. Ann Surg, 145, 857, 1957.

5.   Heath H III, Hodgson SF and Kennedy MA. Primary hyperparathyroidism. Incidence, morbidity, and potential economic impact in a community. N Engl J Med, 302, 189, 1980.

6.   Mandl F. Therapeutischer Versuch bei Ostitis fibrosa generalisata mittels Exstirpation eines Epithelkorperchentumors. Wien Klin Wochenschr, 50, 1343, 1925.

7.   Chuchill ED. Operative treatment of hyperparathyroidism. Ann Surg, 100, 606, 1934.

8.   Cope O, Keynes WM, Roth SI and Castleman B. Primary chief-cell hyperplasia of parathyroid glands: new entity in surgery of hyperparathyroidism. Ann Surg, 148, 375, 1958.

9.   Castleman B and Roth SI. Atlas of Tumor Pathology, Second Series, Fasc. 14, Tumors of the parathyroid glands, 54, 1978.

10.   Paloyan E, Lawrence AM, Baker WH and Straus FH. Near-total parathyroidectomy. Surg Clin North Am, 49, 43, 1969.

11.   Bruining HA. Surgical treatment of hyperparathyroidism. Van Gorcum, Assen , The Netherlands and Charles C Thomas, Springfield, 1971, 30.

12.   Ghandur-Mnaymneh L and Kimura N. The parathyroid adenoma: A histologic definition with a study of 172 cases of primary hyperparathyroidism. Am J Pathol, 115, 70, 1984.

13.   Rudberg C, Akerstrom G, Palmer M, Ljunghall S, Adami HO, Johansson H, Grimelius L, Thoren L and Bergstrom R. Late results of operation for primary hyperparathyroidism. Surgery, 99, 643, 1986.

14.   McGarity WC, McKeown PP and Sewell CW. The role of routine biopsy of all parathyroid glands in primary hyperparathyroidism. Am Surg, 51, 8, 1985.

15.   Cooke TJC, Boey JH and Sweeney CW. Parathyroidectomy: extent of resection and late results. Br J Surg, 64, 153, 1977.

16.   Clark OH, Way LW and Hunt TK. Recurrent hyperparathyroidism. Ann Surg, 179, 729, 1974.

17.   Wells SA, Leight GS, Hensley M and Dilley WG. Hyperparathyroidism associated with the enlargement of two or three parathyroid glands. Ann Surg, 202, 533, 1985.

18.   Bruining HA, Van Houten H, Juttmann JR, Lamberts SWJ and Birkenhager JC. Results of operative treatment of 615 patients with primary hyperparathyroidism. World J Surg, 197, 167, 1981.

.

Chapter 2

DIAGNOSIS OF PRIMARY HYPERPARATHYROIDISM

The diagnostic procedure for primary hyperparathyroidism begins with the exclusion of diseases, other than primary hyperparathyroidism, which are associated with hypercalcemia, and performing laboratory tests which assess the function of the parathyroid glands. Dependence on clinical symptoms to establish the diagnosis of primary hyperparathyroidism is hazardous since they may vary considerably ranging from the absence of symptoms to the presence of severe bone or renal disease. The advanced stages of primary hyperparathyroidism have become rare since the introduction of the multi-channel auto-analyzer. A sevenfold increase in the annual incidence of primary hyperparathyroidism has been noted, with a concomitant decrease in the frequency of urolithiasis from 51 to 4 percent. The proportion of asymptomatic patients has also changed from 18 to 51 percent.(1)

The cornerstone for the diagnosis of primary hyperparathyroidism is hypercalcemia. However, the detection of hypercalcemia may be elusive because of frequent fluctuations of serum calcium between normal and elevated values. Lobaugh et al. studied 14 patients with primary hyperparathyroidism before and after parathyroidectomies.(2) Circadian rhythms of serum ionized calcium were determined before and after parathyroidectomies. The highest concentration of ionized calcium occurred between 0800 pm and 0900 pm, and the lowest, between 0800 and 0900 am. The difference between the highest and lowest values of ionized calcium was 0.08 mmol/L. Parathyroidectomy restored the reciprocal relationship between the levels of ionized calcium and parathyroid hormone which were disturbed before the operations. These data show that specimens drawn at different times throughout the day may be most helpful in establishing the diagnosis of primary hyperparathyroidism in patients with mild disease or borderline biochemical findings.

Assays of serum calcium measure total calcium. However, Moore determined levels of total serum calcium, protein bound calcium and ionized calcium in 42 patients with cirrhosis and 21 healthy persons, and found that approximately 47 percent of serum calcium was ionized, 40 percent was bound to proteins, principally albumin, and the remainder was complexed to citrate and other organic anions. There is general agreement that the ionized form of calcium is the only physiologically important component.(3)

Iqbal showed that after correction of serum calcium to a serum albumin level of 40 g/L resulted in an increase of more than 0.2 mmol/L in 22.7 percent of 198 patients with various diseases and a decrease of more than 0.2 mmol/L in 7.1 percent.(4) Therefore, adjustment for serum levels of albumin is neces-sary for assessment of the "true" calcium level. Total serum calcium can be

adjusted for serum albumin as follows: for every 1 g/L by which the serum albumin exceeds 40 g/L, 0.02 is subtracted from the total. A corresponding correction is made when the serum albumin is less than 40 g/L.(5)

Primary hyperparathyroidism is classically associated with hypophosphatemia since parathyroid hormone reduces the reabsorption of phosphate in the renal tubules. However, it is not uncommon to find normal or elevated levels of phosphate in patients with primary hyperparathyroidism.(6) Juttman et al. found only 56 patients with low serum levels of phosphate among 125 patients with primary hyperparathyroidism.(7)

The reabsorption of bicarbonate in the kidney is decreased by parathyroid hormone leading to a metabolic acidosis which is accompanied by an elevated serum level of chloride. In spite of this phenomenon Shishiba et al. found that 30 percent of 63 patients with primary hyperparathyroidism had a positive, instead of a negative base excess.(8)

Although the serum levels of chloride and phosphate independently do not aid much in the diagnosis of primary hyperparathyroidism, Palmer et al. demonstrated that the chloride / phosphate ratio was helpful in differentiating the hypercalcemia of primary hyperparathyroidism from other forms of hypercalcemia.(9) Broulik and Pacovsky and Juttman et al. reported positive predictive values of 96 and 91 percent respectively by using the chloride / phosphate ratio. (10,7) Shishiba et al. found that the chloride / phosphate ratio was abnormal in only 15 percent of patients with primary hyperparathyroidism.(8)

Serum alkaline phosphatase, which reflects the activity of osteoblastic cells, is only elevated in primary hyperparathyroidism with skeletal changes. In the study of Juttmann et al., the serum alkaline phosphatase was elevated in 34 percent of patients with primary hyperparathyroidism.(7)

In 1967 Chase and Aurbach reported increased urinary excretion of cyclic adenosine monophosphate (cAMP) in patients with primary hyperparathyroidism.(11) In normal individuals half of urinary cAMP is plasma cAMP, which is filtrated in the glomeruli, and the other half is produced by the renal tubuli (nephrogenous cAMP). PTH increases the production of nephrogenous cAMP. Broadus et al. demonstrated a significant correlation between the excretion of cAMP and primary hyperparathyroidism.(12) However, Schweigart et al. considered the assessment of cAMP valueless in establishing the diagnosis of primary hyperparathyroidism.(13) Increased excretion of urinary cAMP is non-specific and can be observed in diabetes mellitus, hyperthyroidism, phaeochromocytomas and tumoral hypercalcemia.

Parathyroid hormone enhances the renal excretion of phosphate by

inhibiting reabsorption of phosphate in the proximal renal tubules. Because of variations in dietary phophate and renal function there is an overlap between the levels of tubular reabsorption of phosphate in normal individuals and patients with primary hyperparathyroidism.(14) Walton and Bijvoet proposed a method to determine the maximal reabsorption of phosphate in terms of renal function (TmP/GFR ratio).(15) The TmP/GFR ratio should be a better indicator of primary hyperparathyroidism than tubular reabsorption of phosphate. However, the TmP/GFR ratio is also altered in hypertension, familial hypocalciuric hypercalcemia and by the adminstration of estrogens and corticosteroids.(16) Menko found low values of the TmP/GFR ratio in 68 percent of patients with primary hyperparathyroidism.(17)

Calcium infusions into normal individuals lower the serum level of parathyroid hormone. Hackeng et al. showed in 12 normal individuals a decrease of the plasma level of intact parathyroid hormone from 2.2 to 1.2 pmol/L after oral ingestion of 1000 mg calcium.(18) In 9 patients with primary hyperparathyroidism Tohme et al. found significantly less suppressibility of intact PTH (N-terminal assay) than in 9 normal persons.(19) In patients with primary hyperparathyroidism the fall of serum levels of parathyroid hormone should also be less after infusion of calcium when compared to normal individuals. However, the degree of autonomy or suppressibility of parathyroid function in primary hyperparathyroidism varies. Upon inducing hypercalcemia Murray et al. found a reduction of plasma parathyroid hormone concentration of 55 percent in 9 patients with parathyroid adenomas and of 50 percent in 3 patients with parathyroid hyperplasia.(20) Reiss and Canterbury observed after infusion of calcium no change in serum levels of parathyroid hormone in 7 patients with adenomas, although serum parathyroid hormone levels in 3 patients with hyperplasia were suppressed to a degree comparable to that in normal individuals.(21) Brown et al. reported the calcium sensitivity of dispersed cells derived from adenomas and hyperplastic parathyroid glands.(22) The release of parathyroid hormone was suppressed by 50 percent or more in cells from 7 of 9 hyperplastic glands and from 8 of 12 adenomas. Cells taken from hyperplastic glands, however, were suppressed by significantly lower concentrations of calcium than those from adenomas and resembled normal parathyroid cells in that respect.

Renal excretion of calcium is determined by a balance between the glomerular filtration of calcium and its reabsorption in the tubules. In primary hyperparathyroidism the relationship between calcium excretion and the serum level of calcium is shifted to the right. Therefore, the calcium excretion can be

normal in patients with mildly elevated levels of serum calcium.(23)

The diagnostic value of biopsies of bone in primary hyperparathyroidism is limited. Delling et al. described the histology of bone biopsies in 391 patients with primary hyperparathyroidism.(24) In more than 45 percent of patients a non-specific increase of osteoid seams, osteoblasts and osteoclasts was observed. Fifty percent showed specific, but very often mild, endosteal fibrosis. Only in 4 percent of patients was there severe fibro-osteoclasis with development of brown tumors. Juttman et al. found increased bone turnover in up to 95 percent of the patients in their study of 125 patients with primary hyperparathyroidism.(7)

Radiologic skeletal abnormalities occur when more than one quarter of the bone (mineral) mass has been lost.(25) Currently, bone disease of such severity is very rare in primary hyperparathyroidism. Therefore, radiologic investigations have lost their value in the diagnosis of primary hyperparathyroidism.

Establishing the diagnosis in patients with hypercalcemia, using readily available biochemical assays, appeared to be improved by applying the technique of discriminant function analysis. Gibb et al. reported a study of 148 patients with hypercalcemia due to primary hyperparathyroidism, malignancies and other causes.(26) The useful variables to discriminate hypercalcemia of primary hyperparathyroidism from tumoral hypercalcemia were, in decreasing order of importance, plasma albumin, plasma chloride, (logaritmic analysis of) calcium excretion and (logaritmic analysis of) plasma gamma GT. Plasma albumin levels had the greatest discriminant power, higher concentrations being more commonly associated with primary hyperparathyroidism. The proportion of patients correctly assigned by this process was nearly 90 percent. Lacher et al. found serum albumin to be the single parameter to discriminate hyperparathyroidism from tumoral hypercalcemia.(27) However, serum albumin alone could only detect about 81 percent of the patients. Discriminant analysis with 20 laboratory tests, including an assay for carboxy terminal parathyroid hormone, could perfectly separate the two groups.

More recently, the measurement of parathyroid hormone has emerged as the optimal method for establishing the diagnosis of primary hyperparathyroidism. After the first description of a radio-immuno assay for parathyroid hormone by Berson and Yalow the role of parathyroid hormone assays was controversial for many years because of variations in the specificity and sensitivity of different methods which employed different antibodies.(28,29) Parathyroid hormone is secreted as a single chain polypeptide of 84 amino acids, although there is in vivo and in vitro evidence of breakdown of the peptide in

parathyroid cells and for the secretion of shorter fragments.(30-32) The intact peptide disappears rapidly from the circulation by cleavage between the residues 33-36 by the Kupffer cells of the liver.(34,35) The biological activity of the hormone is determined by the amino acids 1-34 at the amino-terminal end of the molecule. This amino-terminal fragment has a plasma half life of a few minutes. The remainder of the molecule, containing amino acids 35-84, which is the carboxyl-terminal portion, is devoid of biological activity. The carboxyl-terminal fragment is excreted by the kidney and normally has a plasma half life of approximately one hour.(36)

Gough et al. have reviewed the results of different parathyroid hormone assays.(36) In 438 patients with primary hyperparathyroidism, the results of assays for the carboxyl-terminal type were elevated in 87 percent of the patients, while those of the assays for the amino-terminal type were elevated in 67 percent of 301 patients. In their own series of 204 patients, elevated carboxyl-terminal assay results were observed in 91 percent of patients, and the amino-terminal assay results were elevated in only 24 percent.

Subsequently assays for the mid-region were developed. Blind et al. found the sensitivity of the mid-region assay to be nearly 90 percent.(37) However, in one third of patients with tumoral hypercalcemia elevated mid-region assays were found. Impairment of renal function or interaction of an immunoglobulin with the antibody may have caused the elevated values.(38) The diagnostic value of carboxyl-terminal and mid-region assays is limited, in spite of a satisfactory sensitivity, because they detect biologically inactive fragments. The disadvantage of the amino-terminal assay is that although it can recognize the biologically active part of the molecule, it lacks the sensitivity to measure normal levels.(39)

Recently, a two-site immunochemiluminometric assay for parathyroid hormone has been described.(40) The method relies on the formation of an immune complex of labeled anti-amino-terminal PTH antibody, intact PTH, and solid phase anti-midregion PTH antibody. A chemiluminescent compound is used as a label. Only intact parathyroid hormone is recognized by this system, and carboxyl-terminal fragments do not interfere with the measurement. The ability of the assay to detect levels of intact human PTH as low as 0.8 pmol/L allowed detection of intact PTH in the serum of all normal subjects tested. A clear distinction was found between hypercalcemic individuals, subsequently proven to have primary hyperparathyroidism and those with malignancies. This assay has the potential to detect all cases of primary hyperparathyroidism. The intact parathyroid hormone assay also has an important role in monitoring the

13

treatment of renal bone disease (secondary hyperparathyroidism). Likewise the immunoradiometric assays (IRMA) for intact parathyroid hormone, using a radio-active label, have been reported to identify normal subjects, patients with tumoral hypercalcemia and with primary hyperparathyroidism, and distinguish them from each other.(18,41-44) Care has to be taken to draw the blood samples in the fasting state, as an oral calcium load may lower the level of intact PTH in normal individuals and patients with primary hyperparathyroidism.(18) These assays can detect intact PTH in concentrations as low as 0.6 pmol/L. Since the assays for intact parathyroid hormone may take days to complete, they cannot be done intra-operatively.

A parathyroid hormone related protein (PTHrP) has recently been isolated from tumors associated with tumoral hypercalcemia.(45-49) The PTHrP gene encodes three related PTHrP molecules.(50) All have the same amino acid sequence through residue 139. The amino acid sequence of PTHrP shows partial homology to the sequence in parathyroid hormone from amino acid 1 through amino acid 13, but differs thereafter.(51) Several authors have reported interaction of PTHrP with PTH receptors, resulting in hypercalcemia when infused into animals.(52-57) Burtis et al. introduced two sensitive immuno assays for PTHrP, one of those an immuno radiometric assay. The level of PTHrP was found to be normal or low in primary hyperparathyroidism, while in the majority of patients with tumoral hypercalcemia it was increased. Depending on the clinical setting, assessment of PTHrP may be helpful in the differential diagnosis of primary hyperparathyroidism. On the other hand the level of intact PTH will be suppressed in tumoral hypercalcemia.

In conclusion, the gold standard to establish the diagnosis of primary hyperparathyroidism is the two-site immuno-radiometric assay which measures intact PTH in the fasting state. This assay provides dependable discrimination of patients with different forms of hypercalcemia and as a rule allows a rapid diagnosis of primary hyperparathyroidism.

References

1.   Heath III H, Hodgson SF and Kennedy MA. Primary hyperparathyroidism, incidence, morbidity, and potential impact in a community. N Engl J Med, 302, 189, 1980.
2.   Lobaugh B, Neelon FA, Oyama H, Buckley N, Smith S, Christy M and Leight GS. Circadian rhythms for calcium, inorganic phosphorus, and parathyroid hormone in primary hyperparathyroidism: Functional and practical considerations. Surgery, 1009, 1989.
3.   Moore EW. Studies with ion exchange calcium electrodes: III: The state of serum calcium

in patients with cirrhosis. Gastroenterology, 60, 43, 1971.

4. Iqbal SJ, Ledger S, Giles M, Nanji N. Need for albumin adjustments of urgent total serum calcium. Lancet, II, 1477, 1988.

5. Editorial: Correcting the calcium. Br Med J, 598, 1977.

6. Smit FW. Primaire hyperparathyreoidie, diagnostiek en behandeling. Ed.: Dijkhuizen van Zanten, Groningen, 61, 1989.

7. Juttmann JR, Bruining HA and Birkenhager JC. Diagnostische aspecten van primaire hyperparathyreoidie. Ned Tijdschr Geneesk, 124, 1002, 1980.

8. Shishiba Y, Nakazawa H, Shimuzu T, Ozawa Y, Akiyama H and Matsushita H. Significance of parathyroid hormone (PTH) radioimmunoassay in the diagnosis of primary hyperparathyroidism: an experience based on 63 surgically proven cases. Endocrinol Jpn, 34, 263, 1987.

9. Palmer FJ, Nelson JC and Bacchus H. The chloride-phosphate ratio in hypercalcemia. Ann Int Med, 80, 200, 1974.

10. Broulik PD and Pacovsky V. The chloride phosphate ratio as the screening test for primary hyperparathyroidism. Horm Metab Res, 11, 577, 1979.

11. Chase LR and Aurbach GD. Parathyroid function and the renal excretion of 3'5'-adenylic acid. Proc Natl Acad Sci USA, 58, 518, 1967.

12. Broadus AE, Mahaffey JE, Bartter FC and Neer RM. Nephrogenous cyclic adenosine monophosphate as a parathyroid function test. J Clin Invest, 60, 771, 1977.

13. Schweigart U, Henderkott U, Paterek K, Hartung R, Zilker Th and Bottermann P. Wertigkeit verschiedener Stoffwechselparameter bei der Diagnostik des primaren Hyperparathyreodismus. Med Klin, 7, 281, 1976.

14. Smit FW. Primaire hyperparathyreoidie, diagnostiek en behandeling. Ed.: Dijkhuizen van Zanten, 69, 1989.

15. Walton RJ and Bijvoet OLM. Nomogram for derivation of renal treshold phosphate concentration. Lancet, 309, 1975.

16. Bijvoet OLM. Renal phosphate excretion in man. Folia Med Neerl, 15, 84, 1972.

17. Menko FH, Bijvoet OLM, Fronen LHH, Sandler LM, Adami S, O'Riordan JLH, Schopman W and Heynen G. Familial benign hypercalcemia. Q J Med, 206, 120, 1983.

18. Hackeng WHL, Lips P, Netelenbos JC and Lips CJM. Clinical implications of estimation of intact parathyroid hormone(PTH) versus total immunoreactive PTH in normal subjects and hyperparathyroid patients. J Clin Endocrinol Metab, 63, 447, 1986.

19. Tohme JF, Bilezikian JP, Clemens TL, Silverberg, Shane E and Lindsay R. Suppression of parathyroid hormone secretion with oral calcium in normal subjects and patients with primary hyperparathyroidism. J Clin Endocrinol Metab, 70, 951, 1990.

20. Murray TM, Peacock M, Powell D, Monchik JM and Potts JT, Jr. Non-autonomy of hormone secretion in primary hyperparathyroidism. Clin Endocrinol (Oxford), I, 235,1972

21. Reiss E and Canterbury JM. Primary hyperparathyroidism: Application of radio-immunoassay to differentiation of adenoma and hyperplasia and to preoperative localization of hyperfunctioning parathyroid glands. N Engl J Med, 280, 1381, 1969.

22. Brown EM, Brennan MF, Hurwitz S, Windeck R, Marx SJ, Spiegel AM, Koehler JO, Gardner DG and Aurbach GD. Dispersed cells prepared from human parathyroid glands: distinct calcium sensitivity of adenomas vs. primary hyperplasia. J Clin Endocrinol

Metab, 46, 267, 1978.

23. Nordin BEC and Peacock M. The role of the kidney in serum calcium homeostasis. In: Calcitonin 1969, Proceedings of the second international symposium, London, 21-24 July 1969, Heinemann, London 1970, p. 475.

24. Delling G, Dreyer T, Hesch RD, Schulz W, Ziegler R and Bressel M. Morphologische Veranderungen der Beckenkammspongiosa beim primaren Hyperparathyeodismus and ihre Bedeutung fur die Diagnostik. Klin Wochenschr, 65, 643, 1987.

25. Ziegler R, in Rothmund M. Hyperparathyreodismus, Stuttgart: Thieme Verlag, 1980.

26. Gibb JA, Ogston SA, Paterson CR and Evans JR. Discriminant functions in differential diagnosis of hypercalcemic patients. Clin Chem, 36, 358, 1990.

27. Lacher DA, Baumann RR and Boyd JC. Comparison of discriminant analysis procedures in laboratory differentiation of hypercalcemia. Am J Clin Path, 89, 753, 1988.

28. Berson SA, Yalow RS, Aurbach GD and Potts JT. Immunoassay of bovine and human parathyroid hormone. Proc Natl Acad Sci USA, 49, 613, 1963.

29. Raisz LG, Yajnik CH, Bockman RS and Bower BF. Comparison of commercially available parathyroid hormone immunoassays in the differential diagnosis of hypercalcemia due to primary hyperparathyroidism or malignancy. Ann Int Med, 91, 739, 1979.

30. Silverman R and Yalow RS. Heterogeneity of parathyroid hormone, clinical and physiological implications. J Clin Invest, 52, 1958, 1973.

