Perioperative parathormone assessment during surgery for primary hyperparathyroidism;

Comparison of four techniques

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Abstract

Objective To test four different non-portable PTH assays (Immulite Regular assay, Immulite Turbo assay, Advantage Regular assay and Advantage Turbo assay) as alternative for (expensive) portable PTH assays in predicting surgical outcome in primary hyperparathyroidism.

Methods All consecutive patients with biochemically proven primary hyperparathyroidism were enrolled in this study. A decline of >50% in PTH concentration (in a sample taken 8 minutes after resection) was defined to be predictive of postoperative normocalcemia, a drop of less than 50% to indicate persistent disease. The Immulite Regular assay was considered the reference test for perioperative measurement. We prospectively tested 192 samples from 90 patients by the Immulite Regular and Immulite Turbo assay. In addition, 101 samples from 53 patients were tested with the Advantage Regular and Advantage Turbo assay.

Results In 80 patients postoperatively normocalcemia was correctly predicted after a median drop in PTH concentration of 84.9% (all over 50%, measured with Immulite Regular). When comparing preoperative and postoperative PTH levels, the median decline using the Immulite Turbo assay was 84.0%, in the Advantage Regular assay was calculated to be 78.2% versus 76.1% in Advantage Turbo assay. Comparative analysis of Immulite Regular and Immulite Turbo assays showed that they correlated extremely well (r=0.987), as for Advantage Regular and Advantage Turbo (r=0.993). Results of Immulite Regular and Immulite Turbo assays versus Advantage Regular and Advantage Turbo assays were discordant in three patients (all of whom were normocalcemic on the first postoperative day).

Conclusion Both the Immulite Regular and Immulite Turbo assays were found to be highly accurate and moderately superior to the Advantage Regular and Advantage Turbo assays when drawn at 8 minutes with a 50% guideline. All four alternatives were much less expensive than the portable kits.

Introduction

The goal of surgical treatment in primary hyperparathyroidism (pHPT) is normalization of serum calcium. Since this takes at least 24 hours both surgeon and patient are uncertain about the effect of the exploration until the first postoperative day. With the development of less invasive techniques as alternatives for conventional neck exploration (CNE) in the treatment of pHPT, the need for perioperative assurance about the effect of the exploration has increased. The short half-life (< 3 minutes) of intact parathormone (1-84) (PTH), combined with suppression of PTH secretion for hours after parathyroid adenomectomy, contribute to fast clearance from the circulation. Early studies on intraoperative PTH testing to ascertain the success of unilateral explorations, by Nussbaum [1] and Irvin [2] reported the results of modified immunoradiometric assays (IRMA). Although this test provided results within 25 minutes, it has not been widely accepted. The short half-life of isotopes (and the 'shelf-life' of these intraoperative PTH assays), the necessary safety precautions (including proper disposal of waste products), a false negative rate of 15%, and the associated high costs, initially limited use of the assay to a few specialized centers [2]. Other rapid tests were developed but despite the technological advances of these tests the potential of intraoperative measurement was not realized until the introduction of modified immunochemoluminometric assay kits that can be used in the operating theatre [3 4]. Nowadays, these commercially available portable kits can perform PTH measurements within 15 minutes with sensitivity rates up to 96%, specificity of ~100% and an overall accuracy of 97% [3 5]. It cannot be denied that these tests are highly effective in providing true intraoperative results, but the need for instrument performance checks, for generating calibration curves and for assay quality control before surgery and the high cost preclude their widespread use. In the search for an alternative rapid PTH assay to these portable kits, we have evaluated the accuracy of four non-portable 'quick' tests as predictors of surgical success in primary hyperparathyroidism. The following assays were investigated: 1) the Immulite Regular PTH assay (60-minute incubation), 2) the Immulite Turbo PTH assay (10-minute incubation), 3) the Advantage Regular PTH assay (25-minute incubation), and 4) the Advantage Turbo PTH assay (12-minute incubation).

Methods

Parathormone assays

Parathormone levels in blood were assessed by four different commercially available, non-portable PTH assays. Two manufactured by Diagnostic Products Corporation (DPC, Los Angeles, Ca, USA) and two manufactured by Nichols Institute Diagnostics (San Juan Capistrano, Ca, USA). Both of these companies market a Standard assay (DPC: Immulite Regular assay and Nichols: Advantage Regular), and a more rapid, so-called Turbo assay (DPC: Immulite Turbo assay and Nichols: Advantage Turbo assay).

I) The Immulite Regular and the Immulite Turbo assays are analyzed using the DPC Analyzer. Both assays use a pair of antibodies, respectively directed at the C-terminal part of PTH (immobilized to solid phase) and the N-terminal region. The main difference between these assays is the much shorter time of incubation when the Turbo-mode is utilized, requiring a separate set of reagents. The Immulite Turbo assay can only be utilized in the Turbo-mode of the Immulite software. This results in a less sensitive assay, the Immulite Regular assay having a lower limit of detection for PTH of 0.4 pmol/L whereas the sensitivity of the Turbo equals 1 pmol/L. Interassay variations for both tests during the year 2000 were between 7 and 10%.

After proper calibration of the Analyzer, total time between start of processing an EDTA-plasma sample and the first result is 75 minutes for the Immulite Regular assay and 15 minutes for the Immulite Turbo assay. In the Regular mode 50 µl and in the Turbo mode 100 µl of EDTA-plasma is used.

II) The Advantage Regular Intact PTH and the Advantage Turbo Intact PTH assays, of Nichols Institute Diagnostics. In both variants of these assays identical reagents are used, the only difference in processing is the incubation time and the requirement for special software to operate the Analyzer in the Turbo-mode. The sensitivity of the Advantage Regular assay is 0.5 pmol/L whereas no official data are available for the Advantage Turbo assay but its sensitivity is about 1 pmol/L. Interassay variation measured over the past year of the Advantage Regular assay was approximately 11%, whereas no such data are available for the Advantage Turbo assay.

After proper calibration of the Analyzer, total time between start of processing an EDTA-plasma sample and the first result is approximately 40 minutes in the Advantage mode and 17 minutes in the Turbo mode. Both the Advantage Regular assay and the Advantage Turbo assay use 150 µl of EDTA-plasma.

Patients

All consecutive patients with biochemically proven primary hyperparathyroidism were enrolled in this prospective study after informed consent was obtained. Previous (para)thyroid surgery was considered a criterion for exclusion. In an earlier study we had tested the reliability and applicability (reliability study) of the Immulite Regular assay in 25 patients. A decline of more than 50% in PTH concentration (in a sample taken 8 minutes after resection) was defined to be predictive of postoperative normocalcemia, a drop of less than 50% to indicate persistent disease. Once the Immulite Regular test results were proven to correlate fully with postoperative serum calcium levels, we introduced it to our surgical management protocol [6].

Study design

In the present study the Immulite Regular assay was considered the reference test for perioperative PTH measurement. We prospectively tested 192 samples from 90 patients by the Immulite Regular assay. Once the Immulite Turbo assay became available we tested the same 192 samples with that as well, and compared the results of both methods. In addition, 101 samples from 53 patients were tested with the Advantage Regular assay and the Advantage Turbo assay. Results of these two tests were correlated to postoperative calcium levels as well as to the Immulite Regular assay. In addition, we compared the results of the Advantage Turbo and the Advantage Regular assays.

Protocol

One day prior to surgery the Endocrine laboratories were informed about the scheduled exploration so that such necessary preparations as calibration of the Analyzer could take place. Baseline peripheral plasma samples (3 ml EDTA (k3) Vacutainer®) were taken at the operation room after anesthesia was induced but before exploration. Eight minutes after resection of a suspected adenoma the next sample was taken, transported to the Endocrinology laboratories and subsequently analyzed. After exploration, patients were extubated in the operation room and transported to the recovery unit. Patients were only sent back to the surgical ward if the test results were indicative of successful exploration. If not, they were taken back to theatre, as soon as it was ready, for re-exploration.

Operative success was defined as normalized serum calcium levels on the first postoperative day and 1 week and 1 month later.

Depending on the preoperative imaging results patients underwent either minimally invasive adenomectomy (MIA) or conventional neck exploration (CNE). If a soli-

tary adenoma was visualized, a local procedure through a 2-3 cm incision at the ventral border of the sternocleidomastoid muscle was performed as described elsewhere [7]. After identification and resection of the adenoma the procedure was terminated. If no adenoma was found, however, the exploration was converted into CNE, comprising a systematic, bilateral exploration through a 10-12 cm collar incision, with identification of the four parathyroids and resection of the causative adenoma(s) based on gross morphologic features. If MIA (or CNE) failed the re-exploration consisted of a CNE.

