1.a. Full Title:

The Association Between Renal Dysfunction and Clinical and Subclinical Intracranial Hemorrhage: The ARIC Study

1.b. Abbreviated Title (21 characters):

Renal Failure and ICH

2. Writing Group Members: Josef Coresh, Clifford Jack, Rebecca Gottesman, Thomas Mosley, Kuni Matsushita, Rafael Llinas, Argye Hillis; **open to others**

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. **EBM**

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3. Timeline: Analyses on clinical outcome (intracranial hemorrhage) to begin as soon as manuscript proposal is approved, and on subclinical outcome (cerebral microbleeds) to begin as soon as data collection is complete and gradient echo image analysis is completed. Data collection to be completed by late Summer 2013, with likely image analysis completion a few months after that. Goal for manuscript submission: Spring
2014, with possible submission to 2014 International Stroke Conference (ISC) or other national meeting.

4. **Rationale**

Intracranial hemorrhage (ICH) is a devastating neurologic event that occurs on both a microvascular and macrovascular scale. Cerebral microbleeds are associated with cognitive decline (1-3) and increased risk of symptomatic ICH (4,5). On a larger scale, hemorrhagic transformation of stroke and spontaneous primary ICH often results in neurologic deficits, long-term disability, or death. It is unknown whether all ICH shares common risk factors. The ability to predict who is at highest risk of ICH is important to clinicians, particularly when considering treatment with anticoagulation, which theoretically increases the risk further.

**HEMORRHAGIC TRANSFORMATION IN ACUTE ISCHEMIC STROKE**

Patients with acute ischemic stroke frequently have an indication for anticoagulation (related to the etiology of the stroke itself (eg., afib), or independent of the stroke (eg., DVT)). The introduction of new, potentially safer agents (factor Xa and direct thrombin inhibitors) may lower the threshold for initiating treatment. In one month, 19/40 patients diagnosed with acute ischemic stroke at our institution had reasons to require an anticoagulant. Patients who have had an ischemic stroke are at higher risk for hemorrhagic transformation, especially in the days immediately following the event (6-8). This may be because ischemia leaves the cerebrovasculature friable.

We have acquired preliminary data from a small, retrospective cohort of inpatients to suggest that age, infarct volume, and (even relatively mild) renal impairment are important predictors of hemorrhagic transformation of acute ischemic stroke.

**RENAL DYSFUNCTION AND HEMORRHAGIC TRANSFORMATION**

Our preliminary data suggest that hospitalized stroke patients with renal impairment (estimated glomerular filtration rate [eGFR] <60mL/min) are more likely to experience ICH and poor clinical outcomes. This has also been observed in several small studies (9-11). The concept of “uremic platelets” is well described in end-stage renal disease (12,13), but the exact mechanisms leading to platelet dysfunction are unknown. The role of uremic platelets in mild renal impairment is also unclear. Our preliminary finding that mild renal dysfunction is associated with increased risk of hemorrhagic conversion suggests that even mild renal impairment may affect platelet function.

Renal failure has also been associated with inflammation (14,15). Studies have suggested that chronic inflammation may lead to increased permeability of the blood brain barrier (16,17), and more recently proteins associated with blood brain barrier breakdown have been shown to be associated with increased risk for ICH (18).

**INTRACRANIAL HEMORRHAGE AND SPONTANEOUS CEREBRAL MICROBLEEDS**

Another approach to investigating ICH is to examine factors that lead to increased risk within the general population. ICH can take the form of often subclinical cerebral microbleeds, or larger spontaneous hemorrhages. Accumulation of microbleeds may
result in cognitive decline (1-3), but are often considered asymptomatic. The burden of disease also increases risk of transformation into larger, more clinically apparent bleeds (4,5). This may be amplified by the use of anticoagulants, which is increasing in frequency in our country’s aging population. Investigators for the Rotterdam Study found that hypertension, pulse pressure, and white matter disease were associated with increasing frequency of microbleeds (19). To our knowledge, they did not examine the effect of renal impairment. Several smaller studies have looked at renal failure and found that low GFR, hemodialysis, and proteinuria were associated with greater numbers of microbleeds (20-24).

