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Optimal Discrimination of Mild Hyperparathyroidism with Total Serum Calcium, Ionized Calcium and Parathyroid Hormone Measurements

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ABSTRACT

The serum concentrations of calcium, albumin and parathyroid hormone (PTH) and the plasma levels of ionized calcium were determined in 124 healthy subjects, 89 patients with primary hyperparathyroidism (HPT), 23 of whom had the syndrome of multiple endocrine neoplasia type 1 (MEN-1) and 43 patients who had hypercalcaemia of other causes than HPT (non-HPT), in most cases due to widespread malignancies. The total serum calcium was corrected for the serum albumin concentration (CaM). Healthy females over the age of 50 had higher CaM, than younger females and the women of all ages also had, higher serum PTH levels than males. For all study groups both the intra- and inter-diurnal variations were small for all the studied variables. Discriminant function and optimal discriminatory limits were calculated with the help of computer programs. A consideration of all the individuals in the discriminant analysis, revealed that measurements of CaM alone separated most HPT patients both from the healthy subjects and from the non-HPT patients. However, when only those who had borderline values (defined as CaM between 2.45 and 2.75 mmol/l) were included it turned out that measurements of ionized calcium markedly improved the delineation of mild HPT from the healthy subjects and that, in addition, PTH measurements helped to exclude those with non-HPT hypercalcaemia. The optimal discriminatory levels of serum calcium were calculated as the levels which caused the minimum loss in terms of misclassification when attention was paid to the relative importance of false positive to false negative classifications and to the prevalence of HPT. The optimal discriminatory level for serum calcium for a weighting ratio between false positive to false negative of 1:1, and a prevalence of HPT of 1 %, was calculated to be 2.68 mmol/l and for a prevalence of 50 % 2.56 mmol/l. In the latter situation a weighting ratio of 10:1 for false positive to false negative gave a level of 2.63 mmol/l while a weighting ratio of 1:10 corresponded to an optimal discriminatory level of 2.47 mmol/l.

INTRODUCTION

Primary hyperparathyroidism (HPT) is a common disorder, known to affect one per cent of the population above 60 years of age and occurring at even higher frequencies in older individuals (11,36,49). The high prevalence of the disease calls for accurate screening and diagnostic methods which can easily provide an identification of the diseased patients with a minimal number of misclassifications.

The identification of patients with HPT in the clinical routine relies primarily on the demonstration of hypercalcemia. In a previous study single measurements of total serum calcium were used to calculate optimal discriminating limits for the diagnosis of primary HPT (18). In the present report we have extended this analysis to include measurements of the serum concentrations of parathyroid hormone (PTH) and of ionized calcium, determinations of which have become more widely available during recent years.

Practically all studies dealing with the diagnosis of HPT are based on materials where the majority of patients have marked hypercalcaemia. In clinical practice, however, most patients with suspected HPT nowadays have only mildly elevated serum calcium values (20,50).

The present study therefore devotes particular attention to the potential value of ionized calcium and PTH measurements in the delineation of mild HPT. In many instances there may not be a great need to establish the diagnosis of HPT in such borderline cases. Sometimes, however, a precise classification could be important. For example we recently reported (2) that in the dominantly inherited syndrome of multiple endocrine neoplasia type 1 (MEN-1) HPT is apparently the first manifestation. The demonstration of mild HPT therefore is the earliest opportunity to disclose the carrier of the MEN-1 trait. Patients with recurrent renal stones constitute another group where it is highly desirable to obtain definite evidence for or against HPT despite undecided hypercalcemia.

Another clinical problem in the diagnosis of HPT consists of the exclusion of other causes of hypercalcemia, primarily malignant disorders. Although these are generally evident clinically, additional investigations are sometimes required.

REFERENCE SAMPLE GROUPS

Healthy subjects

From a health survey in Uppsala county 98 apparently healthy individuals were recruited to represent a "healthy reference sample group"; 52 men and 46 women, aged between 16 to 92, with a mean age of 46 ± 20 years (mean ± SD), and equal distribution of the sexes in all age groups. Twenty-six apparently healthy employees from the hospital staff, 10 men and 16 women, aged between 20-60 years, participated in studies of the intra- and, inter-diurnal variations.

Patients with HPT: 89 consecutive patients operated for HPT, 27 men and 62 women, aged between 19-83 with a mean of 60 ± 17 years were studied before operation: 66 of them had sporadic HPT and serum calcium was normalized postoperatively in all cases. The other 23, 16 women and seven men, had HPT as a part of MEN-1, nine of them had persistent or recurrent hypercalcaemia after previous operations. Five of them also had an endocrine tumor of the pancreas, in three cases with liver metastases. Neck exploration confirmed parathyroid hyperplasia. Subtotal or total parathyroidectomy with autotransplantation was performed. Serum calcium returned to the normal range postoperatively in all cases.

Patients with hypercalcemia of other origin than HPT (Non -HPT):

Forty-three patients, 21 men and 22 women, aged 38-78 with a mean age of 60 ± 15 years had hypercalcemia of clinically obvious causes other than HPT.

Malignancy was the most common cause, being encountered in 30 patients, 13 men and 17 women. Renal cancer was seen in seven cases, five had cancer mammae, five had lung cancer, five had myeloma, three cancer of the pancreas, three lymphomas, one had thyroid cancer and one patient had a leiomyosarcoma. In most cases bone metastases were evident. Other causes of hypercalcemia were encountered in 13 patients, six men and seven women. Sarcoidosis was seen in seven patients, whose serum calcium values normalized upon treatment with steroids. Two patients had thyrotoxicosis, and became normocalcemic in response to medical treatment. Immobilization due to tetraplegia was the cause of hypercalcaemia in four patients.

LABORATORY METHODS

Blood specimens were collected between 07.00-09.00 on the morning following an overnight fast. For each patient the mean value of all such measurements was calculated and used as the basal value. For the study of variation during the day, specimens were also collected before meals at 12.00 and 16.00 hours. No diet restrictions apart from a ban on milk and cheese were imposed.

Ionized calcium (CaI): Whole blood was collected anaerobically in 5 ml heparinized tubes and analyzed within a few hours for ionized calcium with an ion-selective electrode (Microlyte, Kone Instruments, Finland). All samples were measured in duplicate. The analyzer has an automatic three point calibration procedure using water standards adjusted for ionic strength and pH. After each sample a middle standard is measured for assessment of drift. The temperature of the electrode block is maintained at $30^{o}C$. In patient samples the average analytical within-run standard deviation was 0.012 mmol/l at the level of 1.15 mmol/l and 0.019 mmol/l at the level of 1.45 mmol/l. The life-span of an electrode is 3-6 months. After change of electrode, the values (n=178) from a reference population of healthy individuals were compared with previous values (n=100) in the same individuals. A difference of 0.01 mmol/l was observed for the mean values, without change in imprecision.

