Assessment of the measurement of canine and feline serum fibroblast growth factor-23 concentrations by automated chemiluminescence immunoassay

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ABSTRACT. This study compared canine and feline fibroblast growth factor (FGF)-23 concentration measurements between automated chemiluminescence assay (CLEIA) and enzyme-linked immunosorbent assay (ELISA). Seventy serum samples each from dogs and cats were evaluated. FGF-23 measurements by CLEIA significantly correlated with those of ELISA in both dogs and cats. The Bland–Altman test showed that FGF-23 between CLEIA and ELISA had fixed and proportional biases, respectively, in both dogs and cats. Measurements by CLEIA were lower than those of ELISA, especially in higher serum FGF-23 concentrations. This study showed that FGF-23 concentrations in dogs and cats can be evaluated by automated CLEIA. However, FGF-23 cannot be directly compared between CLEIA and ELISA.

KEY WORDS: biomarker, cat, dog, mineral metabolism

Fibroblast growth factor (FGF)-23 is a phosphaturic hormone secreted from osteocytes in response to increased blood phosphate and calcitriol levels [1]. FGF-23 binds to the klotho-FGF receptor complex and enhances the urinary excretion of phosphate via the downregulation of the sodium-phosphate cotransporter in the kidneys and the inhibition of calcitriol synthesis, resulting in decreased blood phosphate levels [2]. In humans, the involvement of FGF-23 in various conditions, such as the mineral metabolic disturbance in chronic kidney disease (CKD), autosomal dominant hypophosphatemic rickets, and tumor-induced osteomalacia, has been elucidated [1, 2, 6]. In dogs and cats, blood FGF-23 levels are high in patients with CKD [4, 8, 9]. The reduced glomerular filtration rate in CKD induces phosphate accumulation in the body; thus, to compensate, blood FGF-23 concentrations become elevated. Elevated blood FGF-23 levels appear to occur earlier than hyperphosphatemia [4, 9] and are associated with a shorter survival time in dogs and cats with CKD [3, 10]. In addition, our previous study showed that increased serum FGF-23 concentrations were associated with the risk for the development of hyperphosphatemia and CKD progression in normophosphatemic dogs with CKD [7]. Thus, FGF-23 has been noted as an early indicator of mineral metabolic disorders in CKD and may be useful as a prognostic marker and therapeutic target in dogs and cats with CKD in clinical practice. To date, studies in dogs and cats used enzyme-linked immunosorbent assay (ELISA) to measure blood FGF-23 concentrations [3, 4, 7–10]. However, a higher performance assay may be needed in the clinical setting since measuring FGF-23 using ELISA is time-consuming. Therefore, the present study investigated the performance of an automated chemiluminescence assay (CLEIA) in measuring canine and feline serum FGF-23 concentrations and compared with that of ELISA.

This study used canine and feline serum samples requested for biochemical analysis from external veterinary hospitals and submitted them to FUJIFILM VET Systems Co., Ltd. (Tokyo, Japan). All samples were used with agreement from veterinary hospitals according to the institution's guidance. Serum samples were stored at −20°C.

Measurement of serum FGF-23 concentrations by CLEIA were performed on a fully automated chemiluminescence immunoanalyzer (CL-JACK System, Minaris Medical Co., Ltd., Tokyo, Japan) [11]. This assay is used in a current laboratory service for the measurement of canine and feline serum FGF-23 concentration at FUJIFILM VET Systems Co., Ltd. The assay uses the same antihuman intact FGF-23 mouse monoclonal antibodies (Determiner CL FGF23) as ELISA, as described in our previous studies [8, 9]. Statistical analysis was performed using the SPSS commercial software package (SPSS 24 for Windows, IBM Japan, Ltd., Tokyo, Japan). Normality tests were performed using the Shapiro–Wilk test. Normal distribution data were represented by

mean ± standard deviation (SD). Non-parametric data were represented using the median (inter-quartile rage).