5. Main Hypothesis/Study Question

STUDY QUESTIONS: To determine if renal dysfunction is an independent predictor of 1) spontaneous cerebral microbleeds and 2) spontaneous primary ICH, in ARIC.

Aim 1 (microbleeds): A subset of individuals (n=~2,000) in the ARIC cohort are undergoing brain MRI (as part of the already-funded ARIC-NCS study) including gradient echo (GRE) sequences, with quantification of cerebral microbleeds and hemorrhage. It is estimated that these data will be collected fully as of late Summer 2013, with complete analysis in the 6-12 months after.

Aim 2 (clinical ICH): Stroke, including symptomatic ICH, has already been adjudicated in the ARIC population. This will be evaluated in the entire ARIC cohort (N=15,792) Renal function has been measured using creatinine-based eGFR, creatinine, cystatin C-based eGFR, and cystatin C levels.

Hypothesis- Presence of renal dysfunction will be associated with subsequent symptomatic ICH, as well as the presence and increased number of microbleeds, compared to those with normal renal function. We expect a dose-response type of relationship with more microbleeds- individuals with more severe renal dysfunction (eg., lower eGFR) will have a greater number of microbleeds than individuals with milder renal dysfunction, who in turn will have more microbleeds than individuals with normal renal function. We hypothesize that this relationship is independent of other vascular risk factors that are likely confounders in the anticipated relationship.

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).

Study Design:
This will be a retrospective analysis of prospectively collected cohort data.

Inclusion Criteria: We will perform our analysis using existing data that is being collected for:

1. the 2,000 participants in Stage III (Brain MRI) of the ARIC-NCS study (Aim 1) looking at cerebral microbleeds on MRI.
2. the entire ARIC cohort (Aim 2) looking at clinical adjudication of ICH.
Exclusion Criteria: None. We will include all patients who are currently part of the ARIC cohort and have data available for analysis.

Data Analysis:
We will have two primary hypotheses:

1. Renal dysfunction (measured using creatinine/eGFR and cystatin C in separate models) will be associated with cerebral microbleeds among individuals in the ARIC-NCS imaging cohort (N~2000). We will analyze presence/absence of any microbleeds as a dependent variable in logistic regression analyses, using renal function as the primary predictor, with inclusion of other potential confounders in the multivariable model. Separate models will be constructed for 1) creatinine; 2) creatinine-based eGFR; 3) cystatin C; 4) cystatin C-based eGFR. These will be evaluated cross-sectionally (from visit 5) as well as longitudinally from earlier ARIC visits (including average values over all visits, a time-averaged "cumulative" value, and the proportion of ARIC-studied time spent below an eGFR of 60 mL/min, for example). In addition, we will generate ordinal logistic regression models including number/quartile of microbleeds as the dependent variable, with the same predictors.

2. Renal dysfunction (measured using the same exposures) will be longitudinally associated with clinical intracranial hemorrhage (among the entire ARIC cohort (N=15,792)). Symptomatic ICH, as already classified in ARIC, will be the dependent variable in proportional hazards analyses, with measures of renal function (as defined below) as the primary predictors, and adjustment for other potential confounders. This will be in the entire cohort. Time to first hemorrhage will be evaluated in individuals with ICH, for survival analyses, and time to last hemorrhage-free visit will be used in individuals without ICH.

Covariates of interest for both aims will be from the ARIC baseline visit and follow-up visits: (1-4 for both aims, and including ARIC-NCS/Visit 5 for Aim 1): both systolic and diastolic blood pressure, age, race-center, sex, diabetes, lipid levels, statin use, anti-hypertensive agent use, smoking status, anticoagulant/antiplatelet use, history of prior stroke, and BMI. Blood pressure will be evaluated at each visit as well as a cumulative time-averaged blood pressure measurement. Antihypertensive, anticoagulant, and antiplatelet medication history will be evaluated as covariates and in stratified analyses. Cumulative (total % of ARIC-evaluated time) as well as any use of these medications will be used.

