Study protocol

1. Introduction

Metabolic acidosis is one of the common complications of chronic kidney disease (CKD) and is associated with cardiovascular events, endothelial dysfunction, bone mineral disorders, malnutrition-inflammation, and insulin resistance (Copur et. Al, 2020). It is also associated with CKD progression and recent data suggest hydrogen ions retention exists and leads to progressively kidney dysfunction even in patients in early stages of CKD (2-3a) with normal serum bicarbonate (extended acidosis concept) (Raphael, 2019; Maria M. Adeva-Andany, 2014; Madias 2021). As kidney function declines, although the remaining nephrons enhance their ammonia production, hydrogen ions are retained, and one study reported that hydrogen ions retention was paralleled by a reduction in urinary citrate excretion in CKD, at least when eGFR is higher than 45 mL/min (Goraya, Simoni et al. 2019).

The resulting interstitial acidosis activates complement leading to interstitial inflammation and fibrosis. Additionally, acidosis increases different mediators - endothelin 1, aldosterone, angiotensin II – levels, further enhancing renal fibrosis (Bushinsky, 2018).

In the last two decades, animal and human studies showed that correction of acidosis slows down CKD progression. In a murine model of subtotally nephrectomized rats, calcium citrate decreased glomerular and interstitial fibrosis (Gadola et. al., 2004). Prospective, randomized short-term (4 to 7 day) or long-term (6 to 32 months) trials, including patients in CKD stages as early as stage 2, demonstrated that alkali supplementation with sodium bicarbonate or citrate on top of dietary manipulation (low animal protein or enriched in fruits and vegetable diets) significantly improvement serum bicarbonate and reduced the rate of decline in GFR (Goraya, 2019; Bushinsky, 2018; Susantitaphong et.al, 2012, Raphael, 2016). Studies also indicated that alkali therapy was well tolerated, with few to no adverse events (hypokalemia in 2 long-term studies) (Susantitaphong et.al., 2012).

Current clinical practical guidelines recommend correcting the serum bicarbonate to >22 mEq/l by oral bicarbonate supplementation to maintain serum bicarbonate within the normal range (22-26 mEq/l). Several observational studies suggested that the lowest GFR decline was obtained when serum bicarbonate was 26-28 mEq/l. However, in the CRIC cohort, serum bicarbonate levels over 26 mEq/l were associated with a higher risk of heart failure, and those over 32 mEq/l, with a higher risk for death (Raphael, 2019).

Although data support the hypothesis that alkali therapy preserves kidney function in patients with CKD, evidence from large-scale clinical trials is still necessary before definitive conclusions can be drawn. Moreover, to our knowledge there is no clinical trial comparing sodium citrate with sodium bicarbonate for metabolic acidosis in chronic kidney disease.

2. **Title - SoCiB –** Sodium Citrate versus Sodium Bicarbonate for metabolic acidosis in patients with chronic kidney disease

3. **Type of study -** parallel-design randomized controlled 1:1 trial, single center

4. **Study endpoints**

Primary composite outcome - decline in renal function assessed by changes in eGFR (CKD-EPI equation) and change in serum bicarbonate from baseline (Bs) to the end of the study (EOS).

Secondary outcome - all-cause mortality, a ≥50% reduction in eGFR and ESRD.

Other outcomes - changes in urinary albumin/creatinine ratio (RAC), serum albumin, serum soluble plasminogen activator urokinase receptor (suPAR) and arterial stiffness from Bs to EOS.

Safety endpoints
Percentage of patients who develop along the study:
- blood pressure >140/90 mmHg at EOS along the study period;
- hypervolemia - peripheral edema or dyspnea with crackles or high blood pressure needing initiation or escalation of antihypertensive medication or diuretics;
- hypokalemia < 3 mEq/l;
- serum bicarbonate >28 mEq/L
- calciphylaxis
- digestive symptoms (nausea, vomiting)

