ASSESSMENT OF UTILITY OF STAINING IN THE INTRAOPERATIVE LOCALIZATION OF PARATHYROID GLANDS

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Kierownik: prof. dr hab. M. Drews

Identifying the primary and secondary hyperparathyroidism is a recommendation for the surgical treatment. To streamline the surgical treatment there are applied before- and intra-operative methods of location of parathyroid glands.

The aim of the study was to assess an effectiveness and benefits of intra-operative colouring of parathyroid glands with methylene blue.

Material and methods. In the Department of General and Endocrinological Surgery, Medical University in Poznań in the years 2000-2005 116 patients with hyperparathyroidism (61 with primary and 55 secondary hyperparathyroidism) were operated. At 82 patients a method of indicating parathyroid glands with methylene blue was applied. At all patients there were assessed a duration of the operation, the number of prepared parathyroid glands, and the number of the histopathological tests necessary for their identification, as well as procedures during which thyroid gland were additionally operated. There were compared methods of before- and intra-operative location of the glands.

Results. After a statistical analysis of gathered material there was stated characteristic statistically difference of shortening the duration of operation in the group with colouring in comparison to the group without colouring. There were also reduced amount of intra-operative tests needed for identification of removed glands. The method is considered as significantly streamlining the operation. Limitation of the duration of the treatment reduced the perioperative injury, shortened the period of the hospitalization, and consequently reduced costs of the treatment. Colouring method showed itself to be the most effective method of the location of parathyroid glands. The majority of preoperative methods concerns only ectopic glands.

Conclusions. Colouring with methylene blue is a method facilitating identifications of parathyroid glands in the operative field, shortening the duration of the treatment and at the same time safe, cheap and readily available.

Key words: parathyroid glands, intraoperative colouring, surgical treatment

Usually an attempt to localize parathyroid glands is a first step in preparation for surgical treatment of disorders of these glands and concurrently one of the most serious problems encountered by a surgeon undertaking an operation. Therefore there is an increasing emphasis on research that would enable development of a technique allowing for accurate localization of parathyroid glands. This task is difficult since parathyroid glands are characterized by variable size, shape, distribution and number.

Popular and commonly used imaging modalities for localization of parathyroid glands include ultrasound imaging, computed tomography, magnetic resonance imaging and 99mTc MIBI scintigraphy in the preoperative diagnostics and determination of PTH concentration and intraoperative staining of parathyroid glands with methylene blue as well as a probe with gamma camera, used since recently at some sites (1-4). US imaging localizes the parathyroid glands in 75-85% of cases and is non-invasive and relatively cheap. It is difficult
or even impossible to visualize abnormalities of parathyroid glands located in the posterior neck region, behind the esophagus, retrosternally in the mediastinum. In such cases computed tomography is a much effective modality, while MRI is particularly useful in recurrent cases, after previous operations. This diagnostic modality is able to differentiate between scar tissue and thyroid or parathyroid tissue. New MRI techniques are able to visualize a tumor with diameter less than 1 cm. CT can demonstrate a tumor with diameter less than 0.6 cm. Thallium/technetium scintigraphy is done by scanning the neck after Te/Tc injection and repeated scanning after injection of thallium chloride. This technique is based on different uptake of radioisotopes depending on the nature of the parathyroid tumor and is able to assess function and metabolism of the parathyroid gland, unlike US imaging, CT and MRI which enable differentiation of parathyroid tumors basing on their mass and density. Thallium/technetium scintigraphy identifies approximately 75% of parathyroid tumors (7-11).

Intraoperative staining is a valuable method to localize parathyroid glands. Herlitz was the first to report it in 1968. He used toluidine blue as a staining agent which later turned out to cause many side effects, including cardiac arrhythmias and cardiac arrest. Because of this, further clinical trials with this agent were discontinued. Methylene blue (MB), a thiazide staining agent, was initially used in the treatment of intoxications with methemoglobin-generating agents, because of its ability to cumulate in endocrine gland tissues, was used for the first time by Dudle in 1971 to stain parathyroid glands. Since this time there were reports positively assessing this method in patients with both primary and secondary hyperparathyroidism (12, 13).

Selective venous catheterization (SVC) is an invasive procedure used to determine PTH level in the venous blood taken from mediastinal veins (inferior thyroid veins). This method is reserved for the patients with a history of parathyroid surgery in whom non-invasive techniques provide negative results. SVC combined with on-invasive methods confirms tumor localization and is very useful in patients with multiple endocrinopathies. However this technique is not useful in diagnosing hyperparathyroidism.

