Intraoperative Monitoring of Parathyroid Hormone with a Rapid Automated Assay that is Commercially Available

Pai C. Kao,1 Jon A. van Heerden,2 David R. Farley,2 Geoffrey B. Thompson,2 and Robert L. Taylor1
1 Department of Laboratory Medicine and Pathology, and 2 Division of Gastroenterologic and General Surgery, Mayo Clinic, Rochester, Minnesota

Abstract. The Mayo Clinic was one of the first institutions to develop an in-house rapid parathyroid hormone (PTH) assay that used homegrown antibodies to monitor plasma PTH levels during parathyroidectomy. This PTH assay is economical, but it requires highly trained technologists and an experienced laboratory director and it is difficult to perform in the operating suite. We sought a fully automated multipurpose autoanalyzer with bar code reader to identify different patients’ names and capacity to manage specimens from several patients who are having simultaneous operations. In addition, after complete tumor removal, the plasma PTH level should decrease to <25% of the baseline level; otherwise, it may indicate that the antibodies used in the assay have cross-reacted with long half-life fragments other than intact PTH, which has a half-life of only ~2 min. An automated multipurpose analyzer, the Immulite 1000 with a Turbo PTH kit (DPC), fits these criteria and has replaced our in-house rapid assay. Of 47 patients who had parathyroidectomy for primary hyperparathyroidism and were tested with the new equipment, 45 (96%) had their plasma PTH levels decrease to <25% of the baseline levels. In 41 of 47 patients (87%), the PTH value decreased to <5 pmol/L (provisional reference range) within 20 min after tumor excision. The usefulness of the PTH assay extends from the traditional diagnosis of parathyroid disease to intraoperative monitoring, helping to ensure a higher cure rate. (received 22 October 2001; accepted 29 January 2002)

Keywords: parathormone, parathyroidectomy, intraoperative monitoring

Introduction

In 1994, a rapid (15 min) non-isotopic assay for parathyroid hormone (PTH) was developed with in-house antibodies, based on modifications of our in-house overnight immunochemiluminometric assay (ICMA). The modifications included a 5-fold increase in the concentration of acridinium-labeled signal antibody and an elevation of the incubation temperature from room temperature to 45°C [1]. Two physiological factors, the short half-life of intact PTH molecules (1 to 2 min) and the feed-back suppression of PTH secretion of normal parathyroid glands, make monitoring of PTH during parathyroidectomy possible and useful. During monitoring by the rapid ICMA, plasma PTH levels rapidly decreased and remained low or undetectable for up to 130 min [1].

Also in 1994, Irvin et al, who had previously established the usefulness of a rapid PTH assay for monitoring parathyroidectomy by a modified radioisotopic PTH kit [2,3], developed a rapid (10 min) non-radioisotopic PTH assay by modifying a commercial 18-hr ICMA assay; their assays did not, however, show complete suppression of plasma PTH levels after tumor removal [4]. A guideline of 50% decrease in PTH level was used to suggest complete tumor removal [4]. This modified method was adopted and commercialized (Nichols Laboratories, San Juan Capistrano, CA) as a rapid PTH ICMA kit. A mobile cart, including all necessary equipment, was designed to accompany the rapid
PTH kit for monitoring adjacent to or in the operating room. Today, the commercialized rapid PTH assay for monitoring during parathyroidectomy is available in many hospitals and has facilitated a more focused surgical approach [5-8]. From a laboratorian’s point of view, this assay did not show complete suppression of the plasma PTH level after complete tumor removal [4], indicating that the assay was biochemically suboptimal.

The rapid PTH assay, alone, does not help surgeons a great deal [9,10] because it does not localize the tumor; it simply confirms the complete and successful removal of the abnormal parathyroid gland(s). Histologic analyses are always done at our institution for tumor classification. The combination of a rapid PTH assay with frozen-section histologic analysis has produced only minor improvement in curative operations: although the assay can indicate complete tumor removal and histologic analysis can identify the tumor tissue, neither procedure localizes the parathyroid abnormality in the neck or chest.