31. Flueck JA, Di Bella FP, Edis JA, Kehrwald JM and Arnaud CD. Immunoheterogeneity of parathyroid hormone in venous effluent serum from hyperfunctioning parathyroid glands. J Clin Invest, 60, 1367, 1977.

32. Morissey JJ, Hamilton JW, Macgregor RR and Cohn DV. The secretion of parathyroid hormone fragments 34-84 and 37-84 by dispersed porcine parathyroid cells. Endocrinology, 107, 164, 1980.

33. Hanley DA and Ayer LA. Calcium dependent release of carboxyl-terminal fragments of parathyroid hormone by hyperplastic human parathyroid tissue in vitro. J Clin Endocrinol Metab, 63, 1075, 1986.

34. Zull JE and CHuang J. Characterization of parathyroid hormone fragments produced by cathepsin D. J Biol Chem, 260, 1608, 1985.

35. Martin KJ, Hruska KA, Frietag JJ, Klahr S and Slatopolsky E. The peripheral metabolism of parathyroid hormone. N Engl J Med, 301, 1092, 1979.

36. Gough IR, Thompson NW and Eckhauser FE. The value to the surgeon of parathyroid hormone assays in primary hyperparathyroidism. Aust N Z J Med, 58, 381, 1988.

37. Blind E, Schmidt-Gayk H, Scharla S, Flentje D, Fischer S, Gohring U and Hitzler W. Two-site assay of intact parathyroid hormone in the investigation of primary hyperparathyroidism and other disorders of calcium metabolism compared with a midregion assay. J Clin Endocrinol Metab, 67, 353, 1988.

38. Mallette LE and Nammour H. False-positive elevations of midregion parathyroid hormone: inhibition of radioligand binding by heterophylic antibody in patient serum. In Cohn DV, Martin TJ and Meunier PJ. Calcium regulation and bone metabolism, basic and clinical aspects. Proceedings of the 9th international conference on calcium regulating hormones and bone metabolism. Excerpta Medica, Amsterdam, New York and Oxford, vol. 9, 550, 1987.

16

39. Woodhead JS. The measurement of circulating parathyroid hormone. Clin Biochem, 23, 17, 1990.

40. Brown RC, Aston JP, Weeks I and Woodhead JS. Circulating intact parathyroid hormone measured by a two-site immunochemiluminometric assay. J Clin Endocrinol Metab, 65, 407, 1987.

41. Blind E, Schmidt-Gayk H, Armbruster FP and Stadler A. Measurement of intact human parathyrin by an extracting two-site immunoradiometric assay. Clin Chem, 33, 1376, 1987.

42. Newman DJ and Ashby JP. Clinical and laboratory evaluation of a two-site immunoradio-metric assay for intact parathyroid hormone. Ann Clin Biochem, 25, 645, 1988.

43. Ratcliffe WA, Heath DA, Ryan M and Jones SR. Performance and diagnostic application of a two-site immunoradiometric assay for parathyrin in serum. Clin Chem, 35, 1957, 1989.

44. Frolich M, Walma ST, Paulson C and Papapoulos SE. Immunoradiometric assay for intact parathyroid hormone: characteristics, clinical application and comparison with a radio-immunoassay. Ann Clin Biochem, 27, 69, 1990.

45. Stewart AF, Insogna KL and Burtis WJ. Frequency and partial characterization of adenylate cyclase-stimulating activity in tumors associated with humoral hypercalcemia of malignancy. J Bone Mineral Res, 1, 267, 1986.

46. Burtis WJ, Wu T and Bunch C. Identification of a novel 17,000 dalton parathyroid hormone-like adenylate cyclase-stimulating protein from a tumor associated with humoral hypercalcemia of malignancy. J Biol Chem, 262, 7151, 1987.

47. Moseley JM, Kubota M and Diefenbach-Jagger H. Parathyroid hormone-related protein purified from a human lung cancer line. Proc Natl Acad Sci USA, 84, 5048, 1987.

48. Stewart AF, Wu T, Goumas D, Burtis WJ and Broadus AE. N-terminal amino acid sequence of two novel tumor derived adenylate cyclase stimulating proteins: identificati-on of parathyroid hormone-like and parathyroid hormone-unlike domains. Biochem Biophys Res Commun, 146, 672, 1987.

49. Strewler GJ, Stern PH and Jacobs JW. Parathyroid hormonelike protein from human renal carcinoma cells: structural and functional homology with parathyroid hormone. J Clin Invest, 80, 1803, 1987.

50. Mangin M, Ikeda K, Dreyer BE and Broadus AE. Isolation and characterization of the human parathyroid hormone-like peptide gene. Proc Natl Acad Sci USA, 86, 2408, 1989.

51. Burtis WJ, Brady TG and Orloff JJ. Immunochemical characterization of circulating parathyroid hormone-related protein in patients with humoral hypercalcemia of cancer. N Engl J Med, 322, 1106, 1990.

52. Stewart AF, Elliot J, Burtis WJ, Wu T and Insogna KL. Synthetic parathyroid hormone-like protein (1-74): Biochemical and physiological characterization. Endocrinology, 124, 642, 1989.

53. Stewart AF, Wu T, Burtis WJ, Weir EC, Broadus AE and Insogna KL. The relative potency of a human tumor-derived PTH-like adenylate cyclase-stimulating preparation in three bioassays. J Bone Mineral Res, 2, 37, 1987.

54. Horiuchi N, Caulfield MP and Fisher JE. Similarity synthetic peptide from tumor to parathyroid hormone in vivo and in vitro. Science, 238, 1568, 1987.

55. Kemp BE, Moseley JM and Rodda CP. Parathyroid hormone-related protein of malignancy: active synthetic fragments. Science, 238, 1568, 1987.
56. Stewart AF, Mangin M and Wu T. Synthetic human parathyroid hormone-like protein stimulates bone resorption and causes hypercalcemia in rats. J Clin Invest, 81, 596, 1988.
57. Rabbani SA, Mitchell J, Roy DR, Hendy GN and Goltzman D. Influence of the amino-terminus on in vitro and in vivo biological activity of synthetic parathyroid hormone-like peptides of malignancy. Endocrinology, 123, 2709, 1988.

Chapter 3

LOCALIZATION STUDIES OF PARATHYROID GLANDS

This chapter has been submitted for publication.

`

Localization studies of parathyroid glands play a debatable role in the management of primary hyperparathyroidism. Preoperative localization of enlarged parathyroid glands would potentially facilitate exploration, reduce operation time and increase the success rate of parathyroid surgery. However, the success rate of first operations for primary hyperparathyroidism by experienced surgeons is over 90 percent.[1] Therefore, the necessity for localizing studies seems limited in patients undergoing first explorations of the neck. Nevertheless, in institutes preferring unilateral approaches, imaging studies are of great importance in localizing the side of the enlarged parathyroid gland.[2] Reoperations for persistent or recurrent hyperparathyroidism have less favorable results due to fibrosis and anatomically distorted areas, and lead to increased rates of complications like permanent hypoparathyroidism and injury of the recurrent laryngeal nerve.[3,4] In such patients localization studies could be valuable.

Various techniques of parathyroid localization have been attempted, but most have been relatively unsuccessful. Intravenous and intra-arterial injections with selenomethionine (^{75}Se) [5,6], ^{131}I scanning[7], thermography[8,9], barium swallow[10,11], neck massage before parathyroid hormone measurement[12], intravenous methylene blue injection[13] and thyroid lymphography[14] revealed poor results. Most of these localization techniques will identify only large parathyroid tumors (more than 2 cm in diameter), which account for a low percentage of abnormal parathyroid glands.[15] More sensitive and specific localization techniques have recently become available.

Ultrasonography

High resolution real-time 10 MHz ultrasonography recognizes enlarged parathyroid glands as oval, solid lesions with less echogenecity than the thyroid gland. These aspects should, when combined with the anatomic location of the parathyroid glands, enable the investigator to identify parathyroid tumors. Normal parathyroid glands are generally not visualized sonographically because of their small size and close anatomic relationship to, and isoechogenecity with, the thyroid gland.[16-18]

Graif et al.[19] reported the results of pre-operative high resolution sonography of the neck in 53 patients with primary hyperparathyroidism. Fifty-one of 62 parathyroid tumors (83 percent) were located by ultrasound. The mean length of the identified parathyroid tumors was 16 mm (range: 6 - 35

mm). The largest parathyroid tumor missed on sonography measured 45 mm in its longest axis, and was located in the mediastinum. Distinction of cystic parathyroid tumors from thyroid cysts proved to be difficult. Parathyroid glands located in the mediastinum were not easily identified by ultrasound since the bony thoracic cage covering the mediastinum impedes thorough sonographic examination. The cartaliginous structures of the larynx rendered the upper parathyroid glands less accessible in some patients. According to Randel et al.[20] 10 -15 percent of the parathyroid glands are located in regions inaccessible to ultrasound.

Stein and Wexler[21] reported a sensitivity of real-time 10 MHz ultrasound of 67 percent for adenomas, while the sensitivity for hyperplasia was as low as 36 percent in a study of 27 patients with primary hyperparathyroidism. Parathyroid glands weighing 280 mg or more could be correctly identified by ultrasound. On the other hand, enlarged parathyroid glands weighing up to 3000 mg were also missed by ultrasound of the neck.

Lloyd and Lees[22] showed in a study of 173 patients that the sensitivity of ultrasound to detect abnormal parathyroid glands was profoundly dependent on the experience of the operator of the ultrasound. The most experienced ultrasonologist detected 63 percent of parathyroid adenomas compared with a rate of detection of 20 percent by inexperienced radiologists. The mean weight of detected abnormal parathyroid glands was 2.2 g (0.17 - 15.7 g). The missed enlarged glands had a mean weight of 0.29 g (0.07 - 0.36 g). Failure of detection was related to concurrent abnormalities of the thyroid gland, small sizes of parathyroid tumors or to localizations of parathyroid tumors in the space dorsal to the esophagus or within the thymus, both sites being difficult accessible to ultrasound. All parathyroid glands weighing more than 0.36 g and located in normal anatomic positions were detected by ultrasound.

Kern et al.[23] reported the results of intra-operative ultrasonography. In 39 patients who had reoperations of the neck because of persistent hyperparathyroidism, pre-operative ultrasonography correctly localized 20 of 41 abnormal parathyroid glands. Intra-operative ultrasound visualized 33 of 41 abnormal parathyroids. Five intrathyroidal parathyroid adenomas were correctly identified by intra-operative ultrasound. One false positive finding with ultrasound resulted from visualization of a mediastinal lymph node.

Norton et al.[24] also found intra-operative ultrasound to be superior to pre-operative ultrasound. In 25 patients undergoing reoperations for persistent or recurrent hyperparathyroidism 9 parathyroid tumors were localized preoperatively by ultrasound, while intra-operative ultrasound identified 19 of

25 parathyroid tumors correctly. Intrathyroidal parathyroid glands and the lower parathyroid glands were localized reliably, but only two thirds of the enlarged upper parathyroid glands were detected. The average length of the operative procedures was significantly reduced by 2.8 hours when intra-operative ultrasound was used to localize enlarged parathyroid glands.

Differentiation of posteriorly located thyroid nodules from enlarged parathyroid glands can be difficult with ultrasound alone, since thyroid nodules may show the same pattern of echoes as enlarged parathyroids.[25-27] Fine needle aspiration biopsies have increased the specificity of the detection of pathologic parathyroid glands by ultrasound.

Karstrup et al.[28] examined cytologically the aspirated material from 14 ultrasonographically suspect enlarged parathyroid glands. At operation the presence of parathyroid tumors was confirmed. In 10 of 14 cases parathyroid cells were found at cytology, in the remaining 4 cases only cells of endocrine origin were seen. Analysis for parathyroid hormone content revealed in 19 of 20 parathyroid glands a high content.

In a study of 16 patients by Verbanck et al.[29] 24 aspiration biopsies of 26 retrothyroid nodules showed parathyroid tissue. The cell block technique for histological examination of aspirated small tissue fragments was used which leaves the architecture of the tissue undisturbed, thereby facilitating identification of parathyroid tissue. The smallest nodule aspirated measured 4 x 6 mm. In 6 patients alcoholization of the parathyroid tumors was followed by a fall in serum level of parathyroid hormone.

Kahaly et al.[30] studied 15 patients with primary hyperparathyroidism and 20 patients with thyroid nodules. Highly significant differences for concentrations of parathyroid hormone and human thyroglobulin were measured in the aspirates of enlarged parathyroid glands and thyroid nodules. Immunocytology for parathyroid hormone was positive in 14 of 15 aspirations of parathyroid glands and negative for thyroglobulin in all parathyroids.

Karstrup et al.[31] used a 0.8 mm Sure-cut needle for ultrasound guided fine needle biopsies in 60 parathyroid tumors. The success rate of obtaining tissue for histological diagnosis was 55 percent. No false positive or negative findings were encountered. Although a biopsy taken with a large bore needle, like the Sure-cut needle, facilitates histological diagnosis of the specimen, it is more difficult to obtain sufficient material with a large bore needle from a tumor of soft consistency.

Table 1 summarizes the results of the studies on ultrasonography in parathyroid disease. The sensitivity rates for detection of parathyroid tumors

vary from 36 % to 85 %. The smallest parathyroid gland which can be detected by ultrasonography measures 6 mm in longest axis and weighs 130 mg. Intra-operative ultrasonography seems to be more sensitive than pre-operative ultrasonography of the neck.

Table 1. *Ultrasonography in parathyroid disease*

	Number of Glands	Adenoma or Hyperplasia	Sensitivity %	Specificity %	Size/Weight Detected Glands	Size/Weight Missed Glands
Graif[19]	62	Ad + Hyp	83	98	6-35 mm	10-45 mm
Stein[21]	18	Adenoma	67	96	490-2290 mg	220-460 mg
	16	Hyperplasia	37	100	130-1110 mg	200-3080 mg
Lloyd[22]	35	Adenoma	63	95	170-1570 mg	70-360 mg
Kern[23]	41[*]	Ad + Hyp	49	-	-	-
	41[a]	Ad + Hyp	85	-	-	-
Norton[24]	25[*]	Ad + Hyp	36	-	-	-
	25[a]	Ad + Hyp	76	-	-	-

[*] Pre-operative ultrasound   [a] Intra-operative ultrasound

Computed Tomographic Scanning

Computed tomography scanning was initially used only to find parathyroid tumors located in the mediastinum, but CT scanning of the neck has proved to be valuable as well in localizing pathologic parathyroid glands.

Sommer et al.[32] presented in 1982 a study of 21 patients with primary hyperparathyroidism who had CT scans of the neck pre-operatively. CT images before and after contrast administration were examined. Seventeen of 24 parathyroid tumors were correctly localized by CT scan. Three of the identified tumors were located in the mediastinum. The smallest detected adenoma measured 10 x 8 x 5 mm. Goiters made identification of parathyroid tumors more difficult. Small adenomas located caudally to the thyroid gland amid of the numerous vessels traversing the superior thoracic aperture were difficult to detect with CT scanning, particularly in patients with broad shoulders and short necks.

Krebs et al.[33] reported in a series of 10 patients correct pre-operative localization of 8 of 10 adenomas. There was one false positive finding because a redundant esophagus in one patient was interpreted as an adenoma. Most adenomas were surrounded by hypodense areas, representing fat enveloping the adenomas. Some of the adenomas had an irregular aspect on CT scan

24

although they were round or ovoid in shape at operation. One adenoma measuring 5 x 5 x 14 mm could not be seen on CT-scan. Normal parathyroids were not identified because of their small sizes.

In a study of 63 patients with primary hyperparathyroidism Cates et al.[34] were able to localize pathologic parathyroids correctly in 81 percent. No adenomas were encountered in the mediastinum. Cates et al. used a combination of pre- and post-contrast images. Prior to adminstration of intravenous contrast medium enlarged parathyroid glands are similar in attenuation to muscle and adjacent vessels. However, on contrast enhanced scans differentiating parathyroid tumors from thyroid can be impossible. Therefore, examination of pre- and post contrast images is necessary to distinguish parathyroid tumors from muscle, tortuous vessels and thyroid masses.

Stark et al.[35] compared the diagnostic accuracy of conventional CT-techniques and CT-scanning using a special device, hyperextending the neck and depressing the shoulders, in combination with dynamic CT-scanning and administration of methyl-glucamine diatrizoate (Gastrografin[R]) if the esophagus was not delineated clearly. Hyperextending the neck elevates the thyroid and parathyroid glands and part of the thymus above the manubrium. Streak artifacts related to shoulder position were eliminated. Application of this modified technique resulted in an increase of sensitivity from 51 percent to 78 percent. The mean size of the identified enlarged parathyroid glands was 1.4 x 1.0 x 0.8 cm. False positive findings diminished from 9 percent to zero.

In a study of 51 patients undergoing neck operations for primary or secondary hyperparathyroidism Carmalt et al.[36] found a sensitivity of detecting parathyroid tumors by CT scanning of 77 percent in patients with adenomas. Adenomatous glands greater than 8 mm in long axis were correctly localized in 91 percent. However, glands less than 8 mm in long axis were only identified in 25 percent. The sensitivity for detection of hyperplastic parathyroid glands was 33 percent because hyperplastic glands are usually smaller in size than adenomas.

In a study of 100 patients with primary hyperparathyroidism Krubsack et al.[37] compared the sensitivity of CT-scanning for different regions of the neck. For the area contiguous to the right and left lobe of the thyroid the sensitivity of CT-scanning was 71 percent. The sensitivity for the superior mediastinum was however 46 percent. According to Krubsack this reflected the difficulty of differentiating an ectopic parathyroid tumor from blood vessels or slightly enlarged lymph nodes. In this study 38 percent of the parathyroid

glands weighing less than 250 mg were detected by CT-scanning. Parathyroids weighing more than 1000 mg were detected in 81 %.

Miller et al.[38] performed CT-scans of the neck in 53 patients with proved parathyroid adenomas. Only contrast studies were done. The sensitivity with respect to detecting parathyroid adenomas was 47 percent. Thirty-six percent of the adenomas located in the anterior mediastinum were identified by CT. Parathyroid glands smaller than 0.60 cm^3 in volume were not detected by CT.

In 25 patients with persistent or recurrent hyperparathyroidism by Clark et al.[39] 11 of 25 parathyroid tumors were localized by CT-scanning. The identified glands ranged from 0.6 to 4.0 cm in greatest diameter, whereas tumors not identified by CT ranged from 0.6 to 3.5 cm. Three false positive findings were due to enlarged lymph nodes. Metallic clips left behind at the initial operation were troublesome for the interpretation of CT-scans.

Table 2 summarizes the presented studies. Sensitivity rates vary from 33 - 81 %, while the specificity rates are high in most studies. The smallest parathyroid gland which can be detected by CT-scannning measures 6-8 mm in longest axis. The device which hyperextends the neck and depresses the shoulders, as recommended by Stark et al., seems a valuable asset in CT-scanning of the neck.

Table 2. *CT-scanning in parathyroid disease*

	Number of Glands	Adenoma or Hyperplasia	Sensitivity %	Specificity %	Size/Weight Detected Glands	Size/Weight Missed Glands
Sommer[32]	24	Ad + Hyp	71	-	10-50 mm	10-30 mm
Krebs[33]	10	Adenoma	80	96	-	-
Cates[34]	68	Ad + Hyp	81	-	-	-
Stark[35]	57*	Ad + Hyp	51	96	15x10x8 mm#	-
	23[a]	Ad + Hyp	78	100	14x10x8 mm#	-
Carmalt[36]	27	Adenoma	77	98	-	-
	85	Hyperplasia	33	86	-	-
Krubsack[37]	100	Ad + Hyp	68	92	-	-
Miller[38]	51	Adenoma	47	98	-	-
Clark[39]	25	Ad + Hyp	44	-	6-40 mm	6-35 mm

* Conventional method   [a] Special Device   # Average size

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) offers good soft-tissue contrast without the need for intravenous contrast. It can be used to examine the neck and thoracic inlet in transverse, sagittal or coronal planes of section.[40] MRI of the parathyroid glands was described in 1983 by Stark et al.[41] who performed MRI with a whole body coil of the necks of six patients who had parathyroid tumors. Parathyroid glands of normal sizes were not identified. Streak artifacts of the shoulder, causing problems with interpretation of CT scans, were not encountered, and vascular structures were easily identified. Spin-echo techniques readily separated parathyroid tumors from adjacent muscle, fat and thyroid tissue. MRI can distinguish residual scar from thyroid or parathyroid tissue as well, therefore MRI seems a valuable study in patients undergoing parathyroid reoperations. The spatial resolution of MRI was slightly inferior to that of CT-scanning. In the study by Stark et al. only tumors larger than 1.0 cm were detected by MRI.

The accuracy of high-resolution magnetic resonance imaging using local coils was evaluated by Kneeland et al.[42] who studied 22 patients with primary hyperparathyroidism. The sensitivity for finding parathyroid tumors was 74 percent with a specificity of 88 percent. Two adenomas located in the superior mediastinum were identified by MRI, but there were also two false positive finding in this region. Incorrect diagnoses were due to iso-intensity of parathyroid adenomas and thyroid gland or fat. Using different relaxation times (T1 and T2) minimizes confusing these structures. Another problem in interpretating MR images were ghost artifacts caused by respiratory motion.

Spritzer et al.[43] reported the results of MRI with surface coils in 25 patients with primary hyperparathyroidism. MRI allowed correct localization of 14 of 17 adenomas, 2 of 2 carcinomas and 5 of 8 hyperplastic parathyroid glands. MRI identified 9 of 11 abnormal glands in the 0.5 - 1.0 cm range and 12 of 14 lesions greater than 1.0 cm in diameter. The overall sensitivity and specificity were 77.8 percent and 95.4 percent respectively.

In a MRI study by Tscholakoff et al.[44] in 31 patients with primary hyperparthyroidism comparable rates of sensitivity and specificity, 73 and 90 percent respectively, were found. Seven of thirteen parathyroid tumors smaller than 1.0 cm were correctly localized by MRI. Nineteen tumors exceeding 1.0 cm were all but one identified by MRI. Two of three mediastinal glands were found by MRI.

