

Sensitivity of Fibroblast Growth Factor 23 Measurements in Tumor-Induced Osteomalacia

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Context: Tumor-induced osteomalacia (TIO) is a paraneoplastic syndrome of hypophosphatemia, decreased renal phosphate reabsorption, normal or low serum 1,25-dihydroxyvitamin-D concentration, myopathy, and osteomalacia. Fibroblast growth factor 23 (FGF23) is a phosphaturic protein overexpressed in tumors that cause TIO and is, at least partly, responsible for the manifestations of TIO.

Objective: The objective of this study was to determine the sensitivity of FGF23 measurements in TIO.

Design: FGF23 concentrations were measured on stored samples with three ELISAs.

Setting: This study was conducted at subspecialty referral centers.

Patients: Twenty-two patients with suspected TIO, 13 with confirmed tumors, were studied.

Interventions: There were no interventions in this study.

Main Outcome Measure: FGF23 concentration was the main outcome measure of this study.

Results: Elevated FGF23 concentrations were detected using the Immunotopics C-terminal assay in 16 of 22 TIO patients (for a sensitivity of 73%), the Immunotopics Intact assay in five of 22 patients (sensitivity, 23%), and the Kainos Intact assay in 19 of 22 patients (sensitivity, 86%). In the 13 patients with confirmed tumors, the sensitivity was higher with all assays: 92% for the Immunotopics C-terminal assay, 38% for the Immunotopics Intact assay, and 100% for the Kainos assay.

Conclusion: The Kainos Intact assay was the most sensitive, followed by the Immunotopics C-terminal assay. The findings of normal FGF23 concentrations in some patients with TIO may indicate that FGF 23 is not responsible for the hypophosphatemia in these patients or that FGF23 secretion by some tumors is partially responsive to serum phosphate. Normal FGF23 concentrations should be interpreted in relation to the serum phosphate and 1,25-dihydroxyvitamin-D concentrations. (*J Clin Endocrinol Metab* 91: 2055–2061, 2006)

TUMOR-INDUCED OSTEOMALACIA (TIO) is a paraneoplastic syndrome consisting of hypophosphatemia, decreased renal tubular phosphate reabsorption, inappropriately normal or low serum 1,25-dihydroxyvitamin D concentrations, myopathy, and osteomalacia. Patients typically present with a history of chronic bone pain, fractures, and proximal muscle weakness. Children may present with poor growth and lower extremity deformity resulting from rickets. The tumors are often benign, small, and difficult to locate

with conventional imaging techniques. Fibroblast growth factor 23 (FGF23) is a secreted peptide hormone overproduced by the tumor in patients with TIO (1–4), which inhibits renal phosphate reabsorption resulting in hypophosphatemia (4, 5). Surgical excision results in resolution of hypophosphatemia, osteomalacia, muscle weakness (1, 3, 6–8), and the normalization of plasma FGF23 (1, 3, 6, 7, 9, 10).

FGF23 was identified by positional cloning as the gene responsible for autosomal dominant hypophosphatemic rickets (11). Mutations involve a RXXR amino acid sequence cleavage site and make the protein resistant to cleavage by proteases (5, 11–13). Subsequently, circulating FGF23 was found to be elevated in patients with X-linked hypophosphatemic rickets (XLH), TIO, and fibrous dysplasia in plasma and serum in separate studies (1, 3, 4, 6, 7, 14). These disorders all involve renal phosphate wasting and osteomalacia as central abnormalities. In addition, plasma or serum FGF23 is elevated

First Published Online March 21, 2006

Abbreviations: FGF23, Fibroblast growth factor 23; FRP-4, frizzled-related protein-4; RU, relative unit(s); TIO, tumor-induced osteomalacia; TMP/GFR, tubular maximum reabsorption of phosphate per deciliter of glomerular filtrate; XLH, X-linked hypophosphatemic rickets.

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

in patients with renal failure and correlates positively with serum creatinine and phosphate concentrations (15, 16).

Because FGF23 is a major etiological factor in several diseases of phosphate homeostasis, it is important to develop informative assays for plasma or serum FGF23 that will be clinically and diagnostically useful. Previous studies reporting either plasma or serum FGF23 in TIO have typically used one assay and have not assessed the sensitivity of FGF23 assays. The goal of the current study was to measure FGF23 concentrations using three different assays, comparing their sensitivity in 22 patients with clinical TIO (13 of which had confirmed tumors).

