1.a. Full Title:

High-Risk Lipid Phenotype and Incidence of Coronary Heart Disease and Ischemic Stroke and Cardiovascular Mortality Risk Stratified by Monocyte Count

b. Abbreviated Title (Length 26 characters):

Lipid phenotype and monocytes

2. Writing Group:

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

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3. Timeline: 1yr
4. **Rationale:**

Clinical studies have shown that higher white blood cell counts, and in particular monocyte counts, in the setting of acute coronary syndromes, correlate with worse coronary disease and overall outcomes \(^1\)\(^2\). It has also been shown in the ARIC cohort that asymptomatic adults with higher WBC, monocyte, and granulocyte counts have a higher risk of cardiovascular events and higher mortality \(^3\). Many other cohort studies have also shown an association between WBC and incident CVD. Other studies have further suggested that BMI, HDL and triglycerides may be correlated with monocyte and neutrophil counts \(^4\), but the interaction of high-risk lipid phenotypes and differential WBC counts, particularly monocytes, with CHD occurrence has yet to be studied in a large or racially diverse group.

A low HDL level is a well-accepted risk factor for development of atherosclerosis, though how HDL is atheroprotective still remains unclear. One theory is that HDL reduces CVD risk via its ability to promote cholesterol efflux, which was supported by a recent human study that showed cholesterol efflux capacity was more strongly associated than was HDL level alone with atherosclerosis \(^5\). In addition, our laboratory has shown in animal models that when cholesterol efflux is disrupted, a myeloproliferative phenotype marked by hematopoietic stem cell expansion, leukocytosis, increased monocytes and granulocytes, and systemic foam cell accumulation develops \(^6\)\(^7\). These animals also have accelerated atherosclerosis when placed on a high fat diet, i.e. in the setting of high LDL.

These studies suggest a link between HDL and differential WBC counts, particularly monocytes, which is magnified in the setting of hypercholesterolemia. Moreover, they suggest that an elevated monocyte count may be a simple functional marker of reduced cholesterol efflux. Thus, the presence of elevated monocyte counts in patients with low HDL and high cholesterol may identify a higher risk group, which is even more susceptible to development of cardiovascular events and death.

5. **Main Hypothesis/Study Questions:**

Hypothesis 1: The association of elevated monocyte counts with incident CVD events and mortality is enhanced in the presence of a high-risk lipid profile (HDL ≤ 40mg/dL for men, HDL ≤ 50mg/dL for women and LDL ≥ 160mg/dL); that is, there is an interaction between a high-risk lipid profile and elevated monocyte counts.

Hypothesis 2: The association of elevated white blood cell and neutrophil counts with incident CVD events and mortality is enhanced in the presence of a high-risk lipid profile (HDL ≤ 40mg/dL for men, HDL ≤ 50mg/dL for women and LDL ≥ 160mg/dL); that is, there is an interaction between a high-risk lipid profile and elevated white blood cell and neutrophil counts.

Additional study question: What are the independent correlates of monocytosis in the ARIC cohort?
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:** ARIC Study population-based cohort

**Inclusion/Exclusion:** We propose to include all men and women, aged 45-64 years enrolled in the ARIC Study cohort study from 1987-1989. Exclusion criteria are: history of coronary heart disease or stroke, cancer at baseline, WBC count > 15,000 cells/mm$^3$ or <2,000 cells/mm$^3$, and those missing baseline WBC counts with differential or lipid measurements.

**Outcome Variables:** Incident cardiovascular events (coronary heart disease and ischemic stroke events) and coronary disease death as previously defined and validated by ARIC Study Investigators.

**Data Analysis:**

For Hypothesis 1 & 2:

Monocyte levels will be dichotomized, with the elevated group defined as an absolute monocyte count of \( \geq 0.44 \times 10^9/L \). WBC and neutrophil levels will also be dichotomized with elevated WBC count defined as an absolute WBC count of \( \geq 7 \times 10^9/L \) for whites and WBC count \( \geq 6 \times 10^9/L \) for blacks, and elevated neutrophil count defined as an absolute neutrophil count of \( \geq 4.5 \times 10^9/L \) for whites and WBC count \( \geq 3.5 \times 10^9/L \) for blacks.

Lipid risk will be a categorical variable with the following categories:

1. High-risk profile defined by low HDL and high LDL: HDL \( \leq 40\)mg/dL for men, HDL \( \leq 50\)mg/dL for women and LDL \( \geq 160\)mg/dL
2. Low-risk profile defined by normal HDL and LDL: HDL > 40mg/dL for men, HDL > 50mg/dL for women and LDL < 160mg/dL
3. Isolated low HDL: HDL \( \leq 40\)mg/dL for men, HDL \( \leq 50\)mg/dL for women
4. Isolated high LDL: LDL \( \geq 160\)mg/dL

The model will test the interaction between monocyte, WBC and neutrophil counts and lipid categorical variables. Multivariable adjustment will be made using Cox proportional hazards regression for systolic blood pressure and antihypertensive medications, diabetes, triglycerides, BMI, age, sex, smoking, race, cholesterol lowering medications, physical activity and alcohol intake.

The relative risk from monocytes in the high-risk lipid category will be compared with the relative risk from the monocytes in each of the other lipid risk categories. The statistical significance will be given by the interaction term’s p-value.
While recognizing that the actual analysis will be by Cox proportional hazards modeling, we will tabulate event rates in the different risk categories to look at unadjusted relative risks from monocytes and interaction with lipid categories as follows:

<table>
<thead>
<tr>
<th>Monocytes ≥ 0.44 x 10^9/L</th>
<th>Monocytes ≥ 0.44 x 10^9/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>High-risk lipid profile</td>
<td>Low-risk lipid profile</td>
</tr>
<tr>
<td>Isolated low HDL</td>
<td>Isolated high LDL</td>
</tr>
</tbody>
</table>

**All of above analysis will also be done for WBC and neutrophil counts.**

**For additional study question:**

To assess correlates of monocyte counts, independent samples t-test and the \( x^2 \)-test will be done to compare continuous and categorical variables, respectively, among the two monocyte groups. Variables will include age, sex, race, HDL, LDL, triglycerides, BMI, diabetes, smoking, HTN, alcohol intake and physical activity. We will also use multiple logistic regression to test the independent association of these factors to high/low monocyte levels.

**Methodological Limitations:**

The definition of elevated monocyte count is not standardized as “normal” averages differ among gender, race, and ethnicity. Each laboratory has an internal normal range that is determined by the instrument on which blood samples are run and recommendations from the Clinical and Laboratory Standards Institute. The definition of elevated monocyte count proposed for this study is above the average of different populations studied in several epidemiologic studies, and thus likely is representative of a higher range monocyte level. Additionally, in a previous study of this population, this cutoff was used to define the highest monocytes quartile and was associated with the highest relative risk of incidence of CVD events or mortality. Similar limitations and justification apply to cutoffs for WBC and neutrophil counts. Therefore, the cutoffs as adjusted for race may result in different distributions than population based non-adjusted groups. In order to address this, we propose looking at the distributions of the cutoffs for monocytes, neutrophils and WBC counts for the ARIC population as a whole, by race and by gender to assess whether our stated cutoffs will need to be adjusted.

Also, no formal power analysis was performed. We expect that, given ARIC’s size, we will have power to detect clinically meaningful interactions, but small interactions may be missed.

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

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

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”?    ___ Yes    ____ 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)?


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.
13. References:


