ARIC Manuscript Proposal #2753

PC Reviewed: 5/10/16	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: The clinical impact of TC/HDL-C discordance with LDL-C, non-HDL-C and apoB: The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): TC/HDL-C ratio discordance

2. Writing Group:

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

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3. Timeline: We aim to submit this abstract to the American Heart Association Scientific Sessions 2016, for which we are aiming to submit an abstract to the ARIC publications committee in 4 weeks, and the subsequent manuscript within the following 6 months.

4. Rationale:

The role of cholesterol ratios, reflecting the interaction between individual lipoprotein classes, in determining atherosclerotic cardiovascular disease (ASCVD) risk has been recognized since the early phases of the Framingham Heart Study. In the 1980s, Castelli et al. demonstrated that the ratio of Total Cholesterol to High-Density Lipoprotein Cholesterol (TC/HDL-C), which they called summary estimates, provided a convenient measure of ASCVD risk although they were not intended to replace individual lipid measurements[1]. In their study, the TC/HDL-C ratio had incremental prognostic value when added to individual lipid parameters[1] and thus TC and HDL-C were eventually incorporated in worldwide risk estimation scores[2-4]. Since then, many other studies have consistently demonstrated TC/HDL-C's strong association with cardiovascular risk at a population level.[5-10] In the Women's Health Study, TC/HDL-C was better than low-density lipoprotein cholesterol (LDL-C) and as good as or better than non-HDL-C and apolipoprotein fractions in the prediction of future cardiovascular events.[5] In another Women's Health Study, the net reclassification index for adding either apolipoprotein B (apoB) or LDL particle number (LDL-P) to TC/HDL-C was only 2%.[6] Similar results were demonstrated in the Framingham study,[8] Physicians Health Study,[11] and in statin-treated patients.[7] In a meta-analysis of approximately 900,000 patients with 55,000 vascular deaths, TC/HDL-C was suggested to provide 40% more risk information than non-HDL-C.[12]

However, rather than the conventional approach of evaluating relative population risk signals of lipid parameters, the most clinically-relevant question when considering additional parameters would seem to be at the individual patient level. Specifically, in those who have discordance between lipid parameters, does discordance relate to greater atherosclerosis or greater risk of events? In our recent observational study of 1.3 million patients who underwent Vertical Auto Profile (VAP) testing, we found that significant discordance exists between TC/HDL-C vs. LDL-C and non-HDL-C[13] implying potential additional information carried within the ratio. Those with disproportionately high TC/HDL-C were most commonly men and had a more atherogenic lipid phenotype characterized by lower HDL-C and its subfractions, higher TG, TG/HDL-C and LDL density, comparable to the phenotype of those with insulin resistance who have a prevalence of cholesterol-depleted apoB particles.[14-16] Thus, by including the inverse of HDL-C, a higher TC/HDL-C ratio may indirectly reflect discordance between particle cholesterol content and concentration. This novel concept suggests that TC/HDL-C might reflect lipoprotein particle concentration and size information from the standard lipid profile, a hypothesis that

avoids the contentious conviction of an inverse relationship between HDL-C and ASCVD[17]. A recent analysis showed that TC/HDL-C ratio of <3 was the standard lipid profile parameter which best identified a subgroup with LDL-P of <1000 nmol/L.[18] While clinical outcome studies are needed, initial evidence from our TC/HDL-C discordance analysis indicates that TC/HDL-C, available at no extra cost, may carry additional information related to lipoprotein particle concentration and size not available in cholesterol-based measures, a finding of particular importance when discordance exists within individuals. Therefore, we believe that evaluating the clinical impact of TC/HDL-C discordance may have broad implications for ASCVD risk assessment and treatment particularly in the upcoming era of PCSK-9 inhibitor therapy.

In this prospective, community-based analysis of middle age men and women in the Atherosclerosis Risk in Communities (ARIC) study, we aim to examine the impact of TC/HDL-C discordance with LDL-C and non-HDL-C on cardiovascular events.

5. Main Hypothesis/Study Questions:

Aims:

1) To examine discordance between TC/HDL-C ratio and other lipid measurements, such as LDL-C, non-HDL-C and apoB, in relation to cardiovascular risk.

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: We will examine the discordance in prospective associations between TC/HDL-C ratio and other lipid measurements, and cardiovascular events. The baseline for this analysis will be ARIC Baseline (1987-1989).

Exposures: The exposure of interest will be the TC/HDL-C ratio, Friedewald-estimated LDL-C (calculated as TC minus HDL-C minus triglycerides/5 at triglycerides <400 mg/dl), apolipoprotein B (apoB) and non-HDL-C (calculated as TC minus HDL-C). These variables will be dichotomized by the median value (≥median, and < median).

Outcomes: The primary outcome will be atherosclerotic cardiovascular disease (ASCVD) events, defined as incident coronary heart disease (CHD), fatal CHD, and stroke occurring after Baseline Visit through December 31, 2012 (or most recent follow-up available). Incident ASCVD will be defined as definite or probable nonfatal myocardial infarction or fatal CHD, definite or probable stroke (defined as sudden or rapid onset of neurological symptoms that lasted for 24 hours or led to death in the absence of another cause). As secondary outcomes, we will include total mortality occurring after baseline visit through December 31, 2012 (or most recent follow-up available), as well as measures of subclinical atherosclerosis like ankle-brachial index <0.9 and carotid intima media thickness (cIMT) (4^{th} quartile).

Exclusions: We will exclude individuals with missing data for standard lipid profile at baseline. We will exclude participants who were non-black or non-white, as well as blacks from MN and MD sites due to small numbers. We will exclude individuals with a prevalent ASCVD at baseline.