The aim of this study was to assess utility of parathyroid staining using methylene blue in intraoperative parathyroid localization, in particular:

1. Assessment of effect of this method on duration of the surgical procedure and number of intraoperative histopathological examinations required to confirm resected parathyroid glands.
2. Comparison of effectiveness of preoperative and intraoperative methods determining localization of parathyroid glands.

**MATERIAL AND METHODS**

One hundred sixteen patients with hyperparathyroidism (61 patients with primary hyperparathyroidism and 55 with secondary hyperparathyroidism) underwent surgical treatment in 2000-2005 in the Chair and Department of General, Gastroenterological and Endocrinological Surgery, Medical University in Poznań.

Number of parathyroid glands excised during the procedure, duration of the procedure and number of histopathological examinations required for their identification as well as procedures used to additionally operate on the thyroid gland, were assessed in any patient. Methods of preoperative and intraoperative assessment of the gland localization were compared. US imaging and MIBI scintigraphy were performed before the procedure. During the procedure, methylene blue staining was used in 82 patients.

The disease was diagnosed at Chair and Clinic of Endocrinology, Metabolism and Internal Medicine, Medical University in Poznań (head: prof. dr hab. J. Sowiński, M.D.) and Chair and Clinic of Nephrology, Transplantology and Internal Medicine, Medical University in Poznań (head: prof. dr hab. S. Czekalski, M.D.).

The diagnosis was made on the basis of laboratory tests: concentration of ionized and total calcium was determined as well as preoperative, intraoperative and postoperative parathyroid hormone concentration. Imaging studies were performed: US assessment of the neck and MIBI scintigraphy. The excised parathyroid glands were assessed at Department of Pathological Anatomy, Medical University in Poznań (head: prof. dr hab. P. Majewski, M.D.) to confirm the diagnosis.
Laboratory analyses were performed at the central laboratory of Samodzielny Publiczny Szpital Kliniczny no. 2 in Poznań (head: dr B. Knitter).

The obtained results were subjected to statistical analysis in cooperation with Laboratory of Morphometry and Processing of Medical Images, Medical University in Poznań (head: prof. dr hab. E. Kaczmarek, M.D.).

Patients who were admitted to the Clinic to undergo surgical treatment of hyperparathyroidism, were prepared for the surgery in a typical manner for this type of procedures. In the event of patients with chronic renal failure, date of the surgery was adjusted to previously scheduled dates of hemodialysis sessions.

The patients were operated according to a single schedule: after anesthesia and intubation, an intravenous infusion of 1% MB in 500 ml 0.9% NaCl was started at a dose of 5 mg/kg BW. The rate of infusion was chosen in this way that the first 200 ml was infused within 30 minutes while the remaining 300 ml of the staining agent solution was infused over the next 20-30 minutes (14, 15, 16).

The neck exploration was performed via Kocher’s incision. When the thyroid gland was exposed and its poles were demonstrated, an attempt was made to localize the parathyroid glands at their typical locations, between the thyroid gland and the neck blood vessels.

In majority of the patients, the parathyroid tumor was found at the posterior part of the thyroid capsule, often in the area of the recurrent laryngeal nerve. Less often parathyroid tumors were found at the superior pole of the thyroid gland, in the trachea-esophageal region, along large blood vessels of the neck, in the anterior mediastinum, inside the thymus or in the posterior mediastinum (17-20).

An attempt was made to avoid major bleeding during the surgical procedure because analysis of the tissue color is useful in the differentiation between the parathyroid glands and the thyroid gland, thymus, adjacent lymph nodes and fatty tissue. During intraoperative localization of parathyroid tumors, branches of the inferior thyroid artery were carefully inspected as well as a delicate palpation of the trachea-esophageal groove was performed. Despite the fact that in the primary hyperparathyroidism, a single parathyroid adenoma was found in approximately 85% of cases, an operator made an attempt to identify at least one normal parathyroid gland to compare it with the parathyroid gland suspected of being abnormal. When no superior parathyroid gland was found, the retroesophageal region and posterior mediastinum were carefully inspected. The tissues that were removed during the surgery, underwent histopathological examination to confirm that they had a parathyroid histology. Since 2004 parathyroid hormone was routinely determined intraoperatively. A blood sample is taken at the beginning of the procedure and after resection of each probable parathyroid gland. An operation was considered successful when there was a significant decrease of the hormone concentration by at least 50% after resection of a gland/glands (21-29). When a coexisting nodular goiter was found, adequate thyroid operation was performed. Parathyroid glands stained within variable time period, ranging from 50 to 80 minutes from the start of the staining agent administration. When parathyroid glands were found and resected, short muscles of the neck and platysma were sutured and Redon’s drain was left for one day.