Patients and Methods

Patients

Patients with evidence of an acquired phosphate wasting disorder were recruited from the metabolic bone clinic at Indiana University Hospital (Indianapolis, IN) and from the clinical practices of collaborators. Control subjects were 118 healthy subjects with normal serum calcium, phosphate, and renal function from our study database. The study was approved by the Indiana University-Purdue University Indianapolis and Clarian Institutional Review Board, and written informed consent was obtained from all patients.

Twenty-two patients with confirmed or presumptive diagnosis of TIO underwent measurement of plasma FGF23 concentrations (Table 1). Patient ages ranged from 11–79 yr. Patients 2, 3, 14, and 17 were previously included in a paper by Jonsson *et al.* (3) describing levels of plasma FGF23 measured by the C-terminal Immunotopics assay. Patients 5, 10, 12, and 21 were previously described in case reports (6, 7, 9, 17). Clinical diagnosis of TIO was made by presence of hypophos-

phatemia, renal phosphate wasting, low or inappropriately normal serum 1, 25-dihydroxyvitamin D, proximal muscle weakness, and bone pain. Patients were excluded if they had a family history of hypophosphatemia, XLH, or autosomal dominant hypophosphatemic rickets. Thirteen patients had a confirmed tumor. In 10 patients, tumor removal resulted in improvement of symptoms. In one patient, the tumor was not completely resectable (patient 21), and another patient had an unresectable brain tumor (patient 16), whereas patient 17 refused surgery for a maxillary sinus hemangiopericytoma. Patient 7 had a leg tumor removed without change in his hypophosphatemia.

Blood and urine samples were obtained in the morning after an overnight fast. Blood was collected in EDTA tubes and serum separator tubes, centrifuged, and separated into plasma or serum. Samples were frozen at -80°C until testing. The tubular maximum reabsorption of phosphate per deciliter of glomerular filtrate (TMP/GFR) was estimated using the nomogram method described by Walton and Bijvoet (18) (see Table 1). Most patients were being treated with oral phosphate supplements and calcitriol. Serum and urine chemistries were measured in the respective clinical laboratories used by the clinicians involved in the individual patient's care.

Assays

FGF23 concentrations were measured in each patient using three different two-site enzyme-linked immunosorbent assays: a C-terminal human FGF23 ELISA (Immunotopics, San Clemente, CA), an intact human FGF23 ELISA (Immunotopics), and another intact human FGF23 ELISA (Kainos Laboratories, Tokyo, Japan) (1). The C-terminal assay uses polyclonal antiserum and recognizes two epitopes on the C-terminal side of the RXXR cleavage site and thus recognizes both full-length FGF23 and C-terminal cleavage fragments of FGF23. The Immunotopics Intact assay uses polyclonal antiserum to epitopes on both sides of the cleavage site, whereas the Kainos Intact assay uses two monoclonal antibodies to epitopes on either side of the cleavage site. For the two

TABLE 1. Preoperative values in patients with TIO

| Patient | Sex | Age (yr) | Tumor | Cure | Phosphorous (mg/dl) | Creatinine (mg/dl) | TMP/GFR (mg/dl) | Immunotopics C-terminal (RU/ml) (72.9 \pm 38.2) ^b | Immunotopics Intact (pg/ml) (13.3 \pm 19.0) ^b | Kainos Intact (pg/ml) (29.7 \pm 20.7) ^b |
|----------------------------------|-----|----------|--------------------------------|------|---------------------|--------------------|-----------------|--|--|--|
| 1 | M | 52 | Removed ^a | Y | 2.3 | 1.7 | 1.9 | 706.8 | <1 | 554.9 |
| 2 | F | 62 | | | 2.0 | 1.1 | 1.7 | 281.7 | 35.3 | 213.4 |
| 3 | M | 61 | | | 1.9 | 0.9 | 2.0 | 1,346.5 | 22.3 | 921.2 |
| 4 | M | 79 | | | 1.7 | 1.0 | 1.1 | 24.7 | <1 | 15.5 |
| 5 | F | 14 | Removed ^a | Y | 1.9 | 0.6 | 0.7 | 287.0 | <1 | 359.5 |
| 6 | M | 37 | Removed ^a | Y | 2.1 | 1.4 | | 135.3 | 3.3 | 87.6 |
| 7 | M | 12 | Removed | N | 2.0 | 0.5 | 1.6 | 158.8 | 13.5 | 111.5 |
| 8 | M | 34 | | | 1.2 | 0.9 | 0.7 | 96.6 | 9.4 | 239.1 |
| 9 | F | 55 | | | 1.5 | 0.6 | 1.3 | 95.6 | 10.3 | 39.4 |
| 10 | M | 11 | Removed ^a | Y | 1.5 | 0.5 | 1.1 | 3,143.0 | 87.8 | 402.2 |
| 11 | M | 60 | | | 1.8 | 1.0 | 0.7 | 42.4 | <1 | 107.1 |
| 12 | M | 42 | Removed ^a | Y | 1.7 | 1.1 | 1.2 | 483.6 | 50.5 | 397.6 |
| 13 | F | 36 | Removed ^a | Y | 1.2 | 0.9 | 1.3 | 954.0 | 6.9 | 1,919.8 |
| 14 | F | 44 | Removed ^a | Y | 1.4 | 0.6 | | 1,274.1 | 47.2 | 816.2 |
| 15 | F | 68 | Removed ^a | Y | 0.7 | 1.0 | 0.7 | 277.3 | 61.4 | 1,230.8 |
| 16 | M | 22 | Unresectable ^a | | 2.2 | 0.9 | 0.9 | 357.4 | 62.3 | 442.7 |
| 17 | F | 33 | Biopsy positive ^a | | 1.3 | 0.8 | 0.2 | 1,625.2 | 60.2 | 500.6 |
| 18 | F | 45 | | | 2.3 | 0.7 | 1.7 | 75.1 | 10.8 | 58.9 |
| 19 | M | 50 | Removed ^a | Y | 1.8 | 1.1 | 1.2 | 250.7 | <1 | 146.0 |
| 20 | F | 43 | | | 2.0 | 0.7 | | 255.1 | 41.7 | 363.6 |
| 21 | M | 52 | Partial resection ^a | N | 1.2 | 0.9 | 0.3 | 2,050.8 | 48.2 | 4,450.0 |
| 22 | F | 61 | Removed ^a | Y | 0.8 | 0.5 | | 10,642.5 | 426.6 | 9,320.0 |
| FGF23 values greater than normal | | | | | | | | 16 | 5 | 19 |
| Sensitivity in clinical TIO | | | | | | | | 0.73 | 0.23 | 0.86 |
| Sensitivity, confirmed tumor | | | | | | | | 0.92 | 0.38 | 1.00 |

Phosphorus and creatinine values are in mg/dl. The Immunotopics C-terminal results are in relative units per ml (RU/ml), whereas the Immunotopics Intact and Kainos Intact assay results are in pg/ml. For SI conversions of creatinine to $\mu\text{mol/liter}$, multiply by 88.4, and to convert phosphate to mmol/liter , multiply by 0.32.

^a Subjects with confirmed tumor (n = 13) (patients 1, 5, 6, 10, 12, 13, 14, 15, 16, 17, 19, 21, and 22).

^b For each assay, the mean \pm SD is in *parentheses*.

intact assays, the capture antibody recognizes an epitope on one side of the RXXR cleavage site, whereas the detection antibody recognizes an epitope on the other side; thus, the assays only recognize the intact molecule. The FGF23 assays were performed using stored plasma or serum according to the specified kit directions. Inter- and intraassay coefficients of variation for the three assays as reported by the manufacturers are as follows. The Immunotopics C-terminal FGF23 assay has an intraassay coefficient of variation of 5.0%, an interassay coefficient of variation of 5.0–7.3%, and a lower limit of detection of 3.0 relative units (RU)/ml. The Immunotopics Intact FGF23 assay has an intraassay coefficient of variation of 2.6–4.4%, an interassay coefficient of variation of 6.1–6.5%, and a lower limit of detection of 1.0 pg/ml. The Kainos Intact FGF23 assay has a lower limit of detection of 3 pg/ml and intraassay and interassay coefficients of variation of less than 5%. The C-terminal assay measures FGF23 in RU per milliliter, based on the initial standardization procedures used by the manufacturers (3), whereas both intact assays are standardized to measure FGF23 in picograms per milliliter.

Analysis

Data were analyzed and graphed using Microsoft Excel (Microsoft Corporation, Redmond, WA). Pearson's correlation was calculated between the FGF23 concentrations from each assay and the corresponding serum phosphate. FGF23 values in TIO subjects using different assays were compared on logarithmic graphs to normalize the distribution and compared using Pearson's correlation. The paired Student's *t* test for means was used for comparison between presurgical and postsurgical values.

