Relative Contribution of Various Expressions of cAMP Excretion to Other Indices of Parathyroid Function, as Tested by Discriminant Multivariate Linear Regression Analysis

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We evaluated the relative contribution to the diagnosis of hyperparathyroid disease from current laboratory indices of parathyroid function—plasma calcium (I), phosphate (II), carboxy-terminal (III) and predominantly amino-terminal (IV) radioimmunoassays of parathyron, the urinary excretion ratios of cyclic adenosine monophosphate (cAMP) to creatinine (V) or to glomerular filtrate (VI), and the ratio of the nephrogenous fraction of cAMP to glomerular filtrate (VII)—in 224 subjects: 40 with surgically proven hyperparathyroid disease, the others normoparathyroid. The decreasing order of sensitivity was I > VI > VII > V > III > IV > II; all these indices differed significantly between normoparathyroid and hyperparathyroid patients. The decreasing order of specificity was VIII, VII = I > IV > II > VI. Discriminant multivariate linear regression analysis was performed in a subset of 58 subjects (17 hyper- and 41 normoparathyroid) from the population studied here, chosen because all of the laboratory indices were determined for each subject. The classification accuracy was 98.3% for combining I, VII, and III (r = 0.968), or I and V (r = 0.899), or I and VII (r = 0.869). The other variables did not add to the precision of classification.

Additional Keyphrases: hyperparathyroidism, parathyron, calcium, phosphorus, sensitivity, specificity, urine

The choice of a biological test or set of tests as a diagnostic aid is governed not only by scientific but also increasingly by economic considerations. At an epidemiological level, tests should be sensitive and specific. In this context, "sensitivity" means the frequency of positive results in a population having a disease; "specificity" means the number of negative results in subjects without the disease (I, 2).

These concepts may be applied to the biological tests routinely used in the detection and differential diagnosis of diseases related to calcium metabolism, namely, calcium, phosphate, and various parathyron assays. Hypercalcemia, although very common in hyperparathyroidism, is by no means specific to this disease, being encountered in other situations such as malignancies, vitamin D intoxication, and sarcoidosis. Measurement of parathyron, a popular diagnostic tool since the introduction of its radioimmunoassay by Berner et al. (3), has many drawbacks, including the heterogeneity of the circulating forms of parathyron and the use of various antisera that recognize different determinants of the molecule (4).

In the 1970s, measurement of cyclic adenosine monophosphate (cAMP) excretion was added as a new index of parathyroid function (5–7). Parathyron acting on the renal tubule induces cellular events that lead to an increase in the quantity of cAMP in urine. In fact, the renal contribution to cAMP excretion is essentially all ascribable to the action of parathyron on the renal tubule (8).

Here we have evaluated the sensitivity, specificity, and discriminant power of the different expressions of cAMP excretion, alone or combined with other indices, in the diagnosis of hyperparathyroid disease.

Materials and Methods

Patients

The control group (G-I) consisted of 41 normal volunteers: 24 men, ages 20 to 66 (mean 32.4, SD 12.2) years, and 17 women, ages 22 to 60 (mean 34.8, SD 13.6). Forty patients with surgically proven hyperparathyroidism participated in this study before they underwent operation. This group (G-II) comprised 15 men, ages 36 to 64 (mean 51.8, SD 9.09) years, and 25 women, ages 22 to 78 (mean 57.3, SD 14.7).

Thirty-four parathyroidectomized hyperparathyroid patients (G-III) were included in this study: 16 men, ages 47 to 66 (mean 53.8, SD 8.1) years, and 18 women, ages 32 to 70 (mean 57.2, SD 12.8).

Thirty-three hypercalciuric patients (G-IV)—15 men, ages 27 to 76 (mean 48.6, SD 12.7) years, and 18 women, ages 26 to 74 (mean 51.6, SD 13.2)—were considered as a comparison group, being patients at risk of being hyperparathyroid, but not confirmed after a prolonged follow-up.

