Study Protocol

Effect of Citrate Dialysate on Vascular Calcification

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PROTOCOL TITLE: Effect of Citrate Dialysate on Vascular calcification

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REVISION HISTORY

Revision #	Version Date	Summary of Changes
3	1/20/2022	Increase screening to 150
4	3/6/2022	Increase compensation to $75

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1. Study Summary

Study Title	Effect of Citrate Dialysate on Vascular calcification
Study Design	Interventional, Cross-over
Primary Objective	To determine whether dialysis with citrate reduces vascular calcification
Secondary Objective(s)	
Research Intervention(s)/Interactions	Hemodialysis with citrate dialysate or standard dialysate
Study Population	Female outpatient hemodialysis patients
Sample Size	50
Study Duration for individual participants	2 years
Study Specific Abbreviations/ Definitions	HD: hemodialysis BAC: breast arterial calcification
Funding Source (if any)	Fresenius Research

2. Objectives

To determine whether hemodialysis with citrate slows the progression of vascular calcification.

3. Background

Vascular calcification is a significant problem in dialysis patients that leads to poor outcomes (1-3). Currently there is no therapy that directly addresses this problem. Some of the dialysis solutions (dialysates) currently in use contain citrate, a potent inhibitor of calcium precipitation. In particular, citrate can bind to nascent hydroxyapatite crystals and prevent their propagation (4-5). Blood citrate levels are greater after dialysis with citrate dialysates than with non-citrate dialysates (6), which could slow vascular calcification. However, this has never been investigated. Clinical studies have been limited to the calcification propensity of serum, which decreases with citrate dialysate (7). Administration of citrate to rats with renal failure attenuated vascular calcification (8).

Our research has shown that arterial calcification can be easily detected and reliably measured on routine digital mammograms, with sufficient sensitivity to follow progression (9-13). Mammography is more sensitive in detecting arterial calcification than other imaging and associated with far less radiation and cost. This will allow us to detect differences between citrate and non-citrate dialysates in a safe and convenient way.

4. Study Endpoints

The endpoint will be the difference in the rate of progression of breast arterial calcification (BAC) between dialysis with citrate and non-citrate dialysates.

5. Study Intervention/Investigational Agent

Dialysate is generated during dialysate from two concentrates (acid concentrate and bicarbonate concentrate) mixed with treated water. When used, citrate is provided in the acid concentrate. For this study, patients will undergo hemodialysis using a citrate acid concentrate (Citrasate; Fresenius Medical Care) or non-citrate acid concentrate (Naturalyte; Fresenius Medical Care), each for one year in a cross-over study. Both acid concentrates are approved for this use by the FDA and will be used according to standard procedures without altered dialysis machine settings. Citrasate is the acid concentrate currently in use at all Emory Dialysis units and the final dialysate is pumped to each patient station from a central location. Therefore, the study intervention will be the use of Naturalyte instead of Citrasate. The Naturalyte will be ordered and stored by dialysis personnel and used by the dialysis technicians or nurses. It will be provided locally at each station as individual containers (the dialyzers are designed to use either centrally or locally supplied dialysate) so there is no risk of non-study patients receiving dialysate from Naturalyte acid concentrate.

6. Procedures Involved

Female hemodialysis patients will undergo screening mammography and those who have BAC will be enrolled. This will be a cross-over study in which half the patients will continue with citrate dialysis and the other half will be switched to non-citrate dialysis. After one year, the patients will be switch to the other dialsysis. Dialysis treatments are performed 3 times per week. Mammograms will performed at 6, 12, 18 and 24 months at Emory Healthcare facilities. Patients will have monthly blood draws as part of their routine clinical care and are seen on a weekly basis by the attending nephrologist or nurse practitioner. Data to be collected during the study include:

- measurements of BAC on the mammograms (every 6 months). The mammograms will be interpreted by radiologists.
- plasma citrate, magnesium, and ionized calcium levels immediately before and after dialysis (every 6 months). These will be obtained during routine clinical blood draws and represent an insignificant amount of extra blood.
- routine blood chemistries (monthly as part of clinical protocol)
- dialysis parameters: dialysate composition, anticoagulation, body weight, ultrafiltration volume, vital signs, medications, and dialysis adequacy (automatically captured for each dialysis session in the clinical database)

Minimizing risk: Patients are routinely monitored continuously during dialysis by staff nurses and/or technicians. In addition they will be seen by nurse practitioners weekly and by attending nephrologists monthly.