Results

Reference ranges were determined by testing samples from 118 normophosphatemic individuals with all three assays. This included eight samples from children 2–14 yr. The 110 adult samples were from subjects 20–83 yr. The control mean \pm SD for each assay was as follows: for the Immunotopics C-terminal assay, 72.9 ± 38.2 RU/ml; for the Immunotopics Intact assay, 13.3 ± 19.0 pg/ml; and for the Kainos Intact assay, 29.7 ± 20.7 pg/ml. The normal range for each assay was defined as the mean plus two sds in controls. There was no difference between healthy children and adults for the C-terminal assay using the same 110 healthy adults 20–83 yr (71.8 ± 38.1 RU/ml) and 60 healthy children 1–17 yr (71.1 ± 35.7 RU/ml). The phosphate range in children was 3.6–5.4 mg/dl, appropriate for age, whereas the phosphate range in adults was 2.4–4.4 mg/dl.

The results of the three FGF23 assays in patients with TIO are shown in Table 1. Figure 1 compares the values in TIO patients with the normal ranges. Sensitivity was defined as the ability to detect FGF23 concentrations above the normal range for each assay in subjects with TIO. For the Immunotopics C-terminal assay, 16 of 22 patients had values above the normal range, providing a sensitivity of 73% for TIO. With the Immunotopics Intact assay, five patients had values above the normal range, resulting in 23% sensitivity. With the Kainos assay, 19 patients had values above the normal range, giving a sensitivity of 86%. However, when only patients with confirmed tumors ($n = 13$) were included in this analysis, the sensitivity was 92% for the Immunotopics C-terminal assay, 38% for the Immunotopics Intact assay, and 100% for the Kainos assay.

Although most TIO patients had elevated FGF23 concentrations, some patients had FGF23 concentrations in the normal range. Most patients had concentrations greater than the mean for normal controls. Twenty patients had FGF23 levels

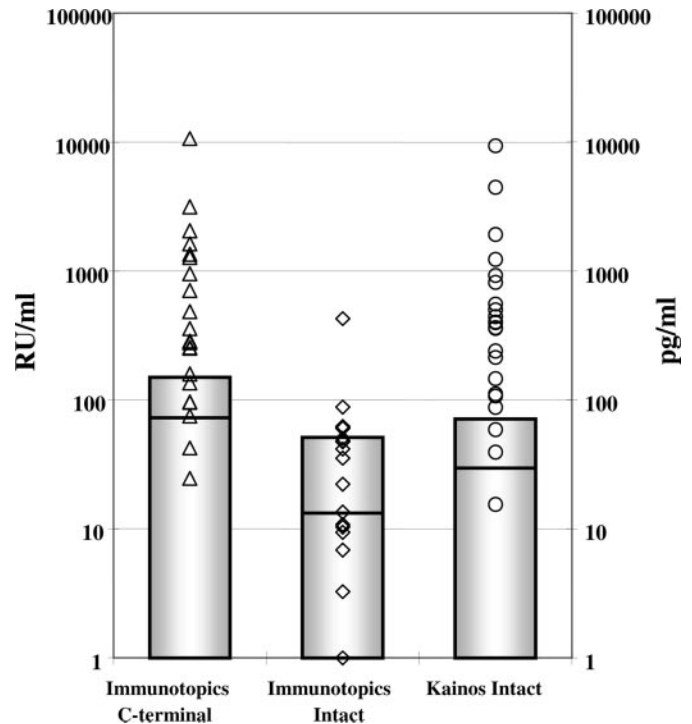


FIG. 1. FGF23 concentrations as measured by three different assays for 22 patients with TIO. The shaded columns indicate the range in healthy controls for each assay, defined as mean + 2 SD. The mean normal value for each assay is indicated by a horizontal bar in the column.

higher than the mean on the Immunotopics C-terminal assay, 12 had FGF23 levels greater than the mean with the Immunotopics Intact assay, and 21 were greater than the mean with the Kainos assay. Five patients had undetectable FGF23 concentrations by the Immunotopics Intact assay. Patients 4, 9, and 18 had FGF23 concentrations in the normal range with all three assays. However, patient 9 had subsequent repeat measurement of FGF23, which was elevated on the Kainos assay (126.0 pg/ml) but not the Immunotopics C-terminal assay (137.6 RU/ml) (the Immunotopics Intact assay was not repeated). Patient 18 had hypophosphatemia and osteomalacia on bone biopsy with normal FGF23 levels on all three assays. Of interest, none of these patients had confirmation of a tumor, but their clinical features were consistent with TIO rather than an inherited disorder or Fanconi's syndrome. Patients 6 and 11 had elevated FGF23 only on the Kainos assay, although patient 6 (in whom a tumor was confirmed) had a C-terminal FGF23 concentration near the upper limit of normal. Patients 1 and 6 also had mild renal insufficiency.