Rapid PTH assays became significant only after they were used in combination with preoperative isotope scanning using technetium-99m–labeled sestamibi and iodine-123 subtraction images, which roughly point out tumor locations. The combination of these new technologies ensures complete removal and has enabled single-gland adenomas to be excised with confidence through a smaller incision with a unilateral approach. This minimally invasive approach, in contrast to a bilateral neck exploration [11], has facilitated outpatient parathyroidectomy and improved cosmesis [12]. This minimal approach shortens operating time, enhances patient recovery, shortens hospitalization, and is cosmetically appealing. The cost-effectiveness of this approach is self-evident.

When we started our study, the only commercially available rapid PTH assay was the Nichols ICMA kit. This kit has some drawbacks: (1) after complete removal of the tumor, PTH levels do not uniformly decrease to a suppressed or undetectable level (the guideline used is a 50% decrease from baseline to suggest complete tumor removal) [4]; (2) it is a manual assay—only the bead washer is an automated step; and (3) each kit is expensive and is used only one time.

While this manuscript was in preparation, three relevant articles were published [13-15]. Two groups of investigators used the Immulite Turbo (Diagnostic Products Corp. (DCA) Los Angeles, CA) assay kit [13,14], which is the same one used in this study, and another group used the Nichols ICMA kit [15]. They confirmed these drawbacks and also gave detailed cost analyses. The DPC kit cost less than $100 per patient, because the reagent can be used for multiple patients on multiple days (within the expiration date); the Nichols kit is for a single use within 1 day, and the cost of reagents alone is $800 [13-15]. We did not include cost analyses in this manuscript, because we did not charge our patients in the evaluation study or in the previous in-house assay. One of the reasons is that the necessity of a rapid PTH assay for monitoring has been a subject of debate, particularly among experienced surgeons who perform bilateral neck exploration procedures [9,10,16,17].

Our in-house rapid PTH assay reagent is quite inexpensive; however, the assay is completely manual. Without a mobile cart with equipment (which Nichols does not sell without purchase of their assay kit), it is difficult to accomplish the assay near the surgical suite [1]. It requires highly trained technologists for operation. Logistically, timely transport of blood specimens from the operating room to the laboratory becomes a major problem. An ideal situation would be to identify a commercial rapid PTH assay that is adapted for use with a multipurpose automated analyzer and can also be used for other routine assays [16]. The focus of this study was, in addition to choosing fully automated equipment with bar code capability, to evaluate the extent to which plasma PTH concentration decreases from baseline following curative parathyroidectomy.

Materials and Methods

Equipment. A multipurpose automated analyzer (Immulite 1000) was equipped with special computer software to run the TurbolIntact PTH kit (DPC, Los Angeles, CA), which is a 50-tube commercially available assay kit for multiple use within the expiration date. A wheeled stainless steel
A cabinet equipped with a centrifuge and other equipment is now commercially available. The Immulite Turbo analyzer can be placed on top of the cabinet for easy access in the operating room. The equipment is fully automatic and has bar code ability for identification of patients and timing of samples; this allows simultaneous monitoring of several patients during operations by different groups of surgeons. The equipment may be switched to the non-Turbo mode to run other laboratory tests.

**Method.** Blood is collected in an EDTA tube and centrifuged to separate the plasma. A minimum of 0.4 ml of plasma is required and is manually delivered into a special sample cup. After this initial manual step, the remainder of the procedure is done automatically, including delivery of 100 µl of the patient’s plasma and incubation with alkaline phosphatase-conjugated, affinity-purified, goat polyclonal anti-PTH (1-34) antibody and a plastic bead coated with affinity purified, polyclonal goat anti-PTH (44-84) antibody. This mixture and the bead are incubated in a special test tube for 6 min at 37°C with intermittent agitation.

Intact PTH in the specimen forms a sandwich between the 2 antibodies and binds on the surface of the plastic bead. After a centrifugal wash to remove the unbound anti-PTH (1-34) antibody, a chemiluminescent substrate (ie, a phosphate ester of adamantyl dioxetane) is added for an additional 4 min of incubation. The chemiluminescence generated by enzymatic reaction of phosphatase is measured in a luminometer. The intensity of the luminescence is directly proportional to the amount of intact PTH present in the plasma.

The chemiluminescence generated by the enzymatic reaction is a long-lasting light, which is different from the few seconds of short flashing of the acridinium ester used in our in-house assay and the manual Nichols commercial kit.

**Validation.** Results determined by the automated Turbo intact PTH assay were compared with results determined by our in-house rapid PTH assay [1].

