The relative ODC mRNA concentration, expressed as number of copies, was higher in tumor tissue than in nontumor tissue (1.41-fold) and showed great variability in both tissues. We observed that in six of nine cases (67%), tumor to nontumor ratios (T:NT) for ODC mRNA expression were >1 (mean T:NT ratio, 3.3). Patients 3, 6, and 7 (33%) showed the most markedly increased ratios (Table 1). Examination of our results, expressed as number of copies of ODC mRNA, showed a notable overlapping of the range of values obtained in the two tissues. For this reason, overexpression cannot be determined by comparing a tumor tissue value with the mean value of all the nontumor samples. However, the ODC mRNA T:NT ratio may be used to establish associations with other prognostic markers (e.g., tumor histology, sex, and age). For example, the two patients with the highest ratios (patients 3 and 6) presented several negative prognostic markers, including age, location of the lesion, metastasis, and plasma tumor markers (Table 1).

Our results obtained with LightCycler technology, expressed as the T:NT ratio (3.35 ± 3.84), were similar to data obtained by Yoshida et al. (2.1 ± 0.9) (2) and Radford et al. (4.19 ± 2.76) (3), using Northern blot analysis. Moreover, the percentage of cases with a ratio >1 (67%) was also in keeping with the results reported by these authors, supporting that increased ODC mRNA may represent an important feature of the neoplastic state in the colon.

In conclusion, the real-time RT-PCR assay for ODC mRNA quantification using the PBGD housekeeping gene as an internal control is a fast and highly reproducible method that is suitable for analysis of small tissue samples. Its ease makes it applicable to large series of patient samples as a routine laboratory method. We believe that studies in larger series with the method described here could provide definitive results as to the role of ODC expression in colon neoplasm.

We thank Dr. Carmen Ricós for suggestions regarding quality control of the method, Roche Diagnostics for technical support, and Celine Cavallo for linguistic advice.

Stability of Novel Plasma Markers Associated with Cardiovascular Disease: Processing within 36 Hours of Specimen Collection, Jennifer K. Pai,1,3* Gary C. Curhan,1,5 Carolyn C. Cannuscio,1 Nader Rifai,5,8 Paul M. Ridker,1,3,6 and Eric B. Rimm1,2,5 (Departments of 1 Epidemiology and Nutrition, Harvard School of Public Health, Boston, MA 02115; Divisions of 3 Preventive Medicine and 4 Cardiology, 5 Channing Laboratory, Department of Medicine, and 6 Center for Cardiovascular Disease Prevention, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA 02115; 7 Merck & Co., Inc., West Point, PA 19422; 8 Department of Pathology, Children’s Hospital Medical Center and Harvard Medical School, Boston, MA 02115; * address correspondence to this author at: Harvard School of Public Health, Department of Epidemiology, 677 Huntington Ave., 9th Floor, Kresge Bldg., Boston, MA 02115; fax 617-566-7805, e-mail jpai@hsph.harvard.edu).

Plasma markers are ideally measured prospectively because marker concentrations may change after diagnosis with disease. In many large prospective studies, such as the Nurses’ Health Study (1) and Health Professionals Follow-up Study (2), blood samples are collected, placed on ice, and mailed back to the central laboratory for processing via overnight or next-day mail service. Because economic constraints typically lead researchers to collect only one sample per study participant (3, 4), it is important to determine the time frame within which markers remain stable and to optimize the methods for handling and processing the sample. Although many studies have examined marker stability after long-term storage, few studies have assessed the impact of transport conditions on whole blood not immediately processed.

We selected a series of lipid and novel inflammatory markers whose concentrations have been shown or have been suspected to influence the risk of cardiovascular disease: C-reactive protein (CRP), fibrinogen, lipoprotein (a) [Lp(a)], apolipoprotein B (apoB), intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), p-selectin, von Willebrand factor (vWF), oxidized LDL (oxLDL), anti-cardiolipin antibodies (aCLAbs), tumor necrosis factor receptors I and II (TNF-R1 and TNF-RII), and matrix metalloproteinase-1 (MMP-1) (5–13). The purpose of this study was to evaluate the stability of selected novel markers of cardiovascular disease under time and temperature conditions that simulated sample transport by mail for up to 36 h before processing.

We included 17 premenopausal women, 25–45 years of age, who had responded to a recruitment advertisement. Blood samples were collected from each woman into three 15-mL Vacutainers containing sodium heparin and placed on ice until processing. The time to processing was defined as the time from when the samples were first placed on ice until they were centrifuged to separate the plasma from the cells, aliquoted into 2-mL tubes, and finally stored in liquid nitrogen (−140 °C) until analysis. One sample was processed immediately and defined as the baseline at 0 h. The second and third samples were
sealed in styrofoam packing materials with a cold pack, as was used in our main study. The second sample was processed 24 h later to simulate overnight mail service, and the third sample was processed 36 h later to simulate next-day mail service. One sample from each time point, from each individual, was then sent to the laboratory for analysis. This project was approved by the Institutional Review Board at the Harvard School of Public Health, and all participants gave informed consent for the blood.