Intra-assay and interassay precision were indicated using the coefficient of variation (CV), which was calculated by dividing the SD by the mean. Intra-assay precision was determined by sequentially measuring FGF-23 concentrations of three different sample concentrations (low, moderate, and high concentrations) and performed five times for each sample within a single assay run. The intra-assay coefficient of variations for canine serum samples with FGF-23 concentrations of 88, 1,093, and 39,621 pg/ml were 1.6, 3.4, and 2.0, respectively. The intra-assay CVs for feline serum samples with FGF-23 concentrations of 337, 939, and 21,746 pg/ml were 2.0, 1.8, and 1.8, respectively.

Inter-assay precision was determined by measuring FGF-23 from three different sample concentrations (low, moderate, and high concentrations) in the same sample once a day for five consecutive working days. The inter-assay CVs for canine serum samples with FGF-23 concentrations of 92, 1,095, and 40,325 pg/ml were 6.0, 7.0, and 5.1, respectively. The inter-assay CVs for feline serum samples with FGF-23 concentrations of 339, 955, and 22,044 pg/ml were 2.9, 3.3, and 4.4, respectively.

A canine serum sample with an FGF-23 concentration of 47,310 pg/ml and a feline serum sample with an FGF-23 concentration of 22,546 pg/ml were used in the dilutional linearity analysis. The mean ± SD of the measured/predicted values were 101.2% ± 4.1% in the canine sample and 105.4% ± 5.0% in the feline sample (Fig. 1).

The stability of serum FGF-23 concentrations in dogs (317 pg/ml) and cats (298 pg/ml) were evaluated by storing three samples for each at room temperature, 4°C, −20°C, and −80°C. The FGF-23 levels at baseline were compared with those obtained after 1, 3, and 7 days. Serum samples stored at −20°C and −80°C were compared with measurements after 29 days. The results for storage stability are shown in Table 1.

The 70 serum samples each from dogs and cats were used to compare between measurements by CLEIA and ELISA. The Sandwich ELISA Kit (MedFrontier FGF23, Minaris Medical Co., Ltd.) was same assay as previous studies [8, 9]. The Spearman rank correlation method showed that serum FGF-23 concentrations by CLEIA significantly correlated with those of ELISA, and the correlation coefficients were 0.995 in dogs and 0.986 in cats (both $P<0.001$) (Fig. 2). The Bland–Altman plot of the FGF-23 measurements between CLEIA and ELISA in dogs and cats are shown in Fig. 3 [5]. Fixed bias was indicated when the 95% confidence interval of the difference between actual and estimated values based on a one-sample *t*-test did not include 0. Proportional bias was indicated when the 95% confidence interval for the slope of the least square regression of differences in means did not include 0. In both dogs and cats, FGF-23 measurements by CLEIA had fixed and proportional biases, respectively. The limit of agreement (LOA) was calculated using the follow equation:

LOA=the mean difference ± 1.96 × SD of the difference.

The LOAs of serum FGF-23 concentrations by CLEIA ranged from −2,001 to 1,097 pg/ml in dogs and from −1,471 to 852 pg/ml in cats (Fig. 3).
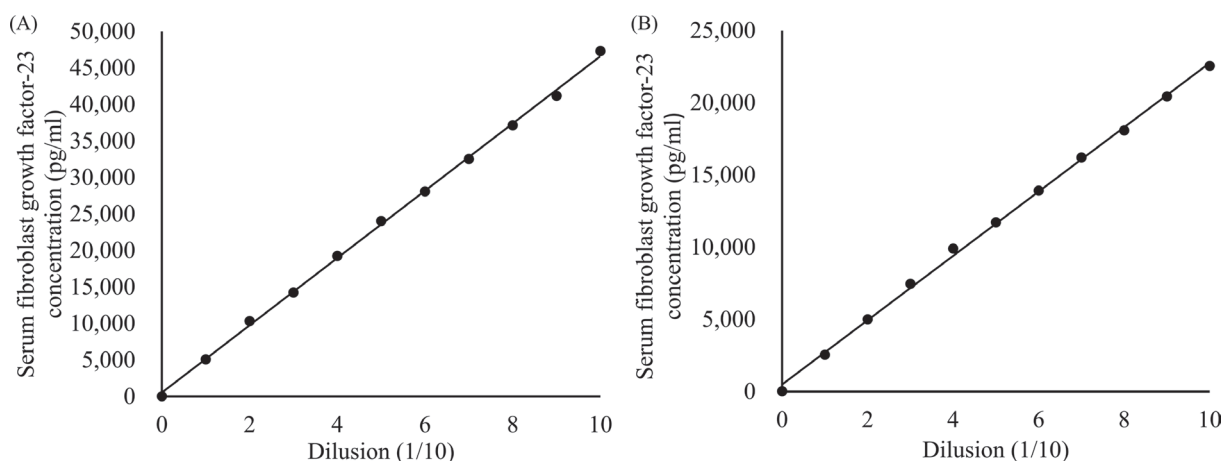
Fig. 1. Dilution linearity in canine serum fibroblast growth factor-23 concentration of 47,310 pg/ml (**A**) and feline serum fibroblast growth factor-23 concentration of 22,546 pg/ml (**B**) measured by automated chemiluminescence immunoassay.