Kier et al.[45] evaluated 24 patients with primary hyperparathyroidism pre-

operatively with MRI using several coils.   Seventeen of 20 adenomas were detected.   Two adenomas were missed because the signals of the thyroid and the parathyroid adenomas were not different.   In the third negative case the surface coil could not be positioned sufficiently since the patient had a short obese neck.   In two patients with hyperplasia only one hyperplastic gland was localized by MRI.   This low yield was attributed by the authors to a technical inferior study in one patient and to the small sizes (117, 123 and 140 mg) of the hyperplastic glands in the other patient.   Four false positive interpretations were caused by co-existent thyroid disease, lymph nodes and ectopic thymus tissue in the lower neck.   The saddle shaped coil provided the best images with sufficient extension to the superior mediastinum.

Table 3 summarizes the results of MRI studies in patients with hyperparathyroidism.   The smallest gland detected by MRI measures 5 mm in longest axis and weighs 100 mg.

Table 3. *Magnetic Resonance Imaging in parathyroid disease*

	Number of Glands	Adenoma or Hyperplasia	Sensitivity %	Specificity %	Size/Weight Detected Glands	Size/Weight Missed Glands
Kneeland[42]	23	Ad + Hyp	74	88	-	-
Spritzer[43]	25	Ad + Hyp	78	95	5-40 mm	5-20 mm
Tscholakoff[44]	34	Ad + Hyp	74	91	5-40 mm	5-20 mm
Kier[45]	20	Adenoma	80	93	100-5000 mg	200-5000 mg
	6	Hyperplasia	17	100	> 2000 mg	117-1300 mg

Thallium - Technetium Scans

The accumulation of Thallium - 201 chloride in a parathyroid adenoma was reported by Fukunaga[46] in 1979.   In a patient with primary hyperparathyroidism a nodule with a diameter of 3 cm revealed on physical examination of the neck.   This nodule was found to be a parathyroid adenoma at operation, weighing 12 g.   The adenoma showed uptake of Thallium-201 pre-operatively.   Thallium-201 appears to behave as an analog of potassium.   Its uptake is inhibited competitively by K^+ and by poisoning the Na-K-ATP'ase dependent membrane pump.[47,48]   Consequently thallium-201 is concentrated by all cells of the body, but preferably by tissues with increased regional blood flow.[49]   Thyroidal tumors, benign or malignant, have been shown to accumulate

an increased amount of Thallium-201. Fukunaga et al. suggested to use combined imaging with Thallium-201 and Technetium-99 or Iodine-123 to localize parathyroid tumors pre-operatively.

Thallium - Technetium subtraction scans of enlarged parathyroid glands are made by injecting Technetium-99 pertechnetate for imaging the thyroidal gland followed by injecting Thallium-201 which accumulates both in thyroid and parathyroid tissue. Computerized subtraction of the technetium images from the thallium images results in a picture showing the parathyroid tumors.

Schoenenberg et al.[50] found 19 true positive scintigrams in 21 patients with adenomas. The mean diameter of the adenomas detected was 1.6 cm. Two adenomas, measuring 3.0 and 2.5 cm in diameter, were missed. Only two of 16 hyperplastic parathyroid glands were discovered by Thallium - Technetium scanning.

In a study by Broughan et al.[51] in 12 of 14 patients, who had had previous neck surgery, the parathyroid pathology was accurately localized by pre-operative scanning. Fourty-two adenomas in 60 patients with adenomas were succesfully localized by subtraction scanning, resulting in a sensitivity of thallium-technetium scans for adenomas of 70 percent. In this study hyperplastic parathyroid glands were present in 12 patients who had a total of 45 enlarged parathyroid glands. Only 16 of the enlarged parathyroids were localized by thallium - technetium scans (36 percent). Four of five intra-thyroidal parathyroid tumors were identified. The rate of false positive findings was 35 percent. Thyroid nodules and motion artifacts were designated as some of the causes.

Clark et al.[39] reported the results of thallium-technetium scanning in 22 patients with persistent or recurrent hyperparathyroidism. Eight studies were positive, eight were negative, and six were false positive. False positive scans were due to enlarged lymph nodes in 3 patients and unknown factors in the other patients.

Carmalt et al.[36] reported thallium-technetium scanning could detect 70 percent of paratyhyroid adenomas greater than 8 mm in long axis. However, adenomas less than 8 mm in long axis were correctly localized in only 25 percent. Forty percent of hyperplastic parathyroid glands greater than 8 mm were detected by thallium-technetium scanning, and 14 percent of hyperplastic parathyroid glands smaller than 8 mm.

Hauty et al.[52] reported an extensive review of the literature on thallium-technetium subtraction scans in 1987. True positive scans were obtained in 247 of 317 patients, i.e. a sensitivity rate of 82 percent. The vast majority of

these patients had adenomas in normal anatomic positions. False negative results were often due to coexisting thyroidal disease. In seven of the reported studies 30 ectopic parathyroid tumors were included. Twenty-five of these 30 ectopic glands were identified by the scintigrams, i.e. a sensitivity of 86 percent. Detection of parathyroid tumors in patients with previous neck explorations was successful in 46 of 66 cases (sensitivity of 70 percent). Localizing hyperplastic parathyroid glands was possible in 77 of 121 hyperplastic parathyroids, accounting for a sensitivity rate of 64 percent. Hyperplastic parathyroid glands weighing less than 300 to 400 mg or with diameters of less than 0.5 cm were not detected by thallium-technetium scans. However, adenomas between 60 and 250 mg were localized. Controversy persists on the issue whether the intensity of uptake of thallium is based on hormonal activity of or blood flow to the parathyroid tumor or is just determined by the size of the parathyroid gland.

Table 4 presents the results of thallium-technetium scans in primary hyperparathyroidism. The sensitivity rates for detecting hyperplastic glands are significantly lower than those for adenomas.

Table 4. *Thallium-Technetium scanning in parathyroid disease*

	Number of Glands	Adenoma or Hyperplasia	Sensitivity %	Specificity %	Size/Weight Detected Glands	Size/Weight Missed Glands
Schoenenberg[50]	21	Adenoma	91	100	-	25-30 mm
	16	Hyperplasia	13	75	-	-
Broughan[51]	60	Adenoma	70	-	-	-
	45	Hyperplasia	36	-	-	-
Clark[39]	22	Ad + Hyp	36	-	8-35 mm	5-35 mm
Carmalt[36]	23	Adenoma	58	98	-	-
	85	Hyperplasia	26	75	-	-
Hauty[52]	317	Ad + Hyp	82	-	-	-
	121	Hyperplasia	64	-	-	< 5 mm

Angiography and venous sampling for parathyroid hormone

Arteriography or venous sampling for parathyroid hormone are used in some institutions as localizing studies of parathyroid tumors, particularly when other localizing studies have failed to find the parathyroid tumor.

Brennan et al.[53] reported in 1981 the results of selective cervical and mediastinal arteriography and selective venous sampling for parathyroid

hormone in patients undergoing reoperation following failed first operations. Selective arteriography localized 49 percent of the parathyroid tumors correctly. Selective venous sampling for parathyroid hormone, which was considered positive if a sample showed a two-fold concentration over average peripheral levels, localized 55 percent of parathyroid tumors correctly. Cerebrovascular or renal complications did not occur in this series. However, in an analysis by Edis et al.[54] of 51 patients, undergoing reoperations, 3 patients experienced significant neurologic complications as the result of angiography.

Miller et al.[55] compared prospectively superselective arterial digital subtraction angiography (DSA) and superselective conventional angiography (CA) in a series of 26 patients with persistent or recurrent hyperparathyroidism, who had undergone negative non-invasive localization studies. Superselective studies included catheterization of both internal thoracic (mammarian) arteries, both inferior thyroid arteries and both common carotid arteries. DSA localized successfully 16 of 26 adenomas (62 percent). Ten of 11 adenomas located in the anterior mediastinum were identified. Conventional angiography showed 10 of 26 adenomas. The greater sensitivity of DSA compared to CA was attributed to increased contrast resolution of DSA-technique. No false positive findings were documented. Complications did not occur.

Edis et al.[54] evaluated the benefits of arteriography and venous sampling for parathyroid hormone in 51 patients undergoing reoperations for persistent or recurrent hyperparathyroidism. Eleven of 20 arteriograms detected the parathyroid tumor correctly. There were three false positive findings with arteriography. The results of venous sampling were no better than the toss of a coin. Correct lateralization was obtained in 4 of 9 venous sampling studies. In the other five patients venous sampling falsely lateralized the tumor to the opposite side of the neck.

Combination of localization studies

Krubsack et al.[37] reported a prospective comparison of Thallium-Technetium scanning (TTS), CT-scanning, ultrasound (US) and magnetic resonance imaging (MRI) in 100 patients with hyperparathyroidism who subsequently had successful parathyroidectomies. Eighty-nine patients had adenomas, and 11 patients had hyperplastic parathyroid glands. TTS had the highest overall sensitivity (73 percent) compared to CT (68 percent), US (55 percent) and MRI (57 percent). Regarding the region of the thymic tongues

and the upper mediastinum, TTS was the most sensitive (90 percent) whereas CT, US and MRI had low sensitivities (46, 44 and 50 percent respectively). For parathyroid tumors weighing less than 250 mg none of the imaging studies could detect more than 50 percent of the tumors. The combination of TTS and CT, or TTS and US was significantly more sensitive than any single imaging technique. The sensitivity of TTS and CT was 90 percent, of TTS and US 85 percent with specificities of 86 and 89 percent respectively. Sensitivity was not significantly improved by combining three or four imaging techniques. In seven patients with hyperplasia, who had no prior neck surgery, imaging studies did not suggest multiple gland disease in any of them.

Conclusions

The rates of detecting pathological parathyroid glands by pre-operative localization studies vary from 13 to 91 percent. Experience of the investigator and application of special imaging techniques are crucially to identify the enlarged parathyroid glands prior to operation. Detection of small parathyroid glands by localization studies remains very difficult. The smallest parathyroid which can be localized measures approximately 5 mm and weighs 100 mg. Therefore, hyperplastic parathyroid glands, which are often small in size, are not identified easily by imaging studies. These small hyperplastic glands can be difficult to find at operation and can lead to persistent hyperparathyroidism.

Since the experienced surgeons achieve cure in more than 90 percent of patients with primary hyperparathyroidism having first operations, pre-operative localization studies are not indicated in these patients. When an unilateral approach is favored, imaging studies are indispensable to localize the side of the parathyroid tumor. In patients with persistent or recurrent hyperparathyroidism imaging studies of parathyroid glands should be recommended.

The non-invasive procedures, i.e. ultrasonography, CT-scanning, thallium-technetium subtraction scanning and magnetic resonance imaging should first be performed. The choice of any single localization technique should depend on the experience available with one of the techniques. Combining two imaging studies increases the rate of detection of abnormal parathyroid glands. In patients who are suspected to have a mediastinal parathyroid tumor, which has not been localized by non-invasive procedures, superselective arterial digital subtraction angiography can be considered to identify the parathyroid tumor.

References

1. Bruining HA, Ausema L, Lameris JS and Birkenhäger J C. Non-invasive parathyroid imaging: a warning. Neth J Med, 29, 153, 1986.
2. Tibblin S, Bondeson AG and Ljungberg O. Unilateral parathyroidectomy in hyperparathyroidism due to single adenoma. Ann Surg, 195, 245, 1982.
3. Bruining HA, Van Houten H, Juttmann JR, Lamberts SWJ and Birkenhäger JC. World J Surg, 5, 85, 1981.
4. Patow CA, Norton JA and Brennan MF. Vocal cord paralysis and reoperative parathyroidectomy, a prospective study. Ann Surg, 203, 282, 1986.
5. Stock JL, Krudy AG, Doppman JL, Jones AE, Brennan MF, Attie MF, Downs RW, Levine MA, Marx SJ, Spiegel AM and Aurbach GD. Parathyroid imaging after intraarterial injections of [^{75}Se] Selenomethionine. J Clin Endocrinol Metab, 52, 835, 1981.
6. Garrow JS and Smith R. The detection of parathyroid tumors by selenomethionine scanning. Br J Radiol, 41, 307, 1968.
7. Mailliard JA. Localization of a parathyroid by ^{131}I scintiscan. JAMA, 204, 177, 1968.
8. Samuels BI, Dowdy AH and Lecky JW. Parathyroid thermography. Radiology, 104, 575, 1975.
9. Samuels BI. The present status of parathyroid thermography. JAMA, 233, 907,1975.
10. Sofianides T, Chang YS, Leary JS and Nichols FX. Localization of parathyroid adenomas by cervical esophagogram. J Clin Endocrinol Metab, 46, 587, 1978.
11. Wang CA. Parathyroid reexploration: a clinical and pathological study of 112 cases, Ann Surg, 186, 140, 1977.
12. Reiss E and Canterbury JM. Primary hyperparathyroidism: Application of radioimmunoassay to differentiation of adenoma and hyperplasia and to preoperative localization of hyperfunctioning parathyroid glands. N Engl J Med, 281, 348, 1969.
13. Dudley NE. Methylene blue for rapid identification of the parathyroids, Br Med J, 3, 680, 1971.
14. Kato T, Hattori T, Miura K, Sato M. Application of thyroid lymphography to preoperative localization of hyperfunctioning parathyroid adenomas. Ann Surg, 179, 378, 1974.
15. Clark OH. Parathyroid localization. Medical Times, 110, 95, 1982.
16. Duffy P, Picker RH, Duffield S, Reeve T and Hewlett S. Parathyroid sonography; a useful aid to preoperative localization. J Clin Ultrasound, 8, 113, 1980.
17. Reading CC, Charboneau JW, James EM, Karsell PR, Purnell DC, Grant CS and Van Heerden JA. High resolution parathyroid sonography. Am J Radiol, 139, 539, 1982.
18. Stark DD, Gooding GAW, Moss AA, Clark OH and Ovenfors CO. Parathyroid imaging: Comparison of high-resolution CT and high-resolution sonography. Am J Radiol, 141, 633, 1983.
19. Graif M, Itzchak Y, Strauss S, Dolev E, Mohr R and Wolfstein I. Parathyroid

sonography: diagnostic accuracy related to shape, location and texture of the gland, B r J Radiol, 60, 439, 1987.

20. Randel SB, Gooding GAW, Clark OH, Stein RM and Winkler B. Parathyroid variants: US evaluation. Radiology, 165, 191, 1987.

21. Stein BL and Wexler MJ. Preoperative parathyroid localization: a prospective evaluation of ultrasonography and thallium-technetium scintigraphy in hyperparathyroidism. Can J Surg, 33, 175, 1990.

22. Lloyd MNH, Lees WR and Milroy EJG. Pre-operative localisation in primary hyperparathyroidism. Clin Radiol. 41, 239, 1990.

23. Kern KA, Shawker TH, Doppman JL, Miller DL, Marx SJ, Spiegel AM, Aurbach GD and Norton JA. The use of high-resolution ultrasound to locate parathyroid tumors during reoperations for primary hyperparathyroidism. World J Surg, 11, 579, 1987.

24. Norton JA, Shawler TH, Jones BL, Spiegel AM, Marx SJ, Fitzpatrick L, Aurbach GD and Doppman JL. Intraoperative ultrasound and reoperative parathyroid surgery: an initial evaluation. World J Surg, 10, 631, 1986.

25. Butch RH, Simeone JF and Mueller PR. Thyroid and parathyroid ultrasonography, Radiol Clin North Am, 23, 57, 1985.

26. Karstrup S and Hegedüs L. Concomitant thyroid disease in hyperparathyroidism. Eur J Radiol, 6, 149, 1986.

27. Rastad J, Lindgren PG, Ljunghall S. Ultrasound scanning for preoperative localisation of parathyroid tumors. Acta Chir Scand, 150, 199, 1984.

28. Karstrup S, Glenthoj A, Torp-Pedersen S, Hegedüs L and Holm HH. Ultrasonically guided fine needle aspiration of suggested enlarged parathyroid glands. Acta Radiol, 29, 213, 1988.

29. Verbanck J, Clarysse J, Loncke R, Segaert M, Van Aelst F and Theunynck P. Parathyroid aspiration biopsy under ultrasonographic guidance. Arch Otolaryngol Head Neck Surg, 112, 1069, 1986.

30. Kahaly G, Krause U, Dienes HP, Schrezenmeir J and Beyer J. Fine-needle biopsy of parathyroid adenomas. Klin Wochenschr, 64, 1176, 1986.

31. Karstrup S, Glenthoj A, Hainau B, Hegedüs L, Torp-Pedersen S and Holm HH. Ultrasound-guided, histological, fine-needle biopsy from suspect parathyroid tumors: success-rate and reliability of histological diagnosis. Br J Radiol, 62, 981, 1989.

32. Sommer B, Welter HF, Spelsberg F, Schere U and Lissner J. Computed tomography for localizing enlarged parathyroid glands in primary hyperparathyroidism. J Comput Assist Tomogr, 6, 521, 1982.

33. Krebs III FJ, Archer CR, Awwad EE and Friedman WH. Preoperative computerized tomographic imaging in hyperparathyroidism. Otolaryngol Head Neck Surg, 97, 351, 1987.

34. Cates JD, Thorsen MK, Lawson TL, Middleton WD, Foley WD, Wilson SD and Krubsack AJ. CT evaluation of parathyroid adenomas: diagnostic criteria and pitfalls. J Comput Assist Tomogr, 12, 626, 1988.

35. Stark DD, Moss AA, Gooding GAW and Clark OH. Parathyroid scanning by computed tomography. Radiology, 148, 297, 1983.

36. Carmalt HL, Gillett DJ, Chu J, Evans RA and Kos S. Prospective comparison of

34

radionuclide, ultrasound, and computed tomography in the reoperative localization of parathyroid glands. World J Surg, 12, 830, 1988.

37. Krubsack AJ, Wilson SD, Lawson TL, Kneeland JB, Thorsen MK, Collier BD,Hellman RS and Isitman AT. Prospective comparison of radionuclide, computed tomographic, sonographic, and magnetic resonance localization of parathyroid tumors. Surgery, 106, 639, 1989.

38. Miller DL, Doppman JL, Shawker TH, Krudy AG, Norton JA, Vucich JJ, Morrish KA, Marx SJ, Spiegel AM and Aurbach GD. Localization of parathyroid adenomas in patients who have undergone surgery. Radiology, 162, 133, 1987.

39. Clark OH, Okerlund MD, Moss AA, Stark D, Norman D, Newton TH, Duh QY, Arnaud CD, Harris S and Gooding GAW. Localization studies in patients with persistent or recurrent hyperparathyroidism. Surgery, 98, 1083, 1985.

40. Stark DD, Clark OH and Moss AA. Magnetic resonance imaging of the thyroid, thymus, and parathyroid glands. Surgery, 96, 1083, 1984.

41. Stark DD, Moss AA, Gamsu G, Clark OH, Gooding GAW and Webb WR. Magnetic resonance imaging of the neck. Radiology, 150, 455, 1984.

42. Kneeland JB, Krubsack AJ, Lawson TL, Wilson SD, Collier BD, Froncisz W, Jesmanowicz A and Hyde JS. Enlarged parathyroid glands: high-resolution local coil MR imaging. Radiology, 162, 143, 1987.

43. Spritzer CE, Gefter WB, Hamilton R, Greenberg BM, Axel L and Kressel HY. Abnormal parathyroid glands: high-resolution MR imaging. Radiology, 162, 487, 1987.

44. Tscholakoff D, Roka R, Hajek PC, Niederle B, Gritzmann N, Neuhold A, Pirich K and Imhof H. Magnet-Resonantztomographie (MRT) als lokalisationsmethode bei Hyperparathyreoidismus. Wien Klin Wochenschr, 11, 357, 1988.

45. Kier R, Blinder RA, Herfkens RJ, Leight GS, Spritzer CE and Caroll BA. MR imaging with surface coils in primary hyperparathyroidism. J Comput Assist Tomogr, 11, 863, 1987.

46. Fukunaga M, Morita R, Yonekira Y, Dokoh S, Yamamoto I, Fujita T, Torizuka K and Kawamura J. Accumulation of ^{201}Tl-chloride in a parathyroid adenoma. Clin Nucl Med, 4, 229, 1979.

47. Mullins LJ and Moore R D. The movement of thallium ions in muscle. J Gen Physiol, 43, 759, 1960.

48. Gehring PJ and Hammond PB. The interrelationship between thallium and potassium in animals. J Pharmacol Exp Ther, 155, 187, 1967.

49. Fine EJ. Parathyroid imaging: its current status and future role. Semin Nucl Med, 4, 350, 1987.

50. Schoenenberger A, Rösler H and Zinger E. J. Preoperative localization of parathyroid tumors by ^{201}Tl/^{99m}Tc subtraction scanning. Eur Urol, 11, 17, 1985.

51. Broughan TA, O'Donnell JK, Kropilak MD and Esselstyn CB. Use of thallium-technetium parathyroid scans. Cleve Clin J Med, 54, 179, 1987.

52. Hauty M, Swartz K, McClung M and Lowe DK. Technetium-thallium scintiscanning for localization of parathyroid adenomas and hyperplasia. Am J Surg, 153, 479, 1987.

53. Brennan MF, Doppman JL, Kurdy AG, Marx SJ, Spiegel AM and Aurbach GD.

Assessment of techniques for preoperative parathyroid gland localization in patients undergoing reoperation for hyperparathyroidism. Surgery, 91, 6, 1981.

54. Edis AJ, Sheedy II PF, Beahrs OH and Van Heerden JA. Results of reoperation for hyperparathyroidism, with evaluation of preoperative localization studies. Surgery, 84, 384, 1978.

55. Miller DL, Chang RC, Doppman JL and Norton JA. Localization of parathyroid adenomas: superselective arterial DSA versus superselective conventional angiography, Radiology, 170, 1003, 1989.

SURGICAL APPROACH AND CLINICAL FOLLOW-UP

IN 693 PATIENTS WITH

PRIMARY HYPERPARATHYROIDISM

Introduction

The optimal surgical treatment of primary hyperparathyroidism is controversial. Bilateral and unilateral approaches have been advocated, and the extent of the resection of parathyroid glands in primary hyperplasia varies. Some endocrine surgeons leave 30-50 mg of parathyroid tissue in situ, while others remove only the enlarged glands. Disagreement persists about the necessity of taking biopsies of all the parathyroid glands. There is confusion in the histological distinction between adenomas and hyperplastic parathyroid glands. Attempts have been made to define these two entities by light microscopy, DNA analysis and electron microscopy, none of which has succeeded.[1-4] Nevertheless, some surgeons rely on the microscopic features of the parathyroid glands to determine the extent of parathyroidectomies. Others use the sizes of the parathyroid glands to differentiate adenomas from hyperplasias.[5] However, the variation in weights of normal parathyroid glands is great and the upper limit of the normal weight is debatable.[6]

It is, therefore, not surprising that a review of the literature concerning the surgical treatment of primary hyperparathyroidism is contradictory. The interpretation of the operative procedures reported in different studies is made more difficult because of the lack of long follow-up studies.

This is an analysis of the results of the operative treatment of a large group of patients with primary hyperparathyroidism who have been treated at two hospitals, operated upon by two surgeons using the same protocol, and followed for a long period of time.

Materials and methods

Six hundred and ninety three patients with primary hyperparathyroidism had their first operations at the University Hospital Dijkzigt in Rotterdam or at the University Hospital in Leiden from 1952 to 1988. Patients with the syndromes of multiple endocrine neoplasias or familial hyperparathyroidism were excluded since in patients with these hereditary disorders the incidence of multiple enlarged parathyroid glands and recurrent hyperparathyroidism is significantly higher than in patients with non-familial hyperparathyroidism.[7]

The charts of all patients were reviewed. Information was available on the diagnostic procedures, the parathyroidectomies, the pathologic examinations of the parathyroid glands, and the post-operative courses.

Two surgeons operated on the majority of the patients. Localization studies were not performed preoperatively. Bilateral approaches through collar incisions of the skin were used. An attempt was made to identify all parathyroid glands. The glands were carefully dissected free to estimate the sizes of the parathyroid glands and to localize the vascular stalks of the parathyroid glands. Small biopsies were taken from all identified parathyroid glands, as far away from the vascular stalks as possible. All biopsies were examined by frozen sections. The identification of parathyroid tissue was the only information required from the pathologist. The interpretations of the microscopic slides of the parathyroid glands, which varied from adenomas, focal hyperplasias, nodular hyperplasias to chief cell hyperplasias, did not determine the extent of the resection of parathyroid tissue. After exposing all parathyroid glands, the glands estimated to weigh more than 40 mg were removed. In cases of enlargement of four glands, remnants estimated to be 40 mg of parathyroid tissue were left in situ. The parathyroid glands which were left behind were marked with clips. The weights of the removed parathyroid glands were recorded. Attempts were made to inspect the thymus in all patients to locate supernumerary or aberrant glands.

The follow-up of patients was carried out with the aid of the National Population Register, which registers the residence of all inhabitants of the Netherlands. The follow-up studies were performed either in the surgical clinics of the University Hospital Rotterdam or by family physicians. The follow-up studies consisted of recording the medications with emphasis on calcium or vitamin D supplementations and two blood samples which were drawn at weekly intervals. Serum levels of calcium, albumin, creatinine and urea were determined. A serum parathyroid hormone (PTH) assay (IRMA for the intact hormone, INCSTAR, Stillwater, Minnesota) was performed when the mean calcium value, corrected for serum albumin, exceeded 2.45 mmol/l (N 2.20 - 2.65 mmol/L) to detect normocalcemic hyperparathyroidism.

During the follow-up studies the quality of the voice was evaluated in all patients. Laryngoscopic examinations were not done.

In this study persistent hyperparathyroidism is defined as post-operative hypercalcemia caused by an enlarged parathyroid gland, which was not identified at the first attempt at a parathyroidectomy. Recurrent hyperparathyroidism is defined as hypercalcemia due to an enlarged gland which was observed to be of normal size during the first operation. Normocalcemic intervals are not parts of these definitions.[8]

Results

Single Gland Disease

Single gland disease is defined as the finding of a single enlarged parathyroid gland with a mass greater than 40 mg and at least one normal parathyroid gland at exploration of the neck. With this definition single gland disease was found in 514 patients.

Because follow-up studies of all 514 patients would be very difficult, a smaller number of patients, which would be statistically meaningful, was selected. The procedure for selection assumed a rate of recurrent hyperparathyroidism, calculated from a review of the literature of 2.5 %.[9] Statistical calculations showed that follow-up studies of 156 patients should be sufficient to detect a reasonable accurate rate of recurrent hyperparathyroidism in 514 patients.[10]

Geographic considerations were important factors in selecting the patients. One hundred sixty patients were selected for follow-up studies. All patients, who were examined in the departments of surgery or internal medicine of the University Hospital Rotterdam because of hypercalcemia after parathyroidectomies, were included in the group.

The mean follow-up was 12.8 years (4 - 35 yrs). The average age at the time of the parathyroidectomies was 47.9 years (12 - 73 yrs). Two thirds of the patients were females.

Four glands were identified in 98 patients. A fifth gland was found in 2 cases, one at the junction of the inferior thyroid artery with the recurrent laryngeal nerve and the other within the thymus.

Table 1. *Number of parathyroid glands identified in 160 patients with single gland disease*

Number of glands	Number of patients	Percentage
5	2	1.3
4	98	61.2
3	38	23.7
2	18	11.3
1	4	2.5

The mean weight of the single enlarged glands was 1556 mg (115 - 16 767 mg).

Temporary hypocalcemia (< 2.20 mmol/L) occurred in 43 patients (22%). Six of these patients had elevated alkaline phosphatases (> 120 U/L), so "bone hunger" was not a major cause of the hypocalcemia. Supplementation of calcium was withdrawn in 17 patients before discharge from the hospital.

Only two patients had permanent hypoparathyroidism after their parathyroidectomies (1.8 %).

In one patient an enlarged parathyroid, weighing 12 000 mg, was removed. Two parathyroid glands of normal sizes were found, and biopsies were taken from these glands. The left upper parathyroid gland was not found.

In the other patient an enlarged parathyroid, weighing 1600 mg, was removed. The upper parathyroid glands were of normal sizes. Biopsies were taken from these glands. The left lower parathyroid gland was not found.

The levels of the serum calcium at the time of the follow-up studies are listed in Table 2. One hundred and fourty two patients had serum calcium values between 2.20 and 2.45 mmol/l, and fourteen, between 2.45 and 2.65 mmol/L (N 2.20 - 2.65 mmol/L). PTH assays were performed in 9 patients with serum calcium levels between 2.45 and 2.65 mmol/L. All were normal.

Table 2. *Serum calcium values corrected for serum albumin at follow-up studies in 160 patients with single gland disease*

Calcium (mmol/L)	Number of patients
<2.20	1
2.20-2.45	142
2.45-2.65	14
>2.65	3

Three patients with single gland disease remained hypercalcemic after the initial parathyroidectomies (1.9 %).

In one patient the left upper parathyroid gland, weighing 70mg, was removed. The right upper parathyroid gland and the left lower gland were of normal sizes. The right lower parathyroid gland was not found. The serum calcium was 2.81 mmol/L after the first exploration of the neck. After 7 months a second operation was done. On the right side, dorsal to the trachea an enlarged parathyroid gland, weighing 400 mg, was found and removed. Post-operatively the serum calcium was normal.

In the second patient the left lower parathyroid gland, weighing 470 mg, was removed at the first operation. The left upper and the right lower glands were of normal sizes. The right

upper parathyroid gland was not identified. Post-operatively, the serum calcium value was 3.10 mmol/L. A second operation was done after 1 week. On the left side, dorsal to the esophagus, an enlarged parathyroid gland, weighing 900 mg, was found. After the removal of this gland, the patient was normocalcemic.

In the third patient, two parathyroid glands were identified at the parathyroidectomy. One gland weighed 1500 mg and was removed. The other gland was normal in size and was left in situ. The upper parathyroid glands could not be found. Five years after the neck exploration the serum calcium was mildly elevated to 2.70 mmol/L. Because the patient was asymptomatic a second operation was not considered. Twenty two years after the parathyroidectomy the serum calcium value was 2.67 mmol/L. Renal function was normal and the patient was asymptomatic.

No instance of recurrent hyperparathyroidism was recorded in patients with single gland disease.

Vocal impairment was not observed in any of the patients with single gland disease.

Multiple Gland Disease

Multiple gland disease was defined as the enlargement of more than one parathyroid gland, each with a mass greater than 40 mg. Histological criteria were not used to define multiple gland disease.

Follow-up information was available on 114 of 179 patients with multiple gland disease. Forty seven patients had died, and the residence of 18 patients was unknown. The mean follow-up was 14.1 years (1 - 29 yrs). The average age at the time of the operations was 48.7 years (15 - 76 yrs). The distribution of females to males was 2:1.

Four parathyroid glands were found in 145 patients (Table 3). In 7 patients a fifth gland was identified. The locations of the glands were as follows: dorsal to the trachea (1), cephalad to the junction of the inferior thyroid artery and the recurrent laryngeal nerve (1), the anterior mediastinum (2) and the thymus (3).

Table 3. *Number of parathyroid glands identified in 179 patients with multiple gland disease*

Number of glands	Number of patients	Percentage
5	7	4.1
4	145	81.1
3	22	12.3
2	5	2.5

Only eighteen patients had 4 enlarged glands (Table 4). One hundred and twenty patients had two enlarged glands. Seventy two patients with 2 enlarged parathyroid glands had an enlarged gland on the left side, and the other, on the right. In forty eight patients, the two enlarged parathyroid glands were found on the same side. The mean weight of the enlarged parathyroid glands in multiple gland disease was 949 mg (45 - 18 000 mg).

Table 4. *Number of enlarged parathyroid glands in 179 patients with multiple gland disease*

Number of enlarged glands	Number of patients	Percentage
5	1	0.8
4	18	9.8
3	40	22.1
2	120	67.3

Temporary hypoparathyroidism required calcium supplementation in 29 patients (25.4%). Four patients had preoperatively elevated serum alkaline phosphatases. Nineteen patients had to continue calcium supplementation for several weeks after discharge from the hospital.

Permanent hypoparathyroidism was observed in 5 patients (4.4%).

In one patient a second exploration of the neck was done because hypercalcemia persisted after the removal of two enlarged upper parathyroid glands. The lower parathyroid glands were not found at the first parathyroidectomy. At the second operation the lower parathyroid glands were identified. One gland weighed 750 mg and was removed. The other was very small but was removed for histological examination.

In the second patient four enlarged parathyroid glands were found at the first operation. Three enlarged glands were removed, and one parathyroid gland, estimated to weigh 160 mg, was left in situ. Persistent hypercalcemia necessitated a second exploration of the neck. Parathyroid tissue was not found at the second operation, but permanent hypocalcemia was the result.

The third patient had four enlarged parathyroid glands. At the initial operation three enlarged glands were removed. The left lower parathyroid gland, estimated to weigh 120 mg, was left in situ. Post-operatively the serum calcium remained elevated. At the second operation, which included a sternotomy, parathyroid tissue was not identified. Because of persistent hypercalcemia a third attempt at parathyroidectomy, which included a thoracotomy, was done, but no parathyroid tissue was found. Eventually a left hemi-thyroidectomy was done and pathological examination showed parathyroid tissue in the the thyroid gland.

In the fourth patient four parathyroid glands were identified at the first operation. The upper parathyroid glands were enlarged and were removed. Their weights were 200 and

400 mg. A second operation was done because of persistent hypercalcemia. At the second operation the right lower lobe of the thyroid gland was removed since the right lower parathyroid gland was not found. Histological examination showed intrathyroidal parathyroid tissue. The left lower parathyroid gland was identified. This parathyroid gland was determined to be minimally enlarged and was removed. The weight was 100 mg.

In the fifth patient three enlarged parathyroid glands were found at exploration of the neck. The upper and the left lower parathyroid glands were removed. The right lower parathyroid gland of normal size was left in situ. A biopsy was taken from this gland.

The serum calcium values of the follow-up studies are shown in Table 5. Hypocalcemia was not recorded in any of the patients because the 5 patients with permanent hypoparathyroidism had adequate supplementation of calcium.

Table 5. *Serum calcium values at follow-up studies in 114 patients with multiple gland disease*

Calcium (mmol/L)	Number of patients
<2.20	0
2.20-2.45	89
2.45-2.65	15
>2.65	10

Eighty nine patients had serum calcium values between 2.20 and 2.45 mmol/L, and fifteen, between 2.45 and 2.65 mmol/L (N 2.20 - 2.65 mmol/L).

Serum parathyroid hormone assays were done in 12 patients with calcium values ranging from 2.45 to 2.65 mmol/L. In two patients elevated PTH values were observed, 69.3 and 113.4 pmol/L respectively (N < 55 pmol/L). These patients probably had normocalcemic hyperparathyroidism. Second operations were not considered because they were asymptomatic. Assays of serum calcium at yearly intervals were advised.

Hypercalcemia after the first parathyroidectomies was recorded in 10 patients with multiple enlarged parathyroid glands. Eight of the patients were documented to have persistent hyperparathyroidism (7.0%).

In one patient two enlarged upper parathyroid glands were removed at the first operation. The lower glands were not identified. After 7 years the serum calcium was 3.10 mmol/L. At the second operation the lower parathyroid glands were found. The right lower gland was very small and was removed for histological examination. The left lower parathyroid gland weighed 750 mg and was removed. Permanent hypoparathyroidism followed. This case is classified as persistent hyperparathyroidism because the enlarged left lower parathyroid gland found at the second operation was not identified at the first one.

In the second patient three enlarged parathyroid glands were found at the initial operation.

The enlarged upper parathyroid glands were removed. The left lower gland with an estimated weight of 80 mg was left in situ. Hypercalcemia persisted. A second exploration of the neck was done 6 months later. The right lower parathyroid gland, weighing 400 mg, was found anterior to the trachea. This gland was removed and the patient became normocalcemic. Twenty five years after the second operation the serum calcium value was 2.35 mmol/L.

The third patient had two enlarged lower parathyroid glands, weighing 3 000 and 4 000 mg, removed at the initial parathyroidectomy. The post-operative serum level of calcium was 2.60 mmol/L. Symptoms of renal calculi persisted. Sporadic mild hypercalcemia was recorded. Two years after the first operation a second attempt at a parathyroidectomy was made. In the stalks of the left and the right thymus, two enlarged parathyroid glands, weighing 300 and 750 mg, were identified. One remnant of 100 mg of parathyroid tissue was left in situ. Sixteen years after the second parathyroidectomies the level of serum calcium was 2.41 mmol/L.

At the initial exploration of the neck in the fourth patient four enlarged parathyroid glands were found. Approximately 250 mg of the right upper parathyroid gland was left in situ. The other parathyroid glands were removed. Hypercalcemia persisted. After six months a second operation, including a sternotomy, was done. A fifth enlarged parathyroid gland was found dorsal to the esophagus. In spite of the removal of this fifth gland mild hypercalcemia persisted (2.70 mmol/L). Oral phosphate treatment was started. Follow-up data were not available.

In the fifth patient 4 enlarged parathyroid glands were identified at the first operation. Three parathyroid glands were removed, and the right lower gland with an estimated weight of 160 mg was left in situ. Persistent hypercalcemia necessitated a second exploration of the neck. Parathyroid tissue was not found, in spite of a sternotomy. However, hypocalcemia developed and sixteen years after the negative exploration of the neck supplementation of calcium and vitamin D was being continued.

In the sixth patient four enlarged parathyroid glands were found at the first operation. The left lower gland, estimated to weigh 120 mg, was left in situ. Hypercalcemia persisted. At the second operation, including a sternotomy, parathyroid glands were not found. During the third attempt at a parathyroidectomy a thoracotomy was done and again parathyroid tissue was not found. Eventually a left hemi-thyroidectomy was done. Histological examination showed parathyroid tissue. Permanent hypoparathyroidism followed.

The seventh patient had two enlarged upper parathyroid glands at the primary operation. These glands were removed. The sizes of the lower glands were estimated to be normal. Hypercalcemia persisted. At the second operation the right lower parathyroid gland was not found. The right lower lobe of the thyroid gland was removed. Pathological examination revealed parathyroid tissue. The left lower parathyroid gland was enlarged and was removed. The weight was 100 mg. The size was probably underestimated at the first operation. Permanent hypoparathyroidism ensued.

The last patient with persistent hyperparathyroidism had enlarged right upper and left lower parathyroid glands at the first operation. The sizes of the other two parathyroid glands were normal. Hypercalcemia persisted after the first operation. At the second operation a sternotomy was done. In the left thymus an enlarged parathyroid gland, weighing 500 mg, was found and removed. The patient became normocalcemic.

Two patients with multiple enlarged parathyroid glands had recurrent

46

hyperparathyroidism (1.8 %).

In one patient two enlarged upper parathyroid glands, weighing 500 and 200 mg, were removed at the first operation. The lower parathyroid glands were of normal sizes.
Post-operative serum calcium levels were normal. Eleven years after the first operation the serum calcium was elevated (2.82 mmol/L). At the second operation the left lower parathyroid gland was enlarged and was removed. The right lower parathyroid gland could not be identified. Normocalcemia ensued.

In the second patient with recurrent hyperparathyroidism 4 parathyroid glands were identified at the first operation. Three glands were enlarged, weighing 250, 500 and 600 mg. The right lower parathyroid gland was normal. Twelve years after the parathyroidectomy the serum calcium was elevated to 3.60 mmol/L. A second operation was not done because of the poor physical condition of the patient. Oral phosphate was given. Nineteen years after the parathyroidectomies the patient died of unknown cause. At autopsy an enlarged right lower parathyroid gland was found.

Vocal impairment was not observed in any of the patients with multiple gland disease.

Discussion

The incidences of parathyroid adenomas (single gland disease) and primary parathyroid hyperplasias (multiple gland disease) depend on the parameters which are used to classify parathyroid glands.

The weights of parathyroid glands are frequently used to differentiate normal from abnormal glands. Normal parathyroid glands are flat. A spherical shape is more frequently observed in enlarged parathyroid glands. However, experience is required to properly estimate the weight of a parathyroid gland. Color and vascularity of the parathyroid glands do not help in differentiating enlarged parathyroid glands from glands of normal sizes. The weights of normal parathyroid glands vary.[11-13] Age, the constitution of the body and chronic illnesses can affect the weights considerably. Dufour and Wilkerson showed that the distribution of the total weights of normal parathyroid glands is significantly skewed to higher values. The 95% interval ranged from 8.2 to 75.0 mg. An upper normal limit of 75 mg has, therefore, been proposed.[6] In our study we have used 40 mg as the upper normal limit based on the upper normal limit of parenchymal weight of parathyroid glands.[12] The parenchymal weight is a better parameter for hormonal activity of parathyroid glands since the fat content varies widely.

The incidence of multiple gland disease in this study is 23.5 %. The sole

criterion used was the presence of two or more enlarged glands, each weighing more than 40 mg. Table 6 shows how the number of patients with multiple enlarged glands changes when different upper limits of normal for weight are used. Edis et al. reported that the incidence of hyperplasia is overestimated by 30 % when a limit of 40 mg, instead of 70 mg is used.[14]   In this study the incidence of hyperplasia would decrease by 3.2% when a limit of 70 mg, instead of 40 mg, is used.

Table 6. *Incidence of hyperplasia using different upper limits of normal parathyroid weight**

Upper limit (mg)	Number of patients	Rate of hyperplasia (%)
40	163	23.5
50	156	22.5
60	146	22.1
70	141	20.3
80	123	17.7
90	113	16.3

*In 16 patients weights were not recorded

Many authors rely on the combination of gross and microscopic features to distinguish adenomas from hyperplastic glands.[15-17] Incidences of hyperplasia ranging from 18 to 58 % have been reported. Cellularity is frequently cited as an important feature of hyperactivity. However, an objective definition of cellularity has never been presented.

Some authors depend entirely on histology to distinguish adenomas from hyperplastic glands.[18,19]   An incidence of hyperplasia of 45 % has been reported. In our opinion, it is inappropriate to use histologic criteria to differentiate adenomas from hyperplastic glands becuase a study on the histology of parathyroid glands in 236 patients with primary hyperparathyroidism has not showed significant different morphologic features in adenomas and primary hyperplasias (vide chapter 5). Therefore, we prefer the concept of single and multiple gland disease which uses the estimated weight of parathyroid glands as the sole criterion to classify parathyroid glands.

The bilateral approach to the neck is most frequently used in parathyroid surgery.[5,20-22] An attempt is made to identify all parathyroid glands during the exploration of both sides of the neck. Proponents of the unilateral approach note that the bilateral approach is associated with significantly longer operative

time and higher incidences of transient and permanent hypoparathyroidism and injury to the recurrent laryngeal nerve.[23,24] Tibblin et al. recorded that the average length of unilateral procedures was 22 minutes shorter when compared to bilateral procedures. In a comparative study by Tibblin et al. the occurrence of post-operative hypocalcemia was studied.[23] After the second post-operative day the serum calcium returned to normal levels in the unilateral group, while in the patients who had bilateral operations with biopsies from normal-sized glands hypocalcemia persisted for several days. Approximately one third of the patients with bilateral operations required temporary supplementation of calcium. At follow-up two of fifty patients with bilateral operations needed vitamin D supplementations.

The occurrence of transient hypocalcemia after bilateral operations is most likely predominantly determined by the taking of biopsies from normal-sized glands. In several studies, rates of transient hypocalcemia ranging from 22 to 48 percent have been reported when biopsies were taken from all parathyroid glands.[14,25] When biopsies were taken only occasionally, the rate of transient hypocalcemia was significantly lower. Transient hypocalcemia was recorded in our study in 22 % of patients with single gland disease and in 25.4 % in multiple gland disease. To prevent transient and permanent hypoparathyroidism biopsies are now taken solely to ascertain the nature of the tissue when gross examination is uncertain.

