IRB Proposal

Title: FGF-23 in Acute Kidney Injury

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## BACKGROUND AND RATIONALE

Renal failure is almost universally accompanied by changes in mineral metabolism (Hruska et al, 1995). Patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD) have high rates of vitamin D deficiency, which contributes to their morbidity and mortality (Wolf et al, 2007). Fibroblast growth factor 23 (FGF-23), a hormone released by osteocytes, plays an important role in phosphorus and vitamin D homeostasis and increased circulating levels are independently associated with mortality in patients with ESRD (Gutierrez et al, 2008).

Similar to CKD and ESRD, acute kidney injury (AKI) is also associated with deficiency of activated vitamin D ($1,25(OH)_2D_3$) in both animal (Gerber et al, 2003) and human studies (Druml et al, 1998; Saha et al, 1993), however, the mechanism is unclear and FGF-23 has not been studied in AKI. Since FGF-23 directly inhibits 1α-vitamin $D_3$ hydroxylase (Gutierrez et al, 2005), a crucial step in $1,25(OH)_2D_3$ synthesis, we hypothesized that elevated levels of FGF-23 might contribute to reduced $1,25(OH)_2D_3$ levels in AKI. To this effect, we recently documented markedly elevated FGF-23 in a single patient with rhabdomyolysis-induced AKI (data not yet published). However, it is not clear whether rhabdomyolysis alone, AKI alone, or the combination of the two were responsible for the elevated levels of FGF-23 observed.

## HYPOTHESES

1. AKI is associated with low $1,25(OH)_2D_3$, irrespective of etiology.
2. AKI is associated with elevated FGF-23, irrespective of etiology.
3. Rhabdomyolysis-induced AKI is associated with significantly higher FGF-23 levels and lower $1,25(OH)_2D_3$ levels than other etiologies of AKI.
4. FGF-23 levels are inversely associated with $1,25(OH)_2D_3$ levels in AKI.
5. Increased FGF-23 levels are directly associated with poorer clinical outcomes in AKI.

## STUDY DESIGN

A case-control study of inpatients with AKI at New York Presbyterian Hospital (NYPH)-Columbia University Medical Center will be performed. We will identify cases of rhabdomyolysis-induced AKI, with rhabdomyolysis being defined as plasma creatinine phosphokinase (CPK) greater than 10,000 IU/L together with a clinical diagnosis consistent with this syndrome (Chang et al, 2004). AKI will be defined in accordance with criteria established by the Acute Kidney Injury Network (Mehta et al, 2007): an abrupt (within 48 hours) absolute increase in serum creatinine ≥ 0.3 mg/dL, a percentage increase in serum creatinine ≥ 50% (1.5-fold from baseline), or documented oliguria ≤0.5 mL/kg/hr for >6 hours. Additional criteria for AKI will include the exclusion of prerenal azotemia, defined as resolution of kidney function within 3 days with the administration of intravenous volume repletion or discontinuation of diuretics, and, if oliguria is used as the sole diagnostic criteria, the exclusion of urinary tract obstruction as the etiology of AKI (Mehta et al, 2007). Two control groups will be used: patients with AKI originating from an etiology other than rhabdomyolysis, and patients with rhabdomyolysis without AKI.

All patients enrolled will have the following laboratory tests measured by venipuncture at entry into the study, and again one week later: plasma concentrations of calcium, albumin, phosphorus, 25-OH Vitamin D, 1,25-OH Vitamin D, intact parathyroid hormone (iPTH), CPK, iron, total iron binding capacity (TIBC), ferritin, and FGF-23.

Additional data to be collected for each patient include the following baseline characteristics and clinical outcomes: age, gender, ethnicity, medical co-morbidities, medications (at home and in the hospital), etiology of AKI, percent change in serum creatinine, dialysis requirement, renal recovery, AKI-free days adjusted for dialysis and death, length of hospital stay, and mortality.

