Collagen Turnover Markers, part of the MESA Elasticity Study

October 1, 2016

The collagen turnover markers Procollagen Type III N­Terminal Propeptide (PIIINP) and Collagen Type I Carboxy­Terminal Telopeptide (ICTP) were assessed as part of the MESA Elasticity study (R01 HL098382, PIs David Jacobs and Daniel Duprez) under the hypothesis that fibrotic changes marked by collagen turnover are correlated with health of arteries and other organs.

The subsample represented in the data file ICTP\_PIIINP\_OCT12016.sas7bdat was therefore selected with the original purpose of studying collagen markers and CVD in relation to measurements of a blood pressure waveform, with test-retest reliability between Exam 1 and Exam 5 approximately 10 years later in a random sample of those selected for baseline measurement and who had both the initial blood pressure waveform and a repeat waveform at Exam 5. This design excluded the 1000 randomly selected MESA participants whose blood sample inventory had been depleted in earlier studies (the exclusion of a random sample leaves a random sample), as well as those who were missing the baseline continuous blood pressure waveform measurement. We included 3286 participants in whom we successfully measured PIIINP or ICTP at either Exam 1 or Exam 5 (see Table for description of variables), namely, all with adjudicated CVD prior to 2011 plus a 56% random sample of all remaining participants.

Data analysis must take these design features into account. In SAS this is accomplished by adding the statement WEIGHT COLLAGENWT, which is available in most PROCs. This effectively upweights the random sample control and reconstructs the full MESA sample, minus the MESA 1000 and those who did not have successful blood pressure waveform measurement at baseline. When the CVD cases that occurred before through Followup 8 are the outcome, the design is case cohort. However, for the most part other outcomes are considered, and questions have arisen concerning proper weighting for this design in proportional hazards regression when the CVD cases that occurred before through Followup 8 are not the outcome. Therefore the recommendation is to use other methods for outcome analysis, but in all analyses to upweight the randomly selected controls.

## Laboratory Measurements

*Markers of Collagen Turnover*

Blood was drawn following a 12 hour fast, and EDTA plasma was stored at −70oC. EDTA plasma PIIINP (µg/L) and ICTP (µg/L) were selected as measures reflecting type III and type I collagen turnover, respectively. Assays were performed in the Molecular Epidemiology and Biomarker Research Laboratory (University of Minnesota, under direction of Myron Gross, PhD) using competitive radioimmunoassay kits (UNIQ #06099, ICTP and UniQ #06098 PIIINP, Orion Diagnostica, Espoo, Finland). In the PIIINP assay, a known amount of labeled PIIINP and an unknown amount of unlabeled PIIINP in the sample compete for the limited number of high affinity binding sites of the antibody. After separating the free antigen the amount of labeled PIIINP in the sample tube is inversely proportional to the amount of PIIINP in the sample. The description of the ICTP assay is parallel to that for PIIINP, using an ICTP specific antibody. The concentrations in unknown samples are obtained from a calibration curve. Coefficients of variation for internal quality control samples during the main runs were 9.3% for PIIINP high control, 16.5% for PIIINP low control, 6.3% for ICTP high control, and 8.8% for ICTP low control. Exam 1 samples were run between September, 2014 and February, 2015. Exam 5 samples, which were included in the original design to study long term stability of the collagen turnover markers, were all run on 3 days during February, 2015; QC sample values were similar to those for days when Exam 1 samples were run. The method was compared to the same methods used at the University of Vermont through exchange of samples; findings were comparable in the two laboratories.