There are no external control standards for ionized calcium, and external quality assessment schemes have stressed the use of protein-enriched solutions (44). In the absence of an external control sample, reconstituted lyophilized control sera (Validate, General Diagnostics) from the same batch - actually not manufactured for this purpose and not always within the reference range for healthy subjects, - have been used as an external control over time with a CV of 1.4 % at 1.20 mmol/l and 2.3 % at 1.60 mmol/l. No effort was taken to improve standardization of water, temperature, pH or gas content (9).

Serum was obtained after clotting and centrifugation, and was either analyzed in the ordinary laboratory routine the same day, or stored at $+4^{o}$ overnight and analyzed the next day.

Total serum calcium (CaT) concentrations were determined by an atomic absorption technique (Perkin-Elmer 3030). The analytical procedure for determination of serum calcium had an average within-run standard deviation of 0.038 mmol/l, and an average between-run standard deviation of

0.022 mmol/l, giving a total analytical standard deviation of 0.044 mmol/l. All values refer to a concentration level of 2.46 mmol/l, and the analytical error was assumed to be the same for all concentration levels expressed as a coefficient of variation (CV=0.018). The analytical bias of the procedure was about -1% compared with the referendum value of the regional external quality assessment program.

Serum albumin was determined by a bromcresol-binding technique and calibrated with purified human albumin solution. The analytical within-run standard deviation was 0.42 g/l and the between-run standard deviation 0.71 g/l, giving a total analytical standard deviation of 0.83 g/l.

Correction of CaT for serum albumin concentration (CaM): As serum calcium is bound to albumin, a correction (modification) of serum calcium values was made for deviations of the actual albumin from the reference mean value of 42 g/l by the following formula used in our laboratory:

$$CaM = CaT - 0.019\ (S\text{-albumin} - 42)\ \ mmol/l$$

Radioimmunoassay of parathyroid hormone (PTH) in serum

Serum specimens were kept at - 20°C until analyzed. The PTH concentration was determined by a radioimmunoassay system employing ^{121}I-labelled bovine PTH (Inolex) and sheep antiserum (S 478) against porcine and bovine PTH. This antiserum reacts with a mid-portion (44-68) of human PTH but has also a high affinity (0.6×10^{13} l/mmol) for intact human PTH (21).

The assay procedure used solid phase-coupled anti-sheep-IgG to separate bound and free labelled PTH. The serum specimens and the antiserum were first incubated for 24 h at 4°C followed by a 48 h incubation with labelled PTH. Microsepharose-coupled horse anti-sheep-IgG (decanting suspension 2, Pharmacia AB, Sweden) was then added and the incubation was prolonged for 3 h. The particles were centrifuged down during 5 min at 2000 g and the pellet was washed once with saline containing 0.5 % Tween-20. Bovine PTH diluted in human sera with low PTH levels was used as laboratory standard and the concentration of PTH in human serum was expressed in arbitrary units (arb U/l). About 2.5 arb U were equivalent to 10 U NIBSC research standard for human PTH 75/479. All specimens were assayed in duplicate. The total assay coefficient of variation in 42 assays was 9.2%, with an average within-assay coefficient of variation of 3.6% at a level of 0.7-0.8 arb U/l.

The disappearance of serum PTH following parathyroidectomy was investigated in 10 patients with adenomatous HPT, basal values of 0.81 - 1.24 arbU/l and a normal kidney function. A mean reduction of 17% was found within the first 15 minutes, which demonstrated a capability of the assay system rapidly to detect changes in serum concentrations (Fig. 1).

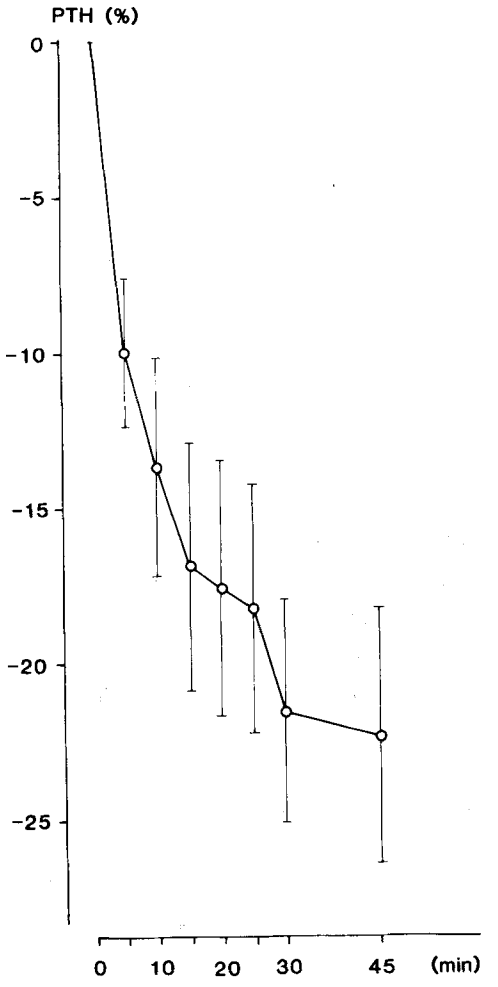

Fig. 1. Reduction of serum parathyroid hormone (PTH) concentrations after parathyroidectomy in 10 patients with adenomatous hyperparathyroidism. (Bars indicate SEM).

COMPUTATIONAL METHODS

All statistical calculations were performed on a BASF 7/73 -IBM/MVS computer system at Uppsala University Data Center. The Statistical Analysis System package (SAS Institute Inc., North Carolina USA) was used for descriptive statistics (means and standard deviations of various reference sample groups; biological intra- and inter-diurnal variation; cross-plotting; statistical goodness-of-fit-tests), linear regression analysis; and non-linear parameter fitting.

Stepwise discriminant analysis was performed with the BMDP program package (Biomedical Computer Programs, P-series, University of California, 1977).

A program for analysis of variance on a LUXOR ABC-80 computer was used to calculate the analytical within-, and between-run variation.

Optimal discriminatory limits were calculated with the help of a program developed at the Unit of Biomedical Systems Analysis (18). This program calculates:

(a) the expected frequency of false negative and false positive outcomes in connection with classification, using a specified discriminatory level;
(b) a measure of loss related to misclassification,
(c) the optimal discriminatory limit, ($\hat{c}$), and
(d) the diagnostic sensitivity, specificity and the predicted value of a positive and negative test result.

Input data to the program are:

(a) frequency distributions of the reference populations; in this case distributions representing healthy individuals, patients with sporadic HPT or HPT as part of MEN-1, as well as patients with non-HPT hypercalcemia;
(b) prevalence of the diseases, expressed as number-ratios;
(c) numerical weights, W1 and W2, representing the relative costs of making misclassifications;
(d) pre-analytical and analytical variation expressed as coefficients of variation: CV_{pre-a} and CV_a, respectively;

The variances of frequency distributions representing the different reference populations are calculated from:

$$S^2_{total} = S^2_{biol} + S^2_{pre\text{-}a} + S^2_a$$

and therefore

$$S^2_{biol} = S^2_{total} - (S^2_{pre\text{-}a} + S^2_a)$$

where S_{biol} is the total biological standard deviation (including intra-, and inter-individual variation); $S_{pre\text{-}a}$ is the pre-analytical standard deviation, i.e. the variation related to specimen handling; and S_a is the analytical standard deviation.