Sixty-three patients (G-V)—25 men, ages 18 to 80 (mean 48.8, SD 16.9) years, and 38 women, ages 19 to 78 (mean 51.5, SD 17.6)—constituted a comparison group of hospitalized patients with various disorders not related to any parathyroid disfunction: nine patients with depression, eight with renal stones, eight with osteoporosis, six with polyarthritis, six with hypertension, five with cardio-pulmonary disorders, four with adenocortical disorders, four with hypothyroidism, three with Paget's disease, two with sarcoidosis, three with acromegaly, two chronic alcoholic patients, one with gastric ulcers, one with gout, and one undergoing exeresis of a maxillary cyst.

Finally, a group of 13 patients (G-VI) (seven men, ages 58 to 74 (mean 67.2, SD 5.1) years, and six women, ages 47 to 84 (mean 65.4, SD 12.7) years) displaying various malignant tumors, with or without bone metastases, participated in this study.

In none of the groups was there any renal failure (concentrations of serum creatinine were <12 mg/L; glomerular

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1 Nonstandard abbreviations: UcAMP, PaAMP, NcAMP, urinary and plasma concentrations and nephrogenous fraction, respectively, of cyclic adenosine monophosphate; Ci-PTH, Ni-PTH, carboxy-terminal and amino-terminal immunoreactive parathyron, respectively.
filtration rate >80 mL/min) or dietary restrictions at the time of the study. Patients taking drugs likely to interfere with the disposition of calcium and phosphate, e.g., diuretics (9), or drugs modifying cAMP excretion, e.g., xanthines (10), were excluded from the study.

Study Protocol

Blood samples were taken in the morning (after overnight fasting) and analyzed for cAMP, creatinine, calcium, phosphate, proteins, and parathyrin. For 60 subjects we estimated parathyrin by two different radioimmunoassays. For 80 of the subjects studied we compared early morning (2-h) and 24-h urine collections, to investigate the influence of the collection schedule on the final estimate of cAMP excretion.

Procedures

Urine was collected without preservative, at ambient temperature. Creatinine, calcium, and phosphate were assayed without delay, whereas cAMP was assayed in aliquots stored at −20°C before assay. We tested the possible degradation of cAMP in sterile or infected urines (containing more than 5 × 10^5 colonies/L) left at ambient temperature for 1, 2, 3, 12, 24, and 36 h. Venous blood samples for creatinine, calcium, phosphate, and total protein assay were collected in plain Vacutainer Tubes (Becton Dickinson) and assayed without delay. Heparinized samples were collected for parathyrin assay, whereas for cAMP assays the samples were collected into tubes containing EDTA as anticoagulant. For the parathyrin and cAMP assays the samples were centrifuged without delay and the supernates maintained at −20°C until assayed.

Creatinine was measured by the Jaffé reaction (11), calcium by the method of Keesler et al. (12), phosphate by the modified Fiske and SubbaRow reaction (13), and total proteins by the biuret reaction described by Failing et al. (14) as adapted to the Technicon AutoAnalyzer (Technicon Instruments Corp., Tarrytown, NY).

For the radioimmunoassays of parathyrin we used the commercial kit of the Institut des Radioelements, Fleurus, Belgium, which recognizes predominantly the carboxy-terminal region of the molecule, and an assay adapted from Arnaud et al. (15) with the Wellcome antibody 211/41, which has predominantly amino-terminal affinities. Both assays have been tested over several years, and were used in a collaborative study to define the Medical Research Council parathyrin standard (15). The present results, however, are expressed in terms of Wilson's parathyrin preparation 1515C001, purchased from Ino lex, Chicago, IL. We assayed cAMP with the kit manufactured by Amersham International, Amersham, Bucks, U.K.

