Title

Clinical performance of a novel chemiluminescent enzyme immunoassay for FGF23

Running title

A Novel CLEIA for FGF23

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Abstract

Introduction: Measurement of fibroblast growth factor 23 (FGF23) has been reported to be clinically useful for the differential diagnosis of chronic hypophosphatemia. However, assays for research use only are available in Japan. Thus, the objective of this study was to examine the clinical utility of a novel and automated chemiluminescent enzyme immunoassay for the measurement of FGF23.

Materials and Methods: Participants were recruited from July 2015 to January 2017 at six facilities in Japan. Thirty-eight patients with X-linked hypophosphatemic rickets (XLH; 15 males, 23 females, age 0–66 years), five patients with tumour-induced osteomalacia (TIO; 3 males, 2 females, age 60–73 years), and twenty-two patients with hypophosphatemia (11 males, 11 females, age 1–75 years) caused due to other factors participated in this study.

Results: With the clinical cut-off value of FGF23 at 30.0 pg/mL indicated in the Diagnostic Guideline of Rickets/Osteomalacia in Japan, the sensitivity and specificity of FGF23-related hypophosphatemic rickets/osteomalacia without vitamin D deficiency (disease group-1) were 100% and 81.8%, respectively, which distinguished it from non-FGF23-related hypophosphatemia (disease group-2). Furthermore, the diagnostic sensitivity of FGF23-related hypophosphatemia with vitamin D deficiency remained at 100%. Among the four patients with FGF23 levels ≥ 30.0 pg/mL in disease group-2, two patients with relatively higher FGF23 values were suspected to have genuine FGF23-related hypophosphatemia, due

to the ectopic production of FGF23 in pulmonary and prostate small cell carcinomas.

Conclusion: The novel FGF23 assay tested in this study is useful for the differential diagnosis of hypophosphatemic rickets/osteomalacia in a clinical setting.

Keywords: rickets, osteomalacia, FGF23, pulmonary small cell carcinoma, prostate small cell carcinoma

Introduction

Chronic hypophosphatemia causes rickets/osteomalacia by impairing the mineralisation of bone matrix. The aetiological factors of hypophosphatemic rickets/osteomalacia include 1) insufficient phosphate intake and malabsorption, 2) renal tubular diseases, 3) impaired actions of vitamin D metabolites, and 4) inappropriately enhanced actions of FGF23 (FGF23-related hypophosphatemic rickets/osteomalacia) [1].

FGF23, produced by osteoblasts/osteocytes, is a hormone that plays a central role in the regulation of blood phosphate concentration [2, 3]. In the renal proximal tubule, FGF23 suppresses phosphate reabsorption and decreases the levels of 1,25-hydroxyvitamin D ($1,25OH_2D$) that promote phosphate absorption in the intestinal tract. Higher levels of FGF23 have been shown to cause several inherited and acquired hypophosphatemic diseases [3, 4]. Among inherited diseases, X-linked hypophosphatemic rickets (XLH) caused by mutations in the *phosphate regulating endopeptidase homolog X-Linked* (*PHEX*) gene is known to be the most common form. On the other hand, tumour-induced osteomalacia (TIO) is a typical acquired hypophosphatemic rickets/osteomalacia caused by phosphaturic mesenchymal tumours producing FGF23.

Since the therapeutic approaches for chronic hypophosphatemia vary depending on

the aetiology, differential diagnosis of the precise underlying conditions of hypophosphatemic rickets/osteomalacia is essential in the clinical setting. Previously, we measured FGF23 levels using an enzyme-linked immunosorbent assay (ELISA) kit (Kainos, Tokyo, Japan) and reported that the levels were high in patients with FGF23-related hypophosphatemic rickets/osteomalacia. On the other hand, FGF23 levels were low or low – normal in patients with chronic hypophosphatemia caused due to other factors, such as Fanconi syndrome and vitamin D deficiency. Thus, there was no overlap in FGF23 levels between patients with these two conditions, when the cut-off value of 30.0 pg/mL was used. This cut-off value for the differential diagnosis of hypophosphatemic rickets/osteomalacia was officially recommended in the guidelines of the Japan Endocrine Society and the Japanese Society for Bone and Mineral Research in 2015 [1,5].

The intact FGF23 assay (Kainos) is a sandwich ELISA which measures only active and full-length FGF23. The assay has been long used in many clinical and basic research in Japan and other countries [6-8]. However, this ELISA has not been approved for medical use in Japan.