In the program the calculations are performed to estimate the "tail-areas" cut-off by a specified discriminatory limit from the different distributions, giving values for the number of false positives (FP) and false negatives (FN).

As previously described (18) the loss is calculated as $A=(W1 \times FP + W2 \times FN)/\phi 0$ where ϕ is the loss under ideal conditions, $CV_a=0$ and $CV_{pre\text{-}a}=0$. Since the weighting factors are given in relative numbers, the loss should be regarded as a relative loss. The calculations are automatically repeated for a number of different values for the discriminatory level, in order to allow determination of the optimal value.

RESULTS

Descriptive statistics:

Table 1 summarizes the mean values for the studied variables in all the five groups of subjects. The mean calcium values were higher in the patients with non-HPT hypercalcaemia than in the HPT patients. The HPT patients had a higher mean value for PTH than all other groups.

There were small, but statistically significant, age- and sex-related differences within the group of normal subjects (Table 2). Women over the age of 50 had higher CaM values than younger females. On the other hand males over the age of 50 showed lower CaT (but not CaM) values than did the younger men. No differences were noted for Cal between the subgroups. Females of all ages had clearly higher values for PTH than males.

The average total intra-diurnal variations (including biological, pre-analytical and analytical) for the different groups are given in Table 3. There were no significant variations over the day in any of these groups for any of the variables studied.

Table 4 gives the intra-individual variations both within and between days. As can be seen in the Table the variations for the calcium measure-

ments were somewhat greater in the hypercalcaemic individuals than in the healthy subjects. Naturally, for all studied variables the inter-diurnal variations were greater than the intra-diurnal but generally the differences were small.

On the basis of these measurements it could be calculated that the biological SD for the Cal values was 0.045 mmol/l for both healthy subjects and patients with HPT while for CaM it was 0.055 mmol/l in both groups. Similarly the biological variation for PTH was calculated to be 0.14 arbU/l in the healthy subjects and 0.09 arbU/l in the patients with HPT.

Table 1. Mean value, standard deviation (SD), and standard error of the mean (SEM) for the measured variables in the different subject groups.

	n	Mean	SD	SEM
Plasma ionized calcium (mmol/l) (Cal)				
Healthy	93	1.203	0.047	0.005
HPT, Sporadic	52	1.400	0.17	0.024
HPT/MEN	12	1.416	0.133	0.038
Non-HPT	43	1.513	0.242	0.037
Total serum calcium (mmol/l) (CaT)				
Healthy	98	2.425	0.082	0.009
HPT, Sporadic	65	2.836	0.287	0.035
HPT/MEN	23	2.745	0.192	0.040
Non-HPT	43	3.103	0.538	0.081
Serum albumin (g/l)				
Healthy	98	43.06	2.86	0.30
HPT, Sporadic	65	38.50	3.83	0.28
HPT/MEN	23	39.78	4.55	0.62
Non-HPT	43	35.32	5.49	0.89
Albumin-modified serum calcium (mmol/l) (CaM)				
Healthy	98	2.415	0.079	0.008
HPT, Sporadic	65	2.908	0.301	0.037
HPT/MEN	23	2.767	0.205	0.043
Non-HPT	43	3.245	0.526	0.079
Serum PTH (arb U/l)				
Healthy	98	0.75	0.17	0.02
HPT, Sporadic	65	1.27	0.68	0.05
HPT/MEN	23	1.20	0.50	0.07
Non-HPT	43	0.80	0.25	0.04

Table 2. Values in healthy subjects separated with regard to age and sex (Mean ± SD).

	AGE		
	< 50 years (n = 50)	> 50 years (n = 48)	All
Cal (mmol/l)			
Men	1.21 ± 0.05	1.20 ± 0.06	1.21 ± 0.05
Women	1.20 ± 0.04	1.21 ± 0.04	1.20 ± 0.04
CaT			
Men	2.45 ± 0.07	2.40 ± 0.08*)	2.43 ± 0.07
Women	2.41 ± 0.10	2.42 ± 0.09	2.40 ± 0.09
Albumin (g/l)			
Men	44.2 ± 2.9	42.2 ± 3.3*)	43.5 ± 3.2
Women	44.0 ± 3.5	41.9 ± 1.8**)	43.0 ± 3.0
CaM			
Men	2.40 ± 0.07	2.40 ± 0.09	2.40 ± 0.07
Women	2.38 ± 0.08	2.43 ± 0.08**)	2.40 ± 0.08
PTH (arb U/l)			
Men	0.67 ± 0.17	0.71 ± 0.14	0.68 ± 0.16
Women	0.82 ± 0.18	0.85 ± 0.14	0.83 ± 0.16***)

*) $p < 0.05$ compared with men < 50 years.
**) $p < 0.05$ compared with women < 50 years.
***) $p < 0.001$ compared with males.

Relationships between Cal and CaM

Both the healthy subjects ($r = 0.33$; $p = 0.001$) and the HPT patients ($r = 0.90$; $p<0.001$) displayed highly significant correlations between the values for CaM and Cal. However, the slope for the regression equation was steeper for the HPT patients than for the healthy subjects (Fig. 2). There were no significant differences as regards the relationships between CaM and Cal (data not shown) between HPT patients and those with other causes of hypercalcaemia . Within the range of CaM values between 2.45 and 2.75 mmol/l there was no significant correlation between the CaM and Cal values for any of the study groups (Fig. 3).

Table 3. Average total intra-diurnal variations in healthy subjects (n = 52), and patients with hyperparathyroidism (n = 50) and other hypercalcaemia (n = 2)).

	Healthy subjects		HPT		Non-HPT	
Hour	Mean	SD	Mean	SD	Mean	SD
	CaI (mmol/l)					
08.00	1.15	0.045	1.40	0.179	1.37	0.145
12.00	1.15	0.042	1.40	0.183	1.36	0.142
16.00	1.14	0.051	1.38	0.198	1.32	0.149
	CaT (mmol/l)					
08.00	2.45	0.102	2.90	0.375	2.80	0.357
12.00	2.45	0.082	2.94	0.388	2.79	0.340
16.00	2.47	0.095	2.90	0.362	2.77	0.304
	Albumin (g/l)					
08.00	42.1	3.1	40.0	3.7	33.7	2.4
12.00	42.9	2.6	40.5	4.2	33.1	3.3
16.00	42.9	3.3	39.5	3.4	33.2	3.7
	CaM (mmol/l)					
08.00	2.46	0.07	2.91	0.39	2.97	0.38
12.00	2.45	0.06	2.98	0.39	2.93	0.35
16.00	2.46	0.06	2.91	0.36	2.91	0.34
	PTH (arb U/l)					
08.00	0.80	0.14	1.16	0.53	0.78	0.27
12.00	0.80	0.14	1.17	0.53	0.78	0.26
16.00	0.81	0.13	1.18	0.55	0.80	0.27

Relationships between CaM and PTH

There were no significant correlations between the CaM and PTH values either in the group of healthy subjects (r = 0.04) or among those with non-HPT hypercalcaemia (Fig. 4).