All 13 biomarkers were assayed by the laboratory of Dr. Nader Rifai (The Children’s Hospital, Boston, MA). apoB and fibrinogen were measured with immunoturbidimetric assays on the Hitachi 911 analyzer (Roche Diagnostics), using reagents and calibrators from Wako (Wako Chemicals USA) and Kamiya Biomedical Co., respectively. CRP and Lp(a) were measured spectrophotometrically on the Hitachi 911 analyzer (Roche Diagnostics), using reagents and calibrators from Denka Seiken. Soluble ICAM-1 and VCAM-1, p-selectin, TNF-RI, TNF-RII, and MMP-1 were measured with ELISAs from R&D Systems. aCLAbs were screened using an ELISA from Alpco (Alpco Diagnostics). oxLDL was measured with an ELISA from Mercodia (Alpco Diagnostics), and vWf was measured with an ELISA from American Diagnostica. All samples were thawed together and analyzed in a single analytical run to minimize the contribution of run-to-run variability. To ensure blinding of laboratory personnel, each sample was assigned a different identification number and placed randomly in the analysis batch with respect to the three different processing times.

All markers were log-transformed to improve normality. The distribution of each marker was expressed as the mean concentration and 95% confidence intervals derived from the SD of all women at each time point. The mean differences were calculated using the paired t-test. For the convenience of the reader, results were transformed back to the original scale, and geometric means, 95% confidence intervals, and differences are presented. The between- and within-person variances were calculated by ANOVA, accounting for repeated measures. We calculated intraclass correlation coefficients (ICCs) by dividing the between-person variance by the sum of the between-and within-person variances (14). ICCs provide the proportion of the total variance that can be explained by the between-person variance.

Shown in Table 1 are the mean and 95% confidence interval for each inflammatory marker at 0, 24, and 36 h and the mean differences over time for 0–24 h and 0–36 h. The mean values for CRP, apoB, oxLDL, ICAM-1, VCAM-1, TNF-RII, and aCLAbs were very stable and statistically consistent up to 36 h until processing. Lp(a) and fibrinogen showed some degradation after initial collection, whereas TNF-RII significantly increased with longer time to processing. The delay in processing also had significant effects on p-selectin, vWF, and MMP-1.

An ICC of 0.99 means that 99% of the variation is explained by between-subject variation and 1% is explained by within-subject variation. Therefore, a low within-subject variation would suggest minimal instability attributable to processing methods. An ICC <0.4 indicates poor reproducibility, 0.4 < ICC < 0.75 indicates fair to good reproducibility, and ICC >0.75 indicates excellent reproducibility (14).

The majority of these biological markers had good to excellent reproducibility, with ICCs of 0.59–0.99. The ICCs for CRP, Lp(a), and aCLAbs were all >0.9 for 0–24 and 0–36 h. Fig. 1 shows the stability of CRP between 0 and 24 h compared with 0 and 36 h. oxLDL, TNF-RI, apoB, TNF-RII, and ICAM-1 all had ICCs that were >0.75 for 0–24 and 0–36 h. VCAM-1 exhibited slightly lower reliability, with ICCs for 0–24 and 0–36 h of 0.59 and 0.61, respectively. Fibrinogen had low ICCs of 0.27 for 0–24 and 0.48 for 0–36 h. p-Selectin and MMP-1 had very poor ICCs of 0.14 and 0.16, respectively, at 0–24 h. The 0–36 h differences for p-selectin and MMP-1, and the 0–24 h differences for vWF yielded negative ICCs, which demonstrates the instability of these markers when not processed immediately.

Few studies have examined time-to-processing conditions up to 36 h. The majority of our markers showed excellent stability and reproducibility when processed within 36 h of collection time and were not significantly different from samples processed immediately after venipuncture. In our study, fibrinogen concentrations did not change significantly up to 24 h, but had changed significantly by 36 h. Two extreme values likely had skewed these results because previous work had demonstrated somewhat more stability. Other groups have reported concentrations that did not vary significantly from baseline for at least 1 week in whole blood stored at 4 °C (15) and changes of <10% after 7 days in plasma stored at 6 °C (16). An additional analysis that excluded those two outlier points improved our intraclass correlation to 0.51 for 0–24 h and 0.53 for 0–36 h. Nonetheless, despite improvements and attempts to standardize assays over time, intra- and interassay CVs for fibrinogen have remained high (17). p-Selectin and vWF showed little stability from 0 to 24 to 36 h. At 4 °C, platelets probably continue to produce selectin (18) and binding of vWF may be impaired (19).