Furthermore, Burosumab, a fully humanized anti-FGF23 antibody, has been developed and reported to be efficient and safe during the clinical studies (phase 2 and 3) on XLH and TIO [9-14]. Thus, the development of a precise FGF23 assay that can be used clinically has become increasingly important.

In some European countries, the other intact FGF23 measurement system (Liaison iFGF23, DiaSorin, Saluggia, Italy) has been approved for clinical use to evaluate the risks in CKD patients [15 – 17].

To meet the increasing demand of intact FGF23 measurement in Japan, an automated and rapid chemiluminescent enzyme immunoassay (CLEIA), Determiner CL FGF23 (Minaris Medical, Tokyo, Japn), referred to as CL-FGF23 hereafter, was developed [~~15~~18]. CL-FGF23 recognizes the same set of epitopes as the intact FGF23 assay (Kainos), but the former applies CLEIA and enables specific detection of intact FGF23, as well as higher sensitivity, wider measurement range, and a shorter measurement time than the intact FGF23 assay (Kainos) [18~~15~~].

In this report, the clinical utility of CL-FGF23 in the differential diagnosis of hypophosphatemic rickets/osteomalacia and chronic hypophosphatemia was prospectively analysed. Since vitamin D deficiency is an important cause of hypophosphatemic rickets/osteomalacia, the primary objective of this study was to compare the FGF23 levels between patients with FGF23-related hypophosphatemia without vitamin D deficiency (disease group-1) and those with hypophosphatemia not related to FGF23 (disease group-2). As vitamin D deficiency is prevalent even among healthy people, the secondary analysis was a comparison of FGF23 levels between all patients with FGF23-related hypophosphatemia with and without vitamin D deficiency and those in disease group-2.

Materials and Methods

Patients

Registration of the participants was conducted for 19 months (from July 2015 to January 2017) in 7 departments at 6 facilities in Japan. The process of registration and selection of the participants is summarized in Fig.1.

Finally, 97 patients diagnosed with hypophosphatemic rickets/osteomalacia and/or chronic hypophosphatemia were enrolled. The diagnostic criterion for ‘chronic hypophosphatemia’ was two consecutive serum inorganic phosphate levels below the age-adjusted reference range of the sites with an interval of 2 weeks or more. Rickets/osteomalacia was diagnosed according to the ‘Diagnostic Manual of Rickets/Osteomalacia’ produced by the Japan Endocrine Society and the Japanese Society for Bone and Mineral Research [1]. Exclusion criteria included patients with impaired renal function (estimated glomerular filtration rate [eGFR] < 60 mL/min/1.73 m^2) and rickets/osteomalacia due to causes other than chronic hypophosphatemia. Twenty patients out of the initially enrolled 97 patients were excluded because they met the exclusion criteria or withdrew from the study.

Next, three endocrinologists from independent institutions of the six research sites

148 and having abundant experience on rickets/osteomalacia assembled and reviewed 77 cases
149 between September 2016 and March 2017. After the review, 12 patients were defined as
150 ‘unclassified’, because at least 1 of the 3 designated specialists opposed the diagnosis of
151 ‘rickets/osteomalacia’ or ‘chronic hypophosphatemia’ in those patients.

152 Finally, the remaining 65 patients were divided into 22 non-FGF23-related (disease
153 group-2) and 43 FGF23-related hypophosphatemia cases by the three specialists. Of those 43
154 patients, 29 cases with 25-hydroxyvitamin D (25OHD) values less than 20 ng/mL were
155 classified as patients with FGF23-related hypophosphatemia and concomitant vitamin D
156 deficiency. Thus, the remaining 14 patients (disease group-1) were classified as FGF23-
157 related hypophosphatemic cases without vitamin D deficiency. Primary analysis comprised
158 patients from groups -1 and -2, whereas secondary analysis comprised those in all patients
159 with FGF23-related hypophosphatemia and group-2.

160 Fasting blood samples from enrolled patients were collected and immediately stored
161 at -20 °C until use. Before initiation of this study and after obtaining written informed
162 consent, approximately 23% (15) of 65 samples from classified patients were frozen and
163 stocked for other investigative purposes. All of these stocked samples were analysed only
164 when additional such consent was obtained.

165 This study was approved by the ethical review board of the six investigational sites,
166 including the Japan Community Health Care Organization (JCHO), Osaka Hospital (approval

no. 006510), Osaka University Hospital (No.157801), Kanagawa Children's Medical Center (approved by institutional IRB on 28 August 2015), Toranomon Hospital (No.15-44), Tohoku University Hospital (No.155001), and the University of Tokyo Hospital (No.2015012-11Y and No.2015013-11Y). After written informed consent was obtained from the participants, the serum samples were analysed and clinical data of these patients were collected.

Assays

Biochemical parameters of serum and urine were measured using an autoanalyser. Intact parathyroid hormone levels were measured using electrochemiluminescence immunoassay or enzyme immunoassay. Levels of $1,25OH_2D$ and 25OHD were measured using radioimmunoassay and competitive protein binding assay (dextran-coated charcoal assay; LSI Medience Corporation, Japan) [~~16~~19], respectively. FGF23 was measured using a CLEIA reagent (CL-FGF23; Minaris Medical, Japan) that recognizes only biologically active and intact FGF23 molecules with a detection range of 5.0~10,000 pg/mL. Basic performance of CL-FGF23 was evaluated and reported elsewhere [~~15~~18]. A previous study indicates that in healthy adults, the reference range of FGF23 measured using the CLEIA reagent is 19.9–52.9 pg/mL (95% CI) with a mean value of 32.8 pg/mL in 396 healthy adults aged from 36 to 67.

186 Statistical analyses

187 Statistical analyses were performed using STATA12 (StataCorp, TX, USA) [~~17~~20].

188 Data are expressed as mean ± S D, and statistical significance was determined using the

189 Kruskal-Wallis test or one-way ANOVA test. Statistical significance was set at $p < 0.05$.

190

Results

The characteristics of patients in group-1 and -2 are summarized in Table 1. In addition, the characteristics of all FGF23-related hypophosphatemic patients with and without vitamin D deficiency are shown (shaded area). In total, 38 patients with XLH (15 males, 23 females, age 0–66 years), 5 patients with TIO (3 males, 2 females, age 60–73 years), and 22 patients with hypophosphatemia caused due to other factors (11 males and 11 females, age 1–75 years) were analysed. In disease group-1 comprising of 14 patients, 11 patients with XLH, 2 patients with TIO, and 6 out of 22 patients in disease group-2 were treated with either active vitamin D3 and/or phosphate.

The distribution of FGF23 values in disease group-1 and -2 and the whole FGF23-related hypophosphatemia as a reference (shaded area) is shown in Fig. 2. In both disease group-1 and reference, FGF23 values in all cases were higher than 30.0 pg/mL, the previously defined cut-off value. However, FGF23 levels in 4 out of 22 cases in disease group-2 were higher than 30.0 pg/mL.

A scatter plot of the serum phosphate and FGF23 values in all patients with FGF23-related hypophosphatemia and disease group-2 (total 65 cases) is shown in Fig. 3. Of the 38 XLH cases, 12 were without vitamin D deficiency (grey circles) and 26 had concomitant

vitamin D deficiency (black circles). Of the 5 TIO cases, 2 were without vitamin D deficiency (grey triangles) and 3 were affected by vitamin D deficiency (black triangles). In contrast, disease group-2 was re-grouped according to the causes of hypophosphatemia, which included 1 case of Fanconi syndrome due to adefovir dipivoxil (asterisk), 3 cases of denosumab-induced hypophosphatemia (open rhombuses), and 18 cases with vitamin D deficiency (open squares). Of the total 65 cases, 39 and 26 patients were ≥ 20 years old (adult patients) and under 20 years old (children), respectively. Thirty-eight XLH patients included 16 children (41%), whereas 22 non-FGF23-related hypophosphatemic cases included 10 children (45.5%). Reference intervals of serum phosphate were higher in younger children, as such, the patients with relatively high serum phosphate levels (3.0 mg/dL and more) comprised child patients despite XLH or vitamin D deficiency (Fig. 3).

The proposed cut-off value (FGF23=30.0 pg/mL) described in the Diagnostic Manual of Rickets/Osteomalacia [1] was applied in Fig. 3, and the clinical performance of CL-FGF23 to differentiate group-1 and -2 was evaluated (Table 2), which revealed that the sensitivity and specificity of CL-FGF23 for the diagnosis of disease group-1 were 100% and 81.8%, respectively. Similarly, positive predictive value (PPV), negative predictive value (NPV), and accuracy were 77.8%, 100.0%, and 88.9%, respectively. Secondary analysis conducted in all patients with FGF23-related hypophosphatemia and disease group-2 revealed that the sensitivity, specificity, PPV, NPV, and accuracy in this cohort were 100%, 81.8%,

91.5%, 100%, and 93.8%, respectively.

Discussion

In the current study, CL-FGF23 successfully differentiated patients with FGF23-related hypophosphatemia from those with the non-FGF23-related variant, using the previously reported cut-off value of 30.0 pg/mL.

Twenty-nine (67.4%) of 43 patients with FGF23-related hypophosphatemia were diagnosed with vitamin D deficiency (< 20 ng/mL). Such patients were excluded from the primary analyses according to the protocol of this study. However, the prevalence of vitamin D deficiency in the adult population in East Asia has been reported to be around 70% [~~18~~21-~~20~~23]. Therefore, we conducted additional secondary analyses to evaluate the diagnostic performance of CL-FGF23 in all patients with FGF23-related hypophosphatemia, including those with vitamin D deficiency. The primary and secondary analyses produced almost the same sensitivity, specificity, PPV, NPV, and accuracy for the diagnosis of FGF23-related hypophosphatemia in this cohort. The PPV and accuracy were higher in the secondary analyses, which indicated that CL-FGF23 is useful for the diagnosis of FGF23-related hypophosphatemia, such as XLH and TIO, regardless of the presence or absence of vitamin D deficiency.

The FGF23 values in 4 out of the 22 cases in disease group-2 were higher than 30.0 pg/mL, and Table 3 shows the clinical background of these 4 cases (#1–4) that were

registered as non-FGF23-related chronic hypophosphatemia caused due to vitamin D deficiency and related to the primary conditions and/or loss of appetite associated with the treatment. Chronic hypophosphatemia is expected to induce negative feedback on the production of FGF23 in osetoblasts/osteocytes, which results in the suppression of serum FGF23 levels. Moreover, #1–4 showed FGF23 levels over 30.0 pg/mL, despite the existence of vitamin D deficiency-associated hypophosphatemia for more than 2 weeks.

In cases #1 and #2, it was suggested that the duration of hypophosphatemia was relatively short, as they did not present any symptoms of rickets/osteomalacia or significant elevation of alkaline phosphatase (ALP). Serum FGF23 levels could not be observed within 6 h, regardless of low or high phosphate load, but they were detected after at least 4 to 5 days with continuous load [~~21~~24,~~22~~25]. Thus, one hypothesis to explain the non-suppression of FGF23 in #1 and #2 was that although they were registered as vitamin D deficiency-associated chronic hypophosphatemia cases, they were recognized during hospitalisation for the treatment of chronic myeloid leukemia and adrenal insufficiency after discontinuation of steroid treatment, and during the fluctuation of their actual serum phosphate levels, by chance, low serum phosphate levels were observed twice within an interval of two weeks or more.

In contrast, # 3 and # 4 showed markedly elevated ALP, suggesting that prolonged hypophosphatemia was sufficient for developing unmineralized bone and bone metastases

contributed to high ALP in case 4. Additionally, FGF23 levels were as high as 71.6 and 469.4 pg/mL in some patients without renal dysfunction, suggesting that these patients had FGF23-related hypophosphatemia that was not detected by the attending clinicians. Case 3 was a patient with pulmonary small cell carcinoma who was hospitalized for chemotherapy. Pulmonary small cell carcinoma is one of the neuroendocrine tumours that can ectopically produce hormones, such as adrenocorticotropic hormone (ACTH) and antidiuretic hormone (ADH) [~~23~~26]. Although very rare, there have been several cases of pulmonary small cell carcinoma accompanied by hypophosphatemic osteomalacia. In 2016, a case of pulmonary small cell carcinoma with acquired hypophosphatemic osteomalacia was reported, which demonstrated high serum FGF23 levels in the patient and ectopic production of FGF23 in the tumour cells analysed using immunohistochemistry [~~24~~27]. It is likely that case #3 was actually suffering from FGF23-related hypophosphatemia due to ectopic overproduction of FGF23 in the pulmonary small cell carcinoma, but we could not obtain the tumour tissues to confirm the same.

Case#4 was also treated with chemotherapy in the hospital for prostate small cell carcinoma. Like pulmonary small cell carcinoma, prostate small cell carcinoma is a neuroendocrine tumour that is uncommonly accompanied by the ectopic secretion of ACTH or ADH [~~25~~28]. Very intriguingly, several cases of prostate small cell carcinoma with acquired hypophosphatemic osteomalacia have been reported, although serum FGF23 levels

have never been assessed in these cases [~~26~~29-~~35~~38]. Unfortunately, pathological examination could not be conducted on patients in case #4. However, the prominently high levels of FGF23 (469.4 pg/mL) and ALP under chronic hypophosphatemic conditions with normal kidney function could only be possible due to FGF23 overproduction. It is likely that FGF23 was produced by prostate small cell carcinoma.

After much contemplation, patients in case# 3 and 4 who were once considered as false-positive cases for FGF23-related hypophosphatemia were finally assumed to be true cases with ectopic production of FGF23 as a paraneoplastic syndrome of neuroendocrine tumours. After this alteration in assumption, the sensitivity and specificity of the diagnostic performance of CL-FGF23 for FGF23-related hypophosphatemia shifted to 100% and 90.0%, respectively, with the cut-off value of 30.0 pg/mL. However, if 44.0 pg/mL of FGF23 was applied as a cut-off value, the two disease groups could be completely distinguished. Thus, albeit with a small sample size, this study demonstrated that with a cut-off value of 44.0 pg/mL, CL-FGF23 can discriminate FGF23-related hypophosphatemia from both the non-FGF23-related variants that are without obvious symptoms of rickets/osteomalacia due to shorter duration of hypophosphatemia as well as canonical non-FGF23-related hypophosphatemia, such as Fanconi syndrome and vitamin D-deficient rickets. Indeed, in this study, when non-FGF23-related hypophosphatemia without obvious skeletal symptoms was excluded from disease group-2, the cut-off value of 30.0 pg/mL provided 100%

discriminating accuracy (data not shown). Thus, for the differential diagnosis of the causes of hypophosphatemic rickets/osteomalacia, the current cut-off value of 30.0 pg/mL FGF23 has sufficient sensitivity and specificity.

Limitations

This was an observational study with a prospective setting, and the sample size was small due to fewer patients with untreated non-FGF23-related hypophosphatemia and FGF23-related hypophosphatemia without vitamin D deficiency. Owing to the small sample size, age, gender, and other demographic parameters could not be matched between the two study groups. Second, while there were other causes for both FGF23-related and non-FGF23-related hypophosphatemia, such patients could not be registered in the current study because of their rarity. Finally, the purpose of this study was to verify the diagnostic performance of CL-FGF23 with a cut-off value (30.0 pg/mL) determined by the existing intact FGF23 assay (Kainos). Therefore, the optimum cut-off value of CL-FGF23 has not been established. The determination of adequate cut-off value for discriminating FGF23-related hypophosphatemia from the non-FGF23-related one will be warranted after the accumulation of clinical data with this new intact FGF23 assay system in the future.

Conclusions

It is evident from this clinical study that measurements with CL-FGF23 using the existing cut-off value of 30.0 pg/mL proposed in the guidelines in Japan demonstrated high sensitivity and specificity. This novel assay system detects only the active form of full-length FGF23 and is clinically beneficial as an *in vitro* diagnostic reagent for the detection of FGF23-related hypophosphatemia. This study also suggested that patients with small cell carcinoma in the lung or prostate can suffer from FGF23-related hypophosphatemia. Thus, this could be an interesting topic for future research to clarify the prevalence of FGF23-related hypophosphatemia in patients with neuroendocrine tumours.

Research funding

This study was supported by Minaris Medical Co., Ltd.

Author contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conceptualisation: NI, KO, and SF.

Formal analysis and investigation: NI.

Writing - original draft preparation: NI, and TK.

Writing - review and editing: NI, and SF.

Funding acquisition: NI, TK, SK, IF, MA, YT, MM, RO, KO, YS, and SF.

Resources: NI, TK, SK, IF, MA, YT, HY, TK, TS, MM, RO, KO, YS, and SF.

Supervision: NI, KO, and SF.

Competing interests

Authors state no conflict of interest.

Informed consent

Informed consent was obtained from all individuals included in this study.

Ethical approval

This study was approved by the ethical review board of the six investigational sites.

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Figure legends

Fig.1 Flow chart of the registration of the participants

The registration flow of this study from the initial enrolment to the final selection of FG23-related hypophosphatemia without vitamin D deficiency (disease group-1), non-FGF23-related hypophosphatemia (disease group-2), and FGF23-related hypophosphatemia with vitamin D deficiency are shown.

*Withdrawal of consent, unmatched to inclusion criteria (higher Cre value, unconfirmed data of chronic hypophosphatemia, etc.), matched to exclusion criteria

** 'Unclassified case' means that the diagnosis of three council members mismatched.

Fig.2 Comparison of FGF23 levels between FGF23-related hypophosphatemic rickets/osteomalacia and non-FGF23-related chronic hypophosphatemia with or without rickets/osteomalacia

FGF23 values measured by CL-FGF23 from disease group-1 and 2 and all FGF23-related hypophosphatemic patients are plotted and compared in the logarithmic expression in Figure 2.

The solid and broken lines indicate serum FGF23 levels of 30.0 pg/mL (current cut-off value) and 44.0 pg/mL, respectively.

Fig.3. Serum phosphate and FGF23 levels in all the participants.

FGF23 levels measured by CL-FGF23 in a logarithmic fashion and serum phosphate levels are plotted in Figure 3.

Grey circles, XLH without vitamin D deficiency; black circles, XLH with vitamin D deficiency; grey triangles, TIO without vitamin D deficiency; black triangles, TIO with vitamin D deficiency; asterisk, Fanconi syndrome due to adefovir dipivoxil, open rhombus, denosumab-induced hypophosphatemia; open square, vitamin D deficiency without FGF23-related hypophosphatemia.

The solid and broken lines indicate serum FGF23 levels of 30.0 pg/mL (current cut-off value) and 44.0 pg/mL, respectively.

Table

Table 1. Characteristics

Parameter		FGF23-related hypophosphatemia without vitamin D deficiency (disease group-1)		FGF23-related hypophosphatemia (reference)		Non-FGF23-related hypophosphatemia (disease group-2)		*P*[c]
	Reference interval[a]	n	mean ± SD	n	mean ± SD	n	mean ± SD	
Gender (male/female; n)		14	8/6	43	16/27	22	11/11	
Age (yr)		14	21 ± 24	43	32 ± 23	22	35 ± 33	0.25
Creatinine (mg/dL), serum	0.4～1.1	14	0.47 ± 0.26	42	0.49 ± 0.19	22	0.42 ± 0.26	0.45
Albumin (g/dL), serum	4.1～5.1	13	4.5 ± 0.4	40	4.4 ± 0.4	22	3.9 ± 0.6	<0.0001
Calcium (mg/dL), serum	8.7～10.3	14	9.4 ± 0.6	42	9.2 ± 0.7	22	8.6 ± 0.8	0.001
Phosphate (mg/dL), serum	Adult: 2.8~4.6	5	2.0 ± 0.5	27	2.1 ± 0.5	12	1.8 ± 0.5	0.32
	Child: 3.3~7.7	9	3.1 ± 0.2	16	3.0 ± 0.7	10	4.0 ± 1.3	0.27
Intact PTH (pg/mL), serum	10～65	11	53.5 ± 25.9	33	89.9 ± 105.8	13	146.5 ± 114.9	0.07
Alkaline phosphatase (U/L), serum (JSCC)	Adult:100~325	5	398 ± 258	27	579 ± 984	12	731 ± 1055	0.56
	Child: 120～1620	9	1418 ± 320	16	1287 ± 375	10	3636 ± 1859	0.0004
1,25(OH)$_2$D (pg/mL), serum	20.0～70.0	11	57.8 ± 25.7	24	63.7 ± 31.8	10	95.7 ± 63.6	0.066
25(OH)D (ng/mL), serum	≥20.0[b]	14	25.6 ± 3.6	43	18.7 ± 5.9	22	14.1 ± 4.8	<0.0001
Causes for FGF23-related hypophosphatemia		XLH (12), TIO (2)		XLH (38), TIO (5)		–		
Causes for hypophosphatemia		–		–		Vitamin D deficiency (18), Denosumab induced (3), Fanconi syndrome (1)		

a. The reference interval for each biochemical parameter is provided in the previous report [37,38].

b. Definition of vitamin D deficiency by a guidelines of the Japanese Society of Pediatric Endocrinology, the Japanese Endocrine Society and the Japanese Society of Bone and Mineral Metabolism **[36, 39, 40]**

c. *P* values are for comparison across all three groups obtained from the Kruskal-Wallis test, one-way ANOVA, as appropriate.

JSCC: Japan Society of Clinical Chemistry method

Table 2. Clinical performance of the FGF23 measurement			
	FGF23-related hypophosphatemia without vitamin D deficiency (Group-1)	Non-FGF23-related hypophosphatemia (Group-2)	Total
Less than the cutoff value of FGF23 (< 30 pg/mL)	0	18	18
Above the cutoff value of FGF23 (≥ 30 pg/mL)	14	4	18
Total	14	22	36

Table 3. Profiles of the patients in group-2 (FGF23 30 pg/mL or more)