As suggested by Broadus et al. (7), we have expressed the urinary excretion of cAMP (a) as a function of urinary creatinine (nanomoles of cAMP per milligram of creatinine), and (b) as a function of the glomerular filtration rate, (nanomoles of cAMP per 100 mL of glomerular filtrate):

\[
\frac{\text{UcAMP} \times D}{\text{Ccr}} \times 100
\]

where UcAMP is the urinary concentration of cAMP (nmol/mL); Ccr is the creatinine clearance (mL/min), and D represents the urine flow rate (mL/min). The nephrogenous fraction of cAMP (NcAMP) is expressed as a function of glomerular filtration rate (nanomoles of NcAMP per 100 mL of glomerular filtrate), as given by the expression:

\[
(\text{UcAMP} \times D) - (\text{PcAMP} \times \text{Ccr}) \times 100
\]

where PcAMP is the concentration of cAMP (nmol/mL) in plasma. Because we did not determine PcAMP in all subjects, we could calculate and report all three expressions for cAMP excretion for only 195 of the subjects.

Statistical Procedures

Student's paired and nonpaired t-tests, means, and standard deviations (SD) were calculated by the methods described by Snedecor and Cochran (17). Sensitivity, specificity, and predictive values for each parameter investigated were calculated as recommended (1, 2) and based, for each variable, on the number of results available for the population under study.

For only 58 subjects (16 from G-I, 17 from G-II, seven from G-III, six from G-IV, nine from G-V, and three from G-VI) did we obtain a complete set of the most relevant indices: calcium, phosphate, carboxy-terminal immunoreactive parathyrin (CI-PTH), amino-terminal immunoreactive parathyrin (NI-PTH), and the three expressions of cAMP excretion. Thus the discriminant regression analyses were limited to these 58 subjects. In the other subjects the missing indices were usually one of the two assays for immunoreactive parathyrin, or sometimes one of the three expressions of cAMP excretion.

In all calculations, the criterion for discrimination was the presence or the absence of hyperparathyroid disease. Univariate and multivariate values allowing the discrimination were calculated as described by Snedecor and Cochran (17, 18) and Anderson (19) for single and multiple linear discriminant regression analysis. The statistical "SWEEP Operator Program" (17) has been adapted to the HP 9830A computer (Hewlett-Packard) for matrix and inverse matrix calculations.

Results

During the evaluation of the cAMP radioimmunoassay, the intra-assay CV was 6% (n = 49 pairs), the interassay CV was 10% (n = 12). We detected no change in the concentration of cAMP in five noninfected urine samples left at room temperature for as long as 36 h (CV 5.5%). Three infected urine samples were kept at room temperature for 24 h without any significant change in cAMP concentrations (t = 0.9521; P > 0.40).

As Figure 1 shows for the 27 subjects in G-I, there is no statistical difference between the cAMP excretion in relation to the duration of the urinary collection. This was true for any of the three expressions of cAMP excretion and for the 53 subjects from other groups for whom we had comparable data (14 in G-II, nine in G-III, nine in G-IV, 15 in G-V, and six in G-VI). Although the three expressions for cAMP excretion differed significantly (p < 0.001) according to sex, this difference was not significant in G-II through G-VI.

Table 1 lists the number of determinations, and the mean and SD for each of the indices investigated, and indicates the significant differences among groups. Results for G-II differed significantly from those for the control group (G-I) for all the indices.

Table 2 shows the results of the calculations of specificity, sensitivity, and predictive value for what we considered to be the most relevant indices to be taken into consideration in the diagnosis of hyperparathyroidism, namely, calcium,
Table 1. Summary of the Indices Investigated