The proponents of the unilateral approach adhere to the concept that primary hyperparathyroidism is caused by enlargement of a single parathyroid gland (adenoma) or by enlargement of all glands (hyperplasia). The unilateral approach is only applicable to single gland disease. When one enlarged and one normal gland are identified on the same side of the neck, single gland disease is believed to be present and the operation is terminated. To differentiate between normal and abnormal glands Wang proposed the density test which measures the difference in total fat content of two parathyroid glands.[24] Low contents of intercellular fat would indicate hormonal hyperfunction. However, detailed studies of normal parathyroid glands have shown wide variations in the amounts of intercellular fat. Seventy five percent of normal glands have been shown to have less than 30 % intercellular fat and, 50 % less than 10 %. With these data, estimations of intercellular fat content have become practically useless as a parameter of normality.[26,27]

Tibblin utilizes the amount of intracellular fat to delineate normal and abnormal parathyroid glands. Normal parathyroid glands contain easily detectable amounts of intracellular fat, while in abnormal glands intracellular fat

is decreased or absent. However, these are not consistent findings. Approximately 10 % of adenomas have significant amounts of intracellular fat, and hyperplastic glands can stain for varying amounts of intracellular fat.[28]

In our opinion, primary hyperparathyroidism can be caused by enlargement of only two or three parathyroid glands. In our material 120 patients had two enlarged glands, and 40, three. Seventy-two of the patients with two enlarged glands had one enlarged parathyroid on the right side of the neck and the other on the left. An unilateral approach in these patients could have resulted in a high incidence of persistent hyperparathyroidism. A study by Wells et al. recorded two or three gland disease in 22 % of all patients, which is in accordance with the incidence (23 %) in our study.[29]

The extent of resection of parathyroid tissue in patients with multiple enlarged glands remains controversial. One group of authors advocates subtotal parathyroidectomies in cases with enlargement of two or more parathyroid glands or in the presence of histological features indicating hyperplasia.[14-18] However, the microscopic criteria for hyperplasia are poorly defined. The motive to perform subtotal parathyroidectomies is the assumption that normal-sized parathyroid glands in multiple gland disease are potentially hyperactive and can cause persistent or recurrent hyperparathyroidism. Another group of authors does not consider normal-sized glands as potentially hyperactive and therefore, selectively removes enlarged glands irrespective of the microscopic findings.[9,29,30] This protocol would be associated with high incidences of persistent and recurrent hyperparathyroidism when normal-sized glands are potentially hyperactive. However, the rate of persistent hyperparathyroidism after subtotal parathyroidectomies varies from 5 to 15 percent, whereas the persistence rate after selective removal of enlarged glands varies from 1 to 4 percent. In our study, which calls for selective removal, the persistence rate was 7.0 %. In one patient with hyperplastic parathyroid glands the second period of hypercalcemia was preceded by a normocalcemic period of 7 years. One can argue that this patient had a recurrence instead of a persistence. However, because the enlarged gland found at the time of the second operation was not identified during the first operation, this patient was classified as having persistent hyperparathyroidism. A statistical significant difference between the rates of persistent hyperparathyroidism in patients with adenomas and patients with hyperplastic parathyroid glands was found (Student t-test, $p < 0.05$). This is not surprising because the chance to miss an enlarged gland is greater when multiple parathyroid glands are enlarged.

Recurrent hyperparathyroidism was reported by other authors in 0.4 to 16

50

% of the patients after subtotal parathyroidectomies, while removing only the enlarged glands was associated with recurrence rates varying between 0.4 and 8 %. In our study two recurrences in patients with hyperplasia were recorded (1.8 %). Recurrent hyperparathyroidism was not observed in patients with adenomas. There was not a significant difference between the rates of recurrence in patients with adenomas and patients with parathyroid hyperplasia (Student t-test, NS). Our definition of recurrent hyperparathyroidism is a reflection of the biological behaviour of parathyroid glands in primary hyperparathyroidism. The lack of a statistical significant difference for the rates of recurrences in adenomas and parathyroid hyperplasias provides some evidence that parathyroid adenomas and parathyroid hyperplasia are part of the same pathological entity.

In conclusion removal of only the enlarged parathyroid glands results in acceptable rates of persistent and recurrent hyperparathyroidism in patients with primary hyperparathyroidism, provided MEN-I, MEN-II and familial hyperparathyroidism are excluded. The histological features of parathyroid glands do not play a role in determining the extent of resection of parathyroid tissue. Therefore, the concept of single and multiple gland disease seems preferable. To prevent transient and permanent hypoparathyroidism a conservative approach to the extent of resection of parathyroid tissue and to the taking of biopsies is preferable.

References

1. Black WC, Utley JR. The differential diagnosis of parathyroid adenomas and chief cell hyperplasia. Am J Clin Path 1967; 49: 761-75.
2. Bowlby LS, DeBault LE, Abraham SR. Flow cytometric DNA analysis of parathyroid glands. Relationship between nuclear DNA and pathologic classifications. Am J Path 1987; 128: 338-44.
3. Cinti S, Colussi G, Minola E, Dickersin GR. Parathyroid glands in primary hyperparathyroidism. An ultrastructural study of 50 cases. Hum Path 1986; 17: 1036-46.
4. Saxe AW, Baier R, Tesluk H, Toerson W. The role of the pathologist in the surgical treatment of hyperparathyroidism. Surg Gynaecol Obstet 1985; 161: 101-5.
5. Bruining HA, Van Houten H, Juttmann JR, Lamberts SWJ, Birkenhager JC. Results of operative treatment of 615 patients with primary hyperparathyroidism. World J Surg 1981; 5: 85-90.
6. Dufour DR, Wilkerson SY. Factors related to parathyroid weight in normal persons. Arch Path Lab Med 1983; 107: 167-72.

7. Lamers CBHW, Froeling PGAM. Clinical significance of hyperparathyroidism in familial multiple endocrine adenomatosis type I (MEA I). Am J Med 1979; 66: 422-24.

8. Bruining HA. Surgical treatment of hyperparathyroidism. Van Gorcum, Assen, The Netherlands and Charles C. Thomas, Springfield 1971: 67-9.

9. Clark OH, Way LW, Hunt TK. Recurrent hyperparathyroidism. Ann Surg 1976; 184: 391-410.

10. Armitage, Second Edition 1987, 165.

11 Alveryd A. Parathyroid glands in thyroid surgery. I: Anatomy of parathyroid glands. Acta Chir Scand 1968; 389; 9-29.

12. Gilmour JR, Martin WJ. The weight of the parathyroid gland. J Path Bact 1937; 44: 431-9.

13. Wang CA. The anatomic basis of parathyroid surgery. Ann Surg 1976; 183: 271-83.

14. Edis AJ, Beahrs OH, Van Heerden JA, Akwari OE. 'Conservative' versus 'liberal' approach to parathyroid neck exploration. Surgery 1977; 82: 466-73.

15. Cooke TJC, Boey JH, Sweeney EC, Gilbert JM, Taylor S. Parathyroidectomy: extent of resection and late results. Br J Surg 1977; 64: 153-9.

16. Paloyan E, Lawrence AM, Baker WH, Straus FH. Near total parathyroidectomy. Surg Clin North Am 1969; 43: 49-54.

17. Rudberg C, Akerstrom G, Palmer M, Ljunghall S, Adami O, Johansson H, Grimelius L, Thoren L, Bergstrom R. Late results of operation for primary hyperparathyroidism in 441 patients. Surgery 1986; 99: 643-52.

18. Haff RC, Armstrong RG. Trends in the current management of primary hyperparathyroidism. Surgery 1974; 75: 715-20.

19. McGarity WC, McKeown PP, Sewell CW. The role of routine biopsy of all parathyroid glands in primary hyperparathyroidism. Am Surg 1985; 51: 8-17.

20. Thompson NW. Endocrine Surgery Update, Thompson NW, Vinik AI. Editors, Grune and Stratton, New York, 1985, 365-374.

21. Edis AJ. Surgical anatomy and technique of neck exploration for primary hyperparathyroidism. Surg Clin North Am 1977; 57: 495.

22. Rothmund M. Hyperparathyreodismus, Rothmund M. Editor, Georg Thieme Verlag, Stuttgart, New York, 1980, 160-165.

23. Tibblin S, Bondeson AG and Ljungberg O. Unilateral parathyroidectomy for solitary parathyroid adenoma. In Fortschritte der endokrinologischen chirurgie, Rothmund M, Kummerle F. Editors, Georg Thieme Verlag, Stuttgart-New York, 1981, 114-119.

24. Wang CA. Surgical management of primary hyperparathyroidism. Curr Probl Surg 1985; 12: 11-13.

25. Kaplan EL, Bartlett S, Sugimoto J and Fredland A. Results of postoperative hypocalcemia to operative techniques: Deleterious effect of excessive use of parathyroid biopsy. Surgery 1979; 86: 462-9.

26. Dekker A, Dunsford HA, Geyer SJ. The normal parathyroid gland at autopsy: The significance of stromal fat in adult patients. J Path 1979; 128: 127-32.

27. Dufour DR, Wilkerson SY. The normal parathyroid revisited: Percentage of stromal fat. Hum Path 1982; 13: 717-22.

28. Bondeson AG, Bondeson L, Ljungberg O, Tibblin S. Fat staining in parathyroid disease -

Diagnostic value and impact on surgical strategy: Clinicopathologic
analysis of 191 cases. Hum Path 1986; 17: 1255-63.

29. Wells SA, Leight GS, Hensley M and Dilley WG. Hyperparathyroidism associated with the enlargement of two or three parathyroid glands. Ann Surg 1985; 202: 533-538.

30. Myers RT. Follow up study of surgically treated primary hyperparathyroidism. Ann Surg 1974; 179: 729-34.

HISTOPATHOLOGY OF SINGLE AND MULTIPLE GLAND DISEASE

IN

PRIMARY HYPERPARATHYROIDISM

Introduction

The pathology of primary hyperparathyroidism remains a controversial and poorly understood subject in spite of considerable interest on the part of pathologists, surgeons, endocrinologists and molecular biologists. Most studies have focused on the morphologic features of the parathyroid glands involved in primary hyperparathyroidism, specifically with regard to the delineation of "adenomas" and "hyperplasias". Since no single criterion has proven irrefutable in making this differentiation various histologic diagnoses have been used ranging from adenomas, double adenomas, pseudo-adenomas, focal hyperplasias, chief cell hyperplasias, water clear cell hyperplasias, adenomatous hyperplasias to nodular hyperplasias. However, some surgeons use the histologic appearance of parathyroid tissue at frozen section to determine the extent of parathyroidectomies: When a hyperplastic gland is reported by the pathologist, 3 or 3 1/2 parathyroids are removed.[1-4]

This study presents the morphologic features of parathyroid glands in 236 patients with primary hyperparathyroidism who had long term follow up studies. The objectives of this study are to document the differences between the histologic features of parathyroid glands of normal sizes, single enlarged and multiple enlarged parathyroid glands, and to determine the possibility of predicting persistent or recurrent hyperparathyroidism and predicting the extent of resection of parathyroid tissue by means of microscopic examination of parathyroid tissue.

Patients and methods

The patients were selected from a registry of 693 patients operated on at the University Hospital Dijkzigt (Rotterdam) or University Hospital in Leiden for primary hyperparathyroidism between the years 1952 - 1989. All the patients had been classified as having single or multiglandular disease based on the findings of the surgeon at the time of operations. Single gland disease was defined as the presence of one enlarged parathyroid gland weighing more than 40 mg. Multiple gland disease was defined as the presence of two or more enlarged parathyroid glands, each weighing more than 40 mg.

One hundred and seventy-nine of the patients had multiple gland disease, and of these, 91 had adequate clinical follow-up and pathologic material to be included in this study. From the remaining 514 patients with single gland disease, a sample large enough to reflect a representative rate of

recurrences was selected. Based on a published average recurrence rate of 2.5 %, it was determined that a sample size of approximately 160 patients would be sufficient to detect recurrent hyperparathyroidism.[5] After eliminating those patients with inadequate pathologic material, this group consisted of 130 patients. Patients with MEN syndromes or familial hyperparathyroidism were not included.

All patients had been operated on by one of two surgeons. A bilateral exploration of the neck, identification and biopsy of all parathyroid glands, and removal of only enlarged glands was the surgical approach. An estimated weight of 40 mg or greater was used as the criterion for enlargement. Follow up studies of the patients in both groups included clinical histories, documentation of medications, calcium or vitamin D supplementations and the determinations of serum levels of calcium, albumin, creatinine, urea and parathyroid hormone (intact hormone) in two blood samples at various times of the day.

All biopsies and intact glands removed from the two groups were examined by two pathologists without knowledge of the operative findings, weights of the glands, gross findings or follow up information. The review was limited only to sections stained with hematoxylin and eosin. The presence or absence of the following microscopic features were recorded: lobularity (Fig. 5.1), microscopic cellularity (Fig. 5.2), chief cells, oxyphil cells or water clear cells (Fig. 5.3), stromal fat, transition of abnormal tissue in the mass into normal parathyroid tissue (Fig. 5.4), a normal rim of parathyroid tissue (Fig. 5.5), a capsule (Fig. 5.5), thick walled blood vessels, nuclear pleomorphism (Fig. 5.6), mitotic activity and blood/lymphatic vascular space involvement. Each gland was categorized as normal, adenoma or hyperplasia based on the criteria of Ghandur-Mnaymneh and Kimura: i.e. a mass is considered as an adenoma if it fulfills the following criteria: absence of fat cells, absence of lobularity, a normal rim and microscopic cellularity.[6] A gland is considered hyperplastic if any one of the following criteria are met: fat cells in the mass, lobular architecture, or a transition of the abnormal tissue in the mass to normal parathyroid tissue. After independent review by two pathologists, a consensus was reached on each case when there were disagreements. If no consensus could be reached, often due to inadequate samples of tissue, the case was considered unclassifiable.

A multidiscriminate analysis was done to assess the value of morphologic features in differentiating between normal parathyroid tissue, single and multiple gland disease.
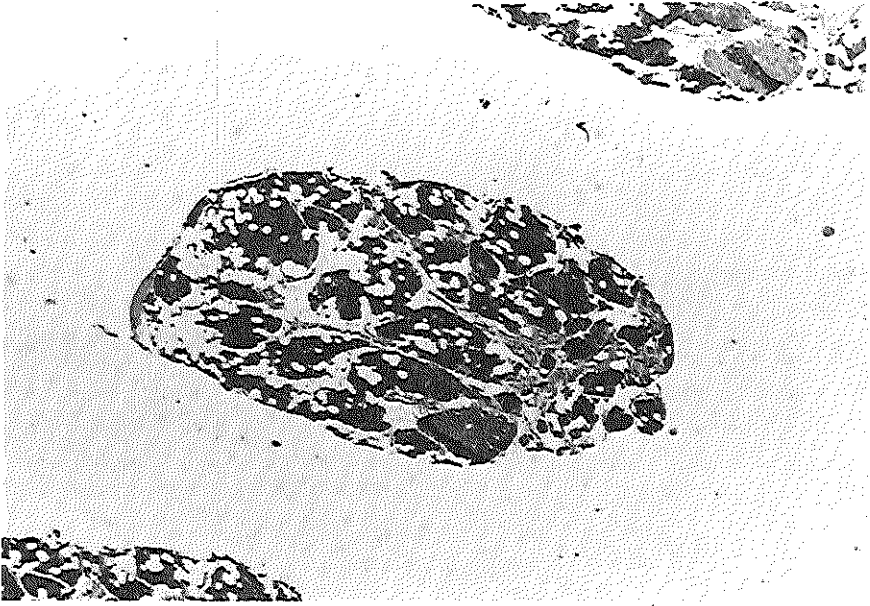
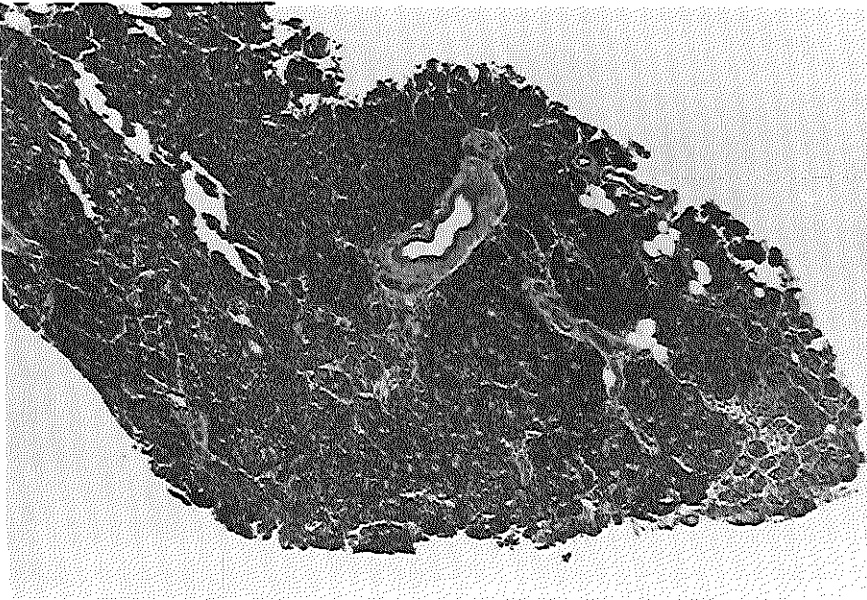Figure 5.1 Normal parathyroid gland with lobular architecture.

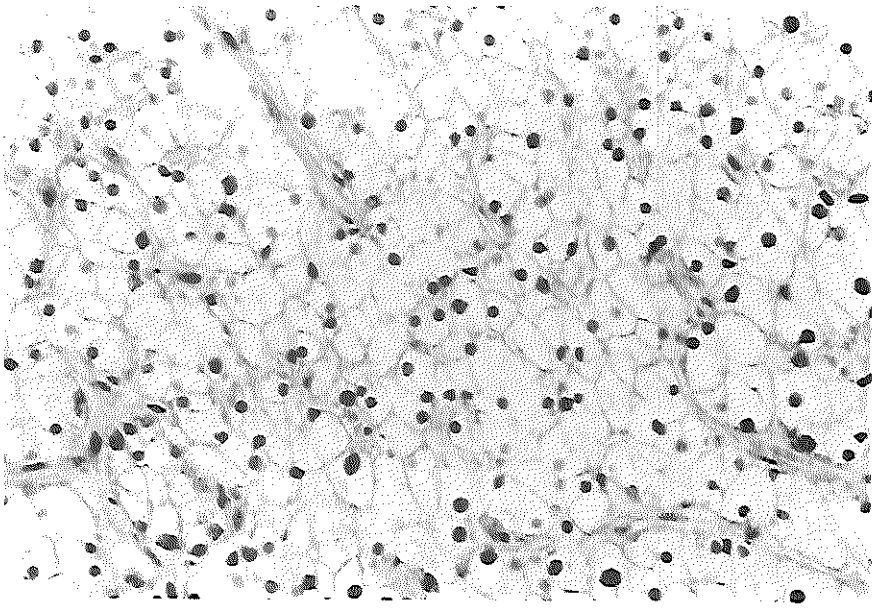Figure 5.2 Parathyroid gland showing microscopic cellularity.

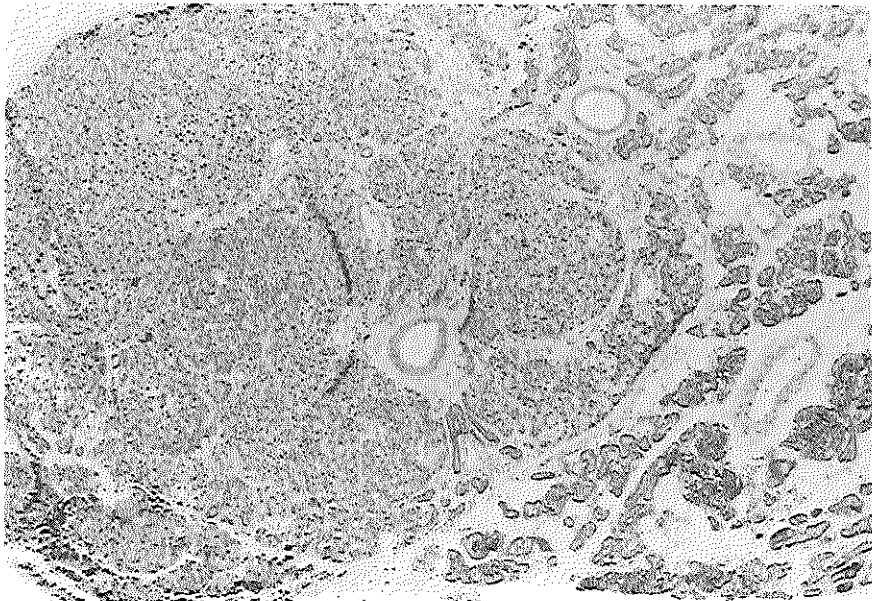Figure 5.3 Water clear cells in a parathyroid gland.

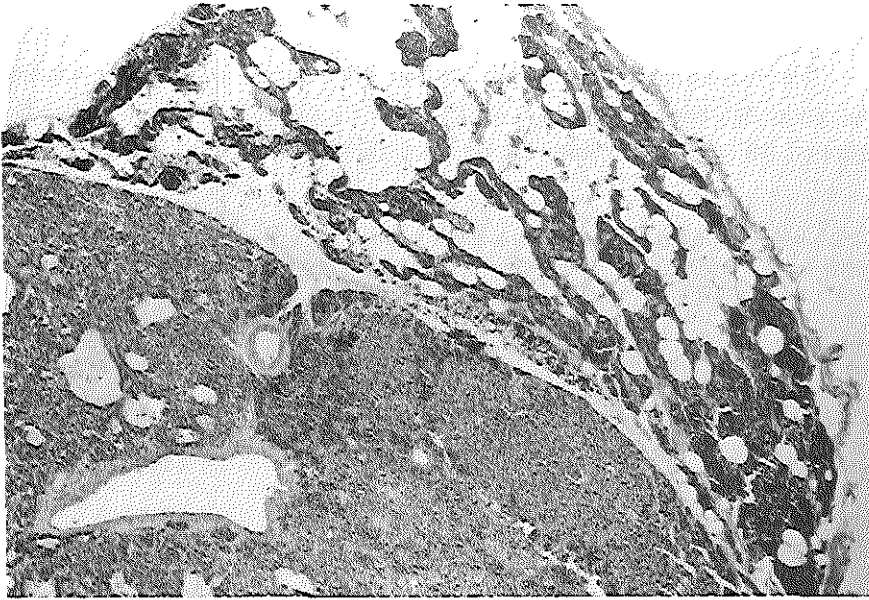Figure 5.4 Transition of cellular parathyroid tissue into normal parathyroid tissue.

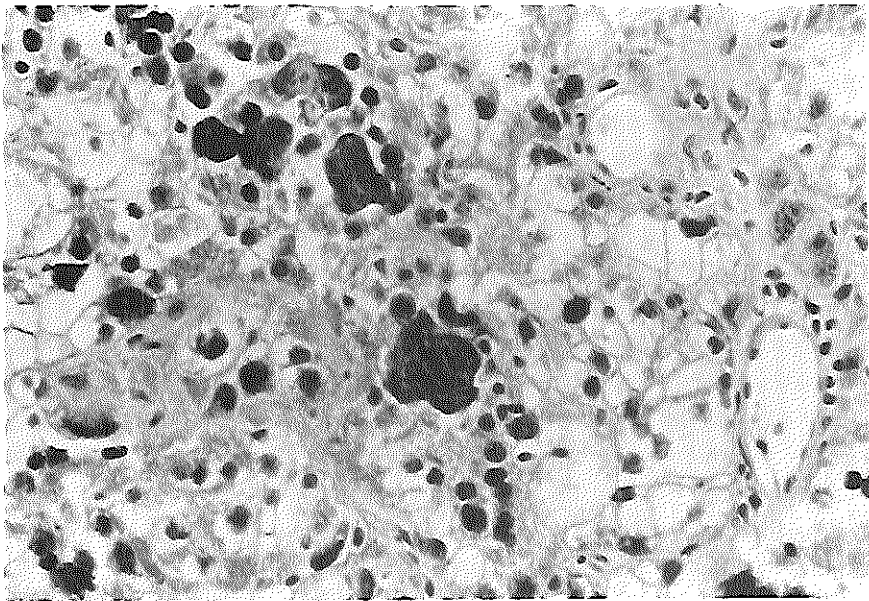Figure 5.5 Capsule separating rim of normal parathyroid tissue and cellular parathyroid tissue.

