Clinical study

Clinical impact of two different intraoperative parathyroid hormone assays in primary and renal hyperparathyroidism

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Abstract

Background: Intraoperative parathyroid hormone (PTH) monitoring predicts successful surgery for primary hyperparathyroidism (pHPT). In renal HPT, intraoperative PTH assays can define whether parathyroid resection is adequate.

Methods: Intraoperative PTH was measured with two different immunometric assays (Immulite Turbo DPC and ADVIA Centaur assay) in 91 patients undergoing parathyroidectomy for primary (n = 57) and renal (n = 34) hyperparathyroidism. PTH was monitored preoperatively, 10, 20, and 30 min after parathyroidectomy and 24 h postoperatively.

Results: Ten minutes after parathyroidectomy, intraoperative PTH dropped into the normal range (<7.6 pmol/l) in 84% of patients with pHPT as measured with the ADVIA Centaur assay (PTH-A), compared with 100% of the samples measured with the Immulite Turbo DPC assay (PTH-I; P = 0.0082). Twenty minutes after parathyroidectomy for secondary HPT, intraoperative PTH decreased to the normal range in 100% measured with PTH-I compared with 50% measured with PTH-A (P = 0.009). Then, 24 h postoperatively, PTH-I and PTH-A levels were within the normal range in all of the successfully treated patients. Both assays correctly identified six patients with persistent disease and another patient with a double adenoma in pHPT.

Conclusions: In patients undergoing parathyroidectomy for primary or renal HPT, PTH levels decreasing to the normal range indicated successful surgery in all of the patients as measured with the PTH-I assay. Comparing the two assays, PTH-I was able to quantify the intraoperative PTH decay more quickly than PTH-A.

European Journal of Endocrinology

Introduction

Primary hyperparathyroidism (pHPT) is found in about 2% of individuals over 55 years and is two to three times more common in women than in men (1). Primary HPT is caused by a single parathyroid adenoma in about 80–85% of the cases. In about 15% multiple gland hyperplasia, double adenomas, or, very rarely, parathyroid carcinomas are diagnosed. The diagnosis of pHPT is confirmed by hypercalcemia (or high-normal serum calcium values) in the presence of elevated levels of intact parathyroid hormone (PTH). Urinary tests for calcium and creatinine can distinguish patients with pHPT from those with benign familial hypercalcemic hypocalciuria. Symptoms of pHPT are bone pain, pathologic fractures, muscle weakness, mood swings, and depression. Parathyroidectomy is the only curative therapy for pHPT (1) and surgery for pHPT is now being performed in an increasing number of patients by using an open (2–4) or endoscopic (5) minimally invasive approach. Instead of routine exploration of all four parathyroid glands, unilateral neck exploration has become the treatment of choice for pHPT in many experienced centers.

Chronic renal failure is one of the most common causes of secondary hyperparathyroidism (sHPT) and tertiary hyperparathyroidism (tHPT). In patients suffering from chronic renal failure phosphorus excretion is inadequate; vitamin D is not sufficiently converted into its active form, the absorption of calcium in the gut is decreased, and therefore hypocalcemia develops. As a result of hypocalcemia, excessive amounts of PTH are secreted, which is associated with hyperplasia of the parathyroid glands. Treatment of renal HPT consists of calcium supplementation, restricting phosphate intake, and administration of vitamin D analogs. If medical treatment is not effective, parathyroidectomy should be considered. Surgical procedures include total parathyroidectomy with autotransplantation or subtotal resection of 3.5 parathyroid glands (6).
Intact PTH consists of a polypeptide chain of 84 amino acids. It is secreted by the parathyroid glands and eliminated mostly via the liver, but it is also passed through the kidneys and stored in the bones. The N-terminal fragment of PTH contains the region that confers bioactivity. Its half-life is only 3–4 min. The C-terminal fragments of PTH (PTH 7–84) have a half-life of several hours and are eliminated by glomerular filtration, thus depending on renal function (7, 8). Since 1991, several quick PTH assays have been established (9–11). Together with improved preoperative localization methods (ultrasound, sesta-MIBI scan), intraoperative PTH measurement enables unilateral surgery for pHPT and to control the success of parathyroidectomy for both primary and renal hyperparathyroidism (11–13).

The objectives of the present study were to compare the pre-, intra- and postoperative results of parathyroidectomy in patients with pHPT and renal HPT by measuring intact PTH levels using two different assays (Immulite Turbo DPC and ADVIA Centaur). To compare the two assays, we focused on the decrease in intraoperative PTH levels with regard to renal function and the accuracy in predicting surgical outcome.

Materials and methods

Between June 2005 and December 2006, 91 consecutive patients (63 females, 28 males, mean 55.3 years) undergoing parathyroidectomy for primary (n = 57) or renal (n = 34) hyperparathyroidism gave informed consent to participate in a prospective study (Fig. 1; Table 1). Among them 70 patients underwent parathyroidectomy at the surgical department of Heidelberg University, and in 21 patient’s parathyroidectomy were performed at Salem Hospital, Heidelberg, Germany. Briefly, inclusion criteria for the study were parathyroidectomy for pHPT, tHPT, or sHPT in patients older than 18 years, and ability to give informed consent. The study was approved by the Ethics Committee of the University Heidelberg, Germany in accordance with the ethical standards of the Helsinki Declaration of 1975.

Surgical technique

Preoperatively in all patients with pHPT, neck ultrasound and a sesta-MIBI scan was performed to localize parathyroid adenomas. For renal HPT only preoperative ultrasound was used to localize hyperplastic parathyroid glands. In patients with previous neck surgery, a selective venous sampling was performed preoperatively.

In 11 patients (19%) with pHPT an open minimally invasive parathyroidectomy was performed. In 46 patients (81%) with pHPT, uni- or bilateral neck exploration was carried out for parathyroid adenoma or hyperplasia. In 28 patients (31%) with additional thyroid disease, a lobectomy or near total thyroidectomy was performed. The medical history of 7 out of the 91 patients (8%) included previous performed neck surgery to treat thyroid disorders. A bilateral neck exploration and total parathyroidectomy with autotransplantation into the tibialis anterior muscle was performed in 34 patients with renal HPT. Preoperatively, 27 patients with sHPT had been treated with chronic hemodialysis. In seven patients with tHPT, kidney transplantation was performed before parathyroidectomy; another three patients received kidney transplants after parathyroidectomy. In 4 out of the 91 study patients (4%), a reoperation was required because of persistent pHPT or tHPT.

In pHPT, a single gland adenoma was found in 55 patients and a double adenoma was found in one patient. In another patient with multiple endocrine neoplasia (MEN) I syndrome, hyperplasia of the parathyroid glands was seen. In renal hyperparathyroidism four parathyroid glands were removed in 31 out of the 34 patients (91%); in three patients (9%) three parathyroid glands were removed. In one of them parathyroidectomy was required because of persistent tHPT.

Blood sampling and measurement of PTH

Blood samples were obtained immediately after inducing general anesthesia and before commencing the operation; 10, 20, and 30 min after removing a parathyroid adenoma in pHPT; or after the removal of the last (in most cases the fourth) hyperplastic
parathyroid gland had been removed in cases of renal HPT and MEN I syndromes. Postoperative blood samples were obtained at 24 h and at 6-month follow-up. Blood specimens were collected in potassium 2.4 ml EDTA tubes and analyzed immediately for intact PTH with the ADVIA Centaur immunoassay (Siemens Medical Solutions, Fernwald, Germany) for intraoperative PTH testing or frozen and analyzed later with the Immulite Turbo DPC assay. Intact PTH levels measured with the ADVIA Centaur immunoassay were given intraoperatively to the surgeons to decide whether to determine or to continue parathyroidectomy. Since increased PTH stability was described for both assays when EDTA plasma tubes were used (14, 15), we used such tubes uniformly for the present study.

Intact PTH was measured with two automated immunoassays: the ADVIA Centaur assay (Siemens Medical Solutions) and the Immulite Turbo DPC assay (DPC Biermann, Bad Nauheim, Germany). The intraoperative PTH assay was used to determine the success and thus the end of the operation. After PTH levels declined intraperatively to 10% of baseline or to the normal range (below 7.6 pmol/l) the operation was finished.

The ADVIA Centaur assay is an automated two-site sandwich immunoassay using direct chemiluminescent technology (16). The assay utilizes two purified polyclonal antibodies that target the N-terminal 1–34 and C-terminal 39–84 regions of the PTH molecule. The incubation time of intact PTH with the ADVIA Centaur assay takes 18 min. The detection limits of the assay are 0.26–7.6 pmol/l.

The Immulite Turbo analyzer, another solid phase two-site chemiluminescent assay DPC, was used for second-line PTH measurement. This assay requires that two enzyme-labeled antibodies specific for the N-terminal (amino acids 44–84) and N-terminal (amino acids 1–34) bind to regions of the intact PTH molecule. The half-life of intact PTH is 2–5 min (7). The incubation time of intact PTH with the described Turbo assay takes 14 min and has an analytical range of 0.31–263 pmol/l. Plasma specimen analyzed with this assay was immediately frozen at −20°C and examined later together with other samples.

Statistical analysis
SAS software (Release 9.1; SAS Institute, Cary, NC, USA) was used for statistical analysis. Descriptive statistics of quantitative parameters (e.g. age, calcium, and PTH levels) are presented as median and range, whereas qualitative parameters (e.g. sex, PTH decrease ≥90%) are presented as absolute and relative rates.

According to their renal function, patients with pHPT and tHPT and patients with sHPT were divided into two independent groups and analyzed separately. Overall, analyses were based on an intention-to-treat cohort of successfully treated patients, which produced varying sample sizes at distinct times. For both groups and the two different PTH assays, three main outcome parameters were tested: i) a decrease in the intraoperative PTH levels to the normal range (yes/no); ii) a decrease in intraoperative PTH ≥90% (yes/no); and iii) differences between the two assays in the relative PTH decrease. All other parameters were tested exploratively. First and second outcome parameters were tested using the McNemar test. The third outcome parameter was tested using the signed-rank test. An effect was considered statistically significant at $P < 0.05$ (two-sided). Standard regression analysis was done using MEDCALC software (MedCalc Software, Mariakerke, Belgium).

### Table 1 Characteristics of 91 patients with parathyroidectomy for primary and renal hyperparathyroidism.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>pHPT (n=57)</th>
<th>tHPT (n=7)</th>
<th>sHPT (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) median</td>
<td>62.9 (25–88)</td>
<td>44.1 (34–57)</td>
<td>53.8 (21–75)</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>49/8 (86%/14%)</td>
<td>3/4 (43%/57%)</td>
<td>11/16 (69%/31%)</td>
</tr>
<tr>
<td>Preoperative creatinine median (mg/dl; normal range: 0.8–1.2 mg/dl)</td>
<td>0.7 (0.4–1.1)</td>
<td>1.36 (0.68–1.7)</td>
<td>5.38 (1.7–7.3)</td>
</tr>
<tr>
<td>Preoperative calcium median (mmol/l; normal range: 2.2–2.6 mmol/l)</td>
<td>2.73 (2.5–3.34)</td>
<td>2.78 (2.6–3.0)</td>
<td>2.57 (2.2–2.9)</td>
</tr>
<tr>
<td>Preoperative PTH-Aa median (pmol/l; normal range: 1.3–7.6 pmol/l)</td>
<td>14.8 (0.9–91.7)</td>
<td>26.6 (16.2–82.9)</td>
<td>91.1 (18.7–251.6)</td>
</tr>
<tr>
<td>Preoperative PTH-Ib median (pmol/l; normal range: 1.3–7.6 pmol/l)</td>
<td>14.6 (0.5–72.6)</td>
<td>32.7 (17.6–76.6)</td>
<td>75.2 (24.3–251.9)</td>
</tr>
<tr>
<td>Primary operation for HPT (%)</td>
<td>54 (95%)</td>
<td>6 (86%)</td>
<td>27 (100%)</td>
</tr>
<tr>
<td>Operation for persistent/recurrent HPT (%)</td>
<td>3 (5%)</td>
<td>1 (14%)</td>
<td>0</td>
</tr>
<tr>
<td>Number of resected parathyroid glands</td>
<td>1 gland: 55 (96%)</td>
<td>3 glands: 1 (14%)</td>
<td>3 glands: 2 (7%)</td>
</tr>
<tr>
<td></td>
<td>2 glands: 1 (2%)</td>
<td>4 glands: 6 (86%)</td>
<td>4 glands: 25 (93%)</td>
</tr>
</tbody>
</table>

- pHPT, primary hyperparathyroidism; sHPT, secondary HPT; tHPT, tertiary HPT.
- PTH measured with the ADVIA Centaur assay.
- PTH measured with the Immulite Turbo DPC assay.
- MEN I syndrome.
**Results**

In patients participating in the study, successful parathyroidectomy was monitored in 85 out of the 91 cases during the first operation and in another patient in a second operation (Fig. 1). Patients with persistent disease were excluded from the evaluation of the intraoperative PTH decrease. The remaining 85 patients with HPT were divided according to their renal function into two study groups with normal (n = 59 patients, pHPT and tHPT) or impaired renal function (n = 26 patients, sHPT).

**PTH measurement in patients with normal renal function (pHPT and tHPT)**

In 57 patients with pHPT and seven patients with tHPT, median preoperative creatinine levels were of 0.7 mg/dl (0.4–1.1 mg/dl) and 1.36 mg/dl (0.68–1.7 mg/dl) respectively, displayed normal renal function. Preoperative serum calcium levels were elevated in 55 out of the 64 patients (86%) with pHPT and tHPT. Median preoperative PTH concentrations in patients with pHPT and tHPT were 14.8 pmol/l (0.9–91.7 pmol/l) as measured with the A VDIA Centaur assay (PTH-A) and 14.6 pmol/l (0.5–76.6 pmol/l) as measured with the Immulite Turbo assay (PTH-I). In all of the 91 patients (pHPT, tHPT, and sHPT), PTH concentrations obtained in each of the two assays are compared in Fig. 2.

Ten minutes after parathyroidectomy, intraoperative PTH-A levels dropped to the normal range in 84% of the patients with pHPT and tHPT compared with 100% of the PTH-I samples measured with the Immulite Turbo DPC assay (P = 0.0082, Table 2). At the same time, a median PTH decrease of 90% or more was found in 16% as measured by PTH-A compared with 50% by PTH-I (P < 0.0001).

Twenty minutes after resection of parathyroid adenoma, PTH-A levels were measured in the normal range in 96% of the patients compared with 100% of the samples tested with the Immulite Turbo DPC assay. In tHPT, PTH levels had normalized in 86% according to PTH-A and in 100% according to PTH-I. Thirty minutes after parathyroidectomy, a normalization of PTH-A in pHPT and tHPT was found in 98%, and in 100% with PTH-I. In pHPT, at all points of intraoperative PTH testing (preoperatively, 10, 20, and 30 min after parathyroidectomy) mean PTH levels by PTH-I were 5.1, 37.8, 48.0, and 47.4% lower than levels by PTH-A. Comparable results were seen in tHPT with mean PTH levels of 11.3, 64.7, and 30% determined by PTH-I, and 28.6% lower than levels by PTH-A.

**Figure 2** Regression parameters for intraoperative PTH measured in 91 samples by two commercial assays (PTH-A and PTH-I).

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parathyroid hyperplasia related to MEN I syndrome, seven parathyroid glands were removed. At follow-up examination, the patient still presented with elevated calcium and PTH levels. Persistent renal HPT was seen in a 75-year-old woman after five parathyroid glands had been removed. In all of the six patients with persistent HPT, PTH levels remained pathologically elevated at the end of the operation and at 24-hour follow-up according to both PTH assays. Clearly elevated PTH levels were seen with both assays in another 64-year-old male patient with pHPT after a large parathyroid adenoma of 2.5 cm (2.3 g) was removed from the carotid sheet. In an ongoing exploration of the other side of the neck a large double adenoma of 2.5 cm (0.5 g) was found in the same position. Since both assays displayed elevated PTH levels in patients with persistent HPT, the intra- and postoperative specificity of both assays was 100%.

Postoperative complications were seen in one patient who had previously undergone neck surgery for thyroid disease: this patient showed a persistent unilateral paresis of the recurrent laryngeal nerve after parathyroidectomy. Therefore, the rate of persistent unilateral recurrent nerve paresis was 1.1%. Two patients with sHPT developed hypoparathyroidism, one of them after kidney transplantation. In both cases, an autotransplantation of frozen parathyroid tissue is planned.

### Discussion

Intraoperative PTH measurement enables unilateral neck exploration for pHPT (2–5, 17) and predicts successful surgery for parathyroid adenoma in primary HPT or confirms the adequate resection of hyperfunctioning parathyroid tissue in renal hyperparathyroidism (12, 13). In patients with chronic renal failure, non(1–84) PTH fragments accumulate, and prolonged degradation of PTH fragments has been observed (18, 19). Renal failure may also slow intraoperative PTH clearance in patients with sHPT (20, 21). Recently, however, new and more accurate PTH assays have been developed which are able to more precisely confirm the adequate resection of hyperfunctioning parathyroid glands in renal HPT (12, 13, 22, 23).

For both primary and renal HPT several intraoperative PTH assays have been described, but the different assays have only been directly compared in a small number of studies (13, 18, 22, 24–27). The Immulite Turbo DPC assay is established for both primary HPT (24, 28) and renal HPT (12). Kao et al. (28) described that the intraoperative PTH value, as measured with the Immulite Turbo DPC assay, decreased to below 5 pmol/l (normal range) within 20 min after resection of parathyroid adenoma in 87% of patients. Amal et al. (16) showed that the ADVIA Centaur assay was able to distinguish clearly between primary

### Tables

#### Table 2
Intra- and postoperative PTH levels after successful parathyroidectomy in 59 patients with pHPT and tHPT.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>PTH-A a</th>
<th>PTH-I b</th>
<th>Agreement of positive results</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraoperative PTH decrease ≥90%</td>
<td>56</td>
<td>9 (16%)</td>
<td>28 (50%)</td>
<td>9 (16%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Intraoperative PTH decrease in the normal range (≤7.6 pmol/l in %)</td>
<td>10 min</td>
<td>45</td>
<td>38 (84%)</td>
<td>45 (100%)</td>
<td>84%</td>
</tr>
<tr>
<td></td>
<td>20 min</td>
<td>42</td>
<td>40 (95%)</td>
<td>42 (100%)</td>
<td>95% ns</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>40</td>
<td>39 (98%)</td>
<td>40 (100%)</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>35</td>
<td>35 (100%)</td>
<td>35 (100%)</td>
<td>–</td>
</tr>
<tr>
<td>pHPT, primary hyperparathyroidism; tHPT, tertiary HPT.</td>
<td>17</td>
<td>aPTH measured with the ADVIA Centaur assay.</td>
<td>bPTH measured with the Immulite Turbo DPC assay.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cFor 12 out of the 59 patients with pHPT and tHPT preoperative PTH levels were already within the normal range.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Table 3
Intra- and postoperative PTH levels after successful parathyroidectomy in 26 patients with sHPT.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>PTH-A a</th>
<th>PTH-I b</th>
<th>Agreement of positive results</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraoperative PTH decrease ≥90%</td>
<td>22</td>
<td>12 (55%)</td>
<td>22 (100%)</td>
<td>12 (55%)</td>
<td>0.0016</td>
</tr>
<tr>
<td>Intraoperative PTH decrease in the normal range (≤7.6 pmol/l in %)</td>
<td>10 min</td>
<td>23</td>
<td>7 (30%)</td>
<td>17 (74%)</td>
<td>6 (26%)</td>
</tr>
<tr>
<td></td>
<td>20 min</td>
<td>22</td>
<td>11 (50%)</td>
<td>22 (100%)</td>
<td>11 (50%)</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>21</td>
<td>13 (62%)</td>
<td>13 (100%)</td>
<td>13 (62%)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>24</td>
<td>24 (100%)</td>
<td>24 (100%)</td>
<td>–</td>
</tr>
<tr>
<td>sHPT, secondary hyperparathyroidism. aPTH measured with the ADVIA Centaur assay. bPTH measured with the Immulite Turbo DPC assay.</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
HPT and non-parathyroid hypercalcemia. In uremic patients with sHPT, however, the ADVIA Centaur assay measured higher PTH concentrations than the classical intact PTH IRMA assay (Ntac-PTH, Diasorin, Stillwater, MN, USA). Twomey et al. (15) showed with the ADVIA Centaur assay that intra-individual PTH differences as large as 25% can exist on the same day between serum and plasma samples of patients suffering from renal failure.