5. **Methods**

Design – parallel-design randomized controlled trial

Estimated number of patients - 50

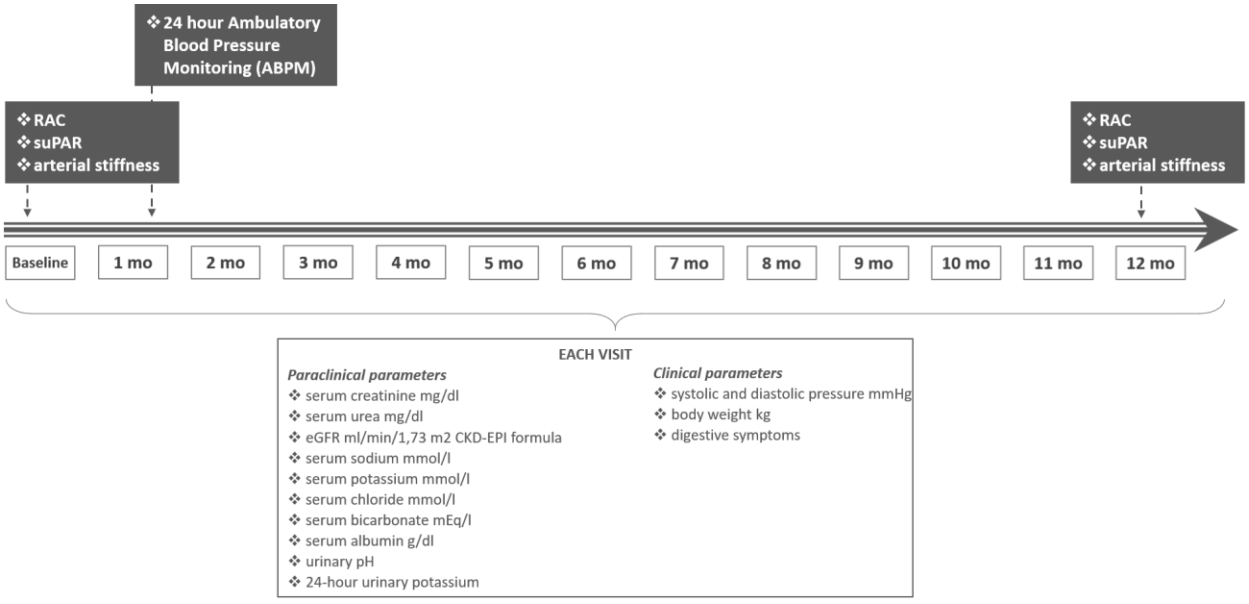
Inclusion criteria
- age > 18 yo
- eGFR 15-45 ml/min/1,73 m^2 CKD EPI
- serum bicarbonate 10-22 mmol/l on to two different ocassion
- ability to travel to study visits
- ability to follow the study treatment regimen
- a wash-out period of one month if previous alkali therapy (such as sodium bicarbonate, sodium citrate, potassium citrate, baking soda etc)

Exclusion criteria

- hipokalemia < 3 mEq/l
- uncontrolled blood pressure (>150/90 mmHg under treatment with more than 3 different classes of antihypertensive drugs, including diuretics)
- heart failure with active class III or IV New York Heart Association, known left ventricular ejection fraction ≤30%, or hospital admission for heart failure within the past 3 months
- hypervolemia of any cause (nephrotic syndrome, liver, or heart failure) considered unsafe for the patient by the PI for the patient
- active hepatic disease
- chronic gastrointestinal disorder (treatment adherence unreliable)
- active malignancy
- pregnancy
- patients taking amilorid or sevelamer
- patients refusing to sign the informed consent