Drug/device use: Naturalyte acid concentrate, which is FDA-approved for this use.

Source records/data collection: All dialysis data is automatically stored in the clinical database. Mammograms are stored in the Emory Healthcare PACS system.

Long-term follow-up: none.

7. Data and Specimen Banking

No specimens will be collected. Data will be available only to the PI and study coordinator. It will only be released to others in a de-identifed form.

8. Sharing of Results with Participants

Results of the mammograms (other than the vascular calcification) will be entered in the medical record, shared with the patients, and reported to the attending nephrologist. Any suspicious findings on mammograms will be pursued per normal clinical protocol by the Breast Imaging Center, which includes contacting and educating the patient and arranging appropriate follow-up (repeat diagnostic mammograms, ultrasound, biopsy).

9. Study Timelines

Duration of an individual participant's participation in the study: 2 years.

Duration anticipated to enroll all study participants: 2 months.

Estimated date for the investigators to complete this study (complete primary analyses): 27 months.

10. Inclusion and Exclusion Criteria

Describe how individuals will be screened for eligibility: mammogram.

Inclusion criteria: adult female hemodialysis patients with BAC on mammograms.

Exclusion criteria: current warfarin use, severe hyperparathyroidism (likely to undergo parathyroidectomy in the next 2 years), difficulty controlling serum calcium (likely to require changes in dialysate calcium concentration in the next 2 years), life expectancy < 2 years, prisoners, or inability to give consent.

11. Vulnerable Populations: none

12. Local Number of Participants

Screened: 150 (estimated)

Enrolled: 124

Completed study: 50 (expected)

13. Recruitment Methods

Describe when, where, and how potential participants will be recruited: at dialysis units during dialysis sessions.

Describe the source of participants: patients undergoing chronic hemodialysis ate Emory Dialysis facilities.

Describe the methods that will be used to identify potential participants: census of current dialysis patients.

Describe materials that will be used to recruit participants: none.

Describe the amount and timing of any payments to participants: $50 for each mammogram to cover time and travel.

14. Withdrawal of Participants

Describe anticipated circumstances under which participants will be withdrawn from the research without their consent: occurrence of exclusion criteria during the study.

Describe any procedures for orderly termination. Patients will be returned to standard care.

Describe procedures that will be followed when participants withdraw from the research: no further data collection upon withdrawal; continued data collection for partial withdrawal (less frequent mammograms).

15. Risks to Participants

1. Citrate dialysis: no risks since this is the current type of dialysis.

2. Non-citrate dialysis: This is the standard type of dialysis in most units and safety is not an issue. Since the citrate dialysate may have some anticoagulant properties, changing to non-citrate dialysate may require an increase in the heparin dose given to minimize the risk of clotting of the dialysis cartridge. This is usually recognized during the dialysis session prior to clotting and is easily managed by adding additional heparin and adjusting future dosing.

3. Mammography: The radiation from a mammogram is minimal, equivalent to 7 weeks of background environmental exposure. Since most, if not all of the women would be getting annual mammograms if not in the study, the extra radiation from the 6 month

and 18 month studies would be equivalent to only 14 weeks of background environmental radiation. There is also the risk that an abnormality will be detected on the mammograms that will require further workup and may cause psychological stress. However, these abnormalities likely would be detected during clinically indicated mammography. There is some discomfort associated with mammography.

16. Potential Benefits to Participants

No direct benefit.