Relationships between FGF23 assays were examined graphically (Fig. 2, A–C), and Pearson's correlations were calculated. The highest correlation occurred between the Immunotopics C-terminal and the Kainos Intact assays with $r = 0.86$ ($P < 0.01$). Comparison of the Immunotopics C-terminal with the Immunotopics Intact assays resulted in $r = 0.65$ ($P < 0.01$), and comparison of the Kainos Intact assay with the Immunotopics Intact assay resulted in $r = 0.59$ ($P < 0.01$).

In light of the higher sensitivity of the Immunotopics C-terminal and Kainos Intact assays, we measured preoperative and postoperative samples with these two assays in nine

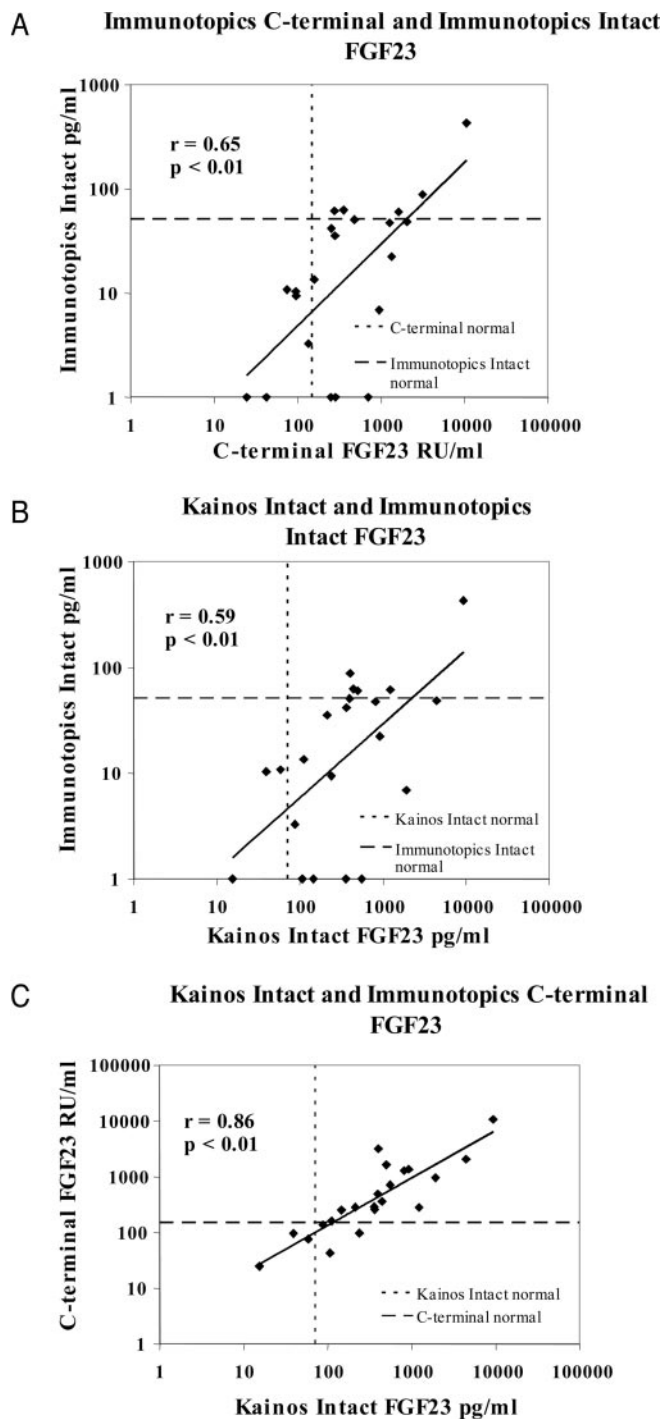


FIG. 2. A–C, Relationship between FGF23 assay results in patients with TIO. The upper limit of normal for each assay is indicated on the graph, defined as mean + 2 SD for normophosphatemic control subjects. $P < 0.01$ for each comparison.

patients who underwent surgery for TIO (Fig. 3, A and B). For this analysis, the closest preoperative sample to the surgery was used. For patients 12, 15, and 21, this was a later sample than the one used in Table 1 and reflected changes in the FGF23 concentration with time. Most postoperative samples were between 1 and 2 d after surgery. All patients had a decrease in their FGF23 concentrations on both the Immu-