Exposures of interest for both aims will include: creatinine/eGFR, cystatin C, and cystatin-C based eGFR. Measurement of creatinine has been and will be collected from visits 1,2,4, and 5 (Stage I) allowing us to trend renal function over time and calculate eGFR. Additional analytes from visit 5 Stages II & III include: cystatin C, cystatin C-based eGFR and urine albumin. Visit 5 results will not be used in the Aim 2 survival analyses since this will not allow for subsequent longitudinal evaluation of associations with ICH. We will not only use eGFR values, but will evaluate slope of eGFR, as well as slope of cystatin C and slope of creatinine.

Outcomes:
1. Neuroimaging (for Aim 1): Cerebral microbleeds—presence, quantity, location on brain MR GRE (NCS MRI visit); or larger radiographically-identified intracranial hemorrhage. Microbleeds on MRI will already be evaluated by Dr. Clifford Jack, as part of the ARIC-NCS study, with rating of presence, number, and location of microbleeds (supratentorial versus infratentorial, cortical versus deep).

2. Surveillance data (for Aim 2): Clinical presence of ICH from cohort surveillance data (adjudicated cases). Non-traumatic intracranial hemorrhage is defined through the adjudication process, and is rated as definite or probable. We will conduct analyses using definite adjudicated ICH. Data is available for up to 2009 at this time for these outcomes.

**Limitations:** The primary limitation is that we may have limited power to evaluate Aim 2. In the ARIC sample, there had been a total of 106 adjudicated “definite” intracerebral hemorrhages through 2008. The mean creatinine was 1.33 mg/dL in individuals with ICH (who had creatinine measured) and 1.17 mg/dL in individuals without ICH (p=0.0006). We therefore anticipate adequate power to show a difference in further adjusted models for renal function and clinical ICH associations. Assuming an estimated prevalence of microbleeds of 10.2% (19) in our ARIC NCS imaging sample (N=2,000; the true prevalence is likely to be higher since individuals with cognitive dysfunction are sampled preferentially), and an estimated mean eGFR of 78.8 mL/min (SD 22.5) in those without microbleeds and 65.2 mL/min in those with microbleeds (SD 19.1) (20), we should have 100% power to detect this difference in eGFR.

Another limitation may be the use of multiple measures of renal dysfunction. While we certainly hope that all measures will yield similar results, we realize that this may not happen. Given past studies demonstrating stronger associations between cystatin C and cystatin C-based eGFR and clinical outcome (25), we will primarily base interpretation on the cystatin C-based results.

7.a. Will the data be used for non-CVD analysis in this manuscript? Y/N

8.a. Will DNA data be used in this manuscript? Y/N

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.cscs.unc.edu/ARIC/search.php](http://www.cscs.unc.edu/ARIC/search.php) Y/N
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)?

Published

Proposals
1. MP #1663- Folsom A, Longstreth, Yatsuya H, Psaty B. Risk factors for hemorrhagic stroke II: A pooled study of CHS and ARIC.
2. MP #1754- Mahmoodi BK, Matsushita K, Astor BC, Gansevoort RT, Coresh J. Association of estimated glomerular filtration rate and albuminuria with ischemic and hemorrhagic strokes.

**Our manuscript will expand on the work of the above proposals by evaluating multiple markers of renal function (including both measurement of creatinine and cystatin C) as well as evaluate renal function as a variable over time for both adjudicated intracerebral hemorrhages AND cerebral microbleeds. We have included several members of these proposals in our writing group.

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  Y/N

11.b. If yes, is the proposal:
A. primarily the result of an ancillary study- A substantial portion of the data used for analysis was obtained through the ongoing ancillary ARIC-Neuro Cognitive Study (ARIC-NCS 2008.06)
B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list numbers)- Cystatin c measurements have been collected as part of the ancillary ARIC- Chronic Kidney Disease Study (ARIC-CKD 2006.16 Longitudinal Study of Predictors and Consequences of Chronic Kidney Disease)

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

12. Manuscript preparation is expected to be completed in three to five years. If a manuscript is not submitted for ARIC review at the end of the 5-years from the date of the approval, the manuscript proposal will expire.
References


