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The Ratio of Parathyroid Hormone as Measured by Third- and Second-Generation Assays as a Marker for Parathyroid Carcinoma

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Background: Parathyroid carcinoma (PCa) is a rare disease that can be difficult to differentiate initially from severe benign parathyroid adenoma. PCa oversecrete the amino form of PTH, which is recognized by third-generation but not by second-generation PTH immunoassays. In normal individuals, the third-generation to second-generation PTH ratio should be less than 1.

Objective: Our objective was to study the utility of the third-generation to second-generation PTH ratio as a means of distinguishing PCa patients (n = 24) from control groups with and without disorders of calcium secretion, including patients on renal hemodialysis (n = 74), postrenal transplantation (n = 60), and primary hyperparathyroidism (PHP; n = 30).

Setting and Design: We conducted a retrospective, laboratory-based study at tertiary referral academic centers.

Results: The mean third-generation to second-generation ratio was 0.58 ± 0.10 in the dialysis patients, 0.54 ± 0.10 in the renal transplant group, 0.54 ± 0.12 in the elderly healthy patients, and 0.68 ± 0.11 in the PHP group. All 245 of these patients presented a PTH third-generation to second-generation ratio of less than 1. In contrast, we observed an inverted third-generation to second-generation PTH ratio of more than one in 20 PCa patients, whereas only four PCa patients had a normal ratio of less than 1.

Conclusions: An inverted third-generation to second-generation PTH ratio occurred in the majority of patients with advanced PCa and was absent in all 245 relevant controls. A third-generation to second-generation PTH ratio higher than 1 had a sensitivity of 83.3% and a specificity of 100% among PHP patients as a marker for PCa. This ratio may be useful to identify patients with PCa earlier and to detect patients either at risk of developing PCa or those in whom recurrence is taking place. **(*J Clin Endocrinol Metab* 95: 0000–0000, 2010)**

Parathyroid carcinoma (PCa) is a rare disease, comprising 0.005% of all cancers (1). Fewer than 400 PCa cases were reported before 1993 (2), and in the U.S. National Cancer Database, only 286 cases have been described over 10 yr (3). PCa accounts for less than 1% of sporadic primary hyperparathyroidism (PHP) and is associated with more severe clinical features than parathyroid adenomas (4). The severe hypercalcemia due to uncontrolled PTH hypersecretion is the main cause of morbidity and death in patients with PCa.

Differentiating PCa from more common parathyroid adenomas is often challenging, particularly because the

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Abbreviations: PCa, Parathyroid carcinoma; PHP, primary hyperparathyroidism.

histopathology of parathyroid tumors can be equivocal (5). Indeed, the definitive diagnosis of PCa is largely made only when recurrence or metastasis occurs. Because surgery remains the only curative treatment for PCa and because better outcomes are associated with complete resection of the tumor at the time of initial surgery, a correct initial diagnosis is important. Hence, a biological marker that could help to reliably distinguish PCa from parathyroid adenoma would be useful.

The first assays used to measure PTH were RIAs. They recognized the full-length PTH_{1-84} but also a large amount of various C-terminal fragments. Since 1987, routine determinations of so-called intact PTH have used various immunometric methods. These immunoassays (commonly termed second-generation PTH immunoassays) use a pair of antibodies, a capture antibody directed against the (39–84) portion of the PTH molecule and a second antibody that recognizes the (13–24) portion of the peptide. They not only recognize PTH_{1-84} but also cross-react to varying degrees (50–100%) with a family of large amino-truncated fragments, of which PTH_{7-84} is the most abundant form (6). Because PTH_{7-84} accumulates in patients suffering from chronic kidney disease, this explains, in part, the PTH overestimation in secondary hyperparathyroidism due to chronic renal failure (7, 8). Another generation of PTH immunoassays appeared in 1999 (third generation). The third-generation PTH immunoassays used similar capture antibodies as the second-generation immunoassays but added an anti-N-terminal antibody directed against the first four amino acids of PTH (9). These immunoassays, therefore, do not measure the PTH_{7-84} fragment. Amino-PTH, a recently recognized form of PTH that is not truncated but modified in the (15–20) region, potentially by phosphorylation of a serine residue at position 17 (10), cross-reacts with these third-generation PTH kits but not with antibodies used in most second-generation immunoassays.

Physiologically, PTH_{7-84} represents 15–50% and amino-PTH less than 10% of circulating PTH_{1-84} (10). Therefore, in healthy individuals, the ratio between the amounts of PTH measured with third- *vs.* second-generation immunoassays, the third-generation to second-generation PTH ratio [$(PTH_{1-84}$ + amino-PTH)/$(PTH_{1-84} + PTH_{7-84})$] will not be greater than 1. Recently, it has been shown that amino-PTH is overproduced in PCa (11, 12) and in some rare cases of severe PHP (13). In such patients, the third-generation to second-generation PTH ratio may be inverted (>1) as compared with normal individuals (≤ 1). Data from small patient series have suggested that the third-generation to second-generation PTH ratio may have clinical utility to discriminate PCa from most cases of benign PHP (12).

The current study was undertaken to assess the third-generation to second-generation PTH ratio in a large population of patients suffering from advanced PCa and compare the findings with relevant populations including patients with PHP.

Patients and Methods

Patients

Twenty-four patients from Europe, the United States, and Australia with advanced PCa were included (Table 1). All had previously undergone surgery and had been admitted to local hospitals for anticancer immunotherapy (14, 15). All underwent assessments of PTH levels before immunotherapy using second- and third-generation immunoassays to derive a third-generation to second-generation PTH ratio.

We recruited a representative comparator group to permit assessments of the third-generation to second-generation PTH ratio as a discriminatory test. The first subgroup was a population of 73 chronic renal failure patients (65.2 ± 16.5 yr; 38% female) undergoing hemodialysis at the Centre Hospitalier Universitaire de Liège. We evaluated 30 patients with PHP (53.8 ± 14.3 yr; 77% female), 60 renal transplant patients (50.6 ± 12.2 yr; 45% female), and 82 consecutive healthy elderly individuals (62.0 ± 8.0 yr; 53% female).

The study was approved by the Institutional Review Board of the Centre Hospitalier Universitaire de Liège, and all patients provided informed consent for immunotherapy treatment.

PTH immunoassays

PTH was determined with the Duo PTH immunoradiometric kit from Scantibodies Laboratory (Santee, CA). This kit includes two different immunoassays: 1) a second-generation PTH immunoassay that recognizes PTH_{1-84} and the non-PTH_{1-84} components but not amino-PTH; 2) a third-generation immunoassay that recognizes PTH_{1-84} and amino-PTH. These two immunoassays were calibrated against the same PTH standard. In our laboratory's experience, the inter- and intraassay coefficients of variation obtained with these two immunoassays are less than 10%.

Statistics

Statistical analysis was carried out by the Medcalc Software (Mariakerke, Belgium). Differences between groups were calculated by the Student's independent-samples *t* test, with *P* value <0.05 indicating a significant difference.

Results

The results of the second-generation and third-generation PTH immunoassays and the third-generation to second-generation ratios are summarized in Table 1. All 245 control subjects had a third-generation to second-generation PTH ratio of less than 1 (Fig. 1). The mean third-generation to second-generation ratio was 0.58 ± 0.10 in dialysis patients, 0.54 ± 0.10 in the renal transplant

TABLE 1. Clinical characteristics in 24 patients with advanced parathyroid cancer

Patient	Sex	Age (yr)	Age at diagnosis (yr)	Serum calcium (mmol/liter)	Intact PTH, 2nd generation (ng/liter)	Whole PTH, 3rd generation (ng/liter)	Ratio	Creatinine (mg/liter)	Metastases, Y/N (location)
1	F	59	42	3.69	261	435	1.66	11.1	Y (lung)
2	M	63	61	3.10	464	544	1.17	15.2	Y (lung)
3[a]	M	36	31	2.89	814	668	0.82	8.8	Y (lymph node)
4	M	58	55	3.13	188	333	1.77	9.7	Y (mediastinum)
5[a]	F	55	53	2.96	452	361	0.80	8.6	N
6	F	65	60	4.62	367	487	1.33	9.7	N
7	M	76	68	3.34	1098	1363	1.24	9.2	Y (lung)
8	M	57	52	4.03	806	1101	1.37	11.8	Y (lung)
9	M	55	49	3.47	828	888	1.07	11.4	Y (lung)
10	M	53	43	3.72	606	768	1.27	13.4	Y (mediastinum, bone)
11[a]	M	70	62	3.40	695	438	0.63	11.8	N
12	M	55	52	4.16	622	691	1.11	15.4	Y (lung)
13	F	66	60	4.30	185	370	2.00	8.3	Y (tracheostomy site)
14	F	58	51	4.10	230	638	2.78	12.7	N
15	M	62	55	2.74	71	99	1.40	17.3	Y (N/A)
16	F	52	43	2.92	272	365	1.34	8.4	Y (N/A)
17	F	42	28	4.56	363	583	1.61	21.2	Y (esophagus)
18	F	50	48	3.57	628	810	1.29	18.0	Y (left thyroid gland)
19	M	68	65	2.98	404	542	1.34	15.6	Y (N/A)
20	F	72	69	4.00	1063	1989	1.87	4.0	Y (esophagus)
21	M	77	68	2.92	197	358	1.82	37.4	N
22	M	58	55	3.10	99	102	1.03	10.1	Y (liver)
23	F	35	34	4.85	747	1076	1.44	12.8	Y (N/A)
24[a]	F	61	58	4.00	145	114	0.79	11.0	N/A

F, Female; M, male; N, no; N/A, not available; Y, yes.

[a] Patients with a third-generation to second-generation PTH ratio (<1).

group, 0.54 ± 0.12 in the elderly healthy patients, and 0.68 ± 0.11 in the PHP group. The mean third-generation to second-generation PTH ratio was similar in the normal elderly and renal transplanted patients. The ratio was significantly higher in the hemodialysis group compared with the renal transplant patients ($P < 0.05$). Patients with parathyroid adenoma had an increased ratio compared with the three comparative control populations ($P < 0.0001$).

An inverted third-generation to second-generation PTH ratio (>1) was seen in 20 PCa patients, whereas four patients had a normal ratio of less than 1 (Fig. 1). The mean third-generation to second-generation ratio (1.40 ± 0.46) was significantly higher among the PCa patients than all control populations. Importantly, the mean third-generation to second-generation ratio in the PCa group was significantly higher than the ratio in PHP patients ($P < 0.0001$).

Discussion

PTH circulates as a mixture of $PTH_{1\text{-}84}$ and various amino-truncated fragments. These fragments not only are products of degradation of $PTH_{1\text{-}84}$ but are also secreted by the parathyroid gland itself. Experimental studies have suggested that $PTH_{7\text{-}84}$ is implicated in the PTH resistance observed in chronic kidney disease by antagonizing the calcemic actions of PTH in rats with normal renal function (16, 17) or by interacting with a novel PTH receptor via the C-terminal region (18, 19). With the advent of the third-generation PTH immunoassays, a new circulating form of PTH, amino-PTH, was discovered. It is still unknown whether this amino-PTH is biologically active, but it has previously been shown to be overproduced in PCa (11, 12) and rarely in cases of severe PHP (13). This overproduction leads to an inversion of the third-generation to second-generation PTH ratio, which is normally less than 1. In the current study, we demonstrate that an inverted third-generation to second-generation PTH ratio was seen in 83% of PCa patients compared with 0% of a series of four relevant control populations.

The current study of the third-generation to second-generation PTH ratio as a marker for PCa is the largest to address this important question in this very rare malignancy. Previously, Caron *et al.* (12) observed an inverted third-generation to second-generation PTH ratio (>1) in a patient with PCa and in only one of 30 and none of 294 osteoporotic patients with and without PHP, respectively. In the PHP patient from that study, the inverted ratio remained after surgery (1.54), and the patient exhibited

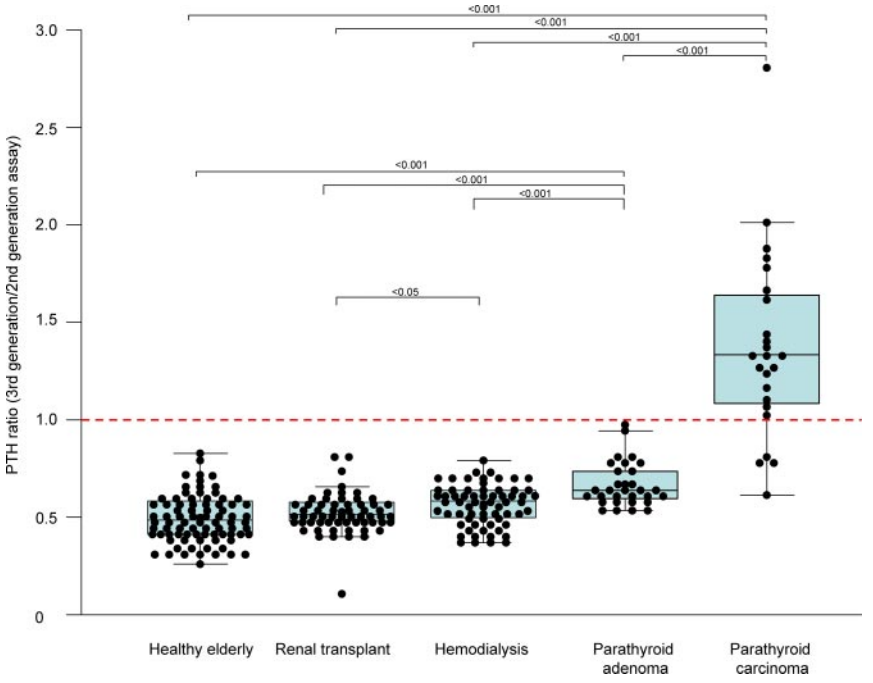

FIG. 1. Box-and-whisker plot of PTH ratio as measured by third- and second-generation PTH immunoassays in the control groups and in patients with parathyroid adenoma and PCa. The *central box* represents the values from the lower to upper quartile (25–75th percentile). The *middle line* represents the median. A *line* extends from the minimum to the maximum value, excluding outside values (values that are above/below the lower quartile ± 1.5 times the interquartile range). The third-generation to second-generation PTH ratio was significantly higher in the hemodialysis group compared with the renal transplanted group ($P < 0.05$). PHP patients had increased ratios compared with the other three control populations ($P < 0.0001$), but this was significantly lower than the ratio observed in PCa patients ($P < 0.0001$).

marginal hypercalcemia over the course of 3 yr follow-up, which suggests continuing unresolved parathyroid gland dysfunction. Blachowicz *et al.* (20) reported similar results with zero of 32 patients with PHP exhibiting an inverted third-generation to second-generation PTH ratio. In a larger study of cinacalcet therapy, Rubin *et al.* (11) reported that of eight patients with PCa studied, four (50%) had an inverted ratio. This study also showed that when cinacalcet lowered PTH, the inverted ratio remained unchanged. This contrasts with surgical treatment when the ratio may revert to less than 1 (13).

In our study, we found the third-generation to second-generation PTH ratio to be systematically less than 1 among a group of 245 subjects that included 73 hemodialysis patients, 60 renal transplant patients, 82 healthy elderly patients, and 30 PHP patients. Assessing the various published data as a whole, the prevalence of an inverted third-generation to second-generation PTH ratio is very low in PHP patients (1.6%; one in 61) and has never been reported in non-PHP controls (0%; zero in 530). In marked contrast, the frequency of an inverted third-generation to second-generation PTH ratio in the PCa population is much higher. Combining the current data with those in the literature, an inverted ratio occurred in 25 of 33 (75.8%) PCa patients reported. We observed a normal PTH ratio in four of 24 PCa patients, whereas Rubin and colleagues noted four of eight such patients (11). Taken together, data from the current study and the published literature indicate that an inverted third-generation to second-generation PTH ratio has a sensitivity of 75.8% and a specificity of 98.9% among PHP patients as a tumor marker for PCa (11, 12, 20).

Our results are derived from patients suffering from advanced PCa. It would be important to evaluate the sensitivity and specificity of the PTH ratio in PCa patients with a less advanced stage of malignancy. A useful application would be the ability to identify patients with PCa at an early stage, when the difficulty in distinguishing these challenging cases from severe benign parathyroid adenoma patients is most pronounced. Also, a prospective longitudinal study is necessary to capture the PTH ratio in a large group of PHP patients presurgically to determine whether those with a persistently inverted PTH ratio are at a greater risk of evolving into PCa. Another crucial point is whether transformation of a severe benign parathyroid adenoma to a carcinoma is associated with a change from a normal to an inverted ratio. Further investigation of the characteristics of PCa patients with a normal ratio (and PHP patients with an elevated ratio) will be necessary to determine whether any refinement can be undertaken to improve the sensitivity above 80%. The nature of the increased ratio and its relationship to the secretion of amino-PTH and other forms of PTH by PCa cells needs to be characterized in absolute terms by detailed molecular chemical analyses. Whether genetic factors such as *HRPT2* mutation status play a role in determining the forms and relative amounts of PTH secreted (and hence the third-generation to second-generation PTH ratio) remains to be determined.

In conclusion, the diagnosis and follow-up of PCa is challenging. Our results, based on a large cohort of PCa patients, shows that an inverted third-generation to second-generation PTH ratio may have clinical utility as a tumor marker for PCa, as suggested in previous smaller series. Further investigations are needed to assess the relationships between an elevated ratio or inversion of a previously normal ratio and the risk of developing PCa or the relapse of a previously treated PCa. The third-generation to second-generation PTH ratio may also be useful

as a follow-up tool in operated patients to identify those with persistent disease. However, the relationship between a third-generation to second-generation PTH ratio higher than 1 and clinical disease characteristics requires further study before its utility as a screening tool and surrogate efficacy measure in PCa can be fully confirmed.

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