1. Full Title: Components of variability in the measurement of NT-pro BNP. The ARIC Study

b. Abbreviated Title (Length 26 characters): Variability in NT-proBNP


The first author, _CLA_ confirms that all the coauthors have given their approval for this manuscript proposal.

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3. Timeline:
As part of ancillary study #2009.15, assay runs and data management are in progress; if approved, a draft of manuscript(s) can be submitted within six months.

4. Rationale:
Brain type natriuretic peptide (BNP) is a cardiac hormone secreted by cardiomyocytes in response to pressure and ventricular volume overload[1, 2]. Levels of BNP and the N-terminal fragment of its prohormone (NT-proBNP) are used for diagnosis and monitoring of patients with heart failure. Also, BNP has emerged as a strong prognostic marker in patients with heart disease [3-8] as well as in general population [9, 10].

In addition to current efforts to measure NT-proBNP in stored serum samples obtained from the fourth ARIC study visit an ancillary study was planned to fill in the gaps in current knowledge of the variability (both measurement and biological) of this biomarker. Similarly, there are very few studies examining stability of this NT-proBNP [11]. Also, only few studies have examined how participant characteristics (e.g. variability (e.g. age, sex, race, body mass index and serum level of biomarker)) influence estimates of variability and also the variation in individuals without heart failure.

We will examine whether participant characteristics (e.g. variability (e.g. age, sex, race, body mass index and serum level of biomarker)) influence the variation in NT-proBNP, in individuals...
without heart failure or coronary artery disease. Briefly, in this study we propose to examine several components of variability (laboratory, process, and biological). Process variability (i.e., variability in blood processing, shipping, and laboratory handling and analysis) will be estimated from replicate plasma samples taken on 112 individuals in the ARIC Carotid MRI (CarMRI) study. Biologic + process variation will be estimated from 55 replicate plasma samples taken 4-8 weeks apart in the CarMRI study. Stability of NT-proBNP will be estimated from ARIC visit 4 specimens.

5. Main Hypothesis/Study Questions:

1. Estimate laboratory variability by analyzing 30 split samples of NT-proBNP from participants with past hospitalization with HF (n = 15) and participants without previous HF hospitalizations (n = 15).
2. Estimate process variability (i.e., variability in blood processing, shipping, and laboratory handling and analysis) from replicate plasma samples taken on 112 individuals.
3. Estimate the biologic + process variation from 55 replicate plasma samples taken 4-8 weeks apart.
4. Estimate variation due degradation:
   i. Short-term degradation will not be assessed since expected to be of small magnitude
   ii. Long-term degradation will be estimated from the different storage times of the specimens.

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 methodological limitations or challenges if present).

Data: Assays done per design described in ancillary study #2009.15 will be used.

Statistical analysis: By treating paired measurements as a random effect in a linear mixed effects model, we were able to partition the total variance ($\sigma^2_{TOT}$) into a between-pair (or between-person, $\sigma^2_{BP}$) and within-pair component of variance. The within-pair variance derived from the first split-sample Sub-study 1 corresponds to the laboratory variability. The within-pair component of variance derived from the within-visit reliability Sub-study 2, in which duplicate samples were obtained from participants on the same day, corresponds to an estimate of variation due to blood collection, processing and laboratory analysis ($\sigma^2_e$). In contrast, the within-pair component of variance derived from the between-visit reliability Sub-study 3, in which duplicate samples were obtained from participants at two separate visits, corresponds to an estimate of the within-person (biologic) variation over time plus method variation ($\sigma^2_{WP} + \sigma^2_e$). The proportion of the total variance attributable to between-person variability, or reliability coefficient ($R = \sigma^2_{BP} / \sigma^2_{TOT}$) can be interpreted as the correlation between paired measurements. The following benchmarks were used for characterization of the adequacy of reliability [12]: slight reliability, 0-0.2; fair reliability, 0.21-0.4; moderate reliability, 0.41-0.6; substantial reliability, 0.61-0.8; almost perfect reliability, 0.81-1.0. Based on our sample sizes of 112 and 55 for the two sub-studies, the 95% confidence interval assuming a moderate reliability of 0.60 will have lower limits of 0.48 and 0.44, respectively. The coefficient of variation (CV) is defined as the square-root of the within-pair component of variance divided by the mean of the paired observations multiplied by 100. CV values greater than 10% are generally considered as cause for concern.
Ideally we would have performed the NT-proBNP assays at the time of specimen collection and then re-analyzed the samples at pre-specified time intervals to evaluate rates of sample degradation. While this is not possible, there are alternative methods that could suggest whether the samples have substantially degraded. For example, the ARIC Carotid MRI study participants contributed specimens over the course of three years during the Visit 4 examination. Since the order of the examination visit was selected at random participant characteristics no not systematically differ by study month it is possible to assess the analyte concentrations by month and analyze the association between times since specimen collection. A roughly flat slope would suggest little degradation, whereas a decreasing slope would suggest sample decay. Similarly, we will plot the percent of samples below the level of detection as an index of degradation through time.

**Limitations:** This study will utilize assays stored since ARIC’s Visit 4 examination; hence we expect some degradation. This may result in measured values below the lower limit of detection for some specimens. This is especially so as the original value in individuals without heart disease is expected to be low, which will introduce some methodological challenges.

It is to be noted that the study sample for our between person variability is not same as laboratory/process variability. Hence, total variance would not be equal their sum of these variance component, however, given the samples were similar in attributes we can assume that this is so. These differences in the sample characteristics will also be explored.

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

b. **NA**

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

8.b. **NA**

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. **No overlaps found.**

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)?

**None**

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? **Yes, 2009.15**

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.

**Agreed**
References: