1.a. Full Title:  Chronic Subclinical Thrombotic Microangiopathy and Chronic Kidney Disease

b. Abbreviated Title (Length 26 characters):  Chronic TMA and CKD

2. Writing Group:
   Writing group members:  Brad Astor, Jerrold Levine, Michael Fischer, James Lash

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal.  _MJF__ [please confirm with your initials electronically or in writing]

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3. Timeline:

This proposal will use available data.  Analyses and manuscript preparation will be performed over the next six months.

4. Rationale:
The incidence and progression of chronic kidney disease (CKD) vary significantly among individuals. While some of these differences are attributed to the presence or absence of known risk factors for kidney disease, an important remainder continues to be poorly explained. In considering these observations, it becomes clear that other potential biologic processes likely exist that contribute to the development and progression of CKD.

Evidence exists to suggest that a progressive decline in kidney function can occur from subclinical microvascular processes such as low-grade thrombotic microangiopathy (TMA), as demonstrated by the presence of kidney biopsy changes consistent with a healing chronic subclinical TMA (1-12). Importantly, these changes can occur in the absence of overt systemic hematologic manifestations of acute TMA, such as disseminated intravascular coagulation (DIC) (1-12). Overt or subclinical TMA has been observed or invoked in a variety of kidney disease settings, including hepatitis C, systemic lupus erythematosus (SLE), post-hematopoietic cell transplant nephropathy, radiation nephropathy, chronic kidney transplant glomerulopathy, and, most prominently, the anti-phospholipid antibody syndrome (3-12).

It also has been speculated that a chronic subclinical TMA may play a role in broader populations, such as those with diabetic and hypertensive kidney disease. For example, among patients with hypertensive nephrosclerosis, it has been observed that blood pressure does not correlate with or predict the extent of vascular lesions (13-15). Furthermore, a recent study has found that individuals with thrombophilias suffer from repeated small chronic thrombotic events within the renal vasculature, leading to vascular scarring (16). Hence, investigators have hypothesized that unrecognized factors for vascular sclerosis, such as prothrombotic and anti-endothelial factors, may lead to a chronic and clinically unapparent TMA (16-17). Similarly, a recent study of diabetic patients found a significant impairment of fibrinolysis, which is an important process in the regulation and/or resolution of TMA (18). The authors posited that these abnormalities lead to ongoing fibrin deposition and endothelial dysfunction, culminating in kidney disease progression and cardiovascular events in diabetics (18). It is also important to note that the beneficial effects of angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) on kidney disease progression and cardiovascular disease are not entirely explained by blood pressure reduction (19-20). It is believed that part of the positive effects of these agents on clinical outcomes derives from their ability to improve endothelial dysfunction and fibrinolysis, two key components of TMA (19-20). Considering this growing body of evidence, we propose that a chronic subclinical TMA may be an unrecognized process that helps to explain CKD disease progression and associated cardiovascular disease in diverse populations. No systemic evaluation of chronic subclinical TMA among individuals with CKD has yet been conducted.

As in acute TMA, chronic microvascular processes (like subclinical TMA) represent a dynamic state with pathologic and reparative processes occurring simultaneously. These processes include i) endothelial cell activation and/or injury, ii) local hypercoagulability, iii) fibrinolysis and remodeling, and iv) platelet activation. We propose that there is a synergistic interdependence between CKD and microvascular processes, with chronic
subclinical renal TMA promoting CKD, and CKD itself favoring the development of local chronic subclinical TMA. This synergistic interaction may contribute to a further decline in kidney function. As listed below, we will investigate the presence of subclinical TMA by assessing the presence of several factors among ARIC participants and studying their association with CKD.

References

5. Main Hypothesis/Study Questions:

**Hypothesis 1:** Factors consistent with chronic subclinical thrombotic microangiopathy (TMA) are: i) more prevalent in individuals with CKD compared with those without CKD; and ii) increasingly prevalent in individuals with severe CKD compared with those with mild CKD.

**Specific Aim 1:** To assess the prevalence of factors consistent with subclinical TMA in subjects with varying degrees of CKD and without CKD.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

The study design will be a cross-sectional analysis of the association between CKD and factors representative of subclinical TMA (endothelial cell activation and/or injury, local hypercoagulability, fibrinolysis and remodeling, and platelet activation).

