QBS values that returned to normal 3 mo after successful surgery (*9*). The most significant abnormalities in that study were seen in the FN and shaft, which consist predominantly of cortical bone (*9*). Prediction of bone loss was possible when using the QBS technique in patients with renal osteodystrophy and secondary hyperparathyroidism (*10*). High QBS values, expressing high bone turnover, predicted rapid bone loss, with a sensitivity of 78% for the LS and 100% for the FN and a specificity of 71% and 78%, respectively. The correlation between QBS and the percentage of change in BMD was better in patients with chronic renal disease in the FN than in the LS, similar to the results in this study. PTH affects cancellous bone less than cortical bone, and this explains the different results for the lumbar vertebrae, although there was a similar trend of change in spinal BMD.

CONCLUSION

Present results indicate that patients with PHPT who have elevated QBS values in the FN show significant bone loss compared with patients with normal bone turnover. The results are similar in patients with symptomatic PHPT and those with asymptomatic disease. QBS, therefore, has the potential for identifying the subgroup of PHPT patients who will lose bone. Measurement of QBS in patients with PHPT assesses bone turnover, and it can predict early rapid cortical bone loss. High QBS values may indicate the need for surgery to prevent irreversible bone loss.

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Technetium-99m-Sestamibi Parathyroid Scintigraphy: Effect of P-Glycoprotein, Histology and Tumor Size on Detectability

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The purpose of this study was to evaluate the effect of P-glycoprotein (P-gp) levels, predominant histology and tumor size on the detectability of parathyroid adenomas with ^{99m}Tc-sestamibi scans. Although previous studies have shown that positivity correlates with tumor size, false-negative studies have been reported with large tumors and true-positives reported with very small tumors. Recent investigations have reported rapid washout of sestamibi in malignant tumors because of high levels of P-gp, similar to that seen with multidrug chemotherapy resistance. Therefore, we postulated that this mechanism might account for false-negative studies in parathyroid tumors. Preliminary reports have suggested that the predominant histological subtype influences positivity on ^{99m}Tc-sestamibi parathyroid scans. **Methods:** We studied 25 patients with surgically confirmed parathyroid adenomas with ^{99m}Tc-sestamibi parathyroid scans. The results of the imaging study were correlated with tissue P-gp levels, predominant histological subtype and size of the surgically removed glands. **Results:** There were 21 true-positive and 4 false-negative results. The size of the adenomas ranged from 0.12 to 8.64 ml. We found no correlation between the results of the dual-phase ^{99m}Tc-sestamibi study and either the predominant cell type or the level of P-gp. Positivity did correlate with the size of the adenoma ($\rho = 0.73$, $p < 0.0001$). We cannot exclude the possibility that P-gp and cell type may still play a role in individual cases, but we suspect that other yet to be determined factors may influence ^{99m}Tc-sestamibi detectability in addition to tumor size.

Key Words: parathyroid; p-glycoprotein; histology; volume

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Dual-phase ^{99m}Tc-sestamibi parathyroid scans are commonly performed in our institution for the preoperative localization of parathyroid adenomas. The overall accuracy has been high, but false-negative studies occasionally occur (*1*). Investigators (*1–3*) have reported a correlation between tumor size and detectability of parathyroid adenomas by sestamibi parathyroid scintigraphy. However, some very small tumors have been

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detected and false-negatives have been reported in large tumors (*4*). This suggests that size may not be the only factor that determines detectability.

P-glycoprotein (P-gp) is a plasma membrane lipoprotein encoded by the human multidrug resistance (MDR) gene. Its expression is believed to increase the efflux of chemotherapeutic drugs from cancer cells, rendering tumors resistant to therapy. Sestamibi, like many cancer drugs, serves as a substrate for P-gp transport. Because some studies have described rapid sestamibi washout in malignant cells with high levels of P-gp (*5,6*), we hypothesized that increased expression of P-gp may be responsible for some false-negative ^{99m}Tc-sestamibi parathyroid studies.

Benard et al. (*7*) reported rapid washout from a large adenoma (7 g) that had an absence of oxyphil cells. They hypothesized that a lack of mitochondria-rich oxyphil cells in parathyroid adenomas causes rapid washout of sestamibi and may lead to false-negative studies. This hypothesis was based on similar conclusions reached by Sandrock et al. (*8*) using ^{201}Tl/^{99m}Tc subtraction studies.

The purpose of our study was to evaluate the effect of P-gp levels, predominant histological subtype and size on the detectability of parathyroid adenomas.