17. Compensation to Participants

$75 by debit card for each mammogram.

18. Data Management and Confidentiality

Data analysis. Rate of BAC progression will be calculated by linear regression of 3 mammograms in each treatment arm: 0, 6, 12 months vs. 12, 18, 24 months. Comparison will be by paired t-testing (or Wilcoxon test in the case of nonparametric distribution). Also, the proportion of women with a decrease in BAC rate during cutrate dialysis will be examined by Fisher's Exact Test. Laboratory values and dialysis parameters will be compared in patients with and without a decrease in BAC rate.

Study size. The goal is a final cohort of 50. The attrition rate among females at Emory Dialysis over the past 9 years has averaged 9.2% per year. However, this is an over-estimate since it likely includes women who would not have qualified for the study. Using a drop-out rate of 10% per year, a starting sample of 62 will be needed. Based on previous data 24, 60% of HD patients will have baseline calcification. Thus, 103 will need to be recruited and screened for BAC.

Power. Our previous studies comparing BAC rates calculated from the first 3 and last 3 mammograms (the study design for this proposal) yielded a standard deviation of 11.9 mm/breast/y. Using this value and the median BAC rate in HD patients of 20.1 mm/breast/y 27, a cohort of 47 women would provide 80% power to detect a 25% change in rate at p=0.05. In two longitudinal studies calculating rates from just two mammograms, we were able to detect a 78% decrease in the rate of BAC after stopping warfarin at p=0.02 in just 13 patients 28 and a 90% decrease after kidney transplantation in only 9 patients (unpublished data).

Subject availability. There are currently 300 female in-center HD patients within Emory Dialysis and it is estimated that at least 80% or 240 will qualify for screening. Therefore, a recruitment rate of 43% will be required. Describe the data analysis plan, including any statistical procedures or power analysis.

Data management. Data collected will be mammograms, research blood assays (citrate, magnesium, ionized calcium), clinical blood assays, and dialysis parameters. All data/specimens will be handled clinically except for samples for research assays, which will be transported to the P.I's laboratory by the research coordinator. Mammograms

will be stored in the Emory Healthcare PACS system, results of research blood assays will be stored in a password-protected file on a HIPAA-compliant server in the Department of Medicine, and results of clinical assays and dialysis parameters will be stored in the Emory Dialysis electronic medical record. Composite data for final analysis will be stored in a password-protected file on a HIPAA-compliant server in the Department of Medicine. Subjects will be assigned unique identifiers, the key to which will be stored as a separate, password-protected file. Access to all data will be limited to the P.I., coinvestigators, and research coordinator. Any transmitted data will not contain any identifiers. Data will be stored until all primary and secondary analyses are completed.

19. Provisions to Monitor the Data to Ensure the Safety of Participants

Patients will be monitored during each dialysis by nurses or technicians, weekly by nurse practitioners, and monthly by attending nephrologists. Clinical data (pre-dialysis labs and anticoagulation) along with intra-dialytic events are already captured per clinical protocol and stored digitally and will be reviewed monthly by the P.I. Oversight of the progress and safety of the trial will be provided by the PI. Adverse events are not anticipated, but any occurring will be documented and reported according to Emory IRB policies and procedures. Cumulative adverse events and study progress summary will be communicated to the IRB at the time of continuing review.

The only potential safety issue relates to the anticoagulant properties of citrate whereby changing to the non-citrate dialysate may increase the risk of clotting of the dialyzer (already a rare occurrence in the outpatient setting). The staff are already trained to recognize the need to increase the heparin dose prior to clotting and this is already captured on the digital dialysis records, which will be reviewed by the study team on a weekly basis for the first month for each subject. If an increased frequency of clotting or need to increase heparin dosing is noted, subsequent subjects will have their heparin dose increased pre-emptively.

Confidentiality will be protected by utilizing a code number as the only identifier for each subject and the master list will be kept under lock and key with access limited to the PI.

The PI will be responsible for reviewing protocol compliance, data collection and verification.