Table 1. Storage stability of serum fibroblast growth factor (FGF)-23 concentrations using chemiluminescence assay in dogs and cats

| Storage period (day)	Canine FGF-23 (pg/ml) recovery percentage				Feline FGF-23 (pg/ml) recovery percentage			
	Room temperature	4°C	−20°C	−80°C	Room temperature	4°C	−20°C	−80°C
1	166 (52%)	263 (82%)	355 (112%)	315 (99%)	241 (81%)	284 (95%)	282 (94%)	300 (101%)
3	135 (43%)	248 (78%)	329 (104%)	322 (102%)	182 (61%)	288 (97%)	290 (97%)	303 (102%)
7	104 (33%)	210 (66%)	339 (107%)	335 (106%)	83 (28%)	278 (93%)	299 (100%)	305 (102%)
28	No data	No data	302 (95%)	302 (95%)	No data	No data	270 (90%)	263 (88%)

The storage stability was represented by recovery percentage, dividing the measurement by the baseline value.

The intra- and interassay CVs of serum FGF-23 concentrations measured by CLEIA were lower than those measured by ELISA in our previous studies [8, 9]. These results are likely because human-induced bias in the automated analyzer assessment is smaller than that in the manual method. Recovery percentages of canine FGF-23 at room temperature and 4°C were lower than those of feline samples. This finding was consistent with our previous studies using ELISA [8, 9]. Our study could not determine the reason why storage stability differed between serum FGF-23 concentration in dogs and cats. Although furin is thought to be involved in the degradation of intact FGF-23, the protease degrading intact FGF-23 in the serum sample is unknown [12]. Therefore, the difference of storage stability between canine and feline FGF-23 observed in the present study might result from species difference of the protease in serum sample. Serum FGF-23 concentrations in both dogs and cats between CLEIA and ELISA showed significantly strong correlations. Therefore, CLEIA using an automated analyzer can effectively evaluate canine and feline serum FGF-23 concentrations. However, the Bland–Altman analysis showed the presence of fixed and proportional biases in the measurements by CLEIA in dogs and cats. In addition, the measurements by CLEIA were lower than those of ELISA, especially in serum samples of higher serum FGF-23 concentrations. These findings are consistent with a study in humans that compared FGF-23 between CLEIA and ELISA [11]. Thus, serum FGF-23 concentration measurements between CLEIA and ELISA cannot be directly compared. Since serum FGF-23 concentrations in dogs and cats with CKD, especially end-stage kidney disease, increases from 100 to 1,000-fold in comparison to those of healthy animals [4, 9], a greater discrepancy between CLEIA and ELISA can occur in dogs and cats with CKD. Therefore, although plasma FGF-23 concentration >3,000 pg/ml was associated with a poor prognosis in cats with CKD in a previous study [3], this cut-off value cannot be extrapolated to FGF-23 using CLEIA. Future study to determine cut-offs of FGF-23 by CLEIA associated with survival time and the CKD progression in dogs and cats with CKD is needed.