Our results for apoB showed no significant changes up to 36 h and were consistent with those reported previously. Hankinson et al. (4) demonstrated stability of apoB in blood chilled (–9 °C) up to 24 and 48 h until time to processing. Although long-term storage has been studied (20, 21), the stability of Lp(a) in samples with a time to processing of up to 36 h has not been reported. We found that Lp(a) concentrations were stable up until 24 h from venipuncture, but less so when processing was delayed up to 36 h.

Although aCLAbs are influenced by storage and thawing procedures (22), our study showed that aCLAbs are stable on ice for up to 36 h. Temperature dependence had been shown for ICAM-1 and VCAM-1 from frozen cells (23). However, neither of those two markers in serum had been explored for time-to-processing stability. Although short-term processing stability had not been assessed for CRP, Kayaba et al. (24) examined 5-year intrapersonal
correlation for CRP and reported reasonable reliability. This suggests that CRP would be stable when plasma is stored at or below ~80 °C. To our knowledge, MMP-1, oxLDL, TNF-RI, and TNF-RII have not been studied for time-to-processing stability, temperature dependence, or long-term stability. MMP-1 showed more variation from 0 to 24 h than any other marker and would not be stable if kept on ice for 24 h.

CRP, oxLDL, apoB, aCLAbs, TNF-RII, ICAM-1, and VCAM-1 would all remain stable if kept on ice for 36 h until processing. TNF-RI, Lp(a), and fibrinogen appear to be stable up to 24 h until processing, whereas p-selectin, vWF, and MMP-1 should be processed immediately.

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References

Table 1. Selected plasma marker valuesa at 0, 24, and 36 h until sample processing.

<table>
<thead>
<tr>
<th>Marker</th>
<th>0 h</th>
<th>24 h</th>
<th>36 h</th>
<th>Paired mean difference (% difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, mg/L</td>
<td>0.58 (0.25–1.33)</td>
<td>0.58 (0.25–1.32)</td>
<td>0.59 (0.26–1.35)</td>
<td>0.092 (0.32)</td>
</tr>
<tr>
<td>TNF-RI, ng/L</td>
<td>963 (909–1020)</td>
<td>1002 (951–1056)</td>
<td>1074 (1015–1136)</td>
<td>0.04 (0.58)</td>
</tr>
<tr>
<td>Fibrinogen, mg/L</td>
<td>4241 (2244–8016)</td>
<td>3650 (1952–6825)</td>
<td>3614 (1974–6616)</td>
<td>1.15 (2.5)</td>
</tr>
<tr>
<td>aCLAbs, units/L</td>
<td>3600 (550–23580)</td>
<td>3780 (580–24629)</td>
<td>4060 (632–26078)</td>
<td>1.51 (2.74)</td>
</tr>
<tr>
<td>ICAM-1, μg/L</td>
<td>239 (227–251)</td>
<td>241 (230–251)</td>
<td>248 (239–258)</td>
<td>0.008 (0.15)</td>
</tr>
<tr>
<td>VCAM-1, μg/L</td>
<td>356 (334–379)</td>
<td>385 (367–404)</td>
<td>381 (362–400)</td>
<td>0.079 (1.34)</td>
</tr>
<tr>
<td>p-Selectin, μg/L</td>
<td>40.5 (37.1–44.2)</td>
<td>67.2 (61.5–73.5)</td>
<td>151.2 (140.8–162.3)</td>
<td>0.501 (13.54)</td>
</tr>
<tr>
<td>vWF, units/L</td>
<td>677 (619–741)</td>
<td>359 (309–416)</td>
<td>535 (483–594)</td>
<td>0.36 (1.36)</td>
</tr>
<tr>
<td>MMP-1, μg/L</td>
<td>0.95 (0.80–1.12)</td>
<td>1.51 (1.33–1.72)</td>
<td>1.39 (0.97–1.94)</td>
<td>0.903 (184.33)</td>
</tr>
<tr>
<td>oxLDL, unit/L</td>
<td>46.8 (44.0–49.8)</td>
<td>47.0 (43.9–50.4)</td>
<td>47.5 (44.4–50.8)</td>
<td>0.005 (0.13)</td>
</tr>
<tr>
<td>Lp(a), mg/L</td>
<td>117 (51.8–265)</td>
<td>105 (44.9–245)</td>
<td>109 (47.9–248)</td>
<td>1.105 (4.49)</td>
</tr>
<tr>
<td>apoB, mg/L</td>
<td>693 (383–1253)</td>
<td>695 (385–1257)</td>
<td>701 (387–1271)</td>
<td>0.03 (0.07)</td>
</tr>
</tbody>
</table>

*aAll markers are log-transformed. Geometric means, 95% confidence intervals, and paired differences of the log means are presented.