20. Provisions to Protect the Privacy Interests of Participants

In addition to the dialysis staff, participants will interact only with the P.I. or study coordinator. The subjects will only be asked to undergo mammograms. There will be no other intrusion of their privacy since all other data will be collected as part of their

clinical care. The P.I. will have complete access to the dialysis records and the Emory Electronic Medical Record. The study coordinator will have access to the dialysis records and will access the Emory Electronic Medical Record solely for the purpose of facilitating and monitoring the scheduling of mammograms.

21. Economic Burden to Participants

None

22. Consent Process

Indicate whether you will you be obtaining consent, and if so describe:

- Where will the consent process take place? Dialysis units
- Any waiting period available between informing the prospective subject and obtaining the consent. Prospective subjects will be given up to two weeks between being informed and obtaining consent.
- Any process to ensure ongoing consent. Subjects will be informed that they can withdraw at any time.
- Please describe:
    - The role of the individuals listed in the application as being involved in the consent process. Providing information and obtaining consent.
    - The time that will be devoted to the consent discussion. As much time as the individual needs.
    - Steps that will be taken to minimize the possibility of coercion or undue influence. Potential subjects will be told that they are free not to participate and that not participating will not affect their care. The P.I. is not involved in the care of any dialysis outpatients.
    - Steps that will be taken to ensure the participants' understanding. The participants understanding will be assessed by asking them to review what they are asked to do for the study.

Non-English-Speaking Participants

- Indicate what language(s) other than English are understood by prospective participants or representatives. Primarily Spanish but could be many others.
- If participants who do not speak English will be enrolled, describe the process to ensure that the oral and written information provided to those participants will be in that language. Indicate the language that will be used by those obtaining consent. For those who do not understand English, consent will be obtained in the presence of a qualified interpreter.

Participants who are not yet adults (infants, children, teenagers) N/A

Cognitively Impaired Adults

- Describe the process to determine whether an individual is capable of consent. Consultation with the attending nephrologist.

Adults Unable to Consent N/A

Waiver or Alteration of Consent Process (consent will not be obtained, required information will not be disclosed, or the research involves deception) N/A

23. Setting

Describe the sites or locations where your research team will conduct the research.

- Identify where your research team will identify and recruit potential participants. Outpatient dialysis units.
- Identify where research procedures will be performed. Emory Dialysis Centers and Breast Imaging Centers
- Describe the composition and involvement of any community advisory board. N/A.

24. Resources Available

Subject availability. We will need to recruit 62 subjects to ensure a final sample of 50 allowing for 10% drop-out per year (patients dying, receiving kidney transplants, or transferring to other units). This rate has averaged 9.2% among female patients at Emory Dialysis over the past 9 years. Based on our previous data, 60% are expected to have breast arterial calcification, meaning that 103 will need to be recruited and screened for BAC. There are currently 300 female in-center HD patients within Emory Dialysis and it is estimated that at least 80% or 240 will qualify for screening. Therefore, a recruitment rate no greater than 43% will be required.

Timeline. Recruitment completed: 1 month
Last patient started: 2 months
Last patient: 26 months
Data analysis completed: 27 months

Facilities. Emory Dialysis consists of 4 large outpatient dialysis centers distributed around the Atlanta metropolitan region and is staffed by nephrologists, nurse practitioners, registered nurses, and dialysis technicians. The P.I. will work closely with the managers at each unit and hold educational meetings with staff to ensure that they are adequately informed.

The Emory Breast Imaging Center, consisting of several locations, is staffed by Emory radiologists and performs over 20,000 screening mammograms per year. Mammograms are accessible in the Emory Healthcare PACS system and reports are accessible in the Electronic Medical Record. The Center has procedures in place to contact patients about abnormal findings and discuss/arrange additional followup. The P.I. works closely with

the Medical Director (Dr. Michael Cohen), who is aware of the protocol and will be the sole radiologist interpreting the mammograms.

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