The outcome of CKD will be assessed by estimated measures of GFR (eGFR). eGFR measures will be based on a serum creatinine values according to simplified Modification of Diet in Renal Disease (MDRD) as follows:

\[
\text{Estimated GFR (ml/min/1.73m}^2) = 186.3 \times (\text{serum creatinine})^{-1.154} \times \text{age}^{-0.203} \times (0.742 \text{ for women}) \times (1.21 \text{ if African American})
\]

Serum creatinine values will be obtained from the original cohort sample at visit 1. Serum creatinine was measured using a modified kinetic Jaffe method. Serum creatinine concentration will be corrected for inter-laboratory differences and calibrated with Cleveland Clinic measurement standards by the addition of 0.18 mg/dl.

We will further use a single serum creatinine value (eGFR calculation) to categorize strata of eGFR (ml/min/1.73m\(^2\)) as follows: \(<15, 15-29, 30-59, 60-89, \text{ and } \geq 90\). These strata are chosen in order to be consistent with the National Kidney Foundation’s (NKF) Kidney Disease Outcomes Quality Initiative (K/DOQI) standardized definitions for CKD. In keeping with the NKF K/DOQI criteria, we will consider a subject to absolutely have CKD if the eGFR is \(< 60 \text{ ml/min/1.73m}^2\). Since we do not have urinary measures of kidney damage (e.g. proteinuria), it is difficult to rule out CKD subjects with higher eGFR values. Therefore, we will also evaluate the prevalence of subclinical TMA factors with all of the eGFR strata.
The following variables will also be required for the analyses:

- **demographic factors** (age, race, sex, center)
- **co-morbid conditions** (hypertension, diabetes status, prior CVD events)
- **anthropometric data** (waist circumference, waist to hip ratio, BMI)
- **smoking status, alcohol intake**
- **medication use** (anti-hypertensive agents, lipid-lowering agents)
- **chemistry values** (lipids, blood glucose, albumin)
- **hematologic values** (hemoglobin, white blood cell count, platelet count)
- **subclinical TMA-related factors**
  - **coagulation factors** (fibrinogen, VII, VIII: C, vWF: Ag, fibrinopeptide A, anti-thrombin III, protein C, lupus anticoagulant, PTT)
  - **platelet activation factors** (beta-thromboglobulin, platelet factor-4, serum thromboxane B2)
  - **fibrinolytic factors** (tissue plasminogen activator antigen, fibrinopeptide B-beta-15-42, fibrinopeptide B-beta-1-42)

All of these above variables (except subclinical TMA-related factors) will be assessed at visit 1 from the original cohort random sample. Demographic factors, comorbid conditions, smoking/alcohol status and medication use will treated as categorical values (yes/no) in this analysis. Anthropometric data, chemistry values, and hematologic values will be treated as continuous variables in the analysis. The subclinical TMA-related factors will be obtained from the cohort random sample at visit. All of these factors will be treated as continuous variable in the analysis. Examination of associations between subclinical TMA-related factors and CKD will include both bivariate and multivariable analyses and will be performed locally by Dr. Astor, using available data.

7.a. Will the data be used for non-CVD analysis in this manuscript?  **X** Yes  ____ No

    b. If Yes, is the author aware that the file ICTDER02 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used?  **X** Yes  ____ No
    (This file ICTDER02 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript?  ____ Yes  **X** No

     b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = “No use/storage DNA”?
    ____ Yes  **X** No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status.
ARIC Investigators have access to the publications lists under the Study Members Area of the web site at:  http://www.cscc.unc.edu/ARIC/search.php

___X___  Yes  _______ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

MS# 349:  Association of coagulation factors with decreased renal function in the ARIC Study; Tate Erlinger, lead

A draft of the above-referenced paper is nearing completion, with Dr. Astor is a co-author. The paper focuses on incident kidney disease (decreased estimated GFR or kidney-related hospitalizations) predicted by hemostatic and inflammation factors, and does not discuss cross-sectional relationships between these factors and baseline kidney function.

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  ____ Yes  ___X__ No

11.b. If yes, is the proposal

___  A. primarily the result of an ancillary study (list number* __________)
___  B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _________ _________ _________)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.  Acknowledged.