**CI, confidence interval.

**Statistically significant changes from the paired t test at *P ≤ 0.001; **P ≤ 0.01; ***P ≤ 0.05.

Fig. 1. Correlation of log-transformed CRP at 0–24 h (○) compared with 0–36 h (●).
Near-Bedside Whole-Blood Cardiac Troponin I Assay for Risk Assessment of Patients with Acute Coronary Syndromes, Fred S. Apple,7 MaryAnn M. Murakami,5 Robert L. Jesse,2 M. Andrew Levitt,9 Alan K. Berger,4 Lesly A. Pearce,5 and Paul Collinson6 (1 Hennepin County Medical Center and University of Minnesota School of Medicine, Department of Laboratory Medicine and Pathology, Minneapolis, MN 55415; 2 Medical College of Virginia, Department of Medicine, Richmond, VA 23225; 3 Alameda County Medical Center–Highland Campus, Department of Emergency Medicine, Oakland, CA 94602; 4 University of Minnesota School of Medicine, Divisions of Cardiology and Epidemiology, Minneapolis, MN 55454; 5 Statistical Consulting, Minot, ND 58703; 6 St. Georges Hospital, Clinical Biochemistry Department, London SW17 0QT, United Kingdom; * address correspondence to this author at: Hennepin County Medical Center, Clinical Laboratories MC 812, 701 Park Ave., Minneapolis, MN 55415; fax 612-904-4229, e-mail fred.apple@co.hennepin.mn.us)

Numerous studies have evaluated cardiac troponin I (cTnI), cardiac troponin T (cTnT), and creatine kinase-MB for risk stratification of acute coronary syndrome (ACS) patients. Two metaanalyses have demonstrated the ability of cTnI or cTnT to predict adverse outcomes (1, 2). A substudy of the FRISC II trial showed that the prognostic value of cTnT in ACS patients could be attributed to its correlation with the underlying severity of coronary artery stenosis (3).

Because of analytical and clinical differences among troponin assays (4–7), the cardiology (8, 9) and laboratory medicine (10) communities have endorsed the need for evidence-based studies before individual assays are accepted into clinical practice. Few studies have investigated the role of point-of-care (POC) testing for assessing adverse outcomes in ACS patients. One study using qualitative POC assays for cTnI and cTnT showed both assays to be independent predictors of cardiac events at 30 days after admission in ACS patients (11).

In a consensus document from the European Society of Cardiology (ESC) and the American College of Cardiology (ACC), myocardial infarction (MI) was redefined as any amount of myocardial necrosis in the presence of myocardial ischemia, as indicated by an increased cardiac troponin (I or T) above the 99th percentile of a reference population (9). Because many troponin assays lack acceptable precision at the 99th percentile cutoff and assay precision may be important for risk stratification, a revised cutoff has been recommended as the index for myocardial damage, as the lowest troponin concentration closest to the 99th percentile that can be measured with 10% imprecision (CV) (5).

We investigated the prognostic value of a whole-blood quantitative POC cTnI assay for risk stratification of ACS patients admitted in routine clinical practice for all-cause death, cardiac death, and cardiac events and evaluated the cutoffs at the 99th percentile and at the 10% CV. This retrospective study was performed at four sites: Hennepin County Medical Center (Minneapolis, MN); Alameda County Medical Center–Highland Campus (Oakland, CA); Medical College of Virginia (Richmond, VA); and Mayday University Hospital (Surrey, United Kingdom). All sites obtained approval for human subject research from their respective institutional review boards.

We enrolled 382 patients consecutively evaluated for ACS. No follow-up information was available for 15 patients, leaving 367 patients for analysis. Whole-blood cTnI measurements by the First Medical Alpha Dx device (Mountain View, CA) (12) were obtained at baseline (admission) and every 4–8 h over 24 h after admission. Specimens were analyzed within 30 min. The baseline and maximum concentrations were used to stratify patients. In addition, whole blood was collected from 402 healthy individuals (187 males and 215 females) to estimate the 99th percentile cutoff for cTnI (0.15 μg/L) determined by nonparametric analysis (8, 13). The median ages for males and females were 48 years (range, 35–69 years) and 46 years (range, 35–67 years), respectively. Reference individuals were not age or sex matched with the ACS population.

Two cutoffs were used for stratification, 0.15 and 0.3 μg/L. The first was at the 99th percentile as recommended in the guidelines of the ESC/ACC (9); the second, recommended by Apple and Wu (5), was the lowest concentration (above the 99th percentile) that gave a 10% CV according to the manufacturer (7). Patient