A transient drop of oxygen saturation to 89% (measured with a pulsoximeter on a tip of an index finger) was found during the surgical procedure. These parameters were normalized within approximately 5-10 minutes after the end of the methylene blue infusion. Bluish color of urine remained until day four after the surgical procedure in patients without renal failure. Due to possible deficiency of serum calcium in the postoperative period, calcium level was monitored daily and calcium supplements and vitamin D were given. The patients left the Clinic on day 4-5 after the surgical procedure. There was no case of postoperative complications. No death occurred after the surgical procedure.

**RESULTS**

The study results were subjected to statistical analysis. Kruskal-Wallis test with Dunn’s test were used for comparisons between all groups. Furthermore, results for primary hyperparathyroidism and secondary hyperparathyroidism were compared using Mann-Whitney’s test. Method sensitivity was compared with Gauss’s test for structural indices. Statistical analysis was performed using STATISTICA software.
Table 1. Duration of the surgical procedure in patients with primary and secondary hyperparathyroidism without methylene blue staining

<table>
<thead>
<tr>
<th>Hyperparathyroidism</th>
<th>Mean duration of surgical procedure</th>
<th>Number of patients</th>
<th>Duration of surgical procedure Standard deviation</th>
<th>Minimum duration of surgical procedure</th>
<th>Maximum duration of surgical procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>180</td>
<td>13</td>
<td>24.66</td>
<td>130</td>
<td>225</td>
</tr>
<tr>
<td>Secondary</td>
<td>196.67</td>
<td>21</td>
<td>24.51</td>
<td>170</td>
<td>260</td>
</tr>
<tr>
<td>Total</td>
<td>190.29</td>
<td>34</td>
<td>25.55</td>
<td>130</td>
<td>260</td>
</tr>
</tbody>
</table>

No statistically significant differences

Duration of the surgical procedure in non-stained groups was on average 190.29 min. The duration of the surgical procedure in patients with primary hyperparathyroidism was on average 180 min. The shortest operation took 130 min, the longest – 225 min. The duration of the surgical procedure in patients with secondary hyperparathyroidism was on average 197 min. The shortest operation took 170 min, the longest – 260 min.

Table 2. Duration of the surgical procedure in patients with primary and secondary hyperparathyroidism with methylene blue staining

<table>
<thead>
<tr>
<th>Hyperparathyroidism</th>
<th>Mean duration of surgical procedure</th>
<th>Number of patients</th>
<th>Duration of surgical procedure Standard deviation</th>
<th>Minimum duration of surgical procedure</th>
<th>Maximum duration of surgical procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>153,0208</td>
<td>48</td>
<td>22.47314</td>
<td>130</td>
<td>240</td>
</tr>
<tr>
<td>Secondary</td>
<td>170</td>
<td>34</td>
<td>22.32677</td>
<td>130</td>
<td>240</td>
</tr>
<tr>
<td>Total</td>
<td>160,0610</td>
<td>82</td>
<td>23.81116</td>
<td>130</td>
<td>240</td>
</tr>
</tbody>
</table>

The difference was statistically significant (p<0.003)

Duration of the surgical procedure in stained group was on average 160.06 min. The duration of the surgical procedure in patients with primary hyperparathyroidism was on average 153.02 min. The shortest operation took 130 min, the longest – 240 min. The duration of the surgical procedure in patients with secondary hyperparathyroidism was on average 170 min. The shortest operation took 170 min, the longest – 240 min. The shortest operation took 130 min, the longest – 260 min. There was a statistically significant difference with regard to duration of the surgical procedure between patients with primary and secondary hyperparathyroidism in the group with methylene blue staining.

Table 3. Comparison of duration of the surgical procedure in patients with primary hyperparathyroidism, with and without methylene blue staining

<table>
<thead>
<tr>
<th>Methylene blue yes/no</th>
<th>Mean duration of surgical procedure</th>
<th>Number of patients</th>
<th>Duration of surgical procedure Standard deviation</th>
<th>Minimum duration of surgical procedure</th>
<th>Maximum duration of surgical procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>No staining</td>
<td>180</td>
<td>13</td>
<td>24.66</td>
<td>130</td>
<td>225</td>
</tr>
<tr>
<td>Staining</td>
<td>153,02</td>
<td>48</td>
<td>22.47</td>
<td>130</td>
<td>240</td>
</tr>
<tr>
<td>Total</td>
<td>158,77</td>
<td>61</td>
<td>25.33</td>
<td>130</td>
<td>240</td>
</tr>
</tbody>
</table>

The difference was statistically significant p< 0.0001

The duration of the surgical procedure in patients with primary hyperparathyroidism, without staining, was on average 180 min. The shortest operation took 130 min, the longest – 225 min. The duration of the surgical procedure in patients with primary hyperparathyroidism, with staining, was on average 153 min. The shortest operation took 130 min, the longest – 240 min. We found a significant decrease of duration of the surgical procedure in patients with primary hyperparathyroidism after administration of methylene blue (p<0.0001).