Figure 5.6 Nuclear pleomorphism in a parathyroid gland.

61

Results

The histologic classification of the 130 cases surgically classified as single gland disease is shown in Table 1. Fifty eight cases were classified as adenomas, 48, as hyperplasia, 18, as unclassifiable and 6, as normal glands.

Table 1. *Histologic classification of parathyroid glands*

	Normal-Sized Glands (%)	Single Enlarged Glands (%)	Multiple Enlarged Glands (%)
Normal	316 (93.2)	6 (4.7)	70 (33.9)
Adenoma	2 (0.6)	58 (44.6)	22 (10.6)
Hyperplasia	10 (2.9)	48 (36.9)	84 (40.9)
Unclassifiable	11 (3.2)	18 (13.8)	30 (14.6)
Total	339	130	206

Two patients with single enlarged parathyroid glands had persistent hyperparathyroidism. One patient had had a single enlarged parathyroid removed which was interpreted as hyperplasia. The other patient had had one enlarged gland removed and a biopsy of one normal sized gland. Both were interpreted as hyperplasia.

Two-hundred and six enlarged parathyroid glands were examined in the 91 patients classified by the surgeons as multigland disease. Eighty-four were histologically classified as hyperplasias, 70, as normal glands, 30, as unclassifiable cases, and 22, as adenomas (Table 1).

Six patients with multigland disease had persistent hyperparathyroidism. In three of these patients all parathyroid glands were classified as normal glands, in the other three, the glands were classified as hyperplasias. Recurrent hyperparathyroidism was observed in two patients. In one patient three enlarged glands (all classified as hyperplasias) were removed at the first operation. Material from the normal sized parathyroid which was left behind was not available. After 19 years hypercalcemia developed. A hyperplastic parathyroid gland was then found at autopsy. The other patient with recurrent hyperparathyroidism had two enlarged upper parathyroid glands which were removed at the first operation. Microscopic slides obtained at this operation were not available. Hypercalcemia developed after 11 years. At reoperation an

enlarged left lower parathyroid gland was found and removed. This gland was classified as hyperplasia.

Biopsies of parathyroids of normal sizes taken from patients with single and multigland disease were combined and analyzed as a separate group. There were 339 glands in this group. Three-hundred and sixteen were classified as histologically normal, 2, as adenomas, 10, as hyperplasias, and 11, as unclassifiable cases (Table 1).

Multidiscriminate analysis revealed that the presence or absence of a normal rim , a capsule, chief or oxyphil cells and nuclear pleomorphism did not contribute to differentiating among normal-sized, single enlarged and multiple enlarged parathyroid glands.[7] The standardized canonical coefficients of these features were all below 0.15. Mitotic activity and blood/lymphatic vascular space involvement were not seen in any case. Comparing normal sized parathyroids and the combined group of single and multiple enlarged parathyroids showed a highly significant difference (Eigenvalue = 1.0992). Microscopic cellularity was the most important differentiating feature (Table 2).

Table 2. *Standardized canonical coefficients of histologic features in comparison of normal-sized and enlarged parathyroid glands**

Microscopic Cellularity	1.230
Clear Cells	0.125
Lack of Stromal Fat	0.140
Lobularity	- 0.104
Transition	0.031
Thick Walled Vessels	0.017

* Positive signs indicate abnormality, negative, normalcy.

Table 3 shows the classification of normal-sized parathyroid glands, single enlarged and multiple enlarged glands into normal and abnormal glands on basis of the multidiscriminate analysis.

Table 3. *Classification of parathyroid glands by multidiscriminate analysis*

	Normal-Sized Parathyroid Glands (%)	Enlarged Parathyroid Glands (%)
Normal Glands	299 (88.20)	67 (19.94)
Abnormal Glands	40 (11.80)	269 (80.06)
Total	339	336

Comparing single and multiple enlarged parathyroids showed a far less significant difference (Eigenvalue = 0.317). Microscopic cellularity, lack of stromal fat, presence of transition and thick walled vessels indicated single gland disease, whereas clear cells and lobularity suggested multiple gland disease (Table 4).

Table 4. *Standardized canonical coefficients of histologic features in comparison of single and multiple enlarged parathyroid glands**

Microscopic Cellularity	0.284
Clear Cells	- 0.192
Lack of Stromal Fat	0.280
Lobularity	- 0.568
Transition	0.439
Thick Walled Vessels	0.254

* Positive signs indicate single gland disease, negative, multigland disease

Table 5 shows the classification of all parathyroid glands into "normal", "adenoma" and "hyperplasia".

Table 5. *Classification of parathyroid glands by multidiscriminate analysis*

	Normal-Sized Parathyroid Glands (%)	Single Enlarged Glands (%)	Multiple Enlarged Glands (%)
"Normal"	299 (88.20)	6 (4.62)	61 (29.61)
"Adenoma"	10 (2.95)	99 (76.15)	62 (30.10)
"Hyperplasia"	30 (8.85)	25 (19.23)	83 (40.29)
Total	339	130	206

Discussion

Traditional dogma in primary hyperparathyroidism holds that the majority of cases (50 - 89 %) are caused by parathyroid "adenomas".[8-15] The percentage of adenomas reported has varied depending on the criteria to define them. Adenomas are reported to occur with equal frequency in all four glands. Microscopically, a monomorphic proliferation of chief cells is most common,

often with some oxyphil and/or transitional cells. Water clear cells are rarely found. Cytologic changes in the chief cells, such as nuclear enlargement and hyperchromasias, also occur. Occasionally, marked nuclear pleomorphism, a benign finding, may be seen. Any architectural arrangement, solid, nodular, trabecular, acinar, or follicular, is possible. The presence of small amounts of stromal fat is acceptable to many authors as long as the other criteria are met.[8,9,13,16-18] Degenerative changes, cysts, fibrosis, hemorrhage, etc., especially in larger ones, are not uncommon. The most important findings in the traditional definition of a parathyroid adenoma is a compressed rim of "normal" parathyroid tissue and most importantly, another biopsy-proven normal gland.[9,11,13,16,19]

The existence of multiple adenomas has been debated for years. Harness et al. recorded 5 patients with multiple adenomas in 300 cases of primary hyperparathyroidism using the following criteria: (1) the finding of more than one enlarged parathyroid gland that shows histologically hyperplasia, (2) operative confirmation that the remaining parathyroid glands are normal in size, consistency, color and that at least one or more are histologically normal, (3) neither clinical evidence nor family history of MEN syndromes or familial hyperparathyroidism and (4) permanent cure of hypercalcemia by excision of the enlarged parathyroid glands.[20] Verdonk and Edis reported 38 patients (1.9 %) with double adenomas in 1,962 patients with primary hyperparathyroidism.[21]

In contrast to adenomas, most series report a minority (15 - 20 %) of primary hyperparathyroidism as caused by hyperplasia.[8,9,21] Usually, all four parathyroid glands are affected, but the enlargements may be assymetrical. Microscopically, a mixture of cells is common, and nodularity is much more common and prominent than in adenomas. Water clear cell hyperplasia is distinctive, but rarely seen at the present time. Stromal fat is usually demonstrable in the gland, but may not be seen in all sections. Architectural arrangements are as varied as those seen in adenomas. It is worth noting, how ever, that nodular patterns can be exaggerated and can result in an appearance similar to an adenoma.

The traditional methods to distinguish an adenoma from hyperplasia rely on a variety of morphologic features. However, no single criterion has proven irrefutable in making this differentiation, and the consequence is the use of the terminology single and multiple gland disease.[22-25] Many early studies on differentiating adenomas and hyperplasias have used either poorly described criteria or poorly sampled parathyroid glands. The marked variation in normal

content of stromal fat, and the acceptance by many authors that stromal fat can be present in adenomas has eliminated this finding as a criterion. The finding of a normal rim of compressed parathyroid tissue, as seen in 50 - 70 % of adenomas, is unreliable since this phenomenon may be reproduced by large dominant nodules in hyperplastic glands. Considerable overlap in the cell types may be seen in both adenomas and hyperplasias, as well as the distribution of cell types, which renders this criterion useless. The most heavily relied upon criterion is the identification of another normal gland. However, problems then arise: the concept that parathyroid hyperplasia may be assymetrical is well accepted and may be marked at times. If one accepts this concept, then the distinction of the minimally enlarged or "microscopically hyperplastic" gland from the normal gland becomes critical. Since there is significant variability in fat content of normal glands, this task may be difficult even when an entire gland is available for examination. This distinction may be impossible when only small biopsies are submitted for frozen sections, which is a common practice. The definition of normality, in this context, may significantly alter the apparent incidences of hyperplasia and adenoma. While some have suggested that "microscopic hyperplasia" and mild enlargement in a "normal gland" found along with an adenoma correlates with higher rates or persistent and recurrent hyperparathyroidism, and that such cases should be classified as examples of hyperplasia, others have emphasized that such distinctions are probably more semantic than real because they correlate poorly with persistent or recurrent hyperparathyroidism.

In addition to the morphologic features, staining for fat has been applied to distinguish hyperplasia from adenoma and normal from abnormal glands. Fat staining is based on the principle that in inactive or functionally normal glands, the cells have abundant and easily demonstrable cytoplasmic fat droplets. The hyperfunctioning gland has little or no cytoplamic fat.[26] The usefulness of this technique has been controversial.[6,26,27] We feel that the significant overlap in cytoplasmic fat content in both hyperactive and normal glands, the methodologic differences between various studies and the subjectivity of interpretation, limit the usefulness of staining for cytoplasmic fat on a routine basis.

For practical purposes, the distinction of normal from abnormal glands, at the present time, may best be done by gross evaluation of the glands at the time of parathyroidectomy. In this study parathyroid glands with estimated weights greater than 40 mg were removed irrespective of the microscopical findings other than the assessment of parathyroid tissue. After a mean follow

66

up of 13.5 years the rates of persistent and recurrent hyperparathyroidism were 3.6 % and 0.7 % respectively. In all patients persistent hyperparathyroidism was caused by missed enlarged glands. Therefore, the rate of recurrent hyperparathyroidism is the only meaningful parameter for normality of parathyroid glands. Since the rate of recurrent hyperparathyroidism was only 0.7 % parathyroid glands weighing 40 mg or less can be considered as functionally normal. Taking forty mg as the upper limit of normal for the total weight of a parathyroid gland was based on the study of Gilmour and Martin.[29] However, Dufour and Wilkerson showed that the distribution of the weights of normal glands was skewed to higher values, with a 95 percent interval ranging from 8.2 to 75.0 mg.[30] In our study only 18 of 336 enlarged parathyroids had weights between 40 and 75 mg. Classifying these glands as normal would not alter the results of this study significantly.

In this study all biopsies and intact glands removed from patients with single and multigland disease were categorized as adenoma or hyperplasia based on the criteria of Ghandur-Mnaymneh and Kimura.[6] Based on the assumption that an adenoma is a clonal growth which would be expected to displace adjacent parathyroid tissue as it grew and not incorporate stromal fat and eliminate the normal lobularity of the gland, they proposed that any lesion that contained stromal fat, had a lobular pattern or showed transition from normal parathyroid tissue to abnormal tissue, was an example of hyperplasia. All three of these criteria had to be absent to consider the lesion an adenoma.

In our study 130 cases of single gland disease, 58 (44.6 %) were histologically classified as adenomas and 48 (36.9 %) as hyperplasias.
The 48 cases with hyperplasia could be classified as examples of "focal hyperplasia". In the series of Ghandur-Mnaymneh and Kimura 129 of 144 cases considered to be hyperplastic showed only single gland enlargement. In their study 44 % of the patients with focal hyperplasia showed post-operatively persistent elevation of levels of serum parathyroid hormone. However, in our study only 2 of 48 cases of "focal hyperplasia" showed persistent hyperparathyroidism.

In contrast to the traditional definition of adenomas a "normal rim of parathyroid tissue" was only present in 31.5 % of the single enlarged glands. In a comparison of single and multiple enlarged glands the presence or absence of a "normal rim" was statistically not significant. Microscopic cellularity, lack of stromal fat, transition and thick walled vessels were more frequent features in single gland disease. However, the standardized canonical coefficients of these features were low.

Of 206 cases with multiple gland disease one third was classified as "normal" and 40.9% as hyperplasia. Lobularity and clear cells were more frequently seen in multigland disease than in single gland disease.

Biopsies of normal-sized parathyroid glands were histologically classified as "normal" in 93.2 %. The specificity of the histological diagnosis of "normal" was only 77 % because one third of the multiple enlarged glands was called "normal" by the pathologists.

As demonstrated by these data, the microscopic classification of abnormal glands as hyperplasia or adenomas correlates poorly with the gross classification of the disease. However, the pathologist can distinguish normal from abnormal parathyroid glands with a fair degree of accuracy. We feel that this distinction should be the primary aim of microscopic examination of frozen and permanent sections.

References

1. Rudberg C, Akerstrom G, Palmer M, Ljunghall S, Adami HO, Johansson H, Grimelius L, Thoren L and Bergstrom R. Late results of operation for primary hyperparathyroidism. Surgery, 99, 643, 1986.

2. McGarity WC, McKeown PP and Sewell CW. The role of routine biopsy of all parathyroid glands in primary hypeparathyroidism. Am Surg, 51, 8, 1985.

3. Cooke TJC, Boey JH and Sweeney CW. Parathyroidectomy: extent of resection and late results. Br J Surg, 64, 153, 1977.

4. Paloyan E, Paloyan D and Pickleman JR. Hyperparathyroidism today. Surg Clin North Am, 53, 21, 1973.

5. Clark OH, Way LW and Hunt TK. Recurrent hyperparathyroidism. Ann Surg, 179, 729, 1974.

6. Ghandur-Mnaymneh L and Kimura N. The parathyroid adenoma. A histopathologic definition with a study of 172 cases of primary hyperparathyroidism. Am J Pathol, 115, 70, 1984.

7. Van de Geer JP. Introduction to multivariate analysis for the social sciences, Freeman WH and Company, San Francisco, 201-254, 1982.

8. Woolner LB, Keating FR Jr and Black BM. Tumors and hyerplasia of the parathyroid glands. Cancer, 5, 1069, 1952.

9. Roth SI. Pathology of the parathyroids in hyperparathyroidism. Arch Pathol, 73, 75, 1962.

10. Castleman B, Schantz A and Roth SI. Parathyroid hyperplasia in primary hyperparathyroidism: A review of 85 cases. Cancer, 38, 1668, 1976.

11. Kay S. The abnormal parathyroid. Human Pathol, 7, 127, 1976.

12. Esselstyn CB Jr, Levin HS, Eversman JJ and Schumacher OP. Reappraisal of parathyroid pathology in hyperparathyroidism. Surg Clin North Am, 54, 443, 1974.

13. Black WC and Utley JR. The differential diagnosis of parathyroid adenoma and chief cell hyperplasia. Am J Clin Pathol, 49, 761, 1968.

14. Hellstrom J and Ivemark BI. Primary hyperparathyroidism-clinical and structural findings in 138 cases. Acta Chir Scand (Suppl), 294, 1, 1962.

15. Block MA, Greenwald K, Horn RC Jr and Frame B. Involvement of multiple parathyroids in hyperparathyroidism.Am J Surg, 114, 530, 1967.

16. Castleman B and Mallory TB. The pathology of the parathyroid gland in hyperparathyroidism. Am J Pathol, 11, 1,1935.

17. Lawrence DAS. A histological comparison of adenomatous and hyperplastic parathyroid glands. J Clin Pathol, 31, 626, 1978.

18. Black WC and Hoff RC. The surgical pathology of parathyroid chief cell hyperplasia. Am J Clin Pathol, 53, 565, 1970.

19. Black BK and Ackerman LV. Tumors of the parathyroid, a review of twenty-three cases. Cancer, 3, 415, 1950.

20. Harness JK, Ramsburg SR, Nishiyama RH and Thompson NW. Multiple adenomas of the parathyroids: Do they exist?. Arch Surg, 114, 468, 1979.

21. Verdonk CA and Edis AJ. Parathyroid "double adenomas": Fact or fiction?. Surgery, 90, 523, 1981.

22. Scholz DA, Purnell DC and Edis AJ. Primary hyperparathyroidism with multiple parathyroid enlargement, review of 53 cases. Mayo Clin Proc, 53, 792, 1978.

23. Edis AJ. Surgical anatomy and technique of neck exploration for primary hyperparathyroidism. Surg Clin North Am, 57, 495, 1977.

24. Purnell DC, Scholz DA and Beahrs OH. Hyperparathyroidism due to single gland enlargement. Arch Surg, 112, 369, 1977.

25. Wells SA Jr, Leight GS and Ross AJ. Primary hyperparathyroidism. Curr Probl Surg, 17, 439, 1980.

26. Bondeson A, Bondeson L, Ljungberg O and Tibblin S. Fat staining in parathyroid disease-diagnostic value and impact on surgical strategy: clinicopathologic analysis of 191 cases. Human Pathol, 5, 381, 1981.

27. Roth SI and Gallagher MJ. The rapid identification of "normal" parathyroid glands by the presence of intracellular fat. Am J Pathol, 84, 521, 1976.

28. Dekker A, Watson CG and Barnes EL Jr. The pathologic assessment of primary hyperparathyroidism and its impact on therapy. A prospective evaluation of 50 cases with oil-red-O-stain. Ann Surg, 190, 671, 1979.

29. Gilmour JR and Martin WJ. The weight of the parathyroid glands. J Pathol Bacteriol, 44, 431, 1937.

30. Dufour DR and Wilkerson SY. Factors related to parathyroid weights in normal persons. Arch Pathol Lab Med, 107, 167, 1983.

DNA ANALYSIS IN PRIMARY HYPERPARATHYROIDISM

Introduction

The goal of parathyroid surgery is to remove all hyperactive or potentially hyperactive parathyroid tissue. The majority of patients with primary hyperparathyroidism is cured by the removal of a single enlarged parathyroid gland (adenoma). Controversy remains on the optimal surgical treatment of multiple enlarged parathyroid glands (hyperplasia). Some authors advocate subtotal parathyroidectomies in multigland disease because they consider all parathyroid glands hyperactive in multigland disease.[1-4] Others remove only enlarged parathyroid glands.[5,6] Microscopical examination of parathyroid tissue has been unable to discriminate between parathyroid adenomas and primary parathyroid hyperplasia.[7] This has led to a quest for objective means to make this differentation.

This study assesses the ability of DNA analysis to discriminate normal parathyroid glands from hyperactive or potentially hyperactive parathyroid glands and to establish if DNA analysis shows differneces in DNA content in single and multigland disease.

Patients and Methods

Patient Selection

The charts of patients who had their first parathyroidectomies for primary hyperparathyroidism at the University Hospital in Rotterdam from March 1974 to August 1980 were reviewed. Patients with multiple endocrine neoplasias or familial hyperparathyroidism were excluded. Information was available on the diagnostic procedures, the parathyroidectomies, the pathologic examinations of the parathyroid glands, and the post-operative courses. Two surgeons operated on the majority of the patients. The surgical approach was to identify all parathyroid glands. The glands were carefully dissected free to estimate the sizes of the parathyroid glands. Normal parathyroid glands are flat. A globular shape is suspect. However, experience is required to properly estimate the weight of a parathyroid gland. Small biopsies were taken of all identified glands. All biopsies were examined by frozen sections. The identification of parathyroid tissue was the only information required from the pathologist. The

73

interpretation of the microscopic slides of the parathyroid glands, which varied from adenomas, focal hyperplasias, nodular hyperplasias to chief cell hyperplasias, did not determine the extent of the resection of parathyroid tissue. The parathyroid glands estimated to weigh more than 40 mg were removed. When four parathyroid glands were enlarged, a remnant of 40 mg of parathyroid tissue was left in situ. The fresh weights of the removed parathyroid glands were recorded. The follow up of patients was carried out with the aid of the National Population Register, which registers the residents of all inhabitants of the Netherlands. The follow up studies were performed either in the surgical clinics of the University Hospital Rotterdam or by family physicians. The follow up studies consisted of recording the medications with emphasis on calcium or vitamin D supplementations and two blood assays which were drawn at weekly intervals. Serum levels of calcium, albumin, creatinine and urea were determined. A serum parathyroid hormone (PTH) assay (IRMA 1-84 for the intact hormone, INCSTAR, Stillwater, Minnesota) was performed when the mean calcium value, corrected for serum albumin, exceeded 2.45 mmol/L (normal 2.20 - 2.65 mmol/L) to detect normocalcemic hyperparathyroidism.

Single gland disease was defined as one enlarged parathyroid gland weighing more than 40 mg, in the presence of the other glands appearing normal in size. Multigland disease was defined as two or more enlarged parathyroid glands each weighing more than 40 mg.

Persistent hyperparathyroidism was defined as postoperative hypercalcemia caused by an enlarged parathyroid gland, which was not found during the first attempt at a parathyroidectomy. Recurrent hyperparathyroidism was defined as hypercalcemia due to an enlarged gland, which was observed to be of normal size during the first operation. Duration of periods of normocalcemia was not part of these definitions.[8] Patients who were hypercalcemic after their first parathyroidectomies and who had not second operations were not classified as having persistent or recurrent hyperparathyroidism.

Specimen Acquisition

The enlarged parathyroid glands and the biopsies of parathyroid glands of normal sizes were fixed in 10% (v/v) neutral buffered formalin (NBF), processed routinely, and embedded in paraffin.

