FGF23 metabolism, a new paradigm for chronic kidney disease

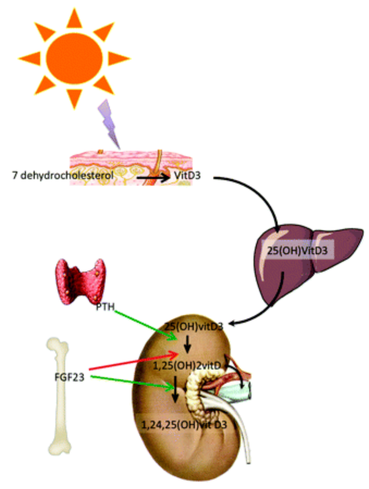
Isabelle Piec, Holly Nicholls, Jonathan C.Y. Tang, Christopher J. Washbourne and William D. Fraser

BioAnalytical Facility, Biomedical Research Centre, Norwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich Research Park, Norwich, Norfolk, UK

Corresponding author: i.piec@uea.ac.uk

Introduction

Fibroblast growth factor-23 (FGF23) is a major regulator of phosphate and vitamin D metabolism often elevated in genetic hypophosphataemic disorders and in chronic kidney disease. In the kidney, FGF23 induces urinary phosphate excretion and reduces synthesis of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) by down regulating 1α-hydroxylase and upregulating 24-hydroxylase activity. We[1] and others[2,3] showed a relationship between FGF23 and various markers of iron status including ferritin, low serum iron being associated with elevated c-terminal (cFGF23) but not intact (iFGF23) FGF23 suggesting a role of iron status in the metabolism of FGF23. Moreover in dyalised patients with chronic kidney disease (CDK), iron administration lowers iFGF23 levels[4].



from Prié and Friedlander CJASN 2010[5]

Objectives:

- Compare the new Biomedica with the routinely used Immunotopics assay for cFGF23.
- Compare concentrations of iFGF23 with cFGF23 in patients with elevated cFGF23.
- Determine ferritin status in patients with elevated cFGF23.

Methods

❖ Samples

Samples with a range of concentrations of cFGF23 (15-6830 RU/mL) were collected in the course of routine care. Samples were anonymised to the researchers at point of access in accordance with generic ethical approval.

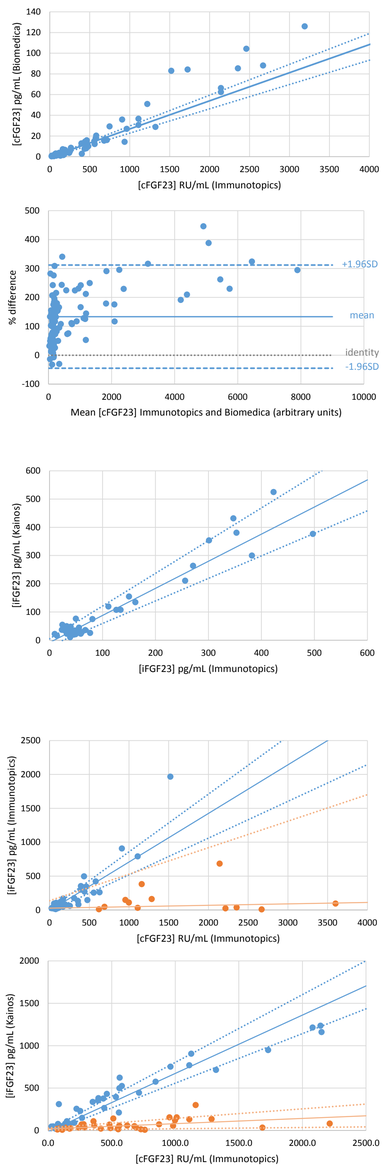
❖ Assays

- c-terminal FGF23 (cat# 60-6100) and intact FGF23 (cat# 60-6600) were both two-site enzyme-linked immunosorbent assay (ELISA) 2nd generation from Immunotopics Inc., CA
- c-terminal FGF23 (cat# BI-20702) was a sandwich enzyme immunoassay from Biomedica.
- intact FGF23 (cat# TCY4000E) was a two-site ELISA from Kainos.
- ferritin (cat# 03737551), urea (cat# 04460715) and creatinine (cat# 04810716) were immunoassay or kinetic colorimetric assays by Roche Diagnostics (Burgess Hill, UK) measured on a COBAS 6000
- 25 hydroxyvitamin D ($25(OH)D_2$ and $25(OH)D_3$) and it's metabolite 24,25 dihydroxyvitamin D_3 ($24,25(OH)_2D_3$) were measured by LC-MS/MS (see poster LB-MO0026 for methodology).

❖ Statistics

- Assay results were compared using Passing Bablock and Bland-Altman analyses.
- Concentrations were compared using one-way ANOVA. Trends were estimated using linear regression analysis
- SPSS for windows version 22.0.0.1 was used and results were considered statistically significant for $p<0.05$ [*$p<0.05$; ** $p<0.01$; ***$p<0.001$].

FGF23 assay comparisons



Passing-Bablock between the different cFGF23 and iFGF23 assays
Line represents the best fit; dotted lines represent 95% confidence interval

❖ The assay currently in routine use to assess FGF23 status is the cFGF23 assay from Immunotopics. We compared results from a newly available kit from Biomedica which showed good correlation ($n = 125$ $r = 0.966$ $p<0.01$) however, a bias became apparent in the highest range of FGF23 as shown on the Bland-Altman plot.

❖ The Intact assay for FGF23 is not in routine use but requests are increasing. We tested kits from Kainos and Immunotopics. Both report values in pg/mL and the results correlated ($n = 48$ $r = 0.963$ $p<0.01$).

❖ Two populations could be defined in which

Group 1) iFGF-23 concentrations correlated with concentrations of cFGF23 (Immunotopics) for both the Immunotopics ($n=43$ $r=0.959$ $p<0.01$) and Kainos kit ($n=43$ $r=0.959$ $p<0.01$) and

Group 2) cFGF23 concentrations were elevated without an elevation of iFGF23.

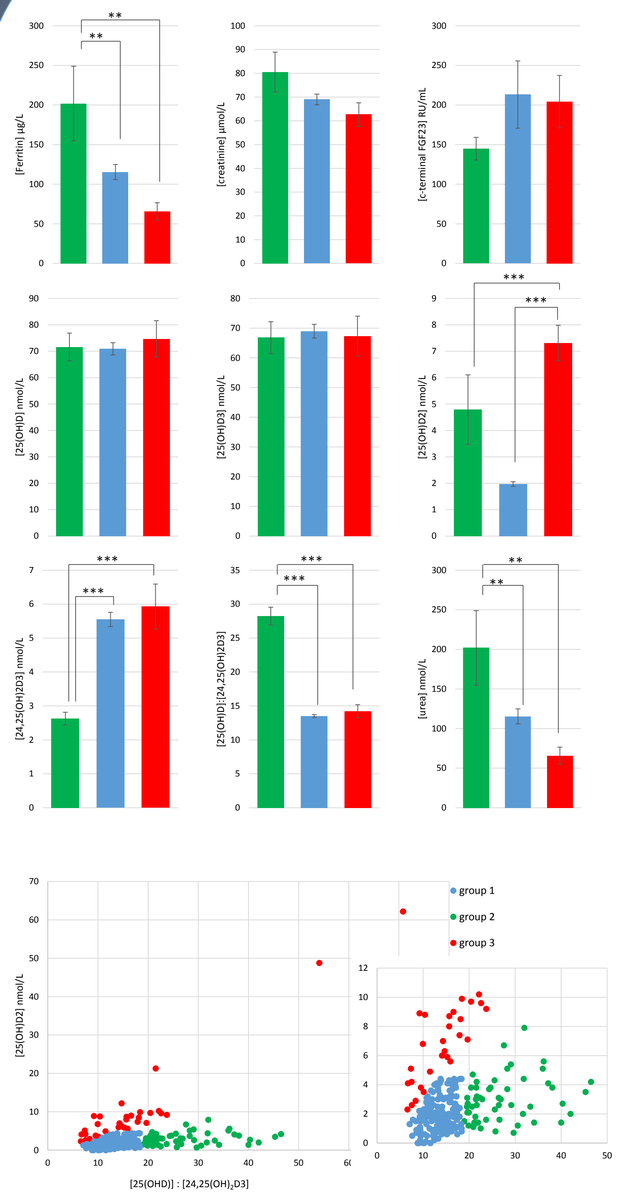
❖ [Ferritin] between the two population were

