Relationship of Technetium Tc 99m Sestamibi Scans to Histopathological Features of Hyperfunctioning Parathyroid Tissue

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Objective: To investigate the histopathological features of pathologic parathyroid specimens associated with negative preoperative technetium Tc 99m sestamibi scans.

Design: Retrospective study.

Setting: Tertiary care center.


Interventions: Surgical exploration and removal of parathyroid adenomas.

Main Outcome Measures: Histopathological characteristics associated with true-positive and false-negative sestamibi scans, including parathyroid specimen weight, size, relative oxyphil and chief cell content, parathyroid hormone staining patterns, cellular architecture, and various immunohistochemical staining patterns.

Results: Twenty-three (24%) of 98 patients with parathyroid adenomas had negative sestamibi scan results. Among 20 age- and sex-matched patients with false-negative results vs 20 patients with true-positive results, tumor size (P = .04) and oxyphil cell content (P = .03) were found to be significantly different.

Conclusion: Parathyroid gland size and oxyphil content are descriptive and predict differences between sestamibi properties of parathyroid adenomas but have no current therapeutic implications for parathyroid surgery.


Primary hyperparathyroidism is a common endocrinopathy reported to affect approximately 1 in 1000 individuals in their 50s, 60s, and 70s. Since the advent of preoperative imaging for the localization of pathologic parathyroid glands, management of primary hyperparathyroidism has evolved significantly. Currently, primary hyperparathyroidism is treated in many clinical settings by a minimally invasive approach instead of the more traditional bilateral neck exploration.

Typically, this minimally invasive approach entails a unilateral exploration of the tracheoesophageal groove and identification of both ipsilateral superior and inferior parathyroid glands. Alternatively, an even less invasive exploration of the neck may be performed, with identification of the hyperfunctioning parathyroid gland by means of radioguidance. In either case, the ability to perform minimally invasive parathyroidectomy has fundamentally resulted from the development of more sophisticated diagnostic modalities, such as scanning with technetium Tc 99m sestamibi.

In addition to preoperative imaging, sestamibi scanning is used intraoperatively to help guide dissection and localization of hyperfunctioning parathyroid tissue. When used in conjunction with an intraoperative gamma probe, this technique allows for a smaller surgical incision over an area of increased radiotracer uptake. This technique also affords reliable intraoperative confirmation that all hyperfunctioning tissue has been adequately resected. For the treatment of sporadic primary hyperparathyroidism, it has been suggested that minimally invasive parathyroidectomy and minimally invasive radioguided parathyroidectomy are more cost-effective than bilateral neck exploration.

The sensitivity of dual-phase sestamibi scanning typically ranges from 62% to 90% for solitary adenomas and 15% to 50% for multiple-gland disease. In cases where sestamibi scans fail to detect any parathyroid disease, it has been found that...
this radiotracer did not completely accumulate in all parathyroid glands to an equal extent. In addition, certain adenomatous glands are reported to have a markedly abnormal capacity for radiotracer efflux to the extracellular compartment, which consequently causes the tracer retention time to approach that of normal parathyroid and/or adjacent thyroid tissue.\(^9\)\(^{13}\) Because tracer uptake and retention are essential for intraoperative localization, this issue has spurred numerous investigations into this phenomenon.\(^13\)

Several theories have been postulated to explain false-negative (FN) sestamibi scan results. Among these, it has been speculated that smaller sizes and weights of adenomas tend to decrease the sensitivity of nuclear imaging.\(^2\)\(^{14\text{-}16}\) In addition, parathyroid oxyphil cells are known to possess a higher mitochondrial content than either thyroid or adjacent tissue. An oxyphil cell predominance should display a greater rate of metabolic activity and hence radiotracer uptake, while a specimen with a chief cell predominance should exhibit minimal metabolic activity and radiotracer uptake. However, at this point, no study has conclusively addressed this issue.\(^8\)\(^{14\text{-}20}\) It is also speculated that FN sestamibi scan results might be associated with the presence of an intracellular protein, P-glycoprotein. This lipoprotein increases efflux of chemotherapeutic drugs from cancer cells. Various studies have suggested that P-glycoprotein may be involved in parathyroid cell efflux of radiotracer from adenomatous glands, ultimately causing premature radiotracer washout.\(^21\)\(^{23}\) Thus, given the numerous theories for the cause of FN sestamibi scan results, this study sought to compare various histopathologic characteristics of parathyroid adenomas that were associated with true-positive (TP) and FN sestamibi scan results.