<table>
<thead>
<tr>
<th></th>
<th>G-I</th>
<th>G-II</th>
<th>G-III</th>
<th>G-IV</th>
<th>G-V</th>
<th>G-VI</th>
</tr>
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<tbody>
<tr>
<td>Calcium, mg/L</td>
<td>96.6 ± 3.8</td>
<td>114 ± 8</td>
<td>94 ± 4</td>
<td>98.3 ± 3.3</td>
<td>97.5 ± 4.6</td>
<td>110 ± 15.5</td>
</tr>
<tr>
<td>Phosphate, mg/L</td>
<td>34.9 ± 4</td>
<td>24.4 ± 5.2*</td>
<td>32.5 ± 6.8</td>
<td>30 ± 3.8°</td>
<td>32.4 ± 8.3</td>
<td>33.5 ± 8.5</td>
</tr>
<tr>
<td>CI-PTH, μg/L</td>
<td>0.545 ± 0.098</td>
<td>0.843 ± 0.188*</td>
<td>0.531 ± 0.112</td>
<td>0.573 ± 0.158</td>
<td>0.554 ± 0.138</td>
<td>0.516 ± 0.086</td>
</tr>
<tr>
<td>NI-PTH, μg/L</td>
<td>0.476 ± 0.129</td>
<td>0.935 ± 0.410*</td>
<td>0.549 ± 0.142</td>
<td>0.548 ± 0.130</td>
<td>0.526 ± 0.136</td>
<td>0.773 ± 0.383</td>
</tr>
<tr>
<td>UcAMP, nmol/mg creatinine</td>
<td>1.94 ± 0.56</td>
<td>4.93 ± 1.85*</td>
<td>2.05 ± 0.75</td>
<td>2.53 ± 0.77°</td>
<td>2.63 ± 1.07°</td>
<td>3.5 ± 0.96°</td>
</tr>
<tr>
<td>UcAMP, nmol/100 mL GF</td>
<td>3.25 ± 0.86</td>
<td>5.78 ± 1.6°</td>
<td>2.94 ± 0.79</td>
<td>3.02 ± 1.14</td>
<td>2.91 ± 0.91</td>
<td>3.48 ± 1.47</td>
</tr>
<tr>
<td>NcAMP, nmol/100 mL GF</td>
<td>1.95 ± 0.49</td>
<td>5.2 ± 2.4°</td>
<td>2.3 ± 0.7</td>
<td>2.47 ± 0.79°</td>
<td>2.57 ± 1.25°</td>
<td>3.29 ± 0.67°</td>
</tr>
<tr>
<td></td>
<td>2.75 ± 0.49</td>
<td>4.9 ± 1.2°</td>
<td>2.46 ± 0.87</td>
<td>2.48 ± 0.99</td>
<td>2.37 ± 0.74</td>
<td>2.52 ± 0.87</td>
</tr>
</tbody>
</table>

Note: *p < 0.001, °p < 0.05. GF, glomerular filtrate.

Ci-PTH, Ni-PTH, phosphate, and cAMP. For each index, these calculations are based on the available data (Table 1). We calculated the cutoff point for each variable, as 2 SD from the mean: mean ± 2 SD for the phosphate, and mean ± 2 SD for the other variables. For the evaluation of the predictive value, we chose a prevalence of hyperparathyroidism of 0.1% according to data in the literature for a general hospital (20), which roughly corresponds to our experience. In our study, the determination of calcium concentration was most predictive of the disease, followed closely by cAMP, then by Ci-PTH and, to a lesser degree, by Ni-PTH assay.

Tables 3, 4, and 5 summarize the results of the discriminant regression analysis. Table 3 presents the independent variables chosen for the monovariate discriminant regression analysis, their respective discriminant values and classification efficiencies, and the statistical significance of their correlation and linear regression relationship.
coefficients. All regression coefficients were statistically significant at least at $p = 0.05$, being the greatest for calcium, the least for age.

Table 4 shows the significance of the partial regression coefficients obtained by coupling either of the expressions of cAMP excretion with the variables calcium, CI-PTH, Ni-PTH, phosphate, and age. Depending on the combination, the coefficients for two or three out of the five variables reached 0.05 significance. The accuracy of classification attained with two of the three combinations (98.3%) far exceeded those seen in Table 3. However, considering all the statistical indices of efficiency of combinations, the best choice would be the combination involving the expression for excretion of nephrogenic cAMP.

By omitting the variables with insignificant partial regression coefficients ($p > 0.05$) we developed the multivariate discriminant analysis summarized in Table 5. Table 5 also shows results for other combinations of independent variables that lead to accurate classification. In the multivariate discriminant analysis only one normoparathyroid patient was erroneously classified; therefore, the overall sensitivity of the combination of two or three concerned variables from Table 5 will be 100%, whereas the specificity will be close to 98%. In comparison, the best results from Table 2, basing the diagnosis on only the variable calcium, were 89% and 94%, respectively.

**Discussion**

It appears from our study that calcemia is one of the best discriminators in the differential diagnosis of hyperparathyroidism. The sensitivity of this measurement for the entire population under study was 89%; the specificity was 94%. Most of loss of specificity was attributable to the small group of patients with malignancy, and might be even lower if a higher proportion of cancer patients were included, as in some studies (21, 22). Nevertheless, plasma calcium missed 11% of our known hyperparathyroid subjects; moreover, normocalcemic hyperparathyroid patients, in proportions similar to or greater than ours, have been reported (5, 23, 24). The apparent gain in sensitivity for calcium in the monovariate discriminant regression analysis is almost entirely due to the exclusion of several of the known hyperparathyroid patients from the latter analysis because of the lack of a complete set of data for these patients (especially for one of the i-PTH assays).

Plasma phosphate, although frequently diminished in hyperparathyroid disease, in our experience shows no better sensitivity, specificity, or greater discriminating efficiency in the multivariate analysis than calcium, parathyrin, or cAMP. The measure of the maximal tubular resorption of phosphates, a discriminator recommended by some (25, 26), clearly separates hypoparathyroid patients from normal ones, but seems less efficient in separating normal patients.
from hyperparathyroidism (26). In our hospital, tubular reabsorption was no better than plasma phosphorus alone as a discriminator (27); moreover, this measure is not easy to perform.

As predictors, parathyrin assays vary in usefulness according to their clinical sensitivities (4). In the present study both types of assays, although highly specific (91% and 96%) failed to detect more than about 60% of the hyperparathyroid patients in the entire population studied, a figure sometimes found by others (7, 25, 28). Although some authors claim to possess more discriminating assays for parathyrin (29, 30), these are not currently available; the same is true for sensitive bioassays (31). Even if the classification accuracy of both parathyrin assays reaches 85% in the monovariate analysis in our restricted group of 58 subjects, this is still less than the classification accuracy of calcium alone, although there is a significant correlation between parathyrin assays and calcium ($r = 0.56$ and 0.68, respectively, between calcium and CI-PTH and Ni-PTH). Recently, parathyrin mid-molecule assays have been recommended for their differentiating efficacy (30). Our experience in that field is limited and has come after the present study.

The measurement of CAMP excretion as a valid index of the parathyroid function, expressed as proposed by Broadsus et al. (7), showed 90% sensitivity for all the three expressions. The best predictive value, for an assumed prevalence of hyperparathyroidism of 0.1%, would be 1.96%, via consideration of NCAMP excretion. In monovariate analysis the classification accuracy of any of the expressions of CAMP excretion lies between that of calcium and parathyrin. Our conclusions concerning this last point, however, might not be valid if patients with renal insufficiency (32), or more patients with malignancy (in whose CAMP excretion might be influenced by other factors than parathyrin) (21), had been included in this study.

From the multivariate analysis we conclude that, although calcium alone has already a high classification accuracy, the addition of parathyrin values and (or) one of the expressions of CAMP excretion increases the classification accuracy to almost 100%.

Multivariate analyses applied to problems similar to ours are rare. One recently published study (22) differed in the choice of the predictors (serum albumin, parathyrin, and plasma chloride), the choice of groups to be differentiated (control vs malignancy), the use of logarithmic instead of linear functions, and the absence of CAMP measurement. In our study the concentrations of proteins and the protein electrophoresis patterns were normal. Our experience with plasma chloride showed only limited usefulness in the differential diagnosis of hyperparathyroidism. Like others (29, 33), we confirm the better discriminating efficacy of CI-PTH over Ni-PTH assays. Because we obtained an excellent correlation coefficient of 0.91 with the linear discriminant function, we made no attempt to use other functions; moreover, statisticians advocate the linear form for diagnostic discriminatory purposes (13, 34).

Finally, the two statistical approaches to validation of the choice of biological indices are not mutually exclusive. Sensitivities and specificities following bayesian theorems provide valuable indices for the choice of cutoff points for the calculation of predictive values. The multivariate discriminant regression analysis provides a basis for the choice of a test or set of tests and hence of the cutoff points that minimize the risk of classification errors.

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References