![Graph](image.png)

Fig. 1. Comparison of results obtained with an automated, commercially available (Immulite Turbo) rapid assay for plasma parathyroid hormone (PTH) with results obtained by our in-house 15-min PTH assay (p < 0.0001, n = 94).
Characteristics of the automatic assay such as precision, sensitivity, and recovery of spiked intact PTH were evaluated.

Blood specimens were collected from 47 patients during the operation at baseline (prior to removing the abnormal parathyroid gland) and 5, 10, and 20 min after parathyroid resection. PTH results were immediately determined by the automated rapid PTH assay. The percentage decrease of PTH concentration from baseline was plotted versus the time post-resection.

Results

Comparison with in-house rapid PTH assay. Ninety-four specimens were analyzed by the Turbo intact PTH assay and the results were compared with those obtained by our in-house rapid PTH assay. The correlation of these 2 assays was good: $y = 1.047x + 0.186; r = 0.968$ (Fig. 1).

Analytical evaluation. Four patient specimens were added to 3 levels of a known amount of intact (1-84) PTH at 13.5, 27.1, and 67.7 pmol/L and then measured by the automated assay. The recovery of added PTH averaged 97, 93, and 103%, respectively. The overall average recovery of the 3 levels was 98%. For linearity studies, 6 specimens were serially diluted 1:2, 1:4, and 1:8. The observed results averaged 86.3% (range 80-105%) of the expected values. In the precision study, the average intra-assay coefficient of variation (CV) was 5.6% at 3.1
pmol/L PTH, and 8.1% at 4.9 pmol/L. The sensitivity of the assay (expressed as 2 SD of replicate assays of the blank sample) was 0.4 pmol/L.

**Intra-operative monitoring evaluation.** Plasma PTH levels decreased to <25% of baseline in 45 of 47 patients who had parathyroidectomy (Fig. 2). One patient had an increase to 443% at 5 min (caused by tumor manipulation) and a subsequent decrease to 86% by 30 min. In another patient, the baseline PTH concentration was 7.2 pmol/L, and decreased to 1.2 pmol/L (17%) at 10 min but increased to 2.4 pmol/L (33%) at 20 min. Thus, 96% of patients had a decrease to <25% of baseline (range, 1%-25%) within 30 min post-resection. All the patients were eucalcemic after surgery.

The progressive decrease of plasma PTH concentration after tumor removal is shown in Fig. 3. In 41 of 47 patients (87%) plasma PTH decreased to <5 pmol/L (reference range, 1-5 pmol/L). In 6 patients, the PTH did not decrease to <5 pmol/L (Table 1). Five of these had a rapid decrease of PTH (>75% from baseline, consistent with cure), but not to the point of achieving a normal PTH value; sampling was stopped early at 10 min in 2 patients and at 15 min in another 2 patients. The sixth patient responded similarly, but the intra-operative PTH levels were extraordinarily high, likely because of manipulation of the tumor.
Our experience indicates that the fully automated PTH assay may be useful for monitoring during surgical exploration of the parathyroid glands. The fully automated equipment, unlike our in-house manual assay, is easy to operate and does not require a highly trained research technologist. The multi-analyzer equipment has bar code ability for positive identification of the patient and timing of specimens. This facilitates the simultaneous monitoring of several parathyroidectomies that are being performed by different surgeons. The analytical equipment is an upgraded multi-assay analyzer with a special computer program for performing intraoperative monitoring of PTH as well as other routine assays, (eg, C-peptide, dehydroepiandrosterone sulfate, estradiol, erythropoietin, and insulin). This multifunction feature is economically advantageous.

Two physiological factors make the intraoperative monitoring of plasma PTH possible. One factor is the short, 2-min half-life of intact PTH; the second is that PTH secretion from the other normal parathyroid glands is suppressed. This suppression period is >130 min, as previously reported [1]. The Nichols assay shows a relatively high level of PTH after successful tumor removal; the cause of the relatively high residual PTH level is likely to reflect cross-reactivities with PTH fragments that are slowly excreted and have a long half-life [4]. The immunoreactive results may include interference from fragments other than intact (1-84) PTH. The guideline that 50% decrease from baseline PTH suggests complete tumor removal seems to include a high background measurement of non-intact PTH; however, it has been widely used in the surgical community. In the present study, with use of the automated DPC assay, 96% of patients had PTH decrease to <75% from baseline instead of only 50%. The difference is mainly due to the different antibodies used in the 2 assays.