Table 4. Average total intra-individual variation within and between days.

	Within-day (SD)	Between-day (SD)
CaI (mmol/l)		
Healthy	0.019	0.027
HPT	0.026	0.031
Non-HPT	0.041	0.060
CaT (mmol/l)		
Healthy	0.043	0.032
HPT	0.054	0.071
Non-HPT	0.050	0.111
Albumin (g/l)		
Healthy	0.221	1.187
HPT	1.502	1.750
Non-HPT	0.992	1.110
CaM (mmol/l)		
Healthy	0.049	0.07
HPT	0.051	0.07
Non-HPT	0.063	0.12
PTH (arb U/l)		
Healthy	0.044	0.16
HPT	0.050	0.12
Non-HPT	0.045	0.05

When all the CaM values for the HPT patients were considered a highly significant positive correlation between CaM and PTH was evident ($r = 0.62$; $p<0.001$) as seen in Fig. 4. There was no significant difference in this respect between HPT associated with MEN-1 and the sporadic form.

When only CaM values below 2.75 mmol/l were considered there was, however, no significant correlation ($r = 0.20$; $p = 0.30$) between the values in the HPT patients (Fig. 5).

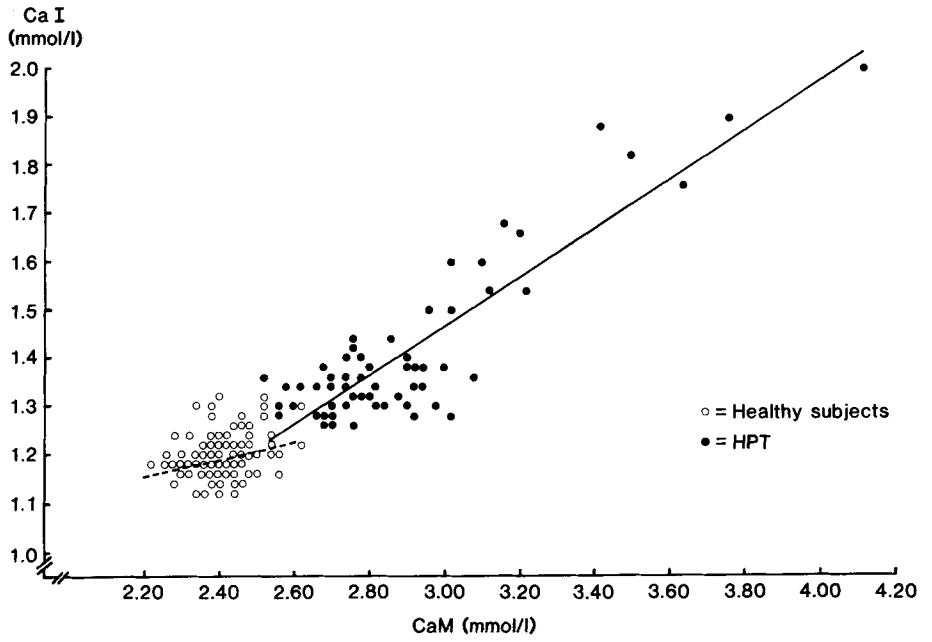

Fig. 2. Relationship between albumin-corrected serum calcium (CaM) and plasma ionized calcium (CaI) concentrations in healthy subjects and patients with hyperparathyroidism (HPT). The interrupted line denotes the regression line for the healthy subjects and the solid line that for the HPT patients.

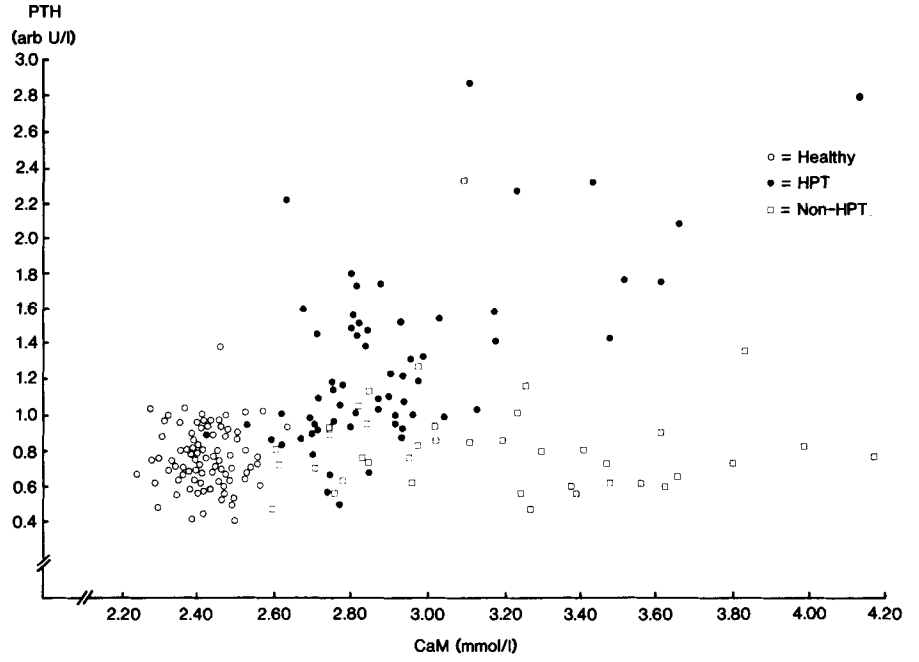

Fig. 3. Relationship between CaM and CaI for values of CaM between 2.45 and 2.75 mmol/l.