In conclusion, since serum FGF-23 concentrations by CLEIA were significantly correlated with the measurements by ELISA, serum FGF-23 concentrations in dogs and cats can be sufficiently evaluated by CLEIA using an automated analyzer. However, especially in higher serum FGF-23 levels, CLEIA can underestimate measurements in comparison with ELISA. Therefore, serum FGF-23 concentrations between CLEIA and ELISA cannot be directly compared, especially in dogs and cats with CKD.
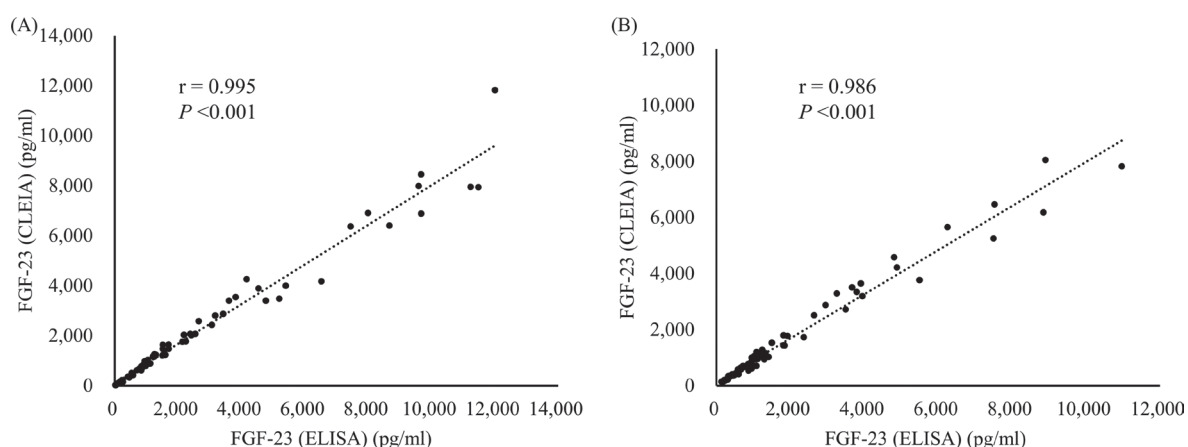
Fig. 2. Scatter plot of serum fibroblast growth factor-23 concentrations with sandwich enzyme-linked immunosorbent assay (ELISA) and automated chemiluminescence immunoassay (CLEIA) in dogs (**A**) and cats (**B**). Dashed lines represent a regression line; r: correlation coefficient.
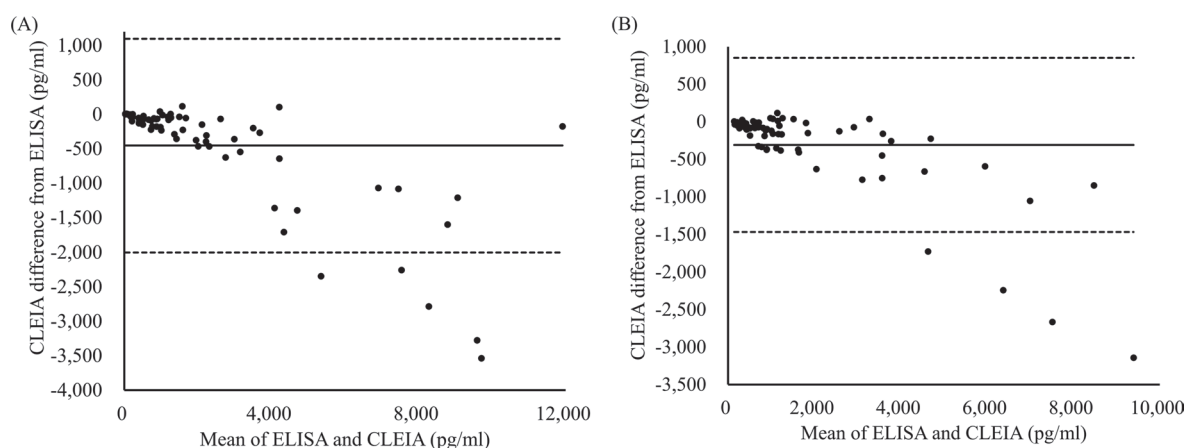
Fig. 3. Bland–Altman plot of serum fibroblast growth factor-23 concentrations with sandwich enzyme-linked immunosorbent assay (ELISA) and automated chemiluminescence immunoassay (CLEIA) in dogs (**A**) and cats (**B**). Dashed lines show limit of agreement for difference.

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