The first criterion important for the surgeon in the operating room is the percentage decrease of plasma PTH levels after parathyroidectomy. We anticipate a 75% drop with this automated assay. A second criterion, and perhaps the most important one with respect to cure, is the return of plasma PTH levels to within the normal range after parathyroid tissue removal. With the automated assay, the PTH level after successful operation decreased to <5 pmol/L in 87% of patients (Fig. 3). Six patients did not have a decrease to <5 pmol/L; nonetheless, all of them were cured. Of these 6 patients, 5 had a PTH decrease of approximately 75% from baseline at either 10 or 15 min. The surgeons had confidence that all the tumor tissue had been removed; no further sample collections were deemed necessary.

The sixth patient, who had a relatively low baseline PTH of 8.8 pmol/L, showed an increase to 39 pmol/L at 5 min (4.4-fold increase). This may be due to manipulation of the tumor. If this manipulative artifact occurred as frequently as reported in one study, the role for intraoperative monitoring by measuring intact PTH would be limited [17]. This pitfall may be avoided by establishing a “new” baseline level at the time of pedicle division in patients who require more tumor manipulation than usual. With use of this guideline in the sixth patient, the “new” baseline was 39 pmol/L at 5 min. Thereafter, this patient had a decrease to 7.6 pmol/L, which represents an 81% drop from the new baseline.

An advantage of the automated rapid PTH assay is random access instead of batchwise analysis, as with other commercial kits. The collection of blood samples for PTH assays was stopped at 10 min in 2

<table>
<thead>
<tr>
<th>Case</th>
<th>Base-line 5 pmol/L</th>
<th>10 pmol/L</th>
<th>15 pmol/L</th>
<th>20 pmol/L</th>
<th>% decrease from baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>20</td>
<td>12</td>
<td>NS†</td>
<td>81</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>44</td>
<td>52</td>
<td>18</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>12</td>
<td>9.3</td>
<td>9</td>
<td>78</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>12</td>
<td>7.6</td>
<td>NS†</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>17</td>
<td>9.4</td>
<td>6.7</td>
<td>73</td>
</tr>
<tr>
<td>6</td>
<td>8.8</td>
<td>39</td>
<td>27</td>
<td>18</td>
<td>14</td>
</tr>
</tbody>
</table>

† NS, no sample was drawn, because the surgeons had confidence that the parathyroid tumor had been completely removed; PTH, parathyroid hormone. * 30-min sample.
patients and at 15 min in 2 patients (Table 1). A surgeon can confidently stop monitoring plasma PTH when the guideline of <25% from baseline has been reached; no further samples are needed.

During the past 25 years, the PTH assay has been improved from an 8-day to a 3-day assay to this current 15-min assay. The clinical applications of the PTH assay have expanded from the diagnosis of hyperparathyroidism to monitoring during parathyroidectomy. With the help of presurgical tumor localization by technetium-99m–labeled sestamibi and iodine-123 subtraction imaging, plus the rapid PTH assay to indicate complete tumor removal, a more focused and directed surgical approach has become feasible. This appears to reduce the extent of operation and the duration of hospitalization. There is a steady movement toward outpatient parathyroidectomy because 85% of patients with primary hyperparathyroidism have only a single tumor [12].

The presurgical image of the tumor location is not always sharp and clear; in addition to the major and primary location, there may be an image indicating a minor and secondary location, sometimes on the opposite side of the neck. Radiologists advise surgeons to remove the tumor at the primary location. If the plasma PTH levels rapidly decrease to an undetectable or almost undetectable level, indicating complete removal of diseased gland, surgeons can ignore the minor and secondary location. If not, surgeons should proceed to the minor and secondary location. A function of the rapid automated PTH assay in monitoring parathyroidectomy is checking the correctness of the presurgical image of tumor location.

If intraoperative PTH assay is not automated, convenient, and economical, intraoperative monitoring of plasma PTH may be replaced by a another new technology of instantaneous radioactivity measurement of removed tumor with a gamma probe [18]. The improvement of PTH immuno-assays illustrates that the adage “Today’s medicine should be obsolete tomorrow” may be correct. The new surgical paradigm should be the “assurance of cure” before the end of operation. Intraoperative PTH assay may make this an exciting reality.

References