**METHODS**

We retrospectively identified 114 patients with primary hyperparathyroidism who had received a preoperative dual-phase sestamibi scan from 1996 to 2001 and were subsequently treated at our institution. In 98 patients, results from preoperative scans, operative reports, and postoperative pathological analysis were available for analysis. Patients were initially included in the study if they met the criterion of a diagnosis of sporadic primary hyperparathyroidism and were scanned at our institution before their surgery. Patients were excluded if they had received any other form of preoperative imaging, such as ultrasound, computed tomography, or magnetic resonance imaging. In addition, patients were excluded if they had any history of previous parathyroid surgery, secondary or tertiary hyperparathyroidism, or multiple endocrine neoplasia type I or II.

Preoperative imaging with sestamibi was performed at our institution by means of double-phase studies interpreted 2 hours after injection of 20 mCi (740 MBq) of technetium Tc 99m sestamibi. Subsequently, these scans were read independently by 2 nuclear medicine specialists who were aware of the patient's putative diagnosis but blinded to all other imaging findings. After the delayed physiologic washout of radiotracer from the thyroid, a diagnostic localization was rendered if any observed focal concentration(s) of sestamibi uptake remained. Patients then proceeded to surgery, with the choice of the operative procedure having been determined by the results of their sestamibi scan and surgeon preference. As scan terminology often differs, we chose to define a TP result as one interpreted as showing solitary disease in any specific neck quadrant, where the location was later confirmed intraoperatively by observation and biopsy. Likewise, an FN result was defined as any equivocal or negative scan result in a patient whose parathyroid disease was later found at surgery and confirmed by biopsy. In addition, at our institution, postoperative pathological analysis of all resected specimens was considered to be the gold standard for identification of all adenomatous tissue. For this study, 2 age- and sex-matched groups comprising 20 patients with FN sestamibi scan results and 20 patients with TP results were chosen at random from our series of 114 patients and compared for physical, cellular, morphologic, and immunohistochemical characteristics of their parathyroid adenomas.

Initial intraoperative frozen and permanent specimens were stained by means of a standard hematoxylin-eosin technique and interpreted by a staff of surgical pathologists. Specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were evaluated by hematoxylin-eosin stain and a panel of antibodies by means of standard avidin-biotin immunoperoxidase techniques (Table 1).

**GROSS, ARCHITECTURAL, AND CYTOLOGIC ASSESSMENT**

The size and weight of all parathyroid adenomas was noted on gross examination. Sections of all tumors were evaluated by a single pathologist (S.K.) who was blinded to the results of all sestamibi scans. The architectural grade of 1 to 5 reflected the increasing presence of follicular features, with 1 indicating solid (0% follicular) and 5 indicating greater than 75% follicular. The cystic quality of the gland was also noted as a percentage of the total gland. The parathyroid gland was considered capsular if a majority of its perimeter had capsular features. Tumors were...
assessed for the presence or absence of a subcapsular rim of normal parathyroid and cellular predominance of oncocytic cell (oxyphilic) content (0%, 1%-25%, 26%-50%, 51%-75%, and >75%) vs chief cell content.

**IMMUNOHISTOCHEMICAL EVALUATION**

The intensity of antibody labeling was evaluated in all tumors by 2 surgical pathologists (S.K. and P.J.B.) who were blinded to the results of all sestamibi scans. Labeling intensity was scored as weak (1+), moderate (2+), or strong (3+). The percentage of cells labeled was graded as 1+ (0%-25%), 2+ (26%-75%), and 3+ (76%-100%). Labeled regions with a predominance of chief cells or oncocytic cells were also noted. The mindbomb homolog 1 (MIB-1) index and proliferation rate was calculated to be 71% for all abnormal parathyroid glands.

Of the 114 patients with documented parathyroidectomies, 98 had received preoperative localization with dual-phase sestamibi scanning before surgery. Of these 98 patients, 83 (85%) were found to have parathyroid adenomas, 13 (13%) hypercellular glands, and 2 (2%) indeterminate specimens. Of the 83 patients with parathyroid adenomas, 59 (71%) had TP sestamibi scan results that correctly localized the side and quadrant of the pathologic gland. Twenty-three patients (28%), however, had FN results, while 1 scan was interpreted as indeterminate. Of the 13 patients with hypercellular glands, 3 (23%) had negative scan results, 1 an indeterminate result, and 9 (69%) TP results. Overall, the sensitivity of dual-phase sestamibi in our patient population was calculated to be 71% for all abnormal parathyroid glands.

Of the 40 randomly selected patients studied, 11 were male and 29 were female. The average age of the men was 58.5 years (range, 27-83 years), and that of the women, 57.6 years (range, 19-86 years). Overall average age was 57.9 years (range, 19-86 years). There were no differences between the 2 groups (FN and TP).