MATERIALS AND METHODS

Patient Population

All ^{99m}Tc-sestamibi parathyroid studies performed at Georgetown University Hospital between December 1992 and March 1997 were identified. Sixty-nine patients had studies for preoperative localization of parathyroid adenomas, and 29 had surgery. All 29 patients had hyperparathyroidism documented preoperatively by increased calcium and parathormone serum levels, and the diagnosis was confirmed by surgical resection and histopathology. Four patients were excluded because hematoxylin-eosin stains could not be obtained in 2 and immunohistochemical staining was not available for the other 2. Fourteen of the 25 patients were women (age range 18–74 yr, mean 56 yr) and 11 were men (age range 23–75 yr, mean 53 yr).

Imaging

Planar and pinhole images were obtained for all patients. Initial images of the chest and neck were acquired 10–15 min after intravenous injection of 25 mCi ^{99m}Tc-sestamibi. First, 10-min images were obtained on a large-field-of-view gamma camera using a 140-keV photopeak with a 15% window and a low-energy, high-resolution collimator. The field of view included the entire neck and chest. Images for a similar time were obtained 1 and 2 hr after injection. Two patients had images taken 3 hr after injection. All patients also had 100,000-count pinhole images of the neck taken immediately after the large-field-of-view images with the thyroid bed in the center of the field of view. One patient (Patient 3) had two studies with ^{99m}Tc-sestamibi and a third study with ^{201}Tl and ^{99m}Tc-pertechnetate.

Image Analysis

Each study was reviewed retrospectively by two nuclear medicine physicians who were blinded to the results of surgery, immunohistochemistry and histopathology. The parathyroid scan images were scored by consensus. A study was interpreted as positive for parathyroid adenoma if it met one of the following criteria: (a) focal uptake with initial intensity (10-min image) greater than or equal to thyroid that clears at the same rate as the thyroid as determined by delayed images (1 and 2 hr and occasionally 3 or 4 hr); (b) focal uptake with initial intensity equal to or greater than thyroid that clears at a slower rate than the thyroid as determined by delayed images; and (c) extrathyroidal focal uptake in neck or mediastinum. All studies were graded as to the certainty of interpretation: 0 (definitely negative), 1 (possibly positive), 2 (probably positive) and 3 (definitely positive). For the purpose of analyzing the data and because the scores were identical, certainty grades of 1, 2 or 3 were considered to be positive studies.

Immunohistochemistry

The procedure for performing immunohistochemical staining for P-gp consisted of cutting 4-μ sections from the specimen that were baked at 60°C for 2 hr. The slide was then taken from xylene to water and steamed to enhance antigen retrieval. Immunohistochemistry was performed. Murine anti-MDR was allowed to incubate with the slides. A biotin-conjugated goat antimouse antibody was added, after which an avidin conjugate was allowed to bond to the biotin. The entire complex was then visualized with the addition of diaminobenzadine, which imparts a brown color localized to the sites of antibody binding (mdr-Ab-1; Calbiochem, Novabiochem International, LaJolla, CA). The level of uptake of the stain was graded from 1 to 4 on the basis of the relative uptake among the specimens, with a high level of P-gp graded as 4, moderate as 3, mild as 2 and 1 for weak or no staining. This system of analysis was based on the consensus recommendations published as a result of a series of multi-institutional trials to assess P-gp in clinical specimens (*9*).

Histologic Interpretation

All slides were evaluated by an experienced pathologist blinded to the results of the imaging study and P-gp level. All formalin-fixed tissues were sectioned (10-μ thickness) and stained with hematoxylin-eosin. The number of chief, clear and oxyphil cells was counted in five randomly selected areas, each 100 × 100 μ. The histologic interpretation was based on a method previously described by Sandrock et al. (*8*).

Statistical Analysis

Statistical analysis was performed to determine if there was a correlation between degree of certainty and P-gp, tumor volume and number of chief, oxyphil and clear cells. Spearman's rank correlation coefficient (ρ) for nonparametric variables was obtained using the software package Stata 5.0 (Stata Corp., College Station, TX). We sought to determine if there was a correlation between P-gp levels, predominant histological subtype and size of parathyroid adenomas and the results of ^{99m}Tc-sestamibi studies.