Covariates: Other covariates that will be further included in models are: age, sex, race, education, physical activity (Baecke questionnaire), BMI (in kg/m^2), hypertension (defined as systolic blood pressure ≥140 mmHg, or diastolic blood pressure≥90 mmHg, or current use of antihypertensive medications), diabetes mellitus (defined as fasting plasma glucose ≥126 mg/dl, or self-reported physician diagnosis of diabetes or use of diabetes medications), smoking status, use of lipid-lowering medication. The ASCVD risk score will be calculated using the Pooled Cohort Risk Equations, published in the 2013 ACC/AHA Guidelines.[19] Smoking status, hypertension, as well as use of lipid lowering medications will be evaluated as time varying covariates.

Main Analyses: We will evaluate the discordance between TC/HDL-C ratio and other lipid measurements (Friedewald LDL-C, non-HDL-C, and apoB), and the prospective association between this discordance and our primary (cardiovascular events) and secondary outcomes. For this purpose, we will conduct the following analyses:

1) We will define and calculate proportions for the following categories:
    a. Discordantly low TC/HDL-C ratio: TC/HDL-C ratio < median and alternative lipid parameter (Friedewald LDL-C, non-HDL-C and apoB) ≥median.
    b. Discordantly high TC/HDL-C ratio: TC/HDL-C ratio ≥ median and alternative lipid parameter (Friedewald LDL-C, non-HDL-C and apoB) <median.
    c. Concordantly low TC/HDL-C ratio: TC/HDL-C ratio and alternative lipid parameter (Friedewald LDL-C, non-HDL-C and apoB) < median.
    d. Concordantly high TC/HDL-C ratio: TC/HDL-C ratio and alternative lipid parameter (Friedewald LDL-C, non-HDL-C and apoB) ≥ median.

2) We will create scatter plots of TC/HDL-C ratio vs. Friedewald LDL-C, non-HDL-C and apoB, splitting the plots into categories shown above.

3) We will identify clinical characteristics across 4 discordant/concordant categories defined above, including age, sex, race, BMI, hypertension, diabetes mellitus, physical activity, education, smoking status, or use of lipid-lowering medications. We will also include laboratory measurements such as apoB, LDL-C, non-HDL-C, triglycerides (TG), TC/HDL-C ratio, TG/HDL-C ratio, C-reactive protein.

4) We will construct Cox proportional hazards models to estimate hazard ratios (95% confidence intervals) for each outcome (primary and secondary) associated with each of the four categories we defined, considering the concordantly low (for both TC/HDL-C and alternative measure) as the reference group. We will perform the following models:
    a. Model 1: adjusted by age, sex and race/center

b. Model 2: Model 1 + smoking status + education + physical activity +BMI+ hypertension + diabetes
c. Model 3: Model 2 + TG + HDL-C
d. Model 4: Model 3 + use of lipid-lowering medication (time-varying)
e. We will additionally evaluate for interaction with ASCVD risk (<5%, 5-7.5%, and ≥7.5%), race, sex, age (<65 and ≥65 years-old).

5) We will calculate incidence rates (per 1000 person-years) of each outcome (primary and secondary) for each of the four categories by using Poisson regressions, and using similar adjustments (Model 1-4) as in point (4).

6) We will reproduce point 1-5 using estimated LDL-C by our novel method instead of Friedewald-estimated LDL-C. [20]

7) We will reproduce point (4) and (5) for individuals with TC/HDL-C<2.5 and ≥2.5 vs. Friedewald LDL-C<70 and ≥70 mg/dl, vs. non-HDL-C<89 and ≥89 mg/dl, and vs. apoB<57.9 and ≥57.9 mg/dl. These cutoff points are equivalent population percentiles of LDL-C = 70 mg/dl that were obtained from NHANES.

8) We will also reproduce (4) and (5) using discordance of 10 percentile units between TC/HDL-C and non-HDL-C, Friedewald LDL-C and apoB.

9) We will show results stratified by:
a. ASCVD risk categories
b. Race
c. Sex
d. Age (<65 and ≥65 years-old)
e. Use of lipid-lowering medication at baseline

Sensitivity Analysis:

- We will reproduce all analyses excluding those on lipid lowering therapy at baseline and at any ARIC visit.

Limitations:

- There is the likelihood for some residual confounding by other risk factors not included in these models.
- Interim initiation of lipid lowering medication likely will modify the association between lipid discordance and ASCVD events. We will use a time-varying approach for "use of lipid lowering medication" status and update this variable for every ARIC visit. Still this variable may be incompletely ascertained. For example, we do not have information about type of statin (low vs. high intensity) or dose or about use of lipid lowering medications between ARIC visits.

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
(This file ICTDER03 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

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)?

There is no overlap. No prior ARIC studies have specifically focused on individual level discordance of TC/HDL ratio with other lipid parameters

Similar ARIC Manuscripts:

2587 (Michos) Vitamin D and change in lipids and incident dyslipidemia
Notes: this looked at lipid changes including TC/HDL ratio, but ASCVD events was not an outcome. Michos is a co-author on both.

1623 (Ndumele) ApoB, Apo A, and standard lipid measures with incident CHD
Notes: This used visit 4 data and did not focus on discordance. Salim Virani was a coauthor on that paper and invited here as well.

1667 (Lopez) Lipid levels, lipid lowering medications and incident AFib
Notes: Afib is not one of our outcomes, and we are focusing on lipid discordance.

2046 (Virani) **Relation of cholesterol and lipoprotein parameters with carotid artery plaque characteristics: the Atherosclerosis Risk in Communities (ARIC) carotid MRI study.**

Notes: We are not using ARIC Carotid data, and S. Virani was invited as a coauthor on this paper

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.

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