Table 4. Comparison of duration of the surgical procedure in patients with secondary hyperparathyroidism, with and without methylene blue staining

<table>
<thead>
<tr>
<th>Methylene blue yes/no</th>
<th>Mean duration of surgical procedure</th>
<th>Number of patients</th>
<th>Duration of surgical procedure Standard deviation</th>
<th>Minimum duration of surgical procedure</th>
<th>Maximum duration of surgical procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>No staining</td>
<td>196.67</td>
<td>21</td>
<td>24.51</td>
<td>170</td>
<td>260</td>
</tr>
<tr>
<td>Staining</td>
<td>170</td>
<td>34</td>
<td>22.33</td>
<td>130</td>
<td>240</td>
</tr>
<tr>
<td>Total</td>
<td>180.18</td>
<td>55</td>
<td>26.42</td>
<td>130</td>
<td>260</td>
</tr>
</tbody>
</table>

The difference was statistically significant (p<0.0001)

The duration of the surgical procedure in patients with secondary hyperparathyroidism, without staining, was on average 196.67 min. The shortest operation took 170 min, the longest – 260 min. The duration of the surgical procedure in patients with secondary hyperparathyroidism, with staining, was on average 170 min. The shortest operation took 130 min, the longest – 240 min. We found a significant decrease of duration of the surgical procedure in patients with secondary hyperparathyroidism after administration of methylene blue (p<0.0001).
Table 5. Duration of the surgical procedure depending on the number of stained parathyroid glands in patients with primary hyperparathyroidism

<table>
<thead>
<tr>
<th>Methylene blue staining</th>
<th>Mean duration of surgical procedure</th>
<th>Number of patients</th>
<th>Duration of surgical procedure</th>
<th>Minimum duration of surgical procedure</th>
<th>Maximum duration of surgical procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>180.00</td>
<td>13</td>
<td>24.66</td>
<td>130</td>
<td>225</td>
</tr>
<tr>
<td>1</td>
<td>160.94</td>
<td>16</td>
<td>32.72</td>
<td>130</td>
<td>240</td>
</tr>
<tr>
<td>2</td>
<td>154.29</td>
<td>14</td>
<td>16.97</td>
<td>130</td>
<td>180</td>
</tr>
<tr>
<td>3</td>
<td>147.50</td>
<td>6</td>
<td>10.84</td>
<td>130</td>
<td>160</td>
</tr>
<tr>
<td>4</td>
<td>143.75</td>
<td>12</td>
<td>10.03</td>
<td>130</td>
<td>155</td>
</tr>
<tr>
<td>Total</td>
<td>158.77</td>
<td>61</td>
<td>25.33</td>
<td>130</td>
<td>240</td>
</tr>
</tbody>
</table>

The difference between “0” and “4” was statistically significant (p<0.003)

0 – average duration of the surgical procedure in patients with primary hyperparathyroidism without methylene blue staining 180 min.
1 – stained parathyroid gland, average duration of the surgical procedure 160.94 min. The shortest surgical procedure took 130 min, the longest – 140 min.
2 – stained parathyroid glands, average duration of the surgical procedure 154.29 min. The shortest surgical procedure took 130 min, the longest – 180 min.
3 – stained parathyroid glands, average duration of the surgical procedure 147.50 min. The shortest surgical procedure took 130 min, the longest – 160 min.
4 – stained parathyroid glands, average duration of the surgical procedure 143.75 min. The shortest surgical procedure took 130 min, the longest – 155 min.