## STATISTICAL ANALYSES

We will compare routine laboratory measurements (calcium, phosphorus, etc) and non-routine laboratory measurements (FGF-23) of both cases and controls with established normal ranges. We will compare mean values between cases and controls in the following manner: unpaired Student's t-test for normally distributed continuous data, Mann-Witney U test for skewed continuous data, and Fisher's exact or the chi-square test for binary variables. Additionally, we will use a logistic regression model, adjusting for potential confounders, to evaluate the relationship between FGF-23 and clinical outcomes. All comparisons will be two-tailed, with $p<0.05$ considered significant.

Power calculation: assuming at least eight patients per year will be admitted to NYPH with rhabdomyolysis-induced AKI (estimated from data warehouse search in Webcis), we intend to enroll 8 such patients, and an additional 16 patients with AKI originating from an etiology other than rhabdomyolysis, for a 1:2 ratio. These sample sizes will provide 80% power to detect a difference in effect size equal to 1.3 times the combined standard deviation. An additional 8 patients with rhabdomyolysis without AKI will serve

as a secondary control group, and will provide 80% power to detect a difference in effect size equal to 1.3 times the combined standard deviation.

**STUDY PROCEDURE**

Patients who agree to participate will undergo venipuncture at the beginning of the study and again seven days later. Whenever possible, timing of venipuncture will be coordinated to coincide with routine daily blood draws to avoid an extra stick. The total amount of extra blood drawn for research purposes will not exceed 6mL per patient at each of the two scheduled draws.

The likely duration of the entire study is one year. The likely duration of the study for any given patient is 1 to 3 weeks (depending on time to renal recovery and hospital length of stay).

**STUDY DRUGS OR DEVICES**

Not applicable

**STUDY QUESTIONNAIRES**

Not applicable

**STUDY SUBJECTS**

| **Inclusion Criteria** | **Exclusion criteria** |
|---|---|
| 1. Age≥18 and ≤70 | 1. Therapy with activated Vitamin D prior to initial measurement of FGF-23 |
| 2. Capacity to give informed consent | 2. history of parathyroid disease, metabolic bone disease, fat malabsorption, or duodenal resection |
| 3. Baseline eGFR>60 mL/min/1.73m$^2$ (MDRD), assessed within the preceding 3months | |

Women and minorities will be included in the study. They will be enrolled randomly, along with all other participants, to accomplish a set of study subjects that is representative of inpatients at NYPH with rhabdomyolysis and non-rhabdomyolysis-induced AKI.

**RECRUITMENT OF SUBJECTS**
NYPH inpatients who are admitted with or who subsequently develop rhabdomyolysis, AKI, or both, will be identified by internal medicine housestaff and/or nephrology fellows, and will be reported to study investigators by means of a 24 hour/day beeper. After confirmation that the patient meets inclusion and exclusion criteria, study investigators will confirm with the patient's primary physician that the patient is suitable for the study and that the patient is willing to discuss the study with the research team. Study investigators will then approach patients, explain the rationale of the study, answer any questions, and ask for informed consent to participate.

**CONFIDENTIALITY OF STUDY DATA**
Data will be de-identified and collected in a password-protected database. Only the study investigators will have access to the database.

**POTENTIAL CONFLICT OF INTEREST**
None

**LOCATION OF THE STUDY**
NYPH-Columbia University Medical Center, Milstein Building

**POTENTIAL RISKS**
Minimal. There is a small possibility that a patient would receive an extra venipuncture.

**POTENTIAL BENEFITS**
Subjects may not benefit directly as a result of participation in the study. The potential benefits to society include elucidating the mechanisms responsible for vitamin D deficiency in AKI, as well as establishing whether FGF-23 is an important biomarker of clinical outcomes in AKI.

**ALTERNATIVE THERAPIES**
Not applicable

**COMPENSATION TO SUBJECTS**
None

**COSTS TO SUBJECTS**
None

**MINORS AS RESEARCH SUBJECTS**
Not applicable

**RADIATION OR RADIOACTIVE SUBSTANCES**
Not applicable

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