Results

Ninety patients with a biochemically proven pHPT were selected for surgery. Their median age was 59 years (range 22-81), with women outnumbering men 62 to 28. The median preoperative calcium level was 2.89 mmol/L (2.55-4.10; normal 2.20-2.60) while the PTH level was 14.1 pmol/L (range: 10-220; normal <8 pmol/L). Based upon preoperative imaging results 64 patients were selected for MIA, 26 for CNE. Two samples (one baseline sample and one postresection sample, total 156 samples) were drawn from 78 patients, three (total 36 samples) from the other 12, in whom multiple gland disease was suspected or to ascertain success after delayed fall in PTH.

In 80 patients postoperative normocalcemia was correctly predicted after a median drop in PTH concentration of 84.9% (all >50 %, measured with Immulite Regular assay). After a thorough exploration (CNE) one patient displayed a drop in PTH concentration of 47.4% at 8 minutes after resection. However, as an exception to the protocol, this patient did not undergo immediate re-exploration because during exploration all parathyroid tissue had been identified (one adenoma and three normal parathyroid glands). The chance of a fifth parathyroid gland was considered, but rejected because of the very low incidence of supernumerary glands. The next day the patient's serum calcium level was normal (2.25 mmol/L).
In two patients falls of 40% and 48%, respectively, suggested surgical failure. Because this decline was so close to the 50% cut-off point we decided to draw a third sample at 30 minutes after resection. These samples showed respective declines of 94% and 99.5% and next day their calcium levels were normal, and this was confirmed during follow-up. Seven patients showed a fall in PTH of less than 50% (falls of 25%, 12%, 2%, increases to 123%, 124%, 140% and 152%). All were re-explored (as is discussed below) and a (second) causative adenoma was found and resected, after which their serum calcium concentrations fell to normal.

Sixty-four patients underwent MIA. Fifty-nine (92%) showed an adequate decline in PTH level and were normocalcemic in the postoperative period. Minimally invasive adenomectomy was followed by an inadequate fall in PTH, requiring immediate re-exploration in two patients at which a 'missed' causative adenoma was found and resected. Both became normocalcemic postoperatively. The other three displayed persistent abnormality and were re-explored at a later stage (these failures were monitored during the 'reliability study'). At re-exploration a (second) adenoma was resected in all of them (multiple gland disease in two, missed adenoma in one) and their serum calcium reverted to normal.

After CNE, 24/26 patients (92%) showed a fall in PTH concentration of more than 50 %, and all were proven to be normocalcemic. The other two patients had persistent disease after a fall of less than 50%, necessitating re-exploration. In the first patient a second adenoma was found in the thymus, while an additional adenoma was localized and subsequently resected from the mediastinum in the second. Both became normocalcemic after re-exploration.

Comparative analyses of PTH assays

Comparative analysis of the results of Immulite Regular and Immulite Turbo assays (192 samples) yielded the following equation:
Immulite Turbo = 0.807 x Immulite Regular + 1.2; r = 0.987 ($p < 0.0001$) (**Figure 1**).
This means that both methods correlated extremely well (r = 0.987), though the test results with the Immulite Turbo assay are somewhat lower (Immulite Turbo = 0.807 x Immulite Regular) than those with the Immulite Regular assay.
Comparing baseline values and postresection values measured by the Advantage Regular and Immulite Regular assays, gave the following equation:
For baseline Advantage Regular = 0.701 x Immulite Regular + 1.0; r = 0.966 and for postresection Advantage Regular = 0.917x Immulite Regular + 0.5; r = 0.930 (**Figure 2**). This implies that the baseline values are 1.4 times higher when measured with Immulite Regular assay, while postresection values are almost equal. As a result the decline in PTH levels is relatively smaller in the Advantage Regular assay.

The results of 101 samples from 53 patients tested with Advantage Regular assay and Advantage Turbo assay showed:
Advantage Turbo = 1.07x Advantage Regular - 0.5; r = 0.993 (**Figure 3**),
meaning that these two techniques also correlate extremely well.

Figure 1 Comparative analysis of Immulite Regular assay and Immulite Turbo assay.