Table 6. Duration of the surgical procedure depending on the number of stained parathyroid glands in patients with secondary hyperparathyroidism

<table>
<thead>
<tr>
<th>Methylene blue staining</th>
<th>Mean duration of surgical procedure</th>
<th>Number of patients</th>
<th>Duration of surgical procedure</th>
<th>Minimum duration of surgical procedure</th>
<th>Maximum duration of surgical procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>196.67</td>
<td>21</td>
<td>24.51</td>
<td>170</td>
<td>260</td>
</tr>
<tr>
<td>1</td>
<td>205</td>
<td>1</td>
<td>0</td>
<td>205</td>
<td>205</td>
</tr>
<tr>
<td>2</td>
<td>192.50</td>
<td>2</td>
<td>10.61</td>
<td>185</td>
<td>200</td>
</tr>
<tr>
<td>3</td>
<td>169</td>
<td>10</td>
<td>29.42</td>
<td>130</td>
<td>240</td>
</tr>
<tr>
<td>4</td>
<td>166.67</td>
<td>21</td>
<td>17.42</td>
<td>140</td>
<td>210</td>
</tr>
<tr>
<td>Total</td>
<td>180.18</td>
<td>55</td>
<td>26.42</td>
<td>130</td>
<td>260</td>
</tr>
</tbody>
</table>

The difference between “0” and “3” (p<0.015) and “0” and “4” (p<0.0005) was statistically significant.

0 – average duration of the surgical procedure in patients with secondary hyperparathyroidism without methylene blue staining 196.67 min.
1 – stained parathyroid gland, average duration of the surgical procedure 160.94 min. The shortest surgical procedure took 130 min, the longest – 140 min.
2 – stained parathyroid glands, average duration of the surgical procedure 154.29 min. The shortest surgical procedure took 130 min, the longest – 180 min.
3 – stained parathyroid glands, average duration of the surgical procedure 147.50 min. The shortest surgical procedure took 130 min, the longest – 160 min.
4 – stained parathyroid glands, average duration of the surgical procedure 143.75 min. The shortest surgical procedure took 130 min, the longest – 155 min.

During the surgical procedure, the resected tissues underwent intraoperative histopathological examination to confirm their parathyroid histology.

Table 7. Comparison of the number of ordered histopathological examinations during procedures without methylene blue staining in patients with primary and secondary hyperparathyroidism

<table>
<thead>
<tr>
<th>Hyperparathyroidism</th>
<th>Average number of histopathological examinations</th>
<th>Number of patients</th>
<th>Number of histopathological examinations</th>
<th>Minimum number of histopathological examinations</th>
<th>Maximum number of histopathological examinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>3</td>
<td>13</td>
<td>1.41</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Secondary</td>
<td>7</td>
<td>21</td>
<td>1.55</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>5.47</td>
<td>34</td>
<td>2.46</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

The difference was statistically significant (p<0.0001)

An average 5.47 histopathological examinations were ordered in a non-stained group. On average 3 examinations, minimum 1, maximum 6 examinations, in patients with primary hyperparathyroidism. On average 7 examinations, minimum 4, maximum 9 examinations, in patients with secondary hyperparathyroidism. A statistically significant difference was found with regard to the number of intraoperative histopathological examinations between patients with primary and secondary hyperparathyroidism, in our study group.
Table 8. Comparison of the number of ordered histopathological examinations during procedures with methylene blue staining in patients with primary and secondary hyperparathyroidism

<table>
<thead>
<tr>
<th>Hyperparathyroidism</th>
<th>Average number of histopathological examinations</th>
<th>Number of patients</th>
<th>Number of histopathological examinations</th>
<th>Minimum number of histopathological examinations</th>
<th>Maximum number of histopathological examinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>2.50</td>
<td>48</td>
<td>1.13</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Secondary</td>
<td>5</td>
<td>34</td>
<td>1.46</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>3.54</td>
<td>82</td>
<td>1.77</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

The difference was statistically significant (p<0.0001)

An average 3.54 examinations were ordered in the stained group. On average 2.5 examinations, minimum 1, maximum 5 examinations, in patients with primary hyperparathyroidism. On average 5 examinations, minimum 3, maximum 9 examinations, in patients with secondary hyperparathyroidism. A statistically significant difference was found with regard to the number of intraoperative histopathological examinations between patients with primary and secondary hyperparathyroidism, in the analyzed study group that received methylene blue (p<0.0001).

Table 9. Comparison of preoperative and intraoperative methods of the parathyroid gland localization. The assessed group included 82 patients in whom a methylene blue staining method was used. All patients underwent US imaging and MIBI scintigraphy before the procedure. During the procedure, results of preoperative diagnoses were verified.