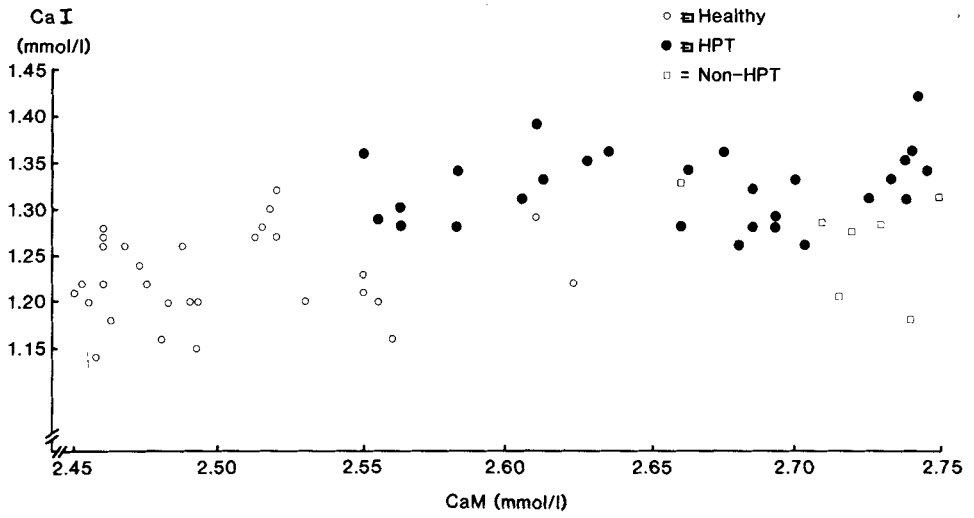

Fig. 4. Relationship between PTH and albumin-corrected serum calcium (CaM) in healthy subjects, patients with HPT and patients with other causes of hypercalcemia (non-HPT).

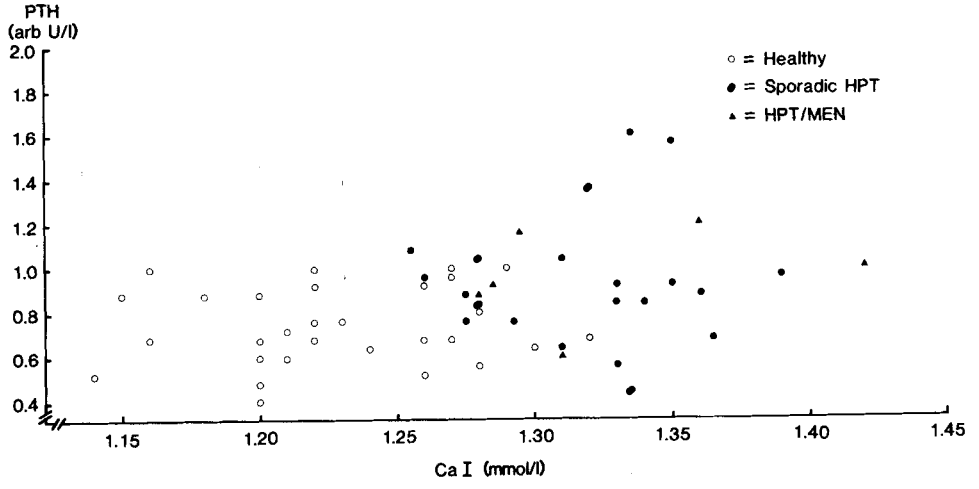

Fig. 5. Relationship between albumin-corrected serum calcium (CaM) and PTH in healthy subjects and HPT patients. Only individuals with CaM values between 2.45 and 2.75 mmol/l are included.

Taken as a group, patients with hypercalcaemia due to other causes than HPT had lower values for PTH for corresponding CaM values than the HPT patients but there was a considerable overlap, particularly for CaM values below 3.0 mmol/l (Fig. 4).

Relationship between Cal and PTH

For the healthy subjects there was no relationship between the Cal and PTH values ($r = 0.15$; $p = 0.15$). For the HPT patients, however, there was a positive correlation ($r = 0.63$; $p<0.001$) when all values were considered, but not in only those with CaM values below 2.75 mmol/l (Fig. 6).

Discriminant analysis

As can be seen from Figs. 2-6 there were overlaps for CaM, Cal and PTH between both healthy subjects and HPT patients as well as between the latter group and those with other causes of hypercalcaemia.

An attempt was therefore made by means of discriminant analysis to find the most efficient combination of the laboratory tests for the separation of HPT from healthy subjects, and to differentiate between HPT and other causes of elevated serum calcium levels.

This analysis was carried out in two steps. In the first, all individuals were included. Thereafter, only those with CaM values between 2.45 and 2.75 mmol/l were evaluated. In both these analyses consideration was given to the age- and sex variations for CaM and PTH in the healthy subjects. The analysis was performed for a prevalence of 50 % and with equal cost-weights given to the false positives and false negatives.

The result of the discriminant function analyses are given in Table 5. In the first part of this analysis, when the whole range of values were included, it appeared that measurements of CaM sufficed to provide an optimal separation of the group of patients with HPT from the healthy subjects. All the healthy subjects had a CaM below 2.657 but 14 patients with HPT were erroneously classified healthy with this limit. In this model neither measurements of Cal nor PTH improved the discriminatory power.

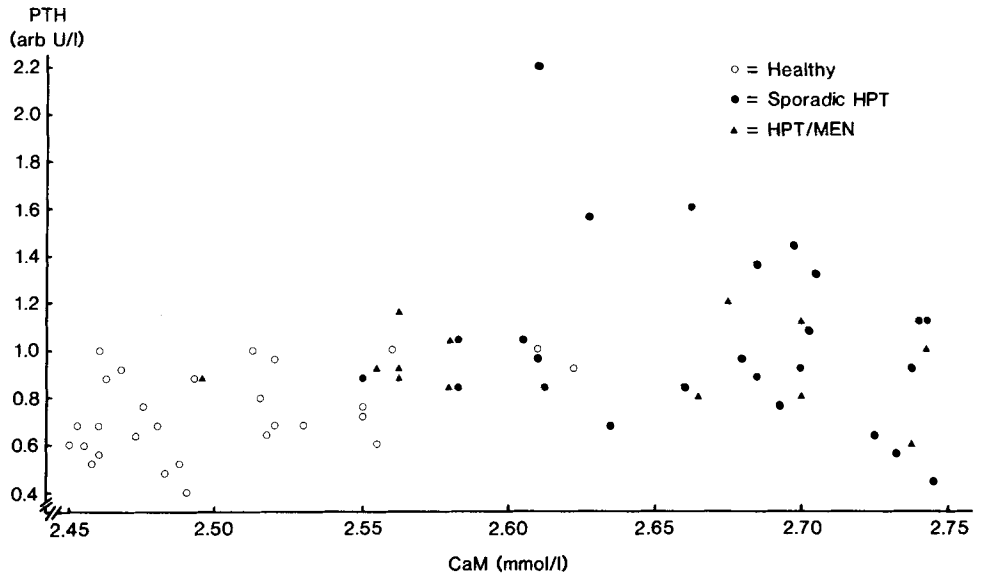

Fig. 6. Relationship between plasma ionized calcium (Cal) and PTH for the same individuals as in Fig. 5.

When, however, the model was restricted to include only subjects with values for CaM between 2.45 and 2.75 mmol/l it appeared that Cal allowed considerable additional separation of the two groups. Thus, the use of the combined measurements resulted in only two misclassifications (Table 5 All).

In both these situations the division of the material with regard to age and sex only caused minor alterations of the classifications.

