ride membrane (Millipore). The membrane was blocked with 10 g/L bovine serum albumin solution in PBS and cut into strips. After washing with T-PBS, strips were incubated overnight at 4 °C with serum samples diluted 1:100 by volume or anti-survivin mAbs (1.0 mg/L; Clone 91618.11; Genzyme/Techné). After washing with T-PBS, strips were incubated for 30 min at room temperature with a 1:1000 dilution of rabbit anti-mouse immunoglobulin or a 1:2000 dilution of rabbit anti-human IgG F(ab)2 conjugated with horseradish peroxidase (DAKO). After washing with T-PBS, strips were developed by use of a diaminobenzidine solution (Sigma). Staining patterns of anti-survivin mAbs (lane A) and sera for two patients with gastric cancer (lanes B and C) and two healthy donors (lanes D and E) are shown in Fig. 1B. Anti-survivin mAb and sera from gastric cancer patients recognized the 32-kDa recombinant survivin protein (Hisn-T7-TAT-myc-survivin). No reactivity was found in sera from the two healthy donors.

We demonstrated anti-survivin antibodies in patients with various gastrointestinal cancers. When anti-p53 antibodies were additionally considered, the overall prevalence of antibody reactivity was not increased. However, determination of survivin antibodies may be useful for detecting gastrointestinal cancers.

Survivin expression has been detected in 53.2% of colorectal cancers (6), and mRNA transcripts encoding survivin were detected in 63.5% of recurrent colorectal cancers by a reverse transcriptase-PCR (8). However, Rohayem et al. (11) reported that 4 of 49 colorectal cancer patients (8.2%) and 11 of 51 sera from lung cancer patients (21.6%) reacted with His-tagged recombinant survivin protein in an anti-survivin ELISA. In the present study, antibody responses against survivin were not always apparent in all patients whose cancers expressed survivin. In addition, survivin expression has been detected in 70.7% of breast cancer patients (10), but antibody responses against survivin were 6.3% (1 in 16) of breast cancer patients (unpublished data). Our results and the reported data suggest that the site of tumor origin influences anti-survivin reactivity.

We found anti-survivin reactivity 1 year after operation in a biliary-tract cancer patient who had no anti-survivin antibodies before or shortly after surgery (data not shown). At the time reactivity was detected, a local recurrence of cancer was found. In addition, anti-survivin reactivity in three biliary-tract cancer patients who had high anti-survivin reactivity before their operations did not show a decrease at 1 week after operation (data not shown). In one of three patients, a local recurrence of cancer was found at the time of writing.

References


EDTA Samples Are Stable for Prothrombin Time Measurement by Combined Thromboplastin Reagent, Juha Horsti (Valkeakoski District Hospital, Tampeare University Hospital Laboratory, 37600 Valkeakoski, Finland; fax 358-58-7435, e-mail juha.horsti@tays.fi)

The prothrombin time (PT) test is used to detect preoperative bleeding tendencies and to monitor anticoagulant therapy (prevention of venous thromboembolism, treatment of deep vein thrombosis, primary prevention of myocardial ischemia, acute myocardial infarction, prothrombotic heart valves, atrial fibrillation, and other indications) (1). Anticoagulant therapy is widely used, and requests for PT tests are increasing continually (2). Two major methods are used worldwide to measure PT, namely the “Quick method” and the Owren method (combined thromboplastin reagent). Properties of these methods were compared in a recent study (3). Most studies in the scientific literature have used the Quick method, and the results do not always correspond to the combined thromboplastin method.

The materials of which evacuated tubes are constructed affect the stability of samples for PT assay. The surface of borosilicate glass activates factor VII at 4 °C, which shortens the coagulation time(s) for PT (4, 5). In polypropylene or siliconized borosilicate glass tubes, surface activation is lower.

According to current recommendations, PT measurement should be made within 2 h for samples stored at room temperature or within 4 h of sample collection for samples stored at 4 °C (6–8), or within 8 h for samples stored at room temperature (9). The use of polypropylene tubes and storing evacuated tubes unopened extends PT stability at room temperature or 4 °C to 24 h (10–12). Baglin and Luddington (2) noted an overall mean difference in the International Normalized Ratio (INR) after 3
days of only 0.05 INR units when they used unopened polypropylene tubes. They did not present individual patient INR results (13), however, only mean values. In another study, the mean PT change after 24 h was relatively small, but individual samples changed >0.5 INR units, and the authors recommended storage of samples for no more than 24 h at room temperature (14).

Horsti (15) showed that EDTA plasma is comparable to a citrate sample for PT measurement by combined thromboplastin reagent. EDTA has advantages as anticoagulant (15). In this study, the aim was to ascertain the stability of EDTA plasma at room temperature for measurement of PT by the Owren method. Assays were performed within 2, 4, or 6 h of sampling in evacuated glass tubes (not siliconized).

We studied 62 (glass tubes) paired patient samples chosen without conscious bias from hospital and health center patients on oral anticoagulant therapy. The baseline measurement commenced as soon as possible within 2 h of blood collection.

The glass sample collection tube (Vacutainer cat. no. 367652; Becton Dickinson) contained 0.072 mL of K₂EDTA at a concentration of 75 g/L. The blood draw was 3.0 mL, thus providing an EDTA concentration of 1.5 mg/mL of blood.

The sample needle (Precision Glide, cat. no. 360213; Becton Dickinson) was 0.8 x 38 mm.

After the samples were collected, all sample tubes were centrifuged at 1560g for 10 min at 20 °C to separate plasma. Sample tube 1 (fresh) was opened within 2 h of collection and analyzed. The same sample tube 1 was analyzed 4 h after collection from the opened tube stored at room temperature. Sample tube 2 was opened 6 h after collection and analyzed.