![Graph showing comparison of methods](image_url)

<table>
<thead>
<tr>
<th>Hyperparathyroidism</th>
<th>Methylene blue</th>
<th>US imaging</th>
<th>Scintigraphy</th>
<th>Number of patients</th>
<th>Method comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>1</td>
<td>46</td>
<td>28</td>
<td>42</td>
<td>staining vs US p&lt;0.0001</td>
</tr>
<tr>
<td>%</td>
<td>95.83</td>
<td>58.33</td>
<td>87.50</td>
<td>48</td>
<td>staining vs scintigraphy ns</td>
</tr>
<tr>
<td>Number</td>
<td>2</td>
<td>30</td>
<td>14</td>
<td>34</td>
<td>staining vs US p&lt;0.0001</td>
</tr>
<tr>
<td>%</td>
<td>88.24</td>
<td>41.18</td>
<td>47.06</td>
<td>34</td>
<td>staining vs scintigraphy p&lt;0.0001</td>
</tr>
<tr>
<td>Number</td>
<td>Total</td>
<td>76</td>
<td>42</td>
<td>58</td>
<td></td>
</tr>
</tbody>
</table>

In primary hyperparathyroidism, US imaging and MIBI scintigraphy were considered successful if they localized at least one parathyroid gland with an adenoma. The same criterion was adopted for the methylene blue staining. In secondary hyperparathyroidism, US imaging and MIBI scintigraphy were considered successful if they localized at least three parathyroid glands. The same criterion was adopted for the methylene blue staining.

**DISCUSSION**

We have not fund in the available literature any report that would analyze duration of the surgical procedure with parathyroid staining with methylene blue. In our study we found a statistically significant difference between duration of surgical procedure performed to treat hyperparathyroidism with and without methylene blue staining. Duration of the procedure was significantly shorter with MB administration. Opportunity of immediate PTH determination essentially eliminated the requirement of waiting for histopathological confirmation of resected parathyroid glands. Currently, after determination of PTH level and finding its significant decrease, the procedure can be finished.

The method of methylene blue staining proved very useful in intraoperative identification of parathyroid glands. Its introduction significantly facilitated assessment of opera-
Assessment of utility of staining in the intraoperative localization of parathyroid glands

The surgical procedure, all patients underwent US imaging, MIBI scintigraphy and PET. Thereafter they underwent operation and the parathyroid glands, resected according to localization clues, underwent histopathological examination. Thirty six parathyroid glands were verified histopathologically in 12 patients with secondary hyperparathyroidism. Twenty five (69%) of them were localized using PET with 11C methionine and 17 (47%) using $^{99m}$Tc-MIBI. PET was positive in 17 out of 18 (94%) cases of adenomas and cancers, while $^{99m}$Tc-MIBI scintigraphy (SPECT) localized only 50% of pathologies. All 8 atypically located adenomas were demonstrated by PET but only 6 out of 8 by $^{99m}$Tc-MIBI scintigraphy (SPECT). Basing on obtained results, the authors emphasize that positron emission tomography is the most accurate method to localize pathological parathyroid glands in secondary hyperparathyroidism and they recommend it when US imaging or $^{99m}$Tc-MIBI scintigraphy (SPECT) are inefficient.

Not all authors are so enthusiastic about scintigraphic methods. Authors from Cleveland (9) reported 22% false negative rate in sestamibi examinations. Among 146 patients undergoing parathyreidectomy, 89 cases of primary hyperparathyroidism were found: 76 solitary adenomas and 13 multinodular lesions – all patients from this group were examined using the assessed technique. Results: sensitivity 77%, specificity 99%, false negative rate 22%. Sensitivity of this diagnostics modality was higher with a solitary adenoma than with multinodular lesions (83-38%). Higher false negative rate was obtained in patients with small tumors and without hypercalcemia.

Intraoperative measurement of blood parathyroid hormone concentration is an examination that supports effectiveness of surgical management. Due to short half-time of this hormone, its concentration rapidly decreases after resection of an overactive gland (33, 34, 27). Polish sites are not worse from foreign sites and this method, which was positively verified in our clinic, was also the subject of reports by teams from Warszawa (35) and Kraków (36, 37, 38). The same technique is used by authors from Spain (Obiols et al.), Brazil (39) and USA (40). The last of cited authors reports on 107 operations performed by a single operator due to primary, secondary and tertiary hyperparathyroidism. Success of

tive field and enabled precise excision of proper tissues and therefore decreased number of resections of incorrect tissues suspected of being parathyroid glands. Lower number of resections limited periprocedural trauma and decreased the number of complications such as intraoperative and postoperative bleedings and injuries of the recurrent laryngeal nerve. Quicker localization of parathyroid glands resulted in shortening of the procedure duration.

Utility of this method and its effect on efficacy of the surgical procedure were confirmed by comparing duration of surgical procedures during which 4, 3, 2 and 1 parathyroid glands were stained. We found that the procedures in which 4 parathyroid glands were stained at once were the shortest ones both in primary and secondary hyperparathyroidism. Operations during which less glands were stained, required longer procedure duration to localize unstained parathyroid glands.