For the differential diagnosis between HPT and other causes of hypercalcemia within the whole range of CaM values it turned out that Cal, PTH, and CaM all contributed to the separation of the two patient groups (Table 5B). The relative importance was greatest for CaM and smallest for PTH. Out of 134 patients 26 were misclassified, most of them having CaM values below 3.0 mmol/l (Fig. 5). When only patients

Table 5. Results of discriminant analysis. Symbols within parentheses denote the measurements chosen by the computer program. The classification into groups A, and B is performed with use of a classification function z = f (CaM, Cal, PTH) as determined in the discriminant analysis for the actual groups. An individual is allocated (classified) to group A for $z<0$ and group B for $z>0$.

A. Discrimination between healthy subjects (A) and patients with HPT (B).

	Classified group A	B
I. All Subjects		
a) All subjects (CaM)[1]		
Correct group A	91	0
Correct group B	14	74
b) Men (CaM)[2]		
Correct group A	50	0
Correct group B	1	15
c) Women < 50 years (CaM, PTH)[3]		
Correct group A	22	1
Correct group B	1	7
d) Women > 50 years (CaM)[4]		
Correct group A	18	0
Correct group B	12	51
II. Subjects with CaM 2.45 - 2.75 mmol/l		
a) All subjects (CaM, Cal)[5]		
Correct group A	29	1
Correct group B	1	31
b) Men (CaM)[6]		
Correct group A	16	0
Correct group B	0	8
c) Women < 50 years (Cal, PTH)[7]		
Correct group A	5	0
Correct group B	0	8
d) Women > 50 years (CaM, Cal)[8]		
Correct group A	8	1
Correct group B	1	19

TABLE 5. (Continued).

B. Discrimination between patients with HPT (B) and other causes of hypercalcaemia (C).

An individual is allocated to group B for $z<0$ and group C for $z>0$.

Classified group	B	C
I. All Subjects		
a) All subjects (CaM, Cal, PTH)[1]		
Correct group B	74	14
Correct group C	12	34
b) Men (CaM)[2]		
Correct group B	14	3
Correct group C	7	14
c) Women < 50 years (PTH)[3]		
Correct group B	7	1
Correct group C	0	5
d) Women > 50 years (CaM, PTH)[4]		
Correct group B	57	6
Correct group C	6	14
II. Subjects with CaM 2.45 - 2.75 mmol/l		
All subjects (Cal)[5]		
Correct group B	22	10
Correct group C	2	5

1) $z = 6.76 \times CaM - 7.13 \times Cal - 3.34 \times PTH$
2) $\hat{c} = 2.967$ (> = group C)
3) $\hat{c} = 0.806$ (> = group B)
4) $z = 4.0 \times CaM - 3.72 \times PTH - 8.64$
5) $\hat{c} = 1.293$ (> = group C)

who had CaM values below 2.75 mmol/l were analyzed the computer programme only selected Cal measurements to separate the two groups most efficiently (Table 5B II).

Optimal discriminatory levels of CaM for screening of HPT

In the reference sample group of healthy subjects the distribution of CaM values was close to Gaussian as judged by statistical goodness-of fit-tests (the Shapiro-Wilks test for $n < 50$) and the Kolmogorov-Smirnow test for $n \geq 50$). In the HPT patient groups, however, the fit was less close. Similar observations were made for the Cal values.

Since it appeared from these calculations and plots that the frequency distributions for the HPT patients were fairly Gaussian over the right-hand side of the curve but not on its extreme left the following procedure was carried out:

The assembled CaM values were reorganized on the assumption that they constituted a part of a Gaussian distribution, where the lowest values had been omitted. A theoretical Gaussian distribution function was then fitted to the truncated frequency distribution giving estimates of the location ($\bar{c}$) and standard deviation (SD).

Figure 7 shows a Gaussian function so fitted using a non-linear parameter estimation procedure for the frequency distributions for CaM in the HPT patients. From this figure it can be seen that the mean value and standard deviation ($\bar{c}$ = 2.81 ± 0.23 mmol/l) for the theoretical distribution are somewhat lower than the corresponding value obtained in a straight forward calculation of the HPT population ($\bar{c}$ = 2.91 ± 0.30 mmol/l).

The optimal discrimatory levels of CaM were calculated from the idealized curves as functions of weighting ratios (false positive or false negative) and for different prevalences (Fig. 8). As can be seen the optimal discriminatory level for a weighting ratio 1:1 varied from 2.73 mmol/l at a prevalence of 0.1 % to 2.56 mmol/l when the prevalence was assumed to be 50%. Furthermore, for a prevalence of 50 % the optimal discriminatory limit was 2.63 mmol/l for a weighting ratio of 10:1 for false positive to false negative, but 2.47 mmol/l for the reverse weighting ratio.

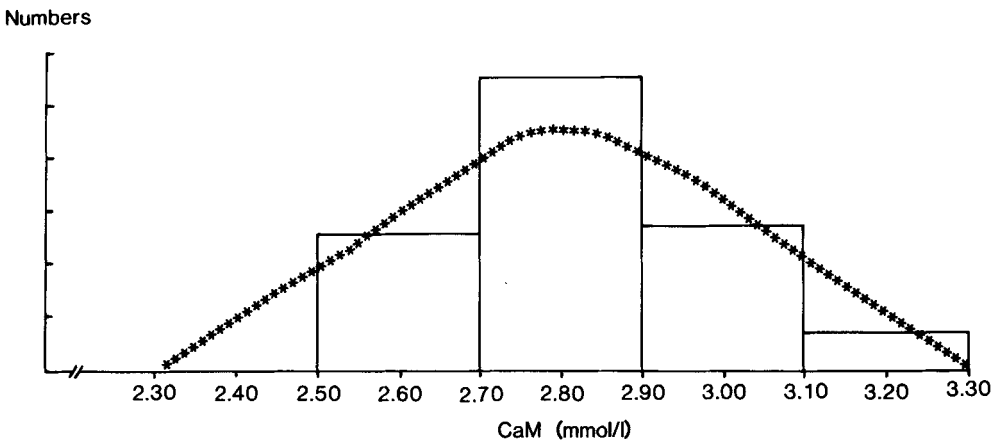

Fig. 7. Histogram for the actual values of CaM in patients with hyperparathyroidism. The fitted theoretical distribution is indicated by dotted lines.