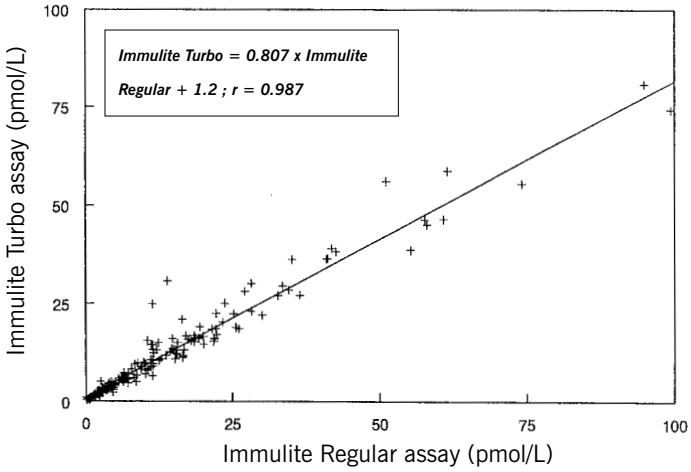


Figure 2 Comparative analysis of Immulite Regular assay and Advantage Regular assay.

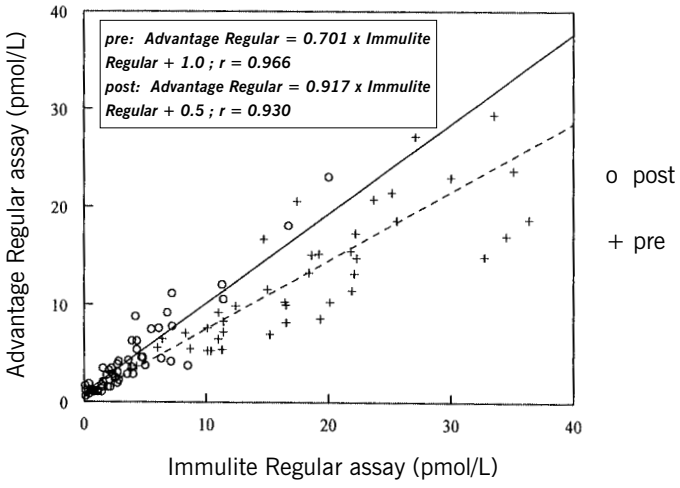
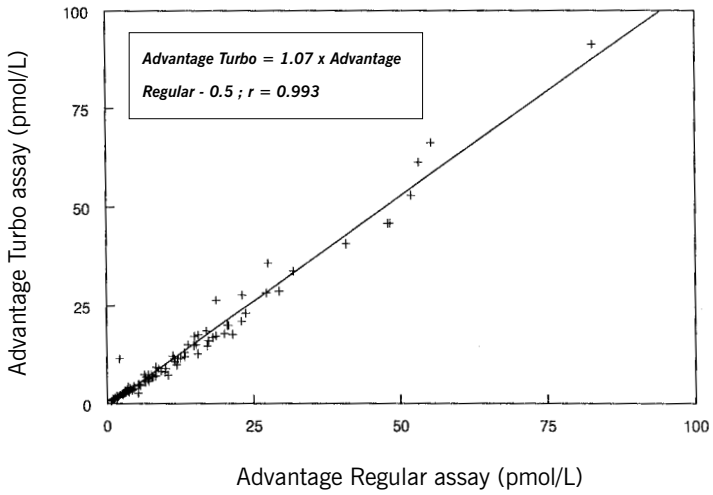


Figure 3 Comparative analysis of Advantage Regular assay and Advantage Turbo assay.



In calculating costs (for all four techniques) the following factors were considered: materials (reagents) used, personal salaries and wages, and overhead costs. We calculated the costs per patient (two assays) to be approximately 100 US dollars.

Discrepancies in parathormone assays

When comparing preoperative and postoperative PTH levels, the median decline using the Immulite Regular assay was 84.9% (range -99.6 to +153.8). Similarly the median decline as judged by the Immulite Turbo assay was 84.0% (- 97 to +153). The only discrepancy between these two methods was a patient in whom the Immulite Regular assay showed a drop of 57.3%, while the Immulite Turbo assay showed a drop of only 48.5%. The postoperative serum calcium, however, returned to normal on day one.