**PHYSICAL AND HISTOLOGIC FEATURES**

Physical measures such as gland size and weight were recorded. The average (±SD) gland weight of parathyroid adenomas with TP and FN sestamibi scan results was 2.08±2.16 g (range, 0.1-8.5 g) and 1.26±1.29 g (range, 0.2-4.0 g), respectively (P=.22). Among patients with TP and FN sestamibi scan results, the size of resected glands ranged from 0.8 to 6.5 cm (mean±SD, 2.53±1.35 cm) for the former and 0.9 to 3.5 cm (1.8±0.57 cm) for the latter (P=.04). However, only 4 of 20 FN specimens were larger than 2 cm, while 12 of 20 TP specimens were larger than 2 cm. Although size and weight were greater in the patients with TP scan results, only size was statistically different between the 2 groups (P=.04).

Histologic comparison was performed on both groups (Table 2). A capsular parathyroid specimen was found in 24 patients and noncapsular parathyroid in 14 (2 were indeterminate). Seventeen glands were solid (0% follicular), 3 were up to 25% follicular, 7 were 26% to 50% follicular, 7 were 51% to 75% follicular, and 2 were greater than 75% follicular. Only 5 glands had cystic features, and most were 26% to 50% follicular. Thirty glands had up to 25% oxyphil cells, 3 glands had 26% to 50% oxyphil cells, 3 glands had 51% to 75% oxyphil cells, and 4 glands had more than 75% oxyphil cells. Of the histologic features, only oxyphil content was significantly different (P=.03) between the 2 groups when compared as high (TP) (≥25%) vs low (FN) (<25%) oxyphil content (Figure 1).

**IMMUNOHISTOCHEMICAL ANALYSIS**

By immunoperoxidase evaluation, parathyroid hormone expression was highest in areas consisting predominantly of chief cells and less intensely expressed in oxyphil cells. Thus, there was a trend toward increased expression of parathyroid hormone in the FN tumors with a low oxyphil cell content. The MIB-1 index and proliferation rate varied among tumors and was generally higher in tumors with greater oxyphil cell content (TP). Thus, there was a trend toward decreased proliferation rate in the sestamibi FN tumors. Sestamibi TP and FN cases were not markedly different when evaluated by immunoperoxidase for (1) chromogranin A, which is a protein present in neurosecretory granules; (2) 2 different antibodies against P-glycoprotein, the product of the multidrug resistance (mdr-1) gene; and (3) protein gene product 9.5, a protein present in nervous system tissue and in neuroendocrine cells (Figure 2).

**COMMENT**

Technetium Tc 99m sestamibi is currently the most popular agent for detecting hyperfunctioning parathyroid tissue. When used for preoperative localization of parathyroid adenomas, sestamibi scans can help guide the surgeon to an area of increased hormonal activity, especially in the reoperative case or a patient with an ectopic gland. It has contributed to the widespread use of minimally invasive procedures that have lower associated rates of morbidity.

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**Table 2. Histologic Comparison of Parathyroid Glands Based on Sestamibi Scan Result**

<table>
<thead>
<tr>
<th>Capsule Feature</th>
<th>No. True Positive</th>
<th>No. False Negative</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsule</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10</td>
<td>14</td>
<td>.33</td>
</tr>
<tr>
<td>No</td>
<td>10</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Follicular architecture, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid (0)</td>
<td>9</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>1-25</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>26-50</td>
<td>3</td>
<td>4</td>
<td>.63</td>
</tr>
<tr>
<td>51-75</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>&gt;75</td>
<td>2</td>
<td>0</td>
<td></td>
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<tr>
<td>Oxyphil cell distribution, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-25</td>
<td>12</td>
<td>18</td>
<td>.03†</td>
</tr>
<tr>
<td>26-50</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>51-75</td>
<td>3</td>
<td>0</td>
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<tr>
<td>&gt;75</td>
<td>3</td>
<td>1</td>
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</table>

*Difference in histologic distribution among true-positive and false-negative scan groups.
†Based on oxyphil score less than 25% and all others 25% or greater.
and mortality, operative time, recovery time, and hospitalization than routine bilateral neck dissections.2-6

Newer surgical techniques for parathyroid surgery, however, are dependent on successful uptake and delayed retention of radiotracer by the tumor. Previous studies of sestamibi localization report sensitivities between 60% and 90% depending on the method of scanning, location of pathologic gland (ectopic vs eutopic), and type of glandular abnormality (hyperplasia vs adenoma).4,5,8-12 Our sensitivity of 71% for the detection of parathyroid abnormality is consistent with these reports. Previous studies8,9 have typically found a higher sensitivity for detecting solitary adenomatous tissue vs multiglandular tissue; the reason for this finding is thought to be that gland sizes are smaller in cases of hyperplasia than in single adenomas.7

Morphologic features such as parathyroid gland size and weight have been previously investigated for their potential effect on localization rates. From these investigations, it has been reported that specimen size and/or weight had significant effects on the results of sestamibi scans.8,14,15,24,25 However, in this study, only specimen size was found to significantly affect the final sestamibi scan outcome. In addition, it appeared that a pathological gland size of less than 2 cm was the threshold value at which sestamibi scans become difficult to interpret. Among our patients, the average weight of parathyroid specimens with a TP and FN scan result also differed. Patients with TP results were found to have slightly larger tumors than patients with FN results. However, because of a large observed variation among specimen weights within both groups, this difference was not statistically significant.