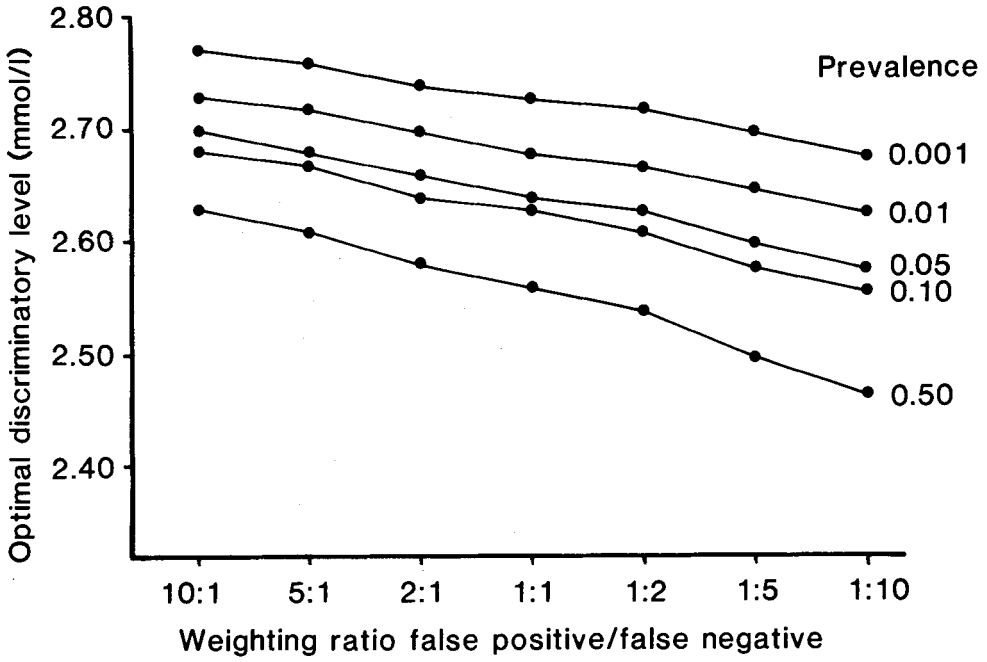

Fig. 8. The optimal discrimatory level for serum calcium (CaM) as a function of weighting-ratio false positive:false negative for different prevalences of hyperparathyroidism.

DISCUSSION

The present study was conducted with two major aims. The first was to establish the extent to which measurements of plasma ionized calcium and serum PTH contributed to the separation of patients with mild HPT from healthy individuals with CaM values in the upper part of the reference range as well as in the differential diagnosis of manifest hypercalcemia. The other was to fix the optimal discriminatory limit for serum calcium in the delineation of mild primary HPT from healthy subjects in the upper part of the reference range.

As a basis for these calculations information was collected for assessment of both intra-individual and inter-diurnal variations for the studied variables.

Diurnal variations

We found only small, statistically insignificant variations of the serum calcium and PTH concentrations through the day. A peak value for serum calcium in the morning has been described (13,25) while an early morning nadir of total calcium and ionized calcium has also been recorded (25,29).

A diurnal pattern of serum immunoreactive PTH has been reported in both normal subjects and patients with HPT (14,25,29,41) and also a relationship between PTH and sleep stages (27,39), found no evidence for rhythmic episodic variation of calcium or PTH during the day.

In the present study little attention was paid to the influence of meals. Although it would be of some benefit to have all specimens for calcium and PTH taken in the morning with the patient fasting it seems that for most practical clinical purposes a random sample during the day is sufficient.

The inter-diurnal variations were only moderately greater than the intra-diurnal variations which were comparatively modest. The majority of these variations could be explained by pre-analytical and analytical variations, i.e. the biological variations were small. The 95 % confidence interval for a single serum calcium value of 2.50 mmol could be calculated to be 2.38 - 2.62 mmol/l. Thus the clinical routine which requires several samples on alternate days might be unnecessary in subjects where the first value is lower than 2.50 mmol/l.

Age and sex variations

The observation of higher CaM values for elderly healthy women agrees with recent findings in a large health survey (36), but is in contrast to some earlier reports where lower values for total calcium in elderly persons were recorded (15,26) or no change with age was observed. However, no sex or age difference was found for CaI in accordance with previous reports (42).

We did not find any significant age-dependent differences for the serum PTH concentrations. Higher PTH levels in older persons have previously been reported (10,16,22,34,47). However, Marcus et al. (34) reported that when the values were corrected for glomerular filtration rate a difference related to age was not significant in healthy subjects.

Clearly significant sex differences were noted in the present study, for serum PTH with a 20 % higher values in females. Similar findings have not previously been generally observed (30). The reasons for the sex differences are not obvious since they relate to both pre- and postmenopausal females. An apparent clinical consequence of the observations that both CaM and PTH are higher in healthy post-menopausal females is that there will be an increased number of diagnosed HPT in this group if identical cut-off points are used as in males and younger females, given the existence of a number of patients with mild HPT who otherwise remain unrecognized. This might to some extent explain the apparently higher prevalence of HPT in postmenopausal women (11).

Delineation of HPT

The availability of measurements of plasma ionized calcium and serum PTH in clinical practice has aroused great expectations for improved diagnosis of HPT, regarding both the differential diagnosis of hypercalcemia and the separation from the upper reference range of healthy subjects.

Several studies have described cases where an elevated plasma ionized calcium was found together with a value within the reference range for total serum calcium, and where subsequent neck exploration revealed HPT (7,23,32). It seems logical that in some patients protein-binding of free calcium varies so that the total serum calcium value might be misleading (37). However, if ionized calcium is substituted for total serum calcium measurements in the clinical routine it seems likely that an overlap will occur between the upper part of the distribution of the reference values of

healthy individuals and the lower part of the corresponding distribution curve for patients with HPT. At least in the present study the overlap between the two populations was of a similar magnitude as regards ionized calcium values and total, albumin-corrected values.

The techniques for measurement of the free, ionized, fraction of serum calcium have only recently become available for general clinical routine use. Many earlier studies were therefore performed for the purpose of developing formulas where this fraction could be estimated from measurements of the total serum calcium and calcium-binding proteins, primarily albumin (12,31,35,38). In clinical practice it has generally been considered convenient to adjust the serum calcium according to the value of the serum albumin from the reference mean value. Such a correction has been demonstrated to improve the diagnosis of HPT (43).

We found, as Brauman et al. (6) that the slope for ionized calcium on total (modified) serum calcium was steeper for the hypercalcaemic patients than for the healthy controls, i.e. the pathological condition leading to a rise in the plasma ionized calcium, did not proportionally increase the fraction bound to albumin. Patients with HPT consequently had higher values for CaI, for corresponding values of CaM.

Measurements of PTH did not completely separate patients with mild HPT from healthy subjects. There is a correlation between the glandular mass, serum PTH and serum calcium in patients with primary HPT. Thus patients with the mildest hypercalcemia have the smallest glands, often with modest hyperplasia, and they also have serum PTH values close to or even within the reference range of healthy individuals (50). Furthermore, at the cellular level patients with the mildest hypercalcemia have a close to normal response to alterations of the ambient calcium concentrations as regards the release of PTH (40,45).

Evaluations of serum PTH concentrations must take into consideration the facts that several fragments of PTH are circulating, and that the relative amount of different fragments may be altered by various disorders (1,4,19). The clinical usefulness of each assay must therefore be established empirically (7).

The antiserum used in the present study has a high affinity for intact PTH. As there was a fairly rapid elimination of immunoreactive hormone from the circulation in patients operated for HPT (Fig. 1) it seems unlikely that fragments with a slow eliminating rate strongly influenced the assay.