#	FGF23 (pg/mL)	Age (yr)	Gender[a]	Pi (mg/dL)	Ca (mg/dL)	ALB (mg/dL)	ALP (U/L)	Intact PTH (pg/mL)	1,25(OH)$_2$D (pg/mL)	25(OH)D (ng/mL)	Cre (mg/dL)	Primary conditions	Accompanying diseases	Registered causes for hypophosphatemia
				2.8-4.6[b]	8.7-10.3[b]	4.1-5.1[b]	100-325[b]	10-65[b]	20-70.0[b]	≥20.0[c]	0.4-1.1[b]			
1	31.9	31	M	1.4	7.2	3.0	336	ND	ND	10.2	0.61	Chronic myelogenous leukemia	NA	Vitamin D deficiency
2	43.9	70	F	1.8	8.2	3.7	119	46.0	65.7	11.0	0.67	Adrenal insufficiency induced by steroid withdrawal	Hypertension, Osteoporosis	Vitamin D deficiency
3	71.6	71	F	1.4	8.9	3.5	2886	20.0	36.3	13.0	0.60	Small cell lung carcinoma	Hypertension, Atrial septal defect, Hyperuricemia	Vitamin D deficiency
4	469.4	68	M	0.8	7.6	3.4	3058	ND	ND	15.1	0.49	Multiple bone metastases from prostate small cell carcinoma	NA	Vitamin D deficiency

ND: Not determined, NA: Not applicable

a. M: men, F: women.

b. The reference interval for each biochemical parameter is provided in the previous report. [37,38]

c. Definition of vitamin D deficiency follows by a guidelines of the Japanese Society of Pediatric Endocrinology, the Japanese Endocrine Society and the Japanese Society of Bone and Mineral Metabolism [36, 39, 40]

Figure1

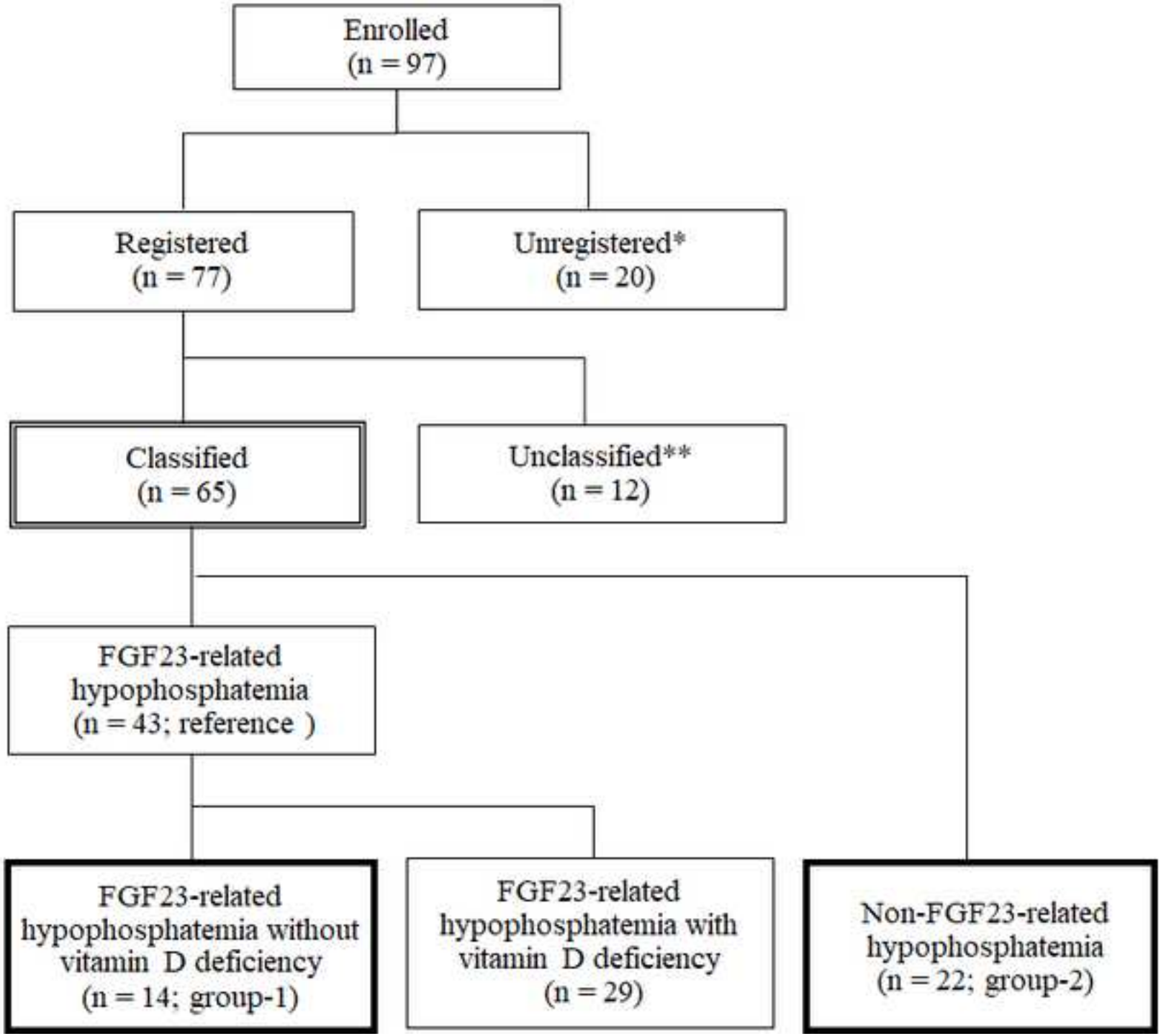

Figure2

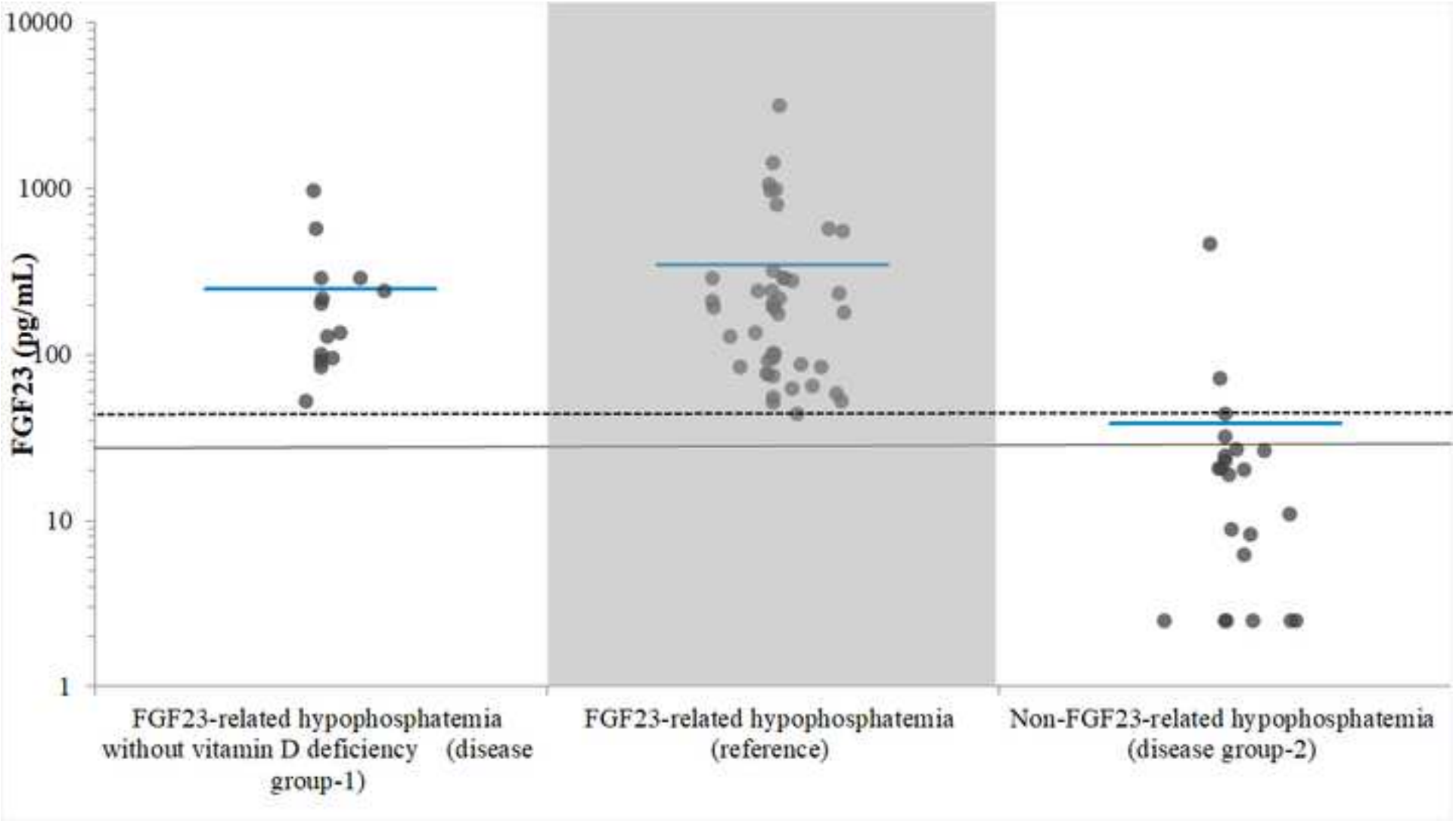

Figure3

Click here to access/download;Figure;CL-FGF23_Fig3_0515.png