Like specimen size, certain histologic features of parathyroid glands were also suspected to be associated with FN sestamibi scan results. Because sestamibi is principally accumulated by mitochondria,17 a distinct cellular predominance within adenomas may affect how well radiotracer is retained. Within the literature, isolated studies have demonstrated that a high oxyphil content in adenomas is

Figure 1. Histologic appearance. Tumors varied in their architectural appearance, being variably follicular (A) or solid (B) and having a preponderance of either chief cells (C) or oxyphils (D) (hematoxylin-eosin, original magnification ×200).
associated with a TP sestamibi scan result. Others, however, have found that no relationship exists between oxyphil predominance and the sestamibi scan interpretation. This latter observation, however, may be partially explained by the fact that numerous studies have used a limited sample size of patients with FN scan results for comparison. As a result, these studies have often failed to attain statistical significance between groups, although in many cases the data suggested a possible relationship.

Carpentier et al reported that a higher oxyphil content (>25%) was associated with an increased late technetium uptake in only 11 cases of primary hyperparathyroidism. Thompson et al failed to demonstrate any significance between sestamibi scan results and the oxyphil content from 14 FN specimens. In this report, all glands containing a high content of oxyphils were found to have TP results, while those with fewer oxyphils had FN results. Parathyroid specimens with greater than 25% oxyphil content were more likely to have a positive sestamibi scan result. In 2 of 20 FN results, the specimens exhibited greater than 25% oxyphil content. Compared with previous reports, our report represents the largest published study that attempted to compare the relationship between FN results and the oxyphil content of pathological specimens.

Follicular architecture and the presence or absence of a parathyroid capsule did not have any relationship to sestamibi scan outcomes. No previous studies, to our

Figure 2. Immunohistochemical evaluation of chief cell–rich regions. There was a greater expression of parathyroid hormone in chief cell–rich areas (A) than in oncocyte-rich areas (not shown). In contrast, chromogranin A (B), protein gene product 9.5 (C), and P-glycoprotein (D) did not consistently label chief cell–rich areas as opposed to oncocyte-rich areas (A and B, DAB chromagen, original magnification ×200; C and D, AEC chromagen, original magnification ×200).
knowledge, have investigated these histologic characteristics. Our hypothesis was that a greater follicular architecture would have decreased cellular density, which might have made a negative sestamibi result more likely. The feature was not statistically significant in this study. Parathyroid capsule is difficult to quantify because of the bias inherent in limited tissue sections that may or may not contain that aspect of the parathyroid gland, as well as iatrogenic artifact resulting from surgical trauma. Parathyroid capsule examination is the preferred method of making the classic pathological diagnosis of parathyroid adenoma because it will show a subcapsular rim of suppressed normal parathyroid gland.

Parathyroid hormone expression was highest in areas of chief cells and less intensely expressed among oxyphil cells. There was a trend toward increased expression of parathyroid hormone in the FN tumors with a low oncocytic cell content. The MI-B1 index and proliferation rate varied among tumors and was generally higher in tumors with greater oncocytic cell content, which are TP tumors. Cases with TP and FN sestamibi results were not consistently distinguished by immunoperoxidase evaluation for chromogranin A, 2 antibodies against P-glycoprotein, and protein gene product 9.5. To our knowledge, this is the first report regarding expression of protein gene product 9.5 in parathyroid tumors. This protein has been identified in peripheral and central nervous system tissues, including some neural tumors, and in neuroendocrine cells, both normal and neoplastic, including cells from the pituitary and pancreatic islets.

CONCLUSIONS

Gland size and oxyphil content were the only statistically significant variables studied that could discriminate between sestamibi-positive and sestamibi-negative adenomas. By immunoperoxidase evaluation, parathyroid hormone expression was highest in areas consisting predominantly of chief cells and less intensely expressed in oncocytic cells. Thus, there was a trend toward increased expression of parathyroid hormone in the tumors with FN results and a reduced oncocytic cell content. The MI-B1 index and proliferation rate varied among tumors and was generally higher in tumors with greater oncocytic cell content in tumors with TP results. Cases with TP and FN sestamibi results were not consistently distinguished by immunoperoxidase evaluation for chromogranin A, 2 antibodies against P-glycoprotein, and protein gene product 9.5. Parathyroid gland size and oxyphil content are descriptive and predict differences between sestamibi scanning properties of parathyroid adenomas but have no current therapeutic implications for parathyroid surgery.

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REFERENCES