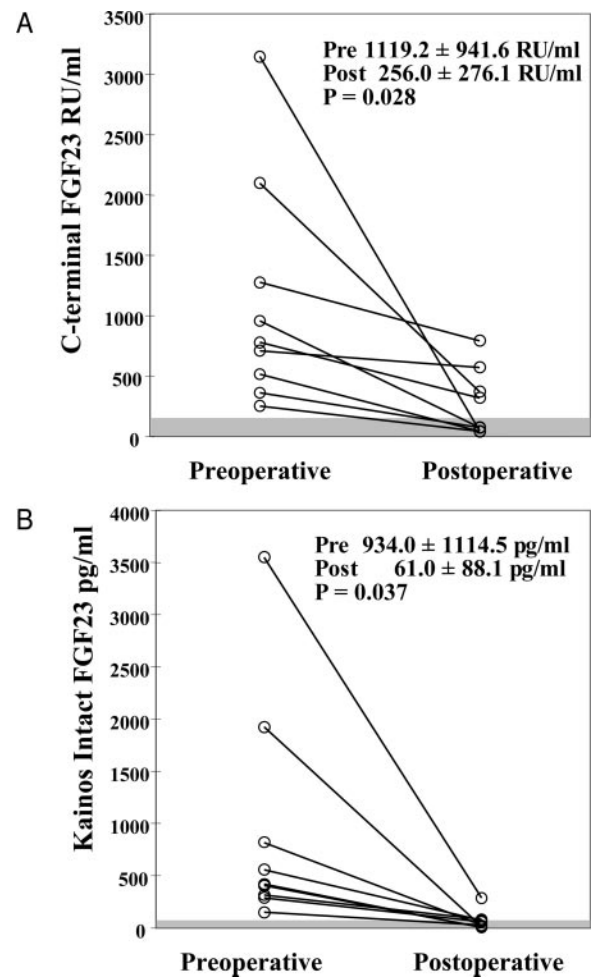


FIG. 3. A and B, Preoperative and postoperative FGF23 concentrations as measured by the Immunotopics C-terminal (A) and the Kainos Intact (B) assays in nine patients who had surgery for TIO. The shaded regions represent the normal ranges for each assay. Patients 1, 5, 10, 12, 13, 14, 15, 19, and 21 are included in this figure. Of these subjects, only patient 21 was not cured by his surgery, and his FGF23 concentrations remained quite elevated. Most postoperative samples are from 1–2 d postoperatively. One patient had the postoperative sample drawn at 2 h. Two other patients had their earliest postoperative samples drawn at 6 and 8 months after surgery.

notopics C-terminal assay and the Kainos Intact assays. By C-terminal assay, the mean preoperative concentration was 1119.2 ± 941.6 RU/ml, and the mean postoperative concentration was 256.0 ± 276.1 RU/ml ($P = 0.028$). By Kainos Intact assay, the mean preoperative concentration was 934.0 ± 1114.5 pg/ml, and the mean postoperative concentration was 61.0 ± 88.1 pg/ml ($P = 0.037$).

Four patients in Fig. 3 continued to have elevated C-terminal results after surgery, whereas two had elevated Kainos Intact assay results. Patient 1 had renal insufficiency and persistent elevations of C-terminal FGF23 after surgery, despite normalization of Kainos Intact FGF23 and phosphate. Patient 14 had a first postoperative sample at 6 months that was elevated on the C-terminal assay but not on the Kainos Intact assay. She was treated with radiation therapy because of pathological features of malignant osteosarcoma. Her subsequent C-terminal FGF23 was normal (82.3 RU/ml), with

normal Kainos assay, and her hypophosphatemia resolved. The only postoperative sample for patient 15 was 2 h post-surgery, which was elevated on both assays. However, she rapidly normalized her phosphate and weaned off all medications. Patient 21 had a partial resection of a tumor, and his FGF23 levels declined but remained elevated on both assays. His phosphate improved but did not normalize.

Despite variability in FGF23 concentrations among patients with similar phosphate concentrations, there was an inverse correlation between phosphate and FGF23 concentrations measured by all three assays. *r* values with phosphate for the Immunotopics C-terminal, Immunotopics Intact, and Kainos assays were -0.50 , -0.50 , and -0.57 respectively ($P < 0.05$).

Discussion

TIO is an acquired disorder characterized by phosphate wasting and impaired vitamin D metabolism. Tumors that cause TIO markedly overexpress FGF23 (2–4, 19, 20). The current study is the largest series of TIO patients with measured circulating FGF23 concentrations and, to our knowledge, the only series to compare the performance of three different FGF23 assays in a phosphate wasting disorder. Although a wide variety of tumors have been reported to be responsible for this paraneoplastic syndrome, recent evidence indicates that the majority (84%) are phosphaturic mesenchymal tumors/mixed connective tissue variant (20). FGF23 concentrations have also been reported to be elevated in other phosphate wasting disorders including XLH (3) and fibrous dysplasia (14). Thus, FGF23 assays may be clinically useful in a variety of patients with disorders of phosphate homeostasis.