Paraffin DNA Method

The paraffin-embedded tissues were deparaffinized and dissociated according to a modification of a method reported by Hedley.[9] Three to four 50 um sections of the paraffin blocks were cut on a standard microtome and deparaffinized with two 3 mL washes of Americlear (Stephens Scientific Division, Oak Ridge, NJ). Tissue sections were rehydrated by sequential 10 minute washes in 3 mL of 100%, 95%, 70%, and 50% (v/v) ethanol, washed in distilled water and held 1 hour to 24 hours in 3 mL of distilled water at room temperature. The tissues were then dissociated in 1 mL of a 1% (w/v) pepsin solution (1100 units/mg protein, Sigma Chemicals, St. Louis, MO) at 37 C for 30 min., vortexing every 5 min. then centrifuged at 1000 x g for 5 min. The cell pellets were resuspended in 1-3 mL Earles Balanced Salt Solution (Sigma Chemicals). The samples were filtered through 150 um metal meshes (Small Parts, Inc. Miami, FL) and recentrifuged at 1000 x g for 5 min. The cell buttons were resuspended in 1 mL propidium iodide staining solution and processed as described in the section on the method of staining.

Method of Staining

Cell suspensions were stained with propidium iodide (PI) (Sigma Chemicals, St. Louis, MO) according to the method described by Bauer.[10] One mL of low salt stain (3.0 g polyethylene glycol (PEG 8000, Fisher Scientific, Pittsburgh, PA), 5 mL of 1 mg/mL PI, 5 mL of 3600 units/mL RNAse A in PBS, 1 mL of 10% (v/v) Triton X-100/PBS, 89 mL of 4 mmol/L Sodium Citrate Buffer) were added to cell pellets from paraffin embedded tissue and gently vortexed. Samples were incubated for 20 min. at 37 C, then 1.0 mL of a high salt stain (3.0 g PEG, 5 mL of 1 mg/mL PI, 1 mL of 10% (v/v) Triton X-100/PBS, 94 mL 400 mmol/L Sodium Chloride) was added to each tube and gently vortexed. Each specimen was filtered through a 60 um nylon mesh (Small Parts, Inc., Miami, FL) into a new polystyrene tube, then analyzed by flow cytometry for DNA content. Staining solutions were prepared in advance and stored at -70 C in 3 mL aliquots until just prior to use.

Flow Cytometry

All samples were analyzed on a Epics-C flow cytometer (Coulter Corporation, Hialeah, FL) at 400 mW of laser power with a 2 W argon ion laser. A 550 nm dichroic short pass mirror and 570 nm long pass mirror were used in front of the red photomultiplier tube (PMT). The signal from the red PMT was split and fed into two amplifiers. One fluorescence amplifier was set at a gain of 5 and the PMT high voltage was adjusted to bring the diploid peak to approximately channel 80 in a 256 channel histogram. The other fluorescence amplifier was set to a gain of 2 to yield a "down-scale" version of the DNA histogram, which was used for visualizing triplets and tetraploid tumors.

The following parameters were collected in list mode on all samples: log forward angle light scatter, log side scatter, red fluorescence, and down-scale red fluorescence. The 256 channel red fluorescence histograms were self-gated above channel 10. All samples were briefly vortexed just prior to running and data were acquired at a flow rate of 100 events per second. A maximum of 10,000 events was acquired for each sample.

Computer Modeling

All histograms were analyzed using Verity:Modfit (Verity Software House, Inc., Topsham, ME), a cell cycle analysis program.[11] The following parameters were calculated and analysed: DNA index, % S-Phase, % G2M, % G2M minus doublets, debris and total events (Fig. 1). DNA index was defined as the ratio of the mean of the G0/G1 peak of the tumor population to the mean of the G0/G1 peak of the normal population. Position is the mean channel number of a modelled Gaussian peak. Populations with a DNA index between 0.9 and 1.1 were considered as DNA diploid populations. DNA tetraploid populations were defined as populations who had > 20 % of the total number of cells at the 4C or G2M region and another peak at 8C. DNA aneuploid populations of cells had DNA indices between 0.9 and 1.1. DNA aneuploid and hypopdiploid populations had indices less than 0.9 , while DNA aneuploid and hyperdiploid populations had indices between 1.1 and 1.9 or greater than 2.1.

Doublets were calculated by interpolating between the singlet and triplet peak heights. The G2M minus the doublets was felt to represent a more accurate assessment of G2M or 4c cells. All results were entered into a database and analyzed using Excel (Microsoft Corporation, Redmond, WA)
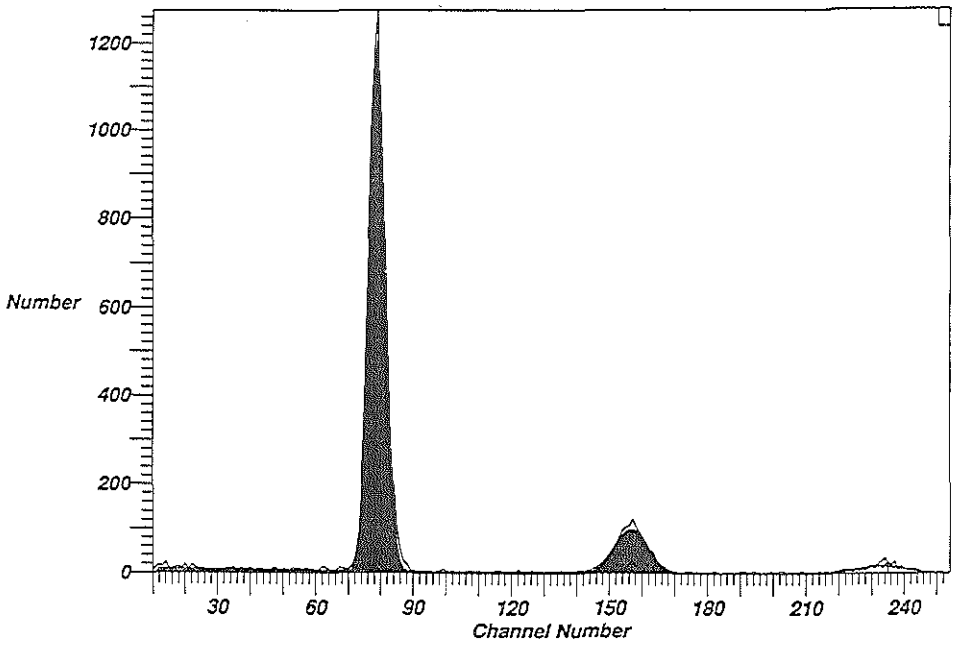
Figure 6.1   Histogram of a parathyroid gland with a DNA diploid population of cells. GO / G1 peak at channel 78 (2c), G2M peak at channel 156 (4c).   S-phase: cells between GO / G1 and G2M peaks. Debris: cells between channel 0 and GO / G1 peak. Triplet peak at channel 234 (6c).

Results

Paraffin embedded material was available for flow cytometric nuclear DNA analysis from 39 patients with single gland disease and 27 patients with multigland disease. The mean age at operation was 47.9 years (19-78 yrs). The ratio of females to males was 2:1.

Ninety-two normal-sized parathyroid glands were subjected to DNA analysis. Seventy-two were taken from patients with single gland disease, and twenty, from patients with multigland disease. Ninety-one of 92 specimens showed cell populations with DNA diploid contents (Table 1.).

Table 1. *DNA pattern in parathyroid glands of patients with primary hyperparathyroidism*

	Diploid	Aneuploid	Tetraploid
Normal-Sized Glands	91	0	1
Single Enlarged Glands	27	8	1
Multiple Enlarged Glands	48	10	1

In one case a DNA tetraploid population (DNA index = 2.05) was found.(Fig. 6.2) This was a biopsy of a normal-sized gland from a patient who had two enlarged glands removed and who developed 14 years after parathyroidectomy an elevated parathyroid hormone level and a serum calcium value of 2.66 mmol/L.
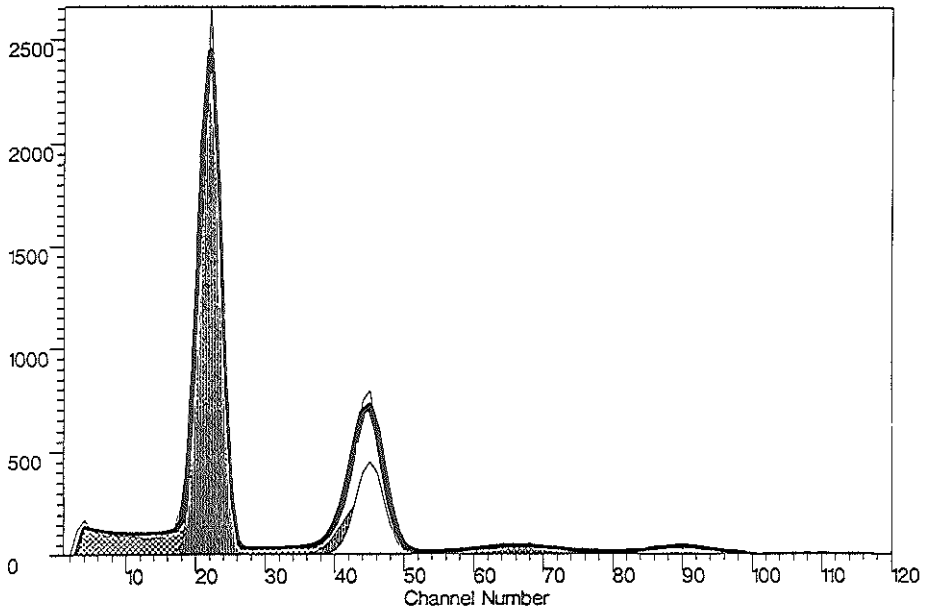
Figure 6.2 Histogram of a normal-size parathyroid gland with population of DNA tetraploid cells. G0 / G1 peak of DNA diploid cells at channel 22. G0 / G1 peak of DNA tetraploid cells at channel 45. G2M peak of DNA tetraploid cells at channel 90.

DNA analysis of 36 enlarged parathyroid glands in patients with single gland disease showed DNA diploid populations in 27 glands (Table 1.). Nine glands showed DNA aneuploid populations. Six were hyperdiploid, and two glands showed both hypodiploid and hyperdiploid populations (Fig. 6.3). A DNA tetraploid population was identified in one single enlarged gland. None of the patients with single gland disease had persistent or recurrent hyperparathyroidism.

79
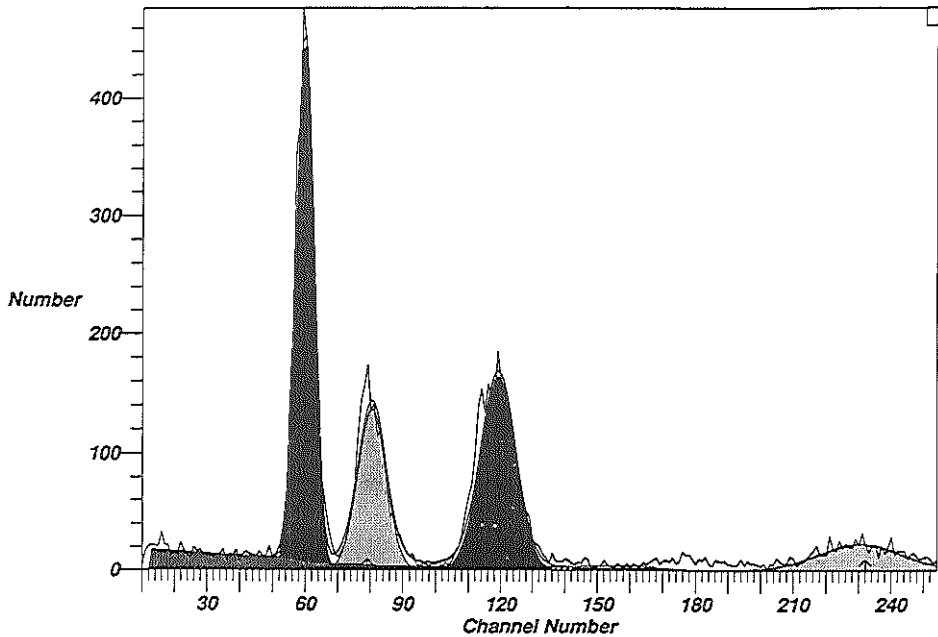
Figure 6.3 Histogram of a single enlarged parathyroid gland with coexistent DNA hypodiploid and hyperdiploid populations of cells at, respectively, channel 60 and 120.

Fifty-nine enlarged parathyroid glands obtained from 27 patients with multigland disease were available for DNA analysis. DNA analysis revealed cell populations with DNA diploid contents in 48 of 59 enlarged glands (Table 1.). Eleven glands contained DNA aneuploid populations. Six of which were hyperdiploid and four, hypodiploid. In one enlarged parathyroid two hyperdiploid populations were found (Fig. 6.4). One aneuploid gland had a DNA tetraploid population. One patient had persistent hyperparathyridism due to a left lower enlarged parathyroid which was missed at the first operation. After removal of this gland normalcalcemia followed. DNA analysis of the left lower gland showed a DNA diploid pattern with 9.2 % of cells at 4C.
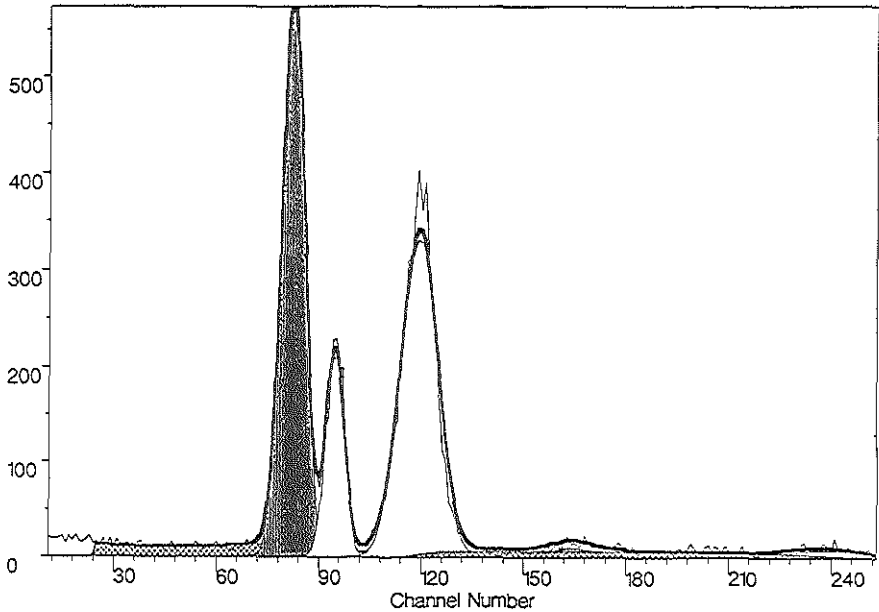
Figure 6.4   Histogram of a multiple enlarged parathyroid gland with two DNA hyperdiploid populations of cells at channel 95 and 120.

The mean weight of single enlarged parathyroid glands with DNA diploid populations was 2,092 mg (115 - 16,676 mg), while the mean weight of single enlarged glands with DNA aneuploid populations was 3229 mg (310 - 12000 mg). A Student t-test revealed no significant difference (t = 0.78).

In multiple gland disease the mean weight of enlarged glands with DNA diploid populations was 2,027 mg (100 - 16,000 mg), while the mean weight of enlarged glands with DNA aneuploidy was 1,150 mg (45 - 18,000 mg). Comparison of the mean weights showed no significant difference (t = 0.80).

A chi-square test was used to compare the incidences of DNA diploid and DNA aneuploid populations of cells in normal-sized parathyroid glands,

single and multiple enlarged glands. The differences were statistically significant when comparing normal-sized glands with enlarged glands in single gland disease (p < 0.001) or multigland disease (p < 0.001). However, there was no statistically significant difference in the incidences of DNA aneuploidy in single and multigland disease.

The Student t-test was used to compare the mean percentages of cells in the S and G2M phases among the three groups (Table 2.). There was a significant difference between the normal-sized and enlarged glands (p < 0.001), but none between the single and multiple enlarged glands.

Table 2. *DNA parameters of parathyroid glands with diploid DNA patterns of patients with primary hyperparathyroidism*

	% S-phase	% G2M	% G2M - doublets
Normal-Sized Glands	1.89 +/- 1.14 (0.6-6.2)	6.94 +/- 3.05 (1.8-15.7)	5.95 +/- 2.08 (1.8-11.9)
Single Enlarged Glands	3.64 +/- 4.00 (0.1-18.5)	10.02 +/- 4.35 (4.9-15.3)	8.03 +/- 2.54 (3.2-14.5)
Multiple Enlarged Glands	3.48 +/- 2.63 (0-12.5)	9.96 +/- 5.53 (1.7-22.2)	8.53 +/- 4.93 (1.7-19.2)

Discussion

The extent of resection of parathyroid tissue in this study was determined by the gross appearance of the parathyroid glands at the exploration of the neck. Only parathyroid glands estimated to weigh more than 40 mg were removed irrespective of the microscopic findings. Using this protocol in 693 patients with primary hyperparathyroidism the rates of persistent and recurrent hyperparathyroidism were respectively 3.6 % and 0.7 % after a mean follow-up of 13.5 years.[12] In this study on the DNA flow cytometric findings in parathyroid glands of patients with primary hyperparathyroidism an attempt was made to correlate DNA patterns to hormonal activity of parathyroid glands and persistent or recurrent hyperparathyroidism.

Irvin and Bagwell attempted to identify histologically undetectable

parathyroid hyperplasia by flow cytometry in 37 patients with parathyroid adenomas.[13] Flow cytometric DNA analysis was done in parathyroid glands obtained from patients without parathyroid disease, normal-sized glands from patients with primary hyperparathyroidism and enlarged parathyroid glands. The percentage of nuclei that fell within the 4C region was referred to as "hyperplastic index". The difference of "hyperplastic index" between normal sized glands and enlarged glands was statistically significant. However, there was a considerable overlap of "hyperplastic index" between the two groups of parathyroids. Twenty-nine percent of the normal-sized parathyroid glands would be classified as enlarged parathyroids using solely the "hyperplastic index". Our study also showed significant differences of % S-phase and % G2M between normal-sized parathyroid glands and enlarged glands in primary hyperparathyroidism. However, there was to much overlap between the two groups to merit use of % S-phase and % G2M to discriminate between normal and abnormal parathyroid glands in individual cases.

In another study Irvin et al. correlated the postoperative levels of serum parathyroid hormone (PTH) and DNA-contents of normal-sized parathyroid glands which were left behind after parathyroidectomies.[14] Percentages of DNA tetraploidy greater than 6 % were considered abnormal based on the percentage of DNA tetraploid cells in normal parathyroid glands from patients without parathyroid disease. Seven of seventeen patients who had follow-up studies had elevated levels of serum PTH. In all these patients the tetraploid DNA content of the normal-sized parathyroids was greater than 6 %. Four patients with normal-sized parathyroids with tetraploid DNA contents greater than 6 % had normal PTH levels. All six patients with less than 6 % of DNA tetraploid cells had normal PTH levels. In our study the mean percentage of G2M of normal-sized parathyroids was 6.94 %. PTH levels were only determined at follow-up when the mean of two serum corrected calcium values exceeded 2.45 mmol/L since normocalcemic hyperparathyroidism was not considered to be likely in patients with serum calcium levels lower than 2.45 mmol/L. PTH assays were done in 6 patients. In one patient an elevated level of PTH was found. In this patient DNA analysis showed a DNA tetraploid population of cells in a biopsy from a normal-sized gland.

The finding of DNA aneuploid populations of cells in single or multiple enlarged parathyroid glands in primary hyperparathyroidism has been reported by several authors. Table 3 and 4 summarize the data of the studies on DNA aneuploidy and tetraploidy in primary hyperparathyroidism.

Table 3. *DNA patterns in single gland disease (adenomas)*

	Number of Glands	% Aneuploidy	% Tetraploidy
Irvin[14]	37	15	-
Harlow[15]	12	25	8
Bowlby[16]	56	5	21
Shenton[17]	39	15	50
Joensuu[18]	54	26	9
Present study	36	22	3

Table 4. *DNA patterns in multiple gland disease (hyperplasia)*

	Number of Glands	% Aneuploidy	% Tetraploidy
Irvin[14]	4	25	-
Bowlby[16]	10	0	20
Present study	59	17	2

DNA aneuploidy is a well recognised feature of human tumors. There is accumulating evidence that ploidy reflects the biological behaviour of a large number of tumors and that diploid tumors in particular have a good prognosis.[19] Tumor ploidy cannot be used as an absolute criterion to distinguish between benign and malignant tumors since the latter may also be diploid. The finding of DNA aneuploidy in benign breast tumors has been reported in a small number of patients who later developed invasive breast cancer.[20,21]

Irvin et al. found rates of DNA aneuploidy in single and multigland disease comparable to our study.[14] In their study the number of parathyroid glands with DNA tetraploid populations of cells was not reported.

Bowlby et al. reported a high incidence of DNA tetraploidy.[16] However, DNA tetraploidy was defined as DNA patterns with greater than 15 % of the cells in the DNA tetraploid region. We feel that a definition of DNA tetraploidy should include another peak of cells at 8C, representing the G2M region of the

84

G0/G1 cells at 4C. A high peak of cells at 4C without a peak at 8C merely reflects high mitotic activity of the analysed tissue. Although Bowlby et al. did not observe recurrent hyperparathyroidism or metastases they suggested that parathyroid glands with DNA aneuploid or DNA tetraploid cells might progress into malignancies. Data on parathyroid glands with DNA aneuploidy or DNA tetraploidy which are left in situ are difficult to obtain. In our study one patient with a DNA tetraploid population of cells in a normal-sized parathyroid gland which was left behind developed recurrent hyperparathyroidism. However, we were unable to delineate DNA aneuploidy or DNA tetraploidy as a premalignant marker.

In the study by Shenton et al. 50 % of the adenomas had more than 20 % of their cells in the G2M region.[17] Both frozen and paraffin embedded parathyroid tissue were used. The contribution of doublets to this high rate of DNA tetraploidy is difficult to establish since corrections for doublets were not done in the study by Shenton et al..

Joensuu and Klemi reported the results of DNA analysis of 164 adenomas of the pituitary, thyroid, parathyroid and adrenal glands.[18] Unequivocal evdence of DNA aneuploidy was found in 29 % of pituitary, 25 % of thyroid, 35 % of parathyroid and 53 % of adrenal adenomas. After a mean follow-up of 3.8 years none of the adenomas had given rise to metastases.