Diagnosing of hyperparathyroidism (HPT) and localization of parathyroid glands basing on biochemical tests, US imaging and $^{99m}$Tc-MIBI is a standard management according to authors of the paper from Gdańsk (30). However, when they used such diagnostic standard in 220 patients with hyperparathyroidism, who subsequently underwent operation, hyperparathyroidism persisted in 8 (6%) out of 132 patients with secondary hyperparathyroidism.

Surgeons from Seattle (31) operated 185 patients with primary hyperparathyroidism in whom they had used US imaging and scintigraphy as diagnostic modalities in the preoperative localization. In 87% of cases, the hyperparathyroidism was caused by a solitary adenoma and in 13% of cases more than one gland was involved. US imaging localized 75% and scintigraphy 83% enlarged parathyroid glands.

Otto et al. from Hannover (32) used two methods to localize parathyroid glands in cases of secondary and tertiary hyperparathyroidism causing diagnostic difficulties as well as in cases of ectopic glands: positron emission tomography (PET) with 11C methionine and single photon emission computed tomography (SPECT) using $^{99m}$Tc methoxyisobutylxirnitrile (MIBI). They examined 30 patients: 16 with primary HPT, 12 with secondary HPT and 2 with recurrence of parathyroid cancer. Before
surgical treatment, confirmed by intraoperative measurement of parathyroid hormone concentration, was achieved in 93.4% patients. To intraoperatively differentiate hyperactive from normally active parathyroid glands, authors from France (43) studied parathyroid hormone concentration at sites indicated by previous localizing studies and/or found during the surgery. They found that in 88% punctuates from overactive glands, parathyroid hormone concentration exceeded 1000 pg/ml, while in 79% of normal glands it was below 1000 pg/ml. The authors concluded that measurement of intra-parathyroid concentration of parathyroid hormone can be useful, in combination with localizing studies, in intraoperative differentiation between normal and hyperactive glands.

Recently radioguided parathyreoidectomy (finding a pathologic parathyroid gland that uptakes radiolabeled tracer with a gamma probe). This method, presented in the literature by various sites, enables targeted operation with high success rate (42, 43, 44). Literature reports are very positive and suggest that it combines benefits related to direct identification of localization of parathyroid glands in the surgical field (provided by the staining method) with an opportunity to intraoperatively assess space outside the operative field (which up to this time has been reserved for scintigraphy) — allowing localization of ectopic parathyroid glands.

Positron emission tomography (PET) with HC-methionine was used to localize abnormal parathyroid glands in St. Thomas Hospital in London (45). The study was performed in 51 patients in whom conventional diagnostic modalities were unable to localize an adenoma. This diagnostic modality had 83% sensitivity and 100% specificity. False negative results were obtained in patients in whom an adenoma was located low in the mediastinum, outsider the searching area. Adenoma is the most common cause of the primary hyperparathyroidism. Its accurate preoperative localization is the key to success of the operation, in particular — targeted operation that can be associated with markedly smaller operative trauma and lower costs of treatment in the postoperative period.

Therefore, despite availability of diagnostic standards, a vivid discussion is ongoing on optimization of imaging of diseased parathyroid glands, in particular when typical diagnostic methods — US imaging and scintigraphy with 99mTc methoxyisobutynitrile (MIBI) are ineffective (46, 47, 48).

Genc et al. (49) compared value of two operative techniques with regard to diagnosis accuracy. They operated a group of 80 unselected patients with primary hyperparathyroidism in whom US imaging or sestamibi scintigraphy demonstrated enlargement of a solitary parathyroid gland. Intraoperative parathyroid hormone concentration was measured in all patients. Forty five patients underwent conventional bilateral neck exploration using an open method, while 35 patients underwent a targeted operation, involving only a gland previously localized by MIBI or US imaging. In the first group, a solitary adenoma was found in 38 patients (84%), two adenomas in 3 (7%) patients, hyperplasia in 3 (7%) while cancer in 1 (2%) patient. In the second group only a single adenoma was found in all patients. After the operation, all patients had normal serum calcium concentration in the mean follow-up of 17 months. The authors concluded that bilateral neck exploration provides higher rate of more accurate diagnosis in 15% of cases, than an operation targeted on a gland indicated by preoperative examinations. They also emphasize that the postoperative follow-up for the recurrence of hyperparathyroidism should last many years. Authors from Pennsylvania, basing on review of literature involving 225 cases of primary hyperparathyroidism, summarized its causes. They included: a solitary adenoma — 88.90%, hyperplasia of multiple glands — 5.74%, two adenomas — 4.14% and cancer of parathyroid gland — 0.74%.