Table 1. Description without case weighting.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **Label** | **N** | **Mean** | **Std Dev** | **Minimum** | **Maximum** |
| **Idno** | **Idno** | 3286 | 5635207 | 1759705 | 3010040 | 8024995 |
| **ICTPmigL1** | **Exam 1 ICTP microg/L** | 3247 | 3.407949 | 1.362961 | 1.12 | 23.45 |
| **PIIINPmigL1** | **Exam 5 PIIINP microg/L** | 3239 | 5.49498 | 1.542453 | 1.32 | 27.71 |
| **PIIINPmigL5** | **Exam 5 PIIINP microg/L** | 444 | 5.365631 | 1.479751 | 2.9 | 14.02 |
| **ICTPmigL5** | **Exam 1 ICTP microg/L** | 483 | 4.226853 | 1.963945 | 1.64 | 18.96 |
| **casecon** | **CVD cases before FU8=1 random control=0** | 3282 | 0.191042 | 0.393182 | 0 | 1 |
| **randomsample** | **random sample excl CVD cases not randomly selected** | 3016 | 1 | 0 | 1 | 1 |
| **collagenwt** | **case weight used with casecon design** | 3282 | 1.63561 | 0.308929 | 1 | 1.785714 |
| **lnICTPmigL1** | **Exam 1 ln(ICTP) ln(microg/L)** | 3247 | 1.167219 | 0.330524 | 0.113329 | 3.154871 |
| **lnPIIINPmigL1** | **Exam 5 ln(PIIINP) ln(microg/L)** | 3239 | 1.670734 | 0.251776 | 0.277632 | 3.321793 |
| **lnICTPmigL5** | **Exam 1 ln(ICTP) ln(microg/L)** | 483 | 1.367818 | 0.361062 | 0.494696 | 2.942332 |
| **lnPIIINPmigL5** | **Exam 5 ln(PIIINP) ln(microg/L)** | 444 | 1.647275 | 0.24955 | 1.064711 | 2.640485 |
| **ICTPmigL51** | **Exam 5 - Exam 1 ICTP microg/L** | 474 | 0.976688 | 2.251582 | -7.81 | 16.52 |
| **PIIINPmigL51** | **Exam 5 - Exam 1 PIIINP microg/L** | 435 | -0.00398 | 1.941298 | -6.92 | 7.85 |
| **ICTPmigL1std** | **Exam 1 ICTP Std Mean 3.3817191 SD 1.7669005** | 3247 | 0.014845 | 0.771386 | -1.28005 | 11.3579 |
| **PIIINPmigL1std** | **Exam 1 ICTP Std Mean 5.4804818 SD 2.0381255** | 3239 | 0.007114 | 0.7568 | -2.04133 | 10.90684 |
| **ICTPmigL5std** | **Exam 1 ICTP Std Mean 4.2325328 SD 2.7008163** | 483 | -0.0021 | 0.727167 | -0.95991 | 5.452969 |
| **PIIINPmigL5std** | **Exam 1 ICTP Std Mean 5.3688262 SD 2.0300520** | 444 | -0.00157 | 0.728923 | -1.21614 | 4.261553 |
| **lnICTPmigL1std** | **Exam 1 ln(ICTP) Std to Mean 1.1606440 SD 0.4349727** | 3247 | 0.015115 | 0.759873 | -2.40777 | 4.584717 |
| **lnPIIINPmigL1std** | **Exam 5 ln(PIIINP) Std to Mean 1.6683147 SD 0.3332161** | 3239 | 0.007261 | 0.755592 | -4.17352 | 4.962181 |
| **lnICTPmigL5std** | **Exam 1 ln(ICTP) Std to Mean 1.3684784 SD 0.4943160** | 483 | -0.00134 | 0.730427 | -1.76766 | 3.183901 |
| **lnPIIINPmigL5std** | **Exam 5 ln(PIIINP) Std to Mean 1.6475988 SD 0.3417455** | 444 | -0.00095 | 0.730221 | -1.70562 | 2.905338 |