In the current study, we compared the sensitivity of three different assays in a large group of patients with TIO. Our data demonstrate that two of the three assays had high sensitivity for TIO, and results were highly correlated. The sensitivity of the Immunotopics Intact assay was poor. There were 14 cases in which the FGF23 concentration in TIO was low or normal using this assay, with elevated FGF23 concentrations using one or both of the other assays. This included eight patients with confirmed tumors. The differences between assays may be due to differential production of full-length or cleavage products of FGF23 in tumors. The average ratio of C-terminal assay to Kainos assay values was 1.51. However, there was considerable variation in this ratio (range, 0.23–3.25), suggesting that the proportion of full-length and cleaved FGF23 is not constant among cases of TIO.

The diagnosis of TIO is difficult and frequently made clinically in patients for whom no other cause of renal phosphate wasting is found. Indeed, many patients have their disorder for years before localization and removal of the causative tumor (including some patients for whom surgery was curative in this series). There is a general bias to only report those cases in which tumor removal completely cures the disorder. To address the larger clinical context, we have also included patients for whom tumors have not yet been found. Thus, we are able to compare FGF23 concentrations in subjects with a clinical diagnosis of TIO as well as in those with a confirmed tumor. Most subjects in whom tumors were

found were cured by removal of the tumor. Our data also demonstrate that patients in whom tumors are localizable are more likely to have elevated FGF23 concentrations. This may be due to greater FGF23 production in larger tumors. However, many subjects without localized tumor also have quite elevated levels.

Tumors that are detected still might not be responsible for TIO, especially if the syndrome is not cured or improved by surgery. Likewise, tumors causing TIO may remain undetected. Our series includes some subjects who were not cured by tumor removal. Patient 7 had a benign leg tumor removed and remained hypophosphatemic. Patient 21 refused amputation for an extensive foot tumor, but debulking resulted in a partial response in FGF23 and phosphate concentrations. Patient 16 had a brain tumor that was not amenable to surgery. A magnetic resonance image of patient 17 revealed an extensive maxillary sinus tumor, and biopsy demonstrated features of hemangiopericytoma, a tumor type associated with TIO. However, if only those 10 patients for whom surgery resulted in clinical cure are analyzed, the sensitivity of the C-terminal assay was 90% and of the Kainos assay was 100%. Smaller tumors may secrete less FGF23 while being more difficult to locate. Because the diagnosis of TIO is otherwise clinical, evaluating FGF23 concentrations both in subjects with and without confirmed tumor is valuable and confirms the role of FGF23 in this phosphate wasting disorder.

Interestingly, patients 1 and 6 had both tumors and renal insufficiency. Patient 6 had mild renal insufficiency with a creatinine of 1.4, elevated full-length FGF23 with the Kainos assay, and high normal results with the C-terminal assay. FGF23 concentrations may have been increased by the presence of renal insufficiency, but his hypophosphatemia resolved after removal of a tumor in his foot. Postoperative FGF23 values were not available. The FGF23 concentration of patient 1 was elevated on the Immunotopic C-terminal and Kainos Intact assays (but undetectable on the Immunotopics Intact assay), and phosphate wasting necessitated treatment with calcitriol and oral phosphate. Six weeks after tumor removal, his serum phosphate, TMP/GFR, and renal function improved. Serum phosphate increased from 2.3 to 3.6 mg/dl, creatinine decreased from 1.7 to 1.3 mg/dl, and TmP/GFR increased from 1.9 to 2.9 mg/dl. After surgery, the intact FGF23 concentration decreased with the Kainos assay but remained elevated when measured by the C-terminal assay. This may suggest persistence of C-terminal fragments of FGF23 due to mild renal impairment in our patient, consistent with the observation of increased circulating FGF23 in patients with renal insufficiency as described by others using the C-terminal FGF23 assay (15, 16). However, elevations of full-length FGF23 (Kainos assay) in the setting of renal failure have also been reported (21–23). Renal failure may contribute to the elevations of FGF23 seen in patients 1 and 6. However, in patient 1, only the intact FGF23 measured by the Kainos assay decreased after surgery. The decrease in the FGF23 concentration with the Kainos assay suggests that this patient had more C-terminal fragments than intact FGF23 postoperatively. The ratio of C-terminal FGF23/Kainos Intact FGF23 was 1.3 preoperatively and 8.2 postoperatively in patient 1. This differs from results reported by Imanishi *et al.*

(Imanishi, Y., personal communication; Ref. 16), who noted no difference in the ratio of circulating intact/C-terminal FGF23 in patients with renal failure compared with control subjects using the Immunotopics Intact and C-terminal assays. This may represent differences between the Immunotopics Intact and Kainos assays, or there may be differences in the rate of decline of full-length or cleavage fragments of FGF23 after tumor removal in the setting of abnormal and changing renal function.