We measured PT (seconds and INR) with an ACL 1000 (Instrumentation Laboratory), a fully automated microcentrifugal analyzer. The coagulation reagent was Nycozyme PT (cat. no. 1002490; International Sensitivity Index, 1.16), and the diluent was citrate-barbiturate buffer (cat. no. 1002881; both from Nycomed Pharma As). Each reaction contained 8 µL of sample, 42 µL of diluent, and 100 µL of reagent. IL Test Reference (cat. no. 97569-00) was used as a calibrator.

The ACL was calibrated with Calibration Reference Plasma (cat. no. 1002437, lot no. 1861000; 100%, INR 1.0; Nycomed Pharma As) and Normal kontrolplasma 160 (NKP 160; cat. no. GHI 160, lot no. Z235P; 100%, INR 1.0; Global Hemostasis Institute). We obtained the following data for ACL PT calibration (n = 6 at every point): 100%, 19.3 s, CV = 0.46%; 50%, 28.2 s, CV = 0.67%; 25%, 42.8 s, CV = 1.07%; R² = 0.997. For ACL quality control during 1 week of routine use of the “pool”, the mean was 17.3 s (n = 46; CV = 2.9%). The within-run CV was 0.65% (n = 10) at a mean of 17.38 s.

For results calculations, we used Microsoft Excel 5.0; the correlation equation for INR was: $y = 1.20x - 0.162; R^2 = 0.998$ (15). Statistical and graphic analysis was by the method of Bland and Altman (13).

The mean baseline value was 2.32 INR, the mean at 4 h after collection was 2.30 INR, and the mean at 6 h after collection was 2.29 INR (Table 1). The mean difference (bias) 4 h after sample collection was 0.02 INR and the ± 2 SD was −0.085 to 0.131 INR (Fig. 1). One sample was out of range, and the difference was 0.22 INR (5%).

The Student t-test 2P value was 0.89 and showed no statistically significant change (P < 0.05). According to the method of Bland and Altman (13), there was no clinically significant difference between results after 4 h.

The mean difference 6 h after sample collection was 0.032 INR, and the ± 2 SD was −0.138 to 0.202. Four samples were out of range, and the differences between results were 0.28 (10%), 0.22 (8%), 0.21 (6%), and −0.15 (4%) INR. The Student t-test 2P value was 0.85 and showed no statistically significant change (P < 0.05). With a probability of 93.5%, there was thus no clinically significant difference between results after 6 h.

Blood samples obtained from 62 patients (glass tubes, not siliconized) showed no statistically significant change in INR values after 4 h (tube opened within 2 h) or 6 h (unopened tube) of storage at room temperature when compared with baseline samples tested within 2 h of collection. According to the Bland-Altman method (13), there was no clinically significant difference for a sample stored for 4 h because 95% of results were within the limits. In the samples stored for 6 h, the changes in four (6.5%) samples were out of limits, and three (5%) were allowed. In previous studies, the allowed clinical change of significance was ≥0.5 INR (10, 12, 14) and <10% (11). In the present study, all out-of-limits differences were

<table>
<thead>
<tr>
<th>Time after sampling</th>
<th>Mean</th>
<th>Difference</th>
<th>± 2 SD limits</th>
<th>Out of 2 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>2.32 INR</td>
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<tr>
<td>4 h</td>
<td>2.30 INR</td>
<td>0.02 (0.9%)</td>
<td>−0.085 to 0.131</td>
<td>1/62</td>
</tr>
<tr>
<td>6 h</td>
<td>2.29 INR</td>
<td>0.03 (1.3%)</td>
<td>−0.138 to 0.202</td>
<td>4/62</td>
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Table 1. Stability of PT in glass tubes (4 and 6 h).

Fig. 1. Comparison of INR results for data obtained at baseline and 4 h after sampling.
The mean difference (bias) was 0.023 INR, and the limits of agreement (± 2 SD) were −0.085 to 0.131 INR.
<0.3 INR at a value of ~3 INR, so we can accept that there is no clinical significance in using samples that have been stored for 6 h in glass tubes. The EDTA sample tubes were not siliconized, and siliconization of tubes in fact improves sample stability.

In the coagulation factor tube, the blood is diluted (1:10) with citrate [0.109 mol/L (3.2%) or 0.129 mol/L (3.8%)], and this changes the sample in an nonphysiologic direction. In the EDTA sample, the blood is in a condition that most resembles physiologic conditions, and this should improve the stability of the sample.

Because the EDTA sample is suitable for many hematologic measurements, its use for coagulation tests affords the possibility of using the same sample for different purposes. This reduces costs, waste, and consumption. The use of the same tube for different purposes is also environmentally sound. After hematologic measurements are made, the sample can be centrifuged to obtain plasma for PT testing. This order of proceeding does not alter the relationship between plasma and cells. In my opinion, this method is suitable for laboratories of different sizes and capabilities for rapid response. The use of the same sample for different kinds of analyses opens up prospects of technical development with analyzers in the future.

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References


Creatine Kinase Isoenzyme MB (CKMB) Controversy: Perimortal Tissue Acidosis May Explain the Absence of CKMB in Myocardium at Autopsy. Marie-Louise L. Boumans,1 Jart H.C. Diris,2 Marius Nap,3 Arno M.M. Muijtjens,4 Jos G. Maessen,5 Marja P. van Dietjien-Visser,6 and Wim T. Hermens7 (1 Cardiovascular Research Institute Maastricht and 4 Department of Educational Development and Research, Maastricht University, 6200 MD Maastricht, The Netherlands; Departments of 2 Clinical Chemistry and 5 Cardiothoracic Surgery, University Hospital Maastricht, 6200 