The median decline in the Advantage Regular assay was calculated to be 78.2% (-92.7 to +125.7) versus 76.1% in Advantage Turbo (-90.6 to +121.6).
The results of the Immulite Regular and Immulite Turbo assays versus the Advantage Regular and Advantage Turbo assays were discordant in three patients (all of whom were normocalcemic on the first postoperative day). In the first patient, the Immulite Regular and Immulite Turbo assays showed declines of 90% and 79%, respectively, while the Advantage Regular and Advantage Turbo assays recorded declines of 44% and 41%. In the second patient declines of 72% and

65% were observed in the Immulite Regular and Immulite Turbo assays. In contrast, rises to 126% and 122% of baseline values were observed using the Advantage Regular and Advantage Turbo assays. The adenoma in this patient, however, was very large and was accidentally fragmented during resection.
In the third patient a delayed fall in PTH was seen: a decrease of 52% was observed with the Immulite Regular and Immulite Turbo assays, but a fall of 9% and a rise of 20% were found by the Advantage Regular and Advantage Turbo assays respectively. However, PTH concentration measured in the 30 minute sample showed concentrations of 5.6% and 9.5% compared with baseline values using Advantage Regular and Advantage Turbo assays.

Discussion

Rapid PTH assay has become essential with the introduction of limited forms of parathyroid surgery. The use of portable non-radioactive kits that can be carried on a trolley has allowed assays to be performed in the operating theatre providing true intraoperative information. As a result, operating time and hospital stay can be reduced, leading to cost savings. Chen et al [8] reported a 50% reduction in hospital charges with outpatient minimally invasive parathyroidectomy. Although the reliability of the portable tests they used was reported to be excellent, the costs (approximately 1,000 US dollar a test) were about one third of mean total hospital charges per patient (3,174 US dollars). In our experience the mean total costs for MIA were calculated to be approximately 1,200 US dollars [9], which included the costs for the Immulite Regular assay (approximately 100 US dollars a test). Although the advantages of a portable kit, i.e. true intraoperative information on surgical outcome, precluding the need for second procedures, are evident, the high costs of such kits outweighs their advantages. The Immulite Regular test gave results within 75 minutes. It correlated in 97% of patients (all but one) with the postoperative serum calcium, and was concluded to be very reliable.

Despite the success of the perioperative PTH assays the following limitation should be noted. In three patients postexcision values fell to near 50% of the preoperative figure. The surgeon judged these patients to be cured and they were not immediately re-explored. This was confirmed in two patients after that a second postexcision sample showed an adequate decline. The cause of delayed fall in PTH concentration was not clear, but a low initial baseline PTH concentration and the manipulation of the adenoma during mobilization are possible reasons [10]. Manipulation of an adenoma may result in release of PTH or of proteins that resemble PTH that can potentially interfere with the assay. In addition, the use of a

50% decline after 8 minutes as a cut-off level does not take in account inter-individual variability in PTH half-life [10 11].

The Immulite Turbo assay was concluded to be a reliable test, which correlated extremely well with the Immulite Regular assay. Only in one patient was a fall of 57.3% shown by the Immulite Regular assay and close to 50% by the Immulite Turbo assay. No other problems were encountered, and it produced results within 15 minutes. Taking into account this and other examples of 'delayed' decline in PTH levels following correct surgery, we believe it is advisable that, in cases of PTH falls close to 50%, to preclude the possibility of a delayed fall, a third sample should be taken before deciding on re-exploration.

From a logistic point of view the procedure might be as follows: 8 minutes after resection the sample will be drawn; delivery to the Endocrine laboratory takes a little under 10 minutes. Since the Analyzer has been calibrated in advance, processing can take place immediately, providing results within 15 minutes. In total, test results become available after approximately 30 minutes. The surgeon can thus await the results with the patient under anesthesia, so avoiding the need for later re-intubation and re-exploration when the initial operation was not successful.

The Advantage Regular and Advantage Turbo assays were concluded to be less accurate than the Immulite Regular and Immulite Turbo assays, though the reasons for this remain unclear. However, we may speculate that differences in standardization of instruments and/or the detection of additional fragments of PTH by the latter two tests might explain the difference. The discrepancies between the methods do not necessarily prevent the use of the Advantage Regular and Advantage Turbo assays as perioperative or intraoperative PTH assays. Using a guideline of 50% decline at 8 minutes produces suboptimal results in both assays, and the test should therefore be prolonged when using Advantage Regular and Advantage Turbo assays.

In conclusion, the use of a perioperative PTH assay is essential in the treatment of primary hyperparathyroidism. Both the Immulite Regular and Immulite Turbo assays were found to be highly accurate and moderately superior to the Advantage Regular and Advantage Turbo assays when drawn at 8 minutes with a 50% guideline. All four alternatives were much less expensive than the portable kits.

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