In this study, we compared, to our knowledge, for the first time the Immulite Turbo DPC assay with the ADVIA Centaur assay for pre-, intra-, and postoperative measurement of intact PTH in patients with primary, secondary, and tertiary HPT. While both assays found only a median difference of 4.7% in preoperative PTH concentrations with marginally higher levels determined by PTH-A levels, the intraoperative PTH decay was clearly different for the two assays. Ten minutes after parathyroidectomy for pHPT/tHPT and sHPT, median PTH-I concentrations declined significantly to below 7.6 pmol/l (normal range) in 100 and 74% of the patients respectively. At the same time point, median concentrations by PTH-A reached the normal range in 84% in pHPT and tHPT and only in 30% in patients with renal failure and sHPT. A complete normalization of intraoperative PTH concentrations was found by PTH-I in all of the patients regardless of renal function 20 min after parathyroidectomy. Intraoperative PTH concentrations below 7.6 pmol/l measured with the ADVIA Centaur assay were found at 30 min in 98% of the patients with pHPT and tHPT and in 62% with sHPT. After 24 h postoperatively, PTH concentrations as determined by both PTH-I and PTH-A had returned to or below the normal range in all of the patients.

The most important aspect of intraoperative PTH measurement for surgery is in deciding to determine the operation for HPT after the removal of a parathyroid adenoma or total parathyroidectomy. Reliable and accurate PTH assays are highly predictive of postoperative outcome and cure of these patients (17, 29). In our study, the Immulite Turbo DPC assay was able to predict significantly whether parathyroidectomy was successful in 98% of the patients with pHPT and tHPT after 10 min and 100% of the uremic patients with sHPT after 20 min. The ADVIA Centaur assay was able to predict successful surgery in 98% of the patients with pHPT and tHPT after 30 min and 100% of the patients with sHPT after 24 h. Comparing the two assays, the ADVIA Centaur assay accurately measured intact PTH levels pre- and postoperatively, but the Immulite Turbo DPC assay intraoperatively quantified a PTH decay faster, especially in uremic patients. Comparing both methods in terms of recovery of the C-terminal fragments of PTH (7–84), which are eliminated by glomerular filtration, Souberbielle et al. (30) described a recovery of synthetic PTH (7–84) in 87.6% as measured with the ADVIA Centaur assay. For the Immulite assay, a recovery of PTH (7–84) was described in 62.7% by Worth et al. (27). The different percentage of measuring PTH (7–84) with both assays showing a higher cross-reactivity for the ADVIA Centaur assay might explain why this assay declined slower into the normal range in patients with impaired renal function compared with the Immulite assay.

Using the ADVIA Centaur system to measure PTH concentrations intraoperatively would have indicated a more extensive search for additional hyperfunctioning parathyroid glands, 10 min after parathyroidectomy in eight patients with pHPT/tHPT and 20 min after parathyroidectomy in ten patients with sHPT. However, longer operation times and extended neck explorations might carry a probable higher risk of intraoperative morbidity. Both assays were highly predictive for multiglandular disease in patients with double adenomas and persistent HPT.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

The authors thank Bettina Danner and Karin Chrenko, Department of Biometry, University of Ulm, Germany for their support with medical statistics.

References

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