They also assessed efficiency of the above mentioned diagnostic methods. Diagnostic sensitivity of 99mTc-sestamibi scintigraphy and US imaging was 88.44% and 78.55%, respectively, for a solitary adenoma, 44.46% and 34.86%, respectively, for hyperplasia of multiple glands and 29.95% and 16.20%, respectively, for hyperplasia of multiple glands and 29.95% and 16.20%, respectively, for hyperplasia of multiple glands and 29.95% and 16.20%, respectively, for hyperplasia of multiple glands and 29.95% and 16.20%, respectively, for hyperplasia of multiple glands and 29.95% and 16.20%, respectively, for hyperplasia of multiple glands and 29.95% and 16.20%, respectively, for the recurrence of hyperparathyroidism.

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On the basis of obtained results, the authors concluded that primary hyperthyroidism is less often caused by a disease of multiple parathyroid glands than previously was thought. Sensitivity of the most common diagnostic modalities in imaging “multi-glandular disease” is low and sensitivity of targeted radioguided parathyroidectomy and unilateral neck revision is higher than was previously reported. Intraoperative measurement of parathyroid hormone concentration was helpful but did not solve all problems during the operation. This paper suggests that efficiency of diagnostic imaging, in particular with multi-glandular disease, is suboptimal. Therefore new solutions that could improve results of localization of pathologic parathyroid glands, are still being searched for. Such solutions include intraoperative staining of parathyroid glands.

Changes in the strategy of treatment of hyperparathyroidism in Polish sites could be reviewed on the basis of three papers from the sites in Gdańsk (30), Cracow (38) and Łódź (50), published in 2005. All papers base on many years of experience and significant number of operated patients, 231, 154 and 143, respectively. Most of the patients operated in Łódź were treated for primary hyperparathyroidism – 58.8% of cases, while in Gdańsk – for secondary hyperparathyroidism (142 per 231). Excision of a single adenoma was the predominant operation in the group of patients with primary hyperparathyroidism in all sites, while in the event of secondary hyperparathyroidism – subtotal parathyroidectomy or total parathyroidectomy with autotransplantation (23%). The most common cause of primary hyperparathyroidism was a solitary adenoma – 90% (28%), 58.3% (30), glandular hyperplasia – 10% (28), 28.6% (30), two adenomas – 3.6% (30) or cancer of the parathyroid gland – 6% (30). The latter number is in the highest range of values reported in the literature.

Initially most of the surgical procedures were performed using an open technique with bilateral neck exploration. Introduction of standardized diagnostic procedures basing on US imaging and MIBI scintigraphy allowed targeted operations with unilateral open exploration (since 1998) and thereafter low invasive open operations (since 2002) and visual track guided operations (since 2003). Introduction of intraoperative determination of parathyroid hormone concentration markedly facilitated surgical procedures, increased comfort of work and efficacy of operations, comparable to Western reports.

Gagner was the first to perform an endoscopic parathyroidectomy in 1996. Miccoli et al. (51) developed further this method and introduced a video assistance. They used it in the time period 1997 – 2003 in 370 (71%) of 520 patients with primary hyperparathyroidism. Twenty four (6.5%) patients were operated under local anesthesia. The procedure was completed successfully, without conversion, in 350 (94%) of cases and its efficacy was confirmed in 353 (98.3%) patients. Authors emphasize that after 6 years of experience, they believe that use of video assistance is as effective as the traditional method, however the postoperative period and cosmetic effects of the surgery are better with the former.

Intraoperative staining of parathyroid glands may markedly facilitate the low invasive procedure, in particular in cases of primary hyperparathyroidism. Methylene blue staining belongs to the most effective, cheapest methods of localization of parathyroid glands during the surgical procedure, while being concurrently safe. Sensitivity of this method in the event of adenomas reaches 100% and is only slightly lower when parathyroid hyperplasia is the cause of hyperparathyroidism. Our results support this conclusion.

CONCLUSIONS

1. Intraoperative staining of parathyroid glands with methylene blue shortened time required to find the glands and therefore duration of the operation.

2. Number of histopathological examinations performed during the procedure was markedly reduced with intraoperative staining of parathyroid glands in secondary hyperparathyroidism. Such phenomenon was not found for primary hyperparathyroidism.

3. Comparison of efficacy of this method with preoperative methods revealed its superiority, in particular in surgical treatment of secondary hyperparathyroidism.

4. High sensitivity of staining parathyroid glands with methylene blue makes this technique a cheap and very precise diagnostic tool.
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Received: 13.10.2009 r.
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