Table 2. Description with case weighting.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **Label** | **N** | **Mean** | **Std Dev** | **Minimum** | **Maximum** |
| **Idno** | **Idno** | 3282 | 5662986 | 2243415 | 3010040 | 8024995 |
| **ICTPmigL1** | **Exam 1 ICTP microg/L** | 3246 | 3.383638 | 1.70611 | 1.12 | 23.45 |
| **PIIINPmigL1** | **Exam 5 PIIINP microg/L** | 3238 | 5.481886 | 1.964969 | 1.32 | 27.71 |
| **PIIINPmigL5** | **Exam 5 PIIINP microg/L** | 441 | 5.360117 | 1.936371 | 2.9 | 14.02 |
| **ICTPmigL5** | **Exam 1 ICTP microg/L** | 480 | 4.228813 | 2.595394 | 1.64 | 18.96 |
| **casecon** | **CVD cases before FU8=1 random control=0** | 3282 | 0.116802 | 0.410828 | 0 | 1 |
| **randomsample** | **random sample excl CVD cases not randomly selected** | 3013 | 1 | 0 | 1 | 1 |
| **lnICTPmigL1** | **Exam 1 ln(ICTP) ln(microg/L)** | 3246 | 1.161122 | 0.419337 | 0.113329 | 3.154871 |
| **lnPIIINPmigL1** | **Exam 5 ln(PIIINP) ln(microg/L)** | 3238 | 1.668537 | 0.3212 | 0.277632 | 3.321793 |
| **lnICTPmigL5** | **Exam 1 ln(ICTP) ln(microg/L)** | 480 | 1.36765 | 0.474488 | 0.494696 | 2.942332 |
| **lnPIIINPmigL5** | **Exam 5 ln(PIIINP) ln(microg/L)** | 441 | 1.646332 | 0.326642 | 1.064711 | 2.640485 |
| **ICTPmigL51** | **Exam 5 - Exam 1 ICTP microg/L** | 474 | 0.988792 | 2.965982 | -7.81 | 16.52 |
| **PIIINPmigL51** | **Exam 5 - Exam 1 PIIINP microg/L** | 435 | 0.012745 | 2.542762 | -6.92 | 7.85 |
| **ICTPmigL1std** | **Exam 1 ICTP Std Mean 3.3817191 SD 1.7669005** | 3246 | 0.001086 | 0.965595 | -1.28005 | 11.3579 |
| **PIIINPmigL1std** | **Exam 1 ICTP Std Mean 5.4804818 SD 2.0381255** | 3238 | 0.000689 | 0.964106 | -2.04133 | 10.90684 |
| **ICTPmigL5std** | **Exam 1 ICTP Std Mean 4.2325328 SD 2.7008163** | 480 | -0.00138 | 0.960967 | -0.95991 | 5.452969 |
| **PIIINPmigL5std** | **Exam 1 ICTP Std Mean 5.3688262 SD 2.0300520** | 441 | -0.00429 | 0.953853 | -1.21614 | 4.261553 |
| **lnICTPmigL1std** | **Exam 1 ln(ICTP) Std to Mean 1.1606440 SD 0.4349727** | 3246 | 0.0011 | 0.964053 | -2.40777 | 4.584717 |
| **lnPIIINPmigL1std** | **Exam 5 ln(PIIINP) Std to Mean 1.6683147 SD 0.3332161** | 3238 | 0.000668 | 0.96394 | -4.17352 | 4.962181 |
| **lnICTPmigL5std** | **Exam 1 ln(ICTP) Std to Mean 1.3684784 SD 0.4943160** | 480 | -0.00168 | 0.959888 | -1.76766 | 3.183901 |
| **lnPIIINPmigL5std** | **Exam 5 ln(PIIINP) Std to Mean 1.6475988 SD 0.3417455** | 441 | -0.00371 | 0.955804 | -1.70562 | 2.905338 |

\*SAS CODE to create variables after assembling the different data flows;

if casecon = **1** then collagenwt = **1**;

else collagenwt = **1**/**0.56**;

lnICTPmigL1 = log(ICTPmigL1);

lnPIIINPmigL1 = log(PIIINPmigL1);

lnICTPmigL5 = log(ICTPmigL5);

lnPIIINPmigL5 = log(PIIINPmigL5);

ICTPmigL51 = ICTPmigL5 - ICTPmigL1;

PIIINPmigL51 = PIIINPmigL5 - PIIINPmigL1;

ICTPmigL1std = (ICTPmigL1 - **3.3817191** )/ **1.7669005**;

PIIINPmigL1std = (PIIINPmigL1 - **5.4804818** )/ **2.0381255**;

ICTPmigL5std = (ICTPmigL5 - **4.2325328** )/ **2.7008163**;

PIIINPmigL5std = (PIIINPmigL5 - **5.3688262** )/ **2.0300520**;

lnICTPmigL1std = (lnICTPmigL1 - **1.1606440** )/ **0.4349727**;

lnPIIINPmigL1std = (lnPIIINPmigL1 - **1.6683147** )/ **0.3332161**;

lnICTPmigL5std = (lnICTPmigL5 - **1.3684784** )/ **0.4943160**;

lnPIIINPmigL5std = (lnPIIINPmigL5 - **1.6475988** )/ **0.3417455**;