RESULTS

The results of the histopathological analysis and image interpretation are presented in Table 1. Twenty-five patients had parathyroid adenomas confirmed at surgery. Twenty-one adenomas were correctly localized on the parathyroid scan (true-positives). Four patients had negative studies, and adenomas were found at surgery (false-negatives). One of these 4 patients (Patient 3) had two studies with ^{99m}Tc-sestamibi and another with ^{201}Tl/^{99m}Tc-pertechnetate, all of which were negative. The size of the adenomas ranged from 0.12 to 8.64 ml (mean 1.97 ml). The four false-negatives (certainty level 0) had tumor sizes ranging from 0.24 to 0.42 ml (mean 0.34 ml) (Table 2). Those with certainty level 1 were 0.12–0.3 ml (mean 0.18 ml). Those with certainty level 2 were 0.27–3.0 ml (mean 1.15 ml), and the patients with adenomas of certainty level 3 had tumor sizes ranging from 0.29 to 8.64 ml (mean 3.85 ml).

Five of the 25 patients had P-gp Grade 4 (highest staining), 3 had Grade 3 (moderate staining), 7 had Grade 2 (mild staining) and 10 had Grade 1 staining (weak or no staining) (Table 2). One of the 5 patients (Patient 3) with a false-negative study had a P-gp grade of 4. However, 4 other patients with similar levels of P-gp had true-positive studies (Patients 1, 2 and 5). Immu-

TABLE 1
Results of Histopathologic Analysis and Image Interpretation

Patient no.	Age (yr)	Sex	P-gp grade	Chief cells (%)	Oxyphil cells (%)	Clear cells (%)	Sestamibi	Certainty level	Volume (ml)
1	18	F	4	94	6	0	TP	3	1.13
2	57	F	4	95	5	0	TP	2	0.27
3	75	M	4	10	90	0	FN	0	0.24
4	48	F	4	98	1	1	TP	3	1.86
5	23	M	4	24	1	75	TP	1	0.12
6	72	F	3	20	0	80	FN	0	0.42
7	74	M	3	60	38	2	TP	3	5.95
8	54	F	3	56	37	7	TP	1	0.12
9	50	M	2	19	81	0	TP	3	0.29
10	74	F	2	93	2	5	FN	0	0.28
11	60	M	2	88	0	12	TP	2	3
12	49	F	2	69	16	15	TP	3	2.25
13	54	M	2	17	0	83	TP	2	0.35
14	57	F	2	100	0	0	TP	3	2.4
15	69	F	2	11	89	0	TP	2	1.54
16	40	M	1	28	20	52	TP	3	2.87
17	29	M	1	0	100	0	TP	2	0.78
18	58	F	1	0	5	95	FN	0	0.42
19	69	F	1	13	4	83	TP	2	0.94
20	52	M	1	0	3	97	TP	3	6
21	48	M	1	0	0	100	TP	3	2.86
22	44	F	1	56	32	12	TP	3	8.64
23	75	M	1	69	16	15	TP	3	1.32
24	50	F	1	98	0	2	TP	1	0.3
25	69	F	1	3	95	2	TP	3	7.2

FN = false-negative; P-gp = P-glycoprotein; TP = true-positive.

nohistochemistry for P-gp was also performed on three normal thyroid specimens, which were all Grade 1.

The percentage of chief, oxyphil and clear cells in the various adenomas are shown in Table 1. Table 2 compares the results with the imaging findings. One study (of Patient 3) had an oxyphil cell predominance. One (of Patient 10) had a chief cell predominance, and 2 (of Patients 6 and 18) had clear cell predominance. Eleven true-positive studies had chief cell predominance, 4 had oxyphil cell predominance and 6 had clear cell predominance.

TABLE 2
Imaging Findings Versus Volume P-Glycoprotein Levels and Histology

Results	Imaging Findings
Volume	Level of certainty
0.24–0.42 ml (mean 0.34 ml)	0
0.12–0.3 ml (mean 0.18 ml)	1
0.27–3.0 ml (mean 1.15 ml)	2
0.29–8.64 ml (mean 3.85 ml)	3
P-glycoprotein levels	
Grade	Sestamibi results
4	1 FN, 4 TP
3	1 FN, 2 TP
2	1 FN, 6 TP
1	1 FN, 9 TP
Predominant histologic type	
Cell type	Sestamibi results
Oxyphil	1 FN, 4 TP
Chief	1 FN, 11 TP
Clear	2 FN, 6 TP

FN = false-negative; TP = true-positive.

Spearman's rank correlation coefficient (ρ) was obtained for the level of certainty versus P-gp level, tumor volume and dominant cell types. The correlation coefficients were as follows: between certainty and tumor volume 0.73 ($p < 0.0001$), P-gp − 0.22 ($p = 0.28$), oxyphil cell counts 0.16 ($p = 0.44$), chief cell counts 0.13 ($p = 0.55$) and clear cell counts −0.07 ($p = 0.74$). The only statistically significant correlation was between certainty and tumor volume.

DISCUSSION

Using ^{99m}Tc-sestamibi, parathyroid adenomas have been localized with high accuracy (*1,2*). False-negative studies are uncommon and have generally been limited to small tumors. Taillefer et al. (*2*) found two false-negative studies in 21 patients with parathyroid adenomas. These adenomas were small; one was 175 mg and the other was 400 mg. A study conducted at our institution (*1*) found one false-negative study in 14 patients. The adenoma was 1 cm in its greatest diameter. Chen et al. (*3*) also had similar findings with true-positive studies, having a mean of 1.29 ml, whereas those with false-negative findings were 0.73 ml. In a study by Johnston et al. (*10*), the smallest gland detected weighed 110 mg, whereas those weighing 90 and 100 mg were not detected.

In one report (*10*), a hyperplastic gland weighing 2 g was not detected. Leslie et al. (*4*) reported false-negative studies in 2 patients in whom large adenomas were found at surgery, but they did not provide details of the size. Benard et al. (*7*) reported rapid washout from a 7-g adenoma that was seen well on initial images but could not be detected on later images. These studies suggest that size is not the only determinant for positive results.

Our results support the concept that size is an important factor in determining positivity on dual-phase studies, because

the mean size of adenomas detected in our study was 1.97 ml, whereas the mean size of adenomas on false-negative studies was 0.34 ml. This theory is supported by our results, which find a significant correlation ($\rho = 0.73$, $p < 0.0001$) between the diagnostic level of certainty and tumor volume. However, it should be noted that we also detected two small adenomas that weighed 0.12 ml (Patients 5 and 8).

We sought to determine if there is an expression of P-gp by parathyroid adenomas and, if so, if there is a variation in the extent of expression and whether high levels of P-gp might result in rapid washout of sestamibi and a false-negative study. Our results show that the expression of P-gp in parathyroid adenomas varies considerably. There was no consistent correlation between the level of P-gp of a specimen and the results of the imaging study. The level of P-gp in normal thyroid specimens corresponds only to mild staining and, therefore, does not explain the faster washout of sestamibi from the thyroid than the parathyroid.

Mitchell et al. (*9*) reported that abnormal parathyroid tissue expresses less P-gp than normal parathyroid glands. However, their staining technique and interpretation differed considerably from ours and did not follow published authoritative recommendations (*11*). Their technique likely resulted in an underestimation of P-gp in adenomas.

A recent report by Benard et al. (*7*) described the rapid washout of ^{99m}Tc-sestamibi from a parathyroid adenoma confirmed to be 4 × 2 × 1.2 cm at surgery. There was a predominance of clear cells in the adenoma, with some chief cells but no oxyphil cells. The authors speculated that the presence of mitochondria-rich oxyphil cells was the critical factor for retention of sestamibi. This hypothesis is based on the data from Sandrock et al. (*8*), who found that abnormal glands detected by scintigraphy had a significantly higher number of oxyphil cells (98 ± 88.9) than false-negative (20.7 ± 32.8) or normal glands (11.7 ± 9.9, $p < 0.001$) and a lower number of clear cells (true-positive 70.9 ± 93.4, false-negative 106 ± 68.2). However, this study used ^{201}Tl and the results may not be valid for sestamibi. Our results do not support their hypothesis. Of the 5 patients who had oxyphil cell predominance, 1 had a false-negative result whereas the other 4 had true-positives. Twelve patients had chief cell predominance, 11 of which had true-positive results, and 1 had a false-negative. Eight patients had clear cell predominance, of which 2 results were false-negative and 6 were true-positive.

CONCLUSION

Our study confirms previous data that the size of a parathyroid adenoma is an important factor in determining positivity on ^{99m}Tc-sestamibi parathyroid studies. We found no significant correlation between the results of the parathyroid scan and the level of P-gp expression or the dominant histological subtype. However, this does not exclude the possibility that these factors may play a role in individual cases. The fact that false-negative studies occasionally occur with large adenomas and that positive studies may be seen with very small adenomas suggests that there may be other yet to be defined factors that affect the detectability of parathyroid adenomas by ^{99m}Tc-sestamibi parathyroid scintigraphy.

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