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FGF23 and cardiovascular damage in anemia with an without chronic kidney disease.

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GENERAL INFORMATION

A. Identifiers:

- Protocol ID PI20/1645

- Title: **FGF23 and cardiovascular damage in anemia with an without chronic kidney disease.**

B. Treatment group description:

Group 1: Patients with iron deficiency anemia with chronic kidney disease, stages 3, 4 and 5 non on dialysis, treated with oral or intravenous iron for 3 months

Group 2: Patients with iron deficiency anemia with normal renal function, treated with oral or intravenous iron for 3 months

C. Sponsor:

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PROTOCOL

1. Objetives:

The General Objective of this research project is to evaluate, through in vivo and in vitro studies, the cardiovascular alterations related to the anemia-induced increase in FGF23production; as well as the identification of possible molecular targets that may be useful in its prevention and / or palliation. This General Objective will be addressed through the following Specific Objectives:

1.- To determine in a population with anemia (due to iron deficiency), with and without chronic kidney disease (CKD), an association between the parameters related to iron metabolism, FGF23 and markers of cardiovascular damage. It will be evaluated:

1.1 The levels of FGF23, intact and C-terminal, before and after treatment.
1.2 The changes in parameters related to mineral metabolism (calcium, phosphorus, magnesium, PTH, vitamin D, soluble Klotho), inflammation and oxidative stress (IL-12, IL-1b, IL-6).
1.3 Markers of cardiovascular damage: BNP, myoglobin, troponin I.
1.4 Iron metabolism parameters (ferritin, transferrin saturation index, hepcidin)
1.5 MicroRNAs and proteomics expression profile.
• Likewise, although it will not use experimental medication, the safety (side effects) of the iron compounds that are administered will be evaluated.

2- To evaluate in vivo, in a murine experimental model of anemia, with or without CKD, the effects of the modulation (inhibition) of triggers of iron deficiency (hepcidin) and of the increase in FGF23 (HF1α), on markers of cardiovascular damage. It will be evaluated:

2.1. Production of intact FGF23 and C-Terminal FGF23.
2.2. Cardiovascular disease markers.
2.3. Proteomic profile and differential localization of proteins in situ in cardiac tissue.

3.- To compare in vivo, in an experimental model of anemia with and without CKD, the effect of different I.V. iron presentations(ferrous sulphate, ferric carboxymaltose and ferric citrate) on FGF23 levels and their cardiovascular impact.

4.-To evaluate in vitro, in cardiomyocytes cultures, in the presence of iron deficiency, the direct effect of FGF23 on the induction of cardiac damage. It will be analyzed:

4.1. Gene and protein expression of markers of cardiac damage (myoglobin, troponin and BNP) and hypertrophy.
4.2. The involvement of the PI3K-AKT / GSK3-B-NFAT and FGFR4 / Raf / MEK / ERK signalling pathways.
4.3. Induction of oxidative stress

5.- To evaluate in vitro, in osteoblasts/osteocytes cultures, the direct effect of ferrous sulphate, ferric carboximaltose, ferric citrate and hepcidin on:

5.1. Production of intact FGF23 and C-terminal FGF23.
5.2. Gene and protein expression of intracellular mediators of FGF23 production: DMP1, GALNT3, Phex, FGFR1 and HF1α.

2. Desing:

2.1: Type: Study in patients: prospective, open, parallel groups.

2.2 Sample size: It is considered that a random sample of 183 individuals for the population of patients with iron deficiency anemia without CKD and 218 for the study to be carried out with patients with anemia and CKD, will be enough to estimate, with a 95% confidence and an accuracy of +/- 5 percentage units, a population percentage that is expected to be around 13% and 15%, respectively. As a percentage of necessary replacements, it is expected to be 5% and 10%, respectively.

3. Study population:

Selection criteria:

This study will include patients who meet all the inclusion criteria and anyone of the exclusion criteria.