Group 1) [ferritin] = 96 ± 18 µg/L

Group 2) [ferritin] = 63 ± 15 µg/L

Difference was not significant.

Vitamin D and FGF23



Graph showing the concentration of Vitamin D2 depending of the ratio metabolite to total vitamin D

❖ 3 groups of patients could be isolated depending on their ferritin status

- Group 1: [ferritin] = 202 ± 47 µg/mL
- Group 2: [ferritin] = 115 ± 9 µg/mL
- Group 3: [ferritin] = 66 ± 11 µg/mL

❖ The lower the ferritin, the lower the creatinine and the urea (and [urea]:[creatinine] ratio).

❖ Group 2 and 3 had higher [cFGF23], although not significant.

❖ In the circulation, vitamin D is present in two forms; predominantly D_3 and to a lesser extent D_2. Overall, no significant difference in 25(OH)D (total or D_3) were observed.

❖ However, while group 2 had a significantly lower $25(OH)D_2$; group 3 had a significantly higher $25(OH)D_2$.

❖ The concentration of $[24,25(OH)_2D_3]$ metabolites was significantly higher in both group 2 and 3 than group 1.

❖ The ratio $[25(OH)D]:[24,25(OH)_2D_3]$ was significantly lower in both group 2 and 3 than group 1.

❖ These populations could be discriminated by plotting $[25(OH)D_2]$ against $[25(OH)D]:[24,25(OH)_2D_3]$ and linear regression analysis showed group 3 was also the only linear population with r^2 of 0.918 ($p<0.001$).

Conclusions

❖ Both Biomedica and Immunotopics assays measure cFGF23 and iFGF23. Biomedica allows calculation of cFGF23 in pg/mL (immunotopics is RU/mL), allowing deduction of actual c-FGF23 concentrations.

❖ Intact FGF23 correlated to c-terminal FGF23 in most cases. However a subpopulation of patients presented with high FGF23 c-terminal without elevation of intact FGF23. This suggest that these patients metabolise FGF23 differently and are not able to clear FGF23 fragments which accumulate over time.

❖ We observed in patients with relatively low ferritin higher levels of cFGF23 which is in accordance with previous studies in FGF23 related diseases. Iron deficiency has been shown to be associated with stimulation of FGF23 transcription and increased concentrations of cFGF23 and iFGF23 in patients with dominant hypophosphatemic rickets while iFGF23 are maintained in controls[2].

❖ Patients with high ferritin/creatinine showed decreased production of vitamin D metabolites which is in accordance with a decrease in hydroxylase activity and lower 1,25(OH)D upon renal failure.

❖ Iron and FGF23 may act in concert to regulate vitamin D status, more studies are needed to make conclusions on the diagnostic value of these results. Iron and/vitamin D supplementation might decrease or increase the observed effects.

References:

1-Durham BH, Joseph F, Bailey LM and Fraser WD. The Association for Clinical Biochemistry; 2007(44):pp463-466. **2**-Imel EA, Peacock M, Grsay AK, Padgett LR, Hui SL and Econs MJ. The journal of Clinical Endocrinology and Metabolism; 2011(96):pp3541-3549. **3**-Imel EA, Gray AK, Padgett LR, Econs MJ. Bone; 2014(60):pp87-92. **4**- Deger SM, Erten Y, Pasaoglu OT, Derici UB, Reis KA, Onec K and Pasaoglu H. Clinical Experimental Nephrology; 2013 (14):pp416-423. 5- Prié D and Friedlander G, Clinical Journal of the American Society of Nephrology; 2010(5):pp1717-1722.