Previous investigations with the same assay have in patients with impaired renal function demonstrated a close correlation between serum levels of PTH, bone resorption surfaces and bone formation rates (34).

A rapid increase in the PTH response to lowering of serum calcium with EDTA has also been observed (3). All these observations indicate that the assay detects mainly intact PTH and only to a lesser extent carboxyl-terminal fragments of PTH which are known to be slowly eliminated from the circulation.

It has been reported that assay system detecting the C-terminal region of PTH have a greater capacity to disclose primary HPT on basal measurements of PTH than do those system which are not capable of detecting rapid changes in secretion (8). From determinations of PTH alone less than half of all patients with HPT were clearly separated from the healthy subjects and the separation against other causes of hypercalcaemia was less than 75 %. When the simultaneously measured calcium concentrations were also considered better separations were obtained, but the delineations were still not complete, particularly in those with mild hypercalcaemia.

PTH is primarily eliminated through glomerular filtration, and impaired renal function will therefore inappropriately raise the PTH levels. Such a rise in PTH levels is more prominent with assays directed towards the carboxy-terminal part of the hormone. In general, with our assay system moderately impaired renal function, without secondary hyperparathyroidism, does not raise the PTH levels above the normal range (34). In patients with hypercalcaemia of other origin than HPT renal function is often impaired (33) and the serum concentrations of PTH may therefore be elevated even above the reference range although the secretion of PTH is in fact suppressed by the hypercalcaemia. A moderate reduction of glomerular filtration, as evidenced by an increase of the serum creatinine values, was present in several of our patients with non-HPT hypercalcaemia, a fact which might explain some of the overlap between the PTH values between the hypercalcaemic patient groups. One patient, with sarcoidosis, with a serum creatinine value around 300 µmol/l even had a markedly elevated PTH value (2.2 arb U/l).

Without performing neck exploration in all hypercalcaemic patients it could not be definitely excluded that some of them did not also have HPT. Patients without malignant disorders, however, displayed normalization of their hypercalcaemia when their underlying disease receded (sarcoidosis,

thyrotoxicosis, immobilisation) and also in most of those with malignancies the clinical picture was characteristic of hypercalcaemia of other origin than HPT.

When the clinical experiences of various research centers are compared the investigated patient populations must also be considered. At our hospital, we have for several years been interested in patients with mild hypercalcaemia and also had a liberal attitude towards surgical treatment of patients with suspected mild HPT. In our consecutive series of patients there are therefore many borderline serum calcium values. The discovery of a great overlap of PTH values between the patients with mild HPT and the healthy reference population should be regarded in this light. If only patients with high serum calcium values, above 2.90 mmol/l, had been considered as having HPT, the diagnostic difficulties with regard to both separation towards the healthy subjects as well as against other causes of hypercalcaemia would have been much less than we now experienced.

Discriminatory analysis

The discriminatory analysis was carried out in two steps, including first all individuals in the two groups to be compared. In a second step only those with borderline CaM values (i.e. 2.45-2.75 mmol/l) were considered. The rationale for the latter restriction was that otherwise patients with the most abnormal biochemical deviations would affect the discriminatory function out of proportion to the desired goal. The clinical problem, obviously, does not consist in separating a HPT patient with a serum calcium value of 3.5 - 4 mmol/l from the healthy population but rather to disclose the mildest form of HPT.

In the statistical analyses it was apparent that measurements of CaM alone could separate the majority of HPT patients from the healthy subjects. This is almost self-explanatory as the majority of HPT patients have clear-cut hypercalcaemia. In such instances additional measurements (of CaI or PTH) cannot allow of further separation. However, when only the borderline individuals were analyzed CaI proved to be a useful additional determination. Although there was a general correlation between CaM (and CaI) and PTH for all the HPT patients the mild parathyroid hyperfunction in those with the mildest hypercalcaemia did not result in significant elevations of the PTH levels and in this area PTH measurements did not provide any further discrimination.

Although there were some age and sex differences for both CaM and PTH these were apparently not such as to improve the discrimination in the present material.

For the differential diagnosis of hypercalcaemia it turned out that measurements of PTH were valuable, particularly in individuals with the highest serum calcium values. In addition Cal values were higher for the HPT patients than for the non-HPT hypercalcaemics, particularly within the lower range of raised serum calcium values. This is most likely explained by an "over-correction" of the CaM values for the decreased serum albumin, often found in patients with hypercalcaemia (5).

Optimal discriminatory limit

Before calculation of the optimal discriminatory limits between the healthy subjects and patients with mild HPT an adjustment of the frequency distribution curve for CaM in the HPT patients was performed. The reason for this manoeuvre was the observation that the calcium values approached a Gaussian distribution except in the lower part where there was a lack of values below 2.60 mmol/l to achieve a bell-shaped curve.

The concept of "normocalcemic primary HPT" has been discussed for several years (23,46,48). Many patients reported to have normocalcemic primary HPT appear to have HPT with a mild, sometimes fluctuating, hypercalcemia (24,28).

However, several observations indicate that HPT could exist also in patients who never display raised serum calcium values. For instance series of patients have been presented where neck exploration has been carried out despite constant normocalcemia (17,23) and where definite HPT has been confirmed histopathologically. In a recent series approximately 40 patients operated at our hospital had serum calcium values between 2.60 and 2.80 mmol/l. HPT could be operatively verified in all patients (50). This suggests that some individuals with serum values below 2.60 mmol/l also have HPT. This discussion of "normocalcemic HPT " only serves as an explanation of the mathematical analysis and does not carry any implications as to whether such patients should be sought or even less which treatment is optimal.

On the basis of these results we could calculate an optimal discriminatory limit for HPT of 2.68 mmol/l, assuming a prevalence of 1% and a weighting ratio of 1:1 between false positives and false negatives. As mentioned

above, however, only positive explorations were performed in consecutive patients with such calcium values. If other prerequisites are correct, this finding indicates that the prevalence of HPT in a general population must be considerably higher than 1%. In support of this view, autopsy studies have found adenomatous HPT in as many as 2.4 % of individuals above the age of 70, and evidence of hyperplastic primary HPT in further 7 % (50). From Fig. 8 it can be seen that the weighting ratio of 1:1 (i.e. equal importance is paid to false positive and false negative classifications) for a serum calcium value of 2.60 mmol/l corresponds to a prevalence in the population of above 10 %.

First degree relatives of patients with the MEN-1 syndrome have a 50 % risk of being carriers of the MEN-1 trait. In a population with a 50 % prevalence of HPT the optimal discriminatory limit for serum calcium in the present study was 2.56 mmol/l if it was considered equally important to avoid falsely positive and falsely negative classifications. It is a moot point whether the weighting ratio in this particular setting is different from other situations where HPT is suspected. Whatever standpoint is taken in the individual clinical situation it is apparent from these considerations that in MEN-1 relatives serum calcium values even within the reference range might be compatible with HPT and therefore necessitate further investigations.

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