Few reports in the literature include correlations of clinical and laboratory parameters with nuclear DNA analyses of parathyroid lesions. Joensuu and Klemi observed that the mean age of patients with DNA aneuploid adenomas was higher at diagnosis.[18] Obara et al. correlated DNA aneuploidy with pre-operative serum calcium and C-PTH levels.[22] DNA aneuploidy was associated with a high serum calcium level, and % S-phase was related to higher pre-operative serum calcium and C-PTH levels.

No attempt was made to correlate the results of nuclear DNA analysis with other parameters, except the weights, in our study. There were no significant differences between the weights of parathyroid glands with DNA diploid and DNA aneuploid contents. Obara et al. also reported no correlation of DNA aneuploidy with the weight of the tumor.[22] In spite of the lack of a correlation of DNA aneuploidy and weight in our study, we noted that in 8 of 10 patients with multigland disease and DNA aneuploidy the DNA aneuploid population of cells was found in the largest parathyroid gland.

In two single enlarged parathyroid glands both hypo-diploid and hyperdiploid populations of cells were found within the same gland, and in one multiple enlarged parathyroid gland two hyperdiploid populations of cells were

identified. These findings suggest a multicellular origin in single and multigland disease. Current concept is that most neoplasms arise from a single cell of origin.[23,24]

Arnold et al. reported the results of two independent molecular genetic studies in parathyroid adenomas and parathyroid hyperplasia.[25] In eight adenomas monoclonal patterns were found, and none of five hyperplastic glands had a monoclonal pattern. A study by Fialkow et al. appears to contradict the findings of Arnold et al..[26] Four parathyroid adenomas from patients with heterozygosity for the X-chromosome-linked enzyme , glucose-6-phosphate dehydrogenase were analysed. Both B and A isoenzymes were found in each adenoma in proportions similar to those observed in normal tissue, indicating that the lesions had multicellular origin. Fialkow et al. concluded that parathyroid hyperplasia and adenomas might be similar biologically.[26]

References

1. Rudberg C, Akerstrom G, Palmer M, Ljunghall S, Adami HO, Johansson H, Grimelius L, Thoren L and Bergstrom R. Late results of operation for primary hyperparathyroidism. Surgery, 99, 643, 1986.
2. McGarity WC, McKeown PP and Sewell CW. The role of routine biopsy of all parathyroid glands in primary hyperparathyroidism. Am Surg, 51, 8, 1985.
3. Cooke TJC, Boey JH and Sweeney CW. Parathyroidectomy: extent of resection and late results. Am J Clin Path, 49, 761, 1967.
4. Haff RC and Armstrong RG. Trends in the current management of primary hyperparathyroidism. Surgery, 75, 715, 1974.
5. Clark OH, Way LW and Hunt TK. Recurrent hyperparathyroidism. Ann Surg, 179, 729, 1974.
6. Wells SA, Leight GS, Hensley M and Dilley WG. Hyperparathyroidism associated with the enlargement of two or three parathyroid glands. Ann Surg, 202, 533, 1985.
7. Nishiyama RH. Pathology of parathyroid tumors. IN: Comprehensive management of head and neck tumors. Vol 2 WB Saunders Company 1987: 1650-60.
8. Bruining HA. Surgical treatment of hyperparathyroidism. Van Gorcum, Assen, The Netherlands and Charles C Thomas, Springfield 1971:67-9.
9. Hedley DW, Friedlander MI, Taylor IW, Rugg CA and Musgrove EA. Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. J Histochem Cytochem, 31, 1333-35, 1983.
10. Bauer KD. Clinical Flow Cytometry Course Part I: Applications and Methods. Heshley Medical Center, Hershy, PA, 1988: Oct 9-14.
11. Bagwell CB. Theory and application of DNA histogram analysis. University of Miami doctoral thesis. ERIC/EDRS ED 299 130, 1979.

12. Bonjer HJ, Bruining HA, Birkenhager JC, Nishiyama RH, Jones MA and Bagwell CB. Single and multigland disease in primary hyperparathyroidism: Clinical follow-up, histopathology and flow cytometric DNA analysis. Accepted for publication in World J Surg, 1992.

13. Irvin GL and Bagwell CB. Identification of histologically undetectable parathyroid hyperplasia by flow cytometry. Am J Surg, 138, 567, 1979.

14. Irvin GL, Taupier MA, Block NL and Reiss E. DNA patterns in parathyroid disease predict postoperative parathyroid hormone secretion. Surgery, 104, 1115, 1988.

15. Harlow S, Roth SI and Marshall RB. FLow cytometric DNA analysis of normal and pathologic parathyroid glands. Modern Pathology, 4, 310, 1991.

16. Bowlby LS, DeBault and Abraham SR. Flow cytometric DNA analysis of parathyroidglands. Relationship between nuclear DNA and pathologic classifications. Am J Path , 128, 338, 1987.

17. Shenton BK, Ellis H, Johnston IDA and Farndon JR. DNA analysis and parathyroid pathology. World J Surg, 14, 296, 1990.

18. Joensuu H and Klemi PJ. DNA aneuploidy in adenomas of endocrine organs. Am J Pathol, 132, 145, 1988.

19. Friedlander ML, Hedley DW and Taylor IW. Clinical and biological significance of aneuploidy in human tumours. J Clin Pathol, 37, 961, 1984.

20. Izuo M, Okagaki T, Richart RM and Lattes R. Nuclear DNA content in hyperplastic lesions of cystic disease of the breast with special reference to malignant alteration. Cancer, 27, 620, 1971.

21. Izuo M, Okagaki T, Richart RM and Lattes R. DNA content in "apocrine metaplasia" of fibrocystic disease of the breast. Cancer, 27, 643, 1971.

22. Obara T, Fujimoto Y, Kanaji Y, Okamoto T, Hirayama A, Ito Y and Kodama T. Flow cytometric DNA analysis of parathyroid tumors. Implications of aneuploidy for pathologic and biologic classification. Cancer, 66, 1555, 1990.

23. Nowell PC. The clonal evolution of tumor-cell populations. Science, 194, 23, 1976.

24. Pierce GB and Speers WC. Tumors as caricatures of the process of tissue renewal:prospects for therapy by directing differentiation. Cancer Research, 48, 1996, 1988.

25. Arnold A, Staunton CE, Kim HG, Gaz RD and Kronenberg HM. Monoclonality and abnormal parathyroid genes in parathyroid adenomas. N Eng J Med, 318,658,1988.

26. Fialkow PJ, Jackson CE, Block MA and Greenawald KA. Multicellular origin of parathyroid "adenomas". N Eng J Med, 297, 696, 1977.

Chapter 7

GENERAL DISCUSSION

The two-site immunoassay which measures intact parathyroid hormone is the gold standard to establish the diagnosis of primary hyperparathyroidism. This assay provides dependable discrimination of patients with different forms of hypercalcemia, and as a rule allows for a rapid diagnosis of primary hyperparathyroidism.

Localization studies of parathyroid glands are unnecessary in first explorations of the neck since the success rate of first parathyroidectomies by experienced surgeons is over 95 %.[1] However, in patients with persistent or recurrent hyperparathyroidism localization studies can be helpful in localizing enlarged parathyroid glands. The sensitivity to detect enlarged parathyroid glands varies considerably and depends greatly on the experience of the investigator. Unfortunately localization studies are unable to detect parathyroid glands smaller than 5 mm or weighing less than 100 mg. Particularly these small glands can be difficult to find at exploration of the neck.

The understanding of the pathology of primary hyperparathyroidism is crucial to determine the optimal surgical treatment of primary hyperparathyroidism. The role of the histological features of parathyroid glands remains controversial. The traditional concept of "parathyroid adenomas" and "hyperplasia" is debatable because histologic features which differentiate "adenomas" and "hyperplasia" consistently have never been reported.[2] In spite of this, the majority of surgeons and pathologists uses this concept, and the extent of resection of parathyroid tissue is determined by the findings at microscopical examination of frozen sections. Several authors state that normal-size parathyroid glands with hyperplastic features are potentially hyperactive and should be removed.[3-5] Unfortunately, the criteria for hyperplasia are poorly described in these studies. The duration of follow-up is usually short or not documented, and persistent or recurrent hyperparathyroidism are not precisely defined. To establish potential hormonal hyperactivity of normal size parathyroid glands long term follow-up studies are necessary. In addition, persistent and recurrent hyperparathyroidism need to be separated properly. Persistent hyperparathyroidism is caused by missed enlarged parathyroid glands at the first parathyroidectomy, and therefore, is a measure for the surgical skill. Recurrent hyperparathyroidism is caused by growth of a parathyroid gland which had a normal-size at the first exploration of the neck, and is therefore, related to the biology of the disease. Excluding patients with multiple endocrine neoplasia (MEN type 1 and 2) and familial hyperparathyroidism, in the present study normal-size parathyroid glands were left behind irrespective of their histological appearances and the number of

enlarged parathyroid glands. After a mean follow-up of 13.5 years the rate of recurrent hyperparathyroidism was 0.7 %. Therefore, we conclude that the only important parameter of hormonal function of parathyroid glands is the size of the gland. The consequence of this observation is the use of the terminology "single and multiple gland disease".

In this study an attempt has been made to correlate histological and flowcytometric findings of parathyroid glands with the gross classification as single or multiple gland disease. The traditional dogma was that a "parathyroid adenoma" is associated with enlargement of a single parathyroid gland and "hyperplasia" with enlargement or rather disease of all parathyroid glands. Since the traditional microscopical criteria for "parathyroid adenoma" and "hyperplasia" had proven to be unable to differentiate these two entities, the criteria recently proposed by Ghandur and Kimura were applied.[6] Normal parathyroid glands were distinguished from abnormal glands fairly accurately by microscopical examination (sensitivity = 93 %, specificity = 77 %). However, the correlation between "parathyroid adenoma" and "hyperplasia" and single and multiple gland disease was poor. A multidiscriminate analysis was also unable to find characteristic histologic patterns in single and multiple gland disease. Flow cytometric nuclear DNA analysis showed similar results as the histological study: significant differences between normal-size and enlarged parathyroid glands but no difference between single and multiple gland disease.

Primary hyperparathyroidism can be caused by enlargement of only two or three parathyroid glands. In this study 120 patients had two enlarged parathyroid glands, and 40 patients had three enlarged parathyroid glands. Only 18 patients had four enlarged parathyroid glands. Seventy-two of the patients with two enlarged parathyroid glands had one enlarged parathyroid on the right side of the neck and the other on the left. A unilateral approach in these patients could have resulted in a high incidence of persistent hyperparathyroidism.

In our opinion, removal of only enlarged parathyroid glands in non-familial primary hyperparathyroidism is associated with acceptable rates of persistent and recurrent hyperparathyroidism. Evidence is lacking that normal-size parathyroid glands are potentially hyperactive. The role of microscopic examination of parathyroid tissue seems to be limited to the distinction of normal from abnormal parathyroid tissue. Flow cytometric DNA analysis can objectively differentiate normal from abnormal parathyroid glands. Although primary hyperparathyroidism can be treated successfully the pathogenesis of primary hyperparathyroidism remains to be elucidated.

References

1. Satava RM, Beahrs OH and Scholz DA. Success rate of cervical exploration for primary hyperparathyroidism. Arch Surg, 110, 625, 1975.

2. Nishiyama RH. Pathology of parathyroid tumors. In: Comprehensive management of head and neck tumors. Vol. 2 WB Saunders Company 1987, 1650.

3. Paloyan E, Lawrence AM, Baker WH and Straus FH. Near-total parathyroidectomy. Surg Clin North Am, 49, 43, 1969.

4. Rudberg C, Akerstrom G, Palmer M, Ljunghall S, Adami HO, Johansson H, Grimelius L, Thoren L and Bergstrom R. Late results of operation for primary hyperparathyroidism. Surgery, 99, 643, 1986.

5. Cooke TJC, Boey JH and Sweeney CW. Parathyroidectomy: extent of resection and late results. Br J Surg, 64, 153, 1977.

6. Ghandur-Mnaymneh L and Kimura N. The parathyroid adenoma: A histologic definition with a study of 172 cases of primary hyperparathyroidism. Am J Pathol, 115, 70, 1984.

Chapter 8

SUMMARY

SAMENVATTING

Primary hyperparathyroidism is a common disease with an incidence of 25 to 28 cases per 100,000 population per year. The clinical picture of primary hyperparathyroidism ranges from absence of symptoms to severe bone and/or renal disease. The etiology and pathogenesis of primary hyperparathyroidism remain poorly understood. As a consequence the surgical treatment varies considerably.

In chapter 1 the historical aspects of the clinical picture, the pathology and the surgical treatment of primary hyperparathyroidism are described. Subsequently the scope of the thesis is presented.

In chapter 2 the diagnostic procedures for primary hyperparathyroidism are reported. The diagnostic value of the assessment of serum calcium, serum phosphate, serum chloride / serum phosphate ratio, serum alkaline phosphatase, urinary excretion of cyclic adenosine mono phosphate (cAMP), maximal reabsorption of phosphate in terms of renal function (TmP / GFR ratio), calcium infusion, renal excretion of calcium, bone histology and radiologic studies of the skeleton are discussed. None of these parameters is highly sensitive or specific for primary hyperparathyroidism. Discriminate analysis of several parameters increases the diagnostic accuracy. The diagnostic value of carboxyl-terminal and mid-region immuno-assays is limited in spite of a satisfactory sensitivity, because these immuno-assays detect biologically inactive fragments of parathyroid hormone. The amino-terminal assay recognizes the biologically active part of the parathyroid hormone, but lacks the sensitivity to measure normal levels of serum parathyroid hormone. Presently the gold standard to establish the diagnosis of primary hyperparathyroidism is the two-site immuno-radiometric assay which recognizes the intact parathyroid hormone, and also detects low levels of serum parathyroid hormone.

In chapter 3 the value of ultrasonography, computed tomography scanning, magnetic resonance imaging (MRI), thallium-technetium subtraction scanning, angiography and venous sampling for parathyroid hormone to localize enlarged parathyroid glands is reviewed. The smallest parathyroid gland which ever has been localized measures 5 mm and weighs 100 mg. The sensitivity of localization studies depends greatly on the experience of the investigator. Combining imaging studies increases the rate of detecting enlarged parathyroid glands. Superselective arterial digital subtraction angiography should be considered in patients suspected to have a mediastinal parathyroid tumor.

In chapter 4 the results of follow-up studies in 693 patients with non-familial primary hyperparathyroidism are reported. All patients had selective removal of enlarged parathyroid glands (estimated weights > 40 mg)

irrespective of their histological appearances. After a mean follow-up of 13.5 years the rates of persistent and recurrent hyperparathyroidism were, respectively, 3.6 and 0.7 %. Evidence was lacking that normal-size parathyroid glands were potentially hyperactive. Transient and permanent hypoparathyroidism occurred in 24 and 2.5 %. Removal of only enlarged parathyroid glands is recommended as the surgical treatment of non-familial primary hyperparathyroidism. Biopsies of parathyroid glands should only be taken sparingly to prevent transient and permanent hypoparathyroidism.

In chapter 5 the histological features of the parathyroid glands of 236 patients with non-familial primary hyperparathyroidism are presented. Normal parathyroid glands were distinguished from abnormal parathyroid glands fairly accurately (sensitivity = 93 %, specificity = 77 %). However, histological classification of enlarged parathyroid glands as "parathyroid adenomas" or "hyperplasia" correlated poorly with the gross classification of single and multiple gland disease. Multidiscriminate analysis of the histological features of the parathyroid glands showed a highly significant difference comparing normal-size parathyroid glands and enlarged parathyroid glands (Eigenvalue = 1.0992). Microscopic cellularity was the most important differentiating feature. Comparing single and multiple enlarged parathyroid glands showed a far less significant difference (Eigenvalue = 0.317).

In chapter 6 the results of flow cytometric nuclear DNA analysis of paraffin embedded parathyroid tissue from 66 patients with non-familial primary hyperparathyroidism are reported. Significant differences for DNA index, % S-phase, % G2M were found comparing normal-size and enlarged parathyroid glands ($p < 0.001$), but none between single and multiple enlarged parathyroid glands.

Primaire hyperparathyreoidie is een frequent voorkomende ziekte met een jaarlijkse incidentie van 25 tot 28 per 100.000. Het klinische beeld is variabel. Sommige patienten zijn asymptomatisch, anderen hebben ernstige skelet- en/of nierafwijkingen. De etiologie en pathogenese van primaire hyperparathyreoidie zijn onduidelijk. Als een gevolg hiervan bestaan er verschillende inzichten ten aanzien van de optimale chirurgische therapie.

In hoofdstuk 1 worden de historische aspecten van het klinische beeld, de pathologie en de chirurgische behandeling van primaire hyperparathyreoidie uiteengezet. Vervolgens wordt de vraagstelling van dit proefschrift geformuleerd.

In hoofdstuk 2 wordt de diagnostiek van primaire hyperparathyreoidie beschreven. De diagnostische waarde van serum calcium gehalte, serum fosfaat , serum chloor / fosfaat ratio, serum alkalische fosfatase, uitscheiding van cyclisch adenosine monofosfaat (cAMP) in de urine, maximale reabsorptie van fosfaat gerelateerd aan de nierfunctie (TmP / GFR ratio), calcium infusie, calcium uitscheiding, bothistologie and rontgen diagnostiek van het skelet worden besproken. Geen van deze parameters is specifiek voor primaire hyperparathyreoidie. Combinatie van diverse parameters vergroot de diagnostische sensitiviteit en specificiteit. Immunoassays voor het carboxyl- of midden gedeelte van het bijschildklier hormoon hebben een hoge sensitiviteit. Deze bepalingen meten echter biologisch niet actieve fragmenten van het bijschildklier hormoon. De immunoassays voor het amino uiteinde van het bijschildklier hormoon herkennen het biologisch actieve gedeelte van het bijschildklier hormoon maar hebben een hoge detectie drempel. De gouden standaard voor de diagnose van primaire hyperparathyreoidie is de two-site immuno-radiometrische bepaling (IRMA) die het gehele bijschidklierhormoon herkent , ook in zeer lage concentraties.

In hoofdstuk 3 wordt de waarde van echografie, CT-scan, nucleaire magnetische resonantie (NMR), thallium-technetium subtractie scan, angiografie en veneuze meting van het bijschildklierhormoon als lokalisatie techniek voor vergrote bijschildklieren besproken. De kleinste bijschidklier die bij pre-operatief onderzoek is waargenomen heeft een diameter van 5 mm en een gewicht van 100 mg. De gevoeligheid van lokalisatie technieken is sterk afhankelijk van de ervaring van de onderzoeker. Combinatie van meerdere lokalisatie studies verhoogt de detectie kans. Superselectieve arteriele digitale subtractie angiografie dient overwogen te worden bij patienten die mogelijk een vergrote bijschildklier in het mediastinum hebben.

In hoofdstuk 4 worden de resultaten van follow-up onderzoek bij 693

patienten met niet-familiaire primaire hyperparathyreoidie gepresenteerd. Bij alle patienten werden alleen de vergrote bijschildklieren (geschat gewicht groter dan 40 mg) verwijderd onafhankelijk van de histologische bevindingen. Na een gemiddelde follow-up van 13,5 jaar werd persisterende hyperparathyreoidie bij 3,6 % van de patienten en recidiverende hyperparathyreoidie bij 0,7 % van de patienten gevonden. Bijschildklieren met een normale grootte leken een normale hormonale functie te hebben en geen aanleiding te geven tot recidiverende hyperparathyreoidie. Tijdelijke en blijvende hypoparathyreoidie kwamen respectievelijk bij 24 en 2,5 % van de patienten voor. Verwijdering van vergrote bijschildklieren bij niet-familiaire primaire hyperparathyreoidie wordt aanbevolen als chirurgische therapie. Een biopsie van een bijschidklier dient alleen genomen te worden als er twijfel bestaat over de aanwezigheid van bijschildklierweefsel.

In hoofdstuk 5 worden de histologische beelden van de bijschildklieren van 236 patienten met niet-familiaire primaire hyperparathyreoidie beschreven. Normale bijschidklieren werden goed onderscheiden van abnormale bijschildklieren (sensitiviteit = 93 %, specificiteit = 77 %). Histologische classificatie van bijschildkleren als "adenomen" of "hyperplasie" correleerde echter niet met de aanwezigheid van een of meer vergrote bijschildklieren. Multidiscriminant analyse van de histologische bevindingen toonde een statistisch significant verschil tussen normale en abnormale bijschildklieren (Eigen-waarde = 1,0992). Microscopische hypercellulariteit vormde het belangrijkste criterium om normale en abnormale bijschildklieren te onderscheiden. Een statistisch significant verschil tussen "single gland disease" en "multiple gland disease" werd niet gevonden (Eigen-waarde = 0.317).

In hoofdstuk 6 worden de resultaten van flow cytometrie van bijschildklieren van 66 patienten met niet-familiaire primaire hyperparathyreoidie beschreven. De DNA-index, het percentage cellen in de synthese fase en het percentage cellen in de G2M fase bleken statistisch significant te verschillen bij vergelijking van normale en vergrote bijschildklieren (p < 0,001). Een statistisch verschil tussen "single gland disease" en "multiple gland disease" werd niet gevonden.

ACKNOWLEDGEMENTS

CURRICULUM VITAE

Hendrik Jacob Bonjer werd geboren op 15 mei 1960 in Leiden. Het voorbereidend wetenschappelijk onderwijs volgde hij op het Rijnlands Lyceum in Oegstgeest. De studie geneeskunde vond plaats aan de Rijksuniversiteit te Leiden van 1978 tot 1985. De eerste praktijkervaring deed hij op als marine arts aan boord van de Hr. Ms. Haarlem, waarna hij op de afdeling chirurgie van het Marine Hospitaal in Overveen werkte (KLTZ R. E. Kleinveld). Na een korte periode op de afdeling chirurgie van het Rode Kruis Ziekenhuis in Den Haag (opleider: Dr. J. J. Hamming) werd in 1987 de opleiding tot algemeen chirurg begonnen in het Academisch Ziekenhuis Dijkzigt in Rotterdam (opleiders: Prof. Dr. J. Jeekel en Prof. Dr. H. A. Bruining). Begin 1990 deed hij wetenschappelijk onderzoek in het Department of Surgical Pathology, Maine Medical Center (R. H. Nishiyama, M. D.) en in het Maine Cytometry Research Institute (E. J. Lovett III, PhD and C. B. Bagwell, M.D., PhD) in Portland, Maine. In mei 1990 werd de opleiding in de algemene chirurgie voortgezet in het St. Clara Ziekenhuis (opleider: Dr. T. I. Yo).

.