In our study, the majority of TIO patients had a measurably elevated serum FGF23 concentration, and the sensitivity for two of the assays (Immunotopics C-terminal assay 73% and Kainos assay 86%) for clinical TIO corresponds well to the immunohistochemistry findings of Folpe *et al.* (20), who found 81% of TIO tumors expressed FGF23. One of the challenges in evaluating TIO is that some patients have clinical and biochemical presentations consistent with TIO, despite a negative search for a tumor using various imaging modalities. When only the patients with surgically confirmed tumors were analyzed, the sensitivity increased to 92% for the Immunotopics C-terminal assay and 100% for the Kainos assay but was only 38% for the Immunotopics Intact assay.

The fact that some patients did not have elevations of FGF23 (with one or more assay) may indicate that there are limitations in currently available assays. Alternatively, in some cases, the clinical picture may not be caused by increased circulating FGF23, and some tumors may express other substances responsible for osteomalacia and phosphate wasting. In this regard, Jan De Beur *et al.* (19) demonstrated that TIO tumors overexpress matrix extracellular phosphoglycoprotein, frizzled-related protein-4 (FRP-4), and other genes. Berndt *et al.* (24) subsequently demonstrated phosphaturic actions to FRP-4 infusions in normal rats. However, the single TIO patient described by Berndt *et al.* did not have an elevated serum FRP-4. In addition, Carpenter *et al.* (25) reported the expression of FGF7 in a tumor responsible for TIO. Although most tumors causing TIO produce FGF23, elevated circulating FGF23 concentrations may not be required to define TIO. Finally, as observed in other endocrine tumors, it is possible that some tumors may continue to be partially responsive to the metabolic state of the patient. Therefore, it may be necessary to interpret FGF23 levels in light of serum phosphate and calcitriol concentrations. In the setting of hypophosphatemia and low calcitriol concentrations, FGF23 levels in the mid- to upper normal range may be inappropriate. Despite hypophosphatemia in all of our TIO patients, only one patient had serum FGF23 levels less than the normal mean with the Kainos assay, and two were less than the normal mean with the C-terminal assay. An appropriate response to hypophosphatemia would be to decrease the production of a phosphaturic hormone (FGF23) to allow for improved renal tubular reabsorption to normalize the phosphate.

Recently, studies have indicated regulation of FGF23 gene expression by 1,25-dihydroxyvitamin D, as well as regulation of 1,25-dihydroxyvitamin D production by FGF23 (26–29). This would emphasize the importance of the common finding that disorders associated with FGF23 excess have inappropriately low or normal 1,25-dihydroxyvitamin D concentrations. Conversely, for appropriate physiological

regulation, FGF23 concentrations should be low in the setting of low 1,25-dihydroxyvitamin D and hypophosphatemia. To accurately correlate the FGF23 concentrations with the phosphate concentrations or 1,25-dihydroxyvitamin D concentrations, these need to be measured simultaneously. Our subjects were on treatment with calcitriol and phosphate at the time of the sampling for FGF23. Consequently, 1,25-dihydroxyvitamin D measurements were not performed on these samples. Future studies should attempt to correlate FGF23 with 1,25 dihydroxyvitamin D concentrations in phosphate wasting disorders and determine the effect, if any, of treatment with calcitriol on FGF23 in these disorders.

In summary, for the Immunotopics C-terminal and Kainos Intact FGF23 assays, FGF23 concentrations were elevated in most patients with a clinical and biochemical presentation consistent with TIO, especially in patients with identifiable tumors. Reliable FGF23 assays have the potential to be clinically useful in the diagnosis and management of disorders of phosphate homeostasis. Additionally, FGF23 concentrations may help determine the cause of hypophosphatemia and may also one day be useful in localizing tumors (30). FGF23 concentrations may need to be interpreted in the context of the serum phosphate and possibly 1, 25-dihydroxyvitamin D concentrations.

Acknowledgments

We thank Dr. Kenneth White for valuable scientific input and comments.

Received September 21, 2005. Accepted March 10, 2006.

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This work was supported by National Institutes of Health Grants R01AR42228, T32 AR 07581-09, and MO1 RR00750.

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