Inclusion Criteria:

- Age. 18-85 years old
- Hemoglobin < 11g/dl
- Serum ferritin < 100 ng/ml or transferrin saturation index < 20%
- Written informed consent

Exclusion Criteria:

- Weight < 50 Kg or BMI < 17
- Acute bleeding > 300 ml, within 72 hours before study inclusion
- Proliferative hematologic disease. Hemochromatosis
- Active infections within 30 days before study inclusion
- Systemic inflammatory illness
- HIV, HCV or HBV infection
- Iron active treatment
- Blood transfusion in the last 90 days before inclusion.
- Cardiovascular hospitalization 30 days before study inclusion
- Anticoagulant treatment with coumarinics
- Chronic liver disease
- Immunosupresive therapy
- Erythropoiesis stimulating agents treatment, radiotherapy or quimiotherapy within 30 days before inclusion
- Scheduled major surgery during study period
- Pregnancy or lactation
- Drugs addiction
- Participation in others clinical trials.

Withdrawal criteria:

1. Informed consent withdrawal.
2. Iron tratment intolerance.
3. Medical decision, based on clinical state.

4. Deviation from the protocol, such as intolerance to the treatment or the appearance of new symptoms or pathologies that discourage its continuity.
5. Loss of follow up.

4. Treatment:

Any oral or intravenous iron formulation, prescribed by the responsable clinician. The investigators will not take part in treatment selection.

5. Study procedures:

The total planned duration of the research project for each patient is 3 months. The outpatients of the nephrology, internal medicine and hematology with the possibility of meeting the requirements will be referred to one of the investigators of the research project who will carry out the following visits and procedures:

V0: After the explanation of the research project and the signing of the informed consent in the first screening visit (V0), the patient's history will be evaluated, anthropometric measurements will be taken, and the existing biochemical data will be studied.

V1: One to two weeks after V0, the patient will be summoned to proceed to take blood samples (one hematimetry tube and 2 biochemistry tubes), collection of the morning urine sample, measurement of hemodynamic parameters, from this moment the patients would begin with the oral or intravenous iron therapy indicated by their responsible doctor.

V2: At month 3 (M3) from the start of the study, blood samples (hematimetry and biochemistry), urine and hemodynamic parameters will be taken. Finishing the study.

Patients who present resistance to oral treatment, maintaining Hb <11 and ferritin <100 ng / ml or TSI <20%, may be transferred to the treatment group with IV iron if so decided by their responsible physicians, V3 will then be programmed.

V3: At month 6 (M6) from the beginning of the study, blood samples (hematimetry and biochemistry), urine and hemodynamic parameters will be taken. Finishing the study.

6. Statistic analysis

Data that follow a Normal distribution will be described as mean ± standard deviation and non-Normal data will be described as median with interquartile range. The primary end point will be compared between the two treatment groups using an unpaired Student's t-test (possibly after log transformation if the data does not follow a Normal distribution) and as an intention to treat. Changes and differences between groups in secondary end points will be analyzed using paired and unpaired Student's t tests or Wilcoxon and Mann-Whitney tests for continuous variables, and Chi-square or Fisher's exact test for dichotomous variables. Linear mixed models will be used to analyze variables with repeated measures. Linear and logistic regression analyzes with adjustment for various covariates will be applied to the baseline data to investigate any association with the change in various parameters, as well as any association with clinical events.

7. Workplan

The total planned duration of the research project is 1 YEAR, from the inclusion of the first patient:

7.1- Start of the study: After obtaining the favorable opinion of the CEI, it is expected to include the first patient in the first quarter of 2022.
7.2- Period of recruitment / inclusion of patients: 6 months (between January 2022 and June 2022).
7.3- Treatment period: each patient included will receive treatment for 3 months (January 2022-January 2023).
7.4- Completion of the study: last contact of the last patient included.
7.5- Statistical analysis and writing of the final report.