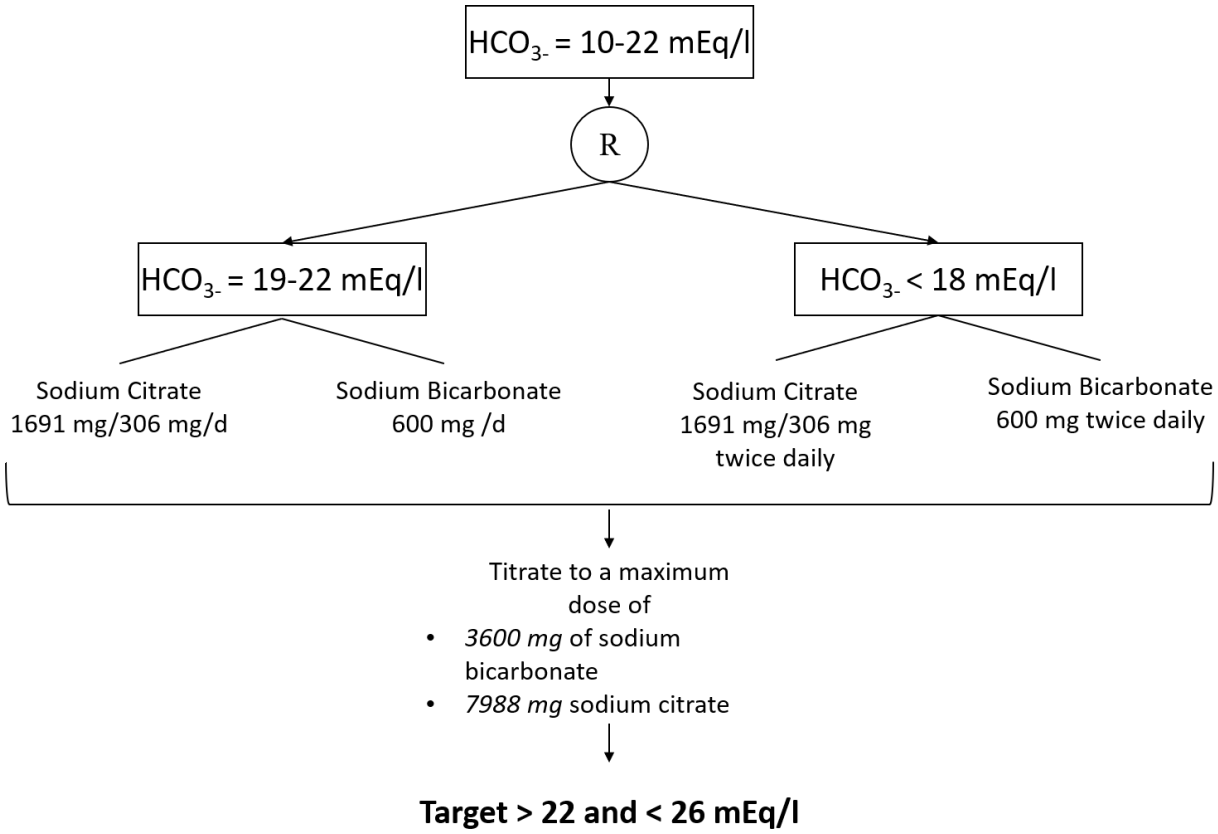
Study procedures

Study visits will be monthly for one year



6. **Medication** after obtaining written informed consent (day 0) each patient will be randomized 1:1 to one of the treatment groups. Based on their level of serum bicarbonate, subjects will receive either high doses or low doses of sodium bicarbonate, respective sodium citrate, as it follows: if serum bicarbonate is 19-22 mEq/l start sodium bicarbonate 600 mg/d or sodium citrate 1691 mg/306 mg/d and if serum bicarbonate is under 18 mEq/l start sodium bicarbonate 600 mg twice daily or sodium citrate 1691 mg/306 mg twice dialy. If serum bicarbonate is still under the target value at next visits, increase sodium bicarbonate by one

tablet to a maximum dose of 3600 mg or increase sodium citrate taking the oral solution twice, thrice or four times per day to a maximum dose of 7988 mg.



7. Statistical analyses. Study duration

Main evaluation parameter: variation (Δ) in eRFG and serum bicarbonate at the end of each intervention (from Bs to the EOS).

The number of patients required for each study group was estimated using the nQuery clinical trial platform (https://www.statsols.com/nquery), having as main objective the comparison of the difference of serum bicarbonate observed at the end of the each treatement group. In a previous pilot study (12) a difference of 2.1 mmol / L (95% CI 0.34-3.86mmol / L) was shown between the variation of serum bicarbonate in the group with sodium bicarbonate versus placebo. Based on these data, for testing non-inferiority of Citronac KD versus sodium bicarbonate (statistical significance of test t <0.05 and statistical power of 95% to reject the null hypothesis) a number of 25 patients in each group was calculated. Considering an 80% adherence, the estimated number of patients to be included in each group is 30.

Data will be analyzed using IBMÒ SPSSÒ Version 23 and Microsoft Office Professional ExcelÒ2003. The normality of continuous variables will be tested by the Shapiro-Wilk test. The parameters with normal distribution will be expressed as mean and standard deviation, and those

with asymmetric distribution, through median and confidence interval. To compare the means or medians between the repeated measurements of a variable on the same subject, the t-paired test and the Wilcoxon signed rank sum test will be used. Qualitative parameters will be described in percentages and the comparison between the two groups of patients will be made with the Chi2 test or Fisher's Exact Test, for a small number of subjects analyzed. The differences resulting from the comparisons will be considered statistically significant at an accepted threshold of 95%, ie $p = 0.05$.