ARIC Manuscript Proposal #4393

PC Reviewed: 01/09/24	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Incidence of venous thromboembolism in participants with clonal hematopoiesis of indeterminate potential (CHIP): The Atherosclerosis Risk in Communities Study

b. Abbreviated Title (Length 26 characters): CHIP and VTE in ARIC

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

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3. Timeline:

The data needed for this analysis is available. We plan to complete the analysis and draft the manuscript for publication within 6 months

4. Rationale:

Clonal hematopoiesis (CH) becomes increasingly common with age.(1) The subset of CH with a driver mutation in one of the genes implicated in hematologic malignancies with variant allele frequency (VAF) of at least 2% in the absence of known hematologic malignancy or other clonal disorders is called clonal hematopoiesis of indeterminate potential (CHIP).(2) CHIP was initially described as a risk factor for hematologic (particularly myeloid) malignancies (HM). Additionally, multiple studies have demonstrated increased mortality and elevated risk of cardiovascular disease, largely among middle-aged adults.(3)

Multiple studies confirmed an increased odds of coronary artery disease, (4-8) stroke, (4) and heart failure (9, 10) in participants with CHIP. Reports have suggested an increased risk for thromboembolic events in individuals with CHIP. In a population of participants with unprovoked venous thromboembolism, 20% (12 of 61) were found to have CHIP.(11) Preliminary results from UKBB showed that large clones of CHIP (VAF ≥10%) in *DNMT3A, TET2,* and *ASXL1* were associated with an increased risk for venous thromboembolism (HR 1.60, 95% CI 1.04–2.46, p=0.032) and pulmonary embolism (HR 1.80, 95% CI 1.08–3.05, p=0.025) (12). The increased risk for venous thromboembolism and pulmonary embolism reported in *Jak2*^{V617F} mice(13) was also reported in cohort studies, in which individuals carrying *JAK2*-CHIP had ~3 times increased risk for venous thromboembolism. (14)

We will use the ARIC cohort to investigate the association of CHIP with VTE and study the CHIP-specific risk factors of VTE.

5. Main Hypothesis/Study Questions:

Aim 1: Studying the association of CHIP with incident VTE
Aim 2: Determine CHIP specific risk factors associated with VTE independent of traditional clinical risk factors

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 / Inclusion:

- ARIC participants with exome sequencing and CHIP ascertainment will be assessed for incident VTE events

- The proposed analysis will be done separately in 2 different cohorts of participants with CHIP ascertainment (~20 years apart):
    - Younger cohort: the first sequencing and CHIP calling is done for 10,881 participants (mostly during V1-V3); prevalence of CHIP ~10%
    - Older cohort: the second sequencing and CHIP calling is done in 5,000 participants (mostly during V5); prevalence of CHIP ~25%

Exclusions:

- No exome sequencing available for CHIP calling
- Sex-mismatched sequencing samples
- Hematologic malignancy before CHIP ascertainment
- Participants with prior history of VTE before index date (inaccurate)

Outcomes:

- All outcome data will be derived from prospectively collected and adjudicated VTE data from investigators at UMN
- Primary outcome: Incident VTE as defined by pulmonary embolism (PE), lower extremity deep vein thrombosis (DVT) from index date until last follow-up
- Secondary outcome: unprovoked VTE

Exposure:

- All exposures and confounders will be assessed separately in the younger and older cohorts
- Participants with CHIP/CCUS is defined by the presence of a somatic mutation in a myeloid neoplasm driver gene (eg, DNMT3A, TET2, ASXL1, JAK2, TP53) at a variant allele fraction (VAF) of ≥2% in an individual without a diagnosed hematologic disorder
- CHIP will be determined using exome sequencing (ES) of blood DNA using the GATK MuTect2 (15) somatic variant caller based on the 74 prespecified driver sequence variations in genes known to promote clonal expansion of hematopoietic stem cells. (4, 5, 16) A conventional variant allele frequency (VAF) of >2% will be used to identify CHIP. and those with VAF >10% will be considered large clones. CHIP calling will be conducted at BROAD institute.
- Participants with CHIP/CCUS will be further stratified by VAF % (2-5%, 5-10%, 10-20%, >20%) and gene mutated in subgroup analysis

Covariates:

- Demographics: age, sex, and race
- Comorbidities: smoking, obesity, renal disease, and solid organ cancer
- Medication: antiplatelet, and anticoagulants

Statistical Analyses:

- Descriptive analysis: We will describe the characteristics of participants by CHIP mutation (yes vs. no). Continuous variables will be presented median and interquartile range, and categorical variables will be presented number and

percentage. The difference in characteristics between CHIP+ (~10%) vs. CHIP- (~90%) participants will be tested by independent t-test or Mann-Whitney U test based on normality and equal variance of continuous variables, and by Fisher's exact test for categorical variables.

- Incidence: To calculate the incidence rate of VTE per 1,000 person-year, we will divide the number of VTE events by the sum of person-years of all participants during the follow-up (starting from CHIP ascertainment time). The difference in incidence rate between the two groups will be tested by Poisson distribution methods. We will further plot the cumulative incidence of VTE between CHIP+ vs. CHIP- group by using competing risk method with death and hematologic cancer development as competing risks. The difference in cumulative incidence of VTE will be tested by Gray's test. Separate analyses on incidence will evaluate unprovoked and provoked VTE separately.
- We will analyze the multivariable Fine-Gray competing risk model to calculate the adjusted sub-distribution hazard ratio (sHR) for VTE in CHIP+ vs. CHIP- group after adjusting for age, sex, race*center, smoking, baseline solid organ malignancy, and anticoagulant use. Separate models will assess unprovoked VTE vs provoked VTE.

Sensitivity Analyses:

- Subgroup/sensitivity analyses: Multiple subgroup and sensitivity analyses will be performed. Subgroup analyses will be done in participants with different VAF thresholds and different genes with driver mutations.
- The prevalence of CHIP is expected to ~10% (100 in 10,000 participants) and higher in the V5 cohort (~25% in ~5,000 participants); however, since age is associated with both VTE and CHIP, it is important to test our hypothesis at both time points if one has more than 1 CHIP assessment done. Therefore, we will perform separate analyses using sequencing data at V2-3 and V5 as a starting point, comparing the findings in different age groups. We prefer this approach over treating the evolution of CHIP over 20 years as a time-varying covariate as the latter approach necessitates accurate capture of other time-varying confounders over time, which may add to the complexity and scope of the proposed analysis.

<u>Limitations</u>:

- Small number of events in the subgroup of CHIP
- Separate analyses in V1-3 and V5 cohorts of participants

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

b. If Yes, is the author aware that the file ICTDER03 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? Done.
(This file ICTDER 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

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"? Confirmed

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

Yes

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)?

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

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)* (1998.03, 2017.24**)**

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

12a. 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.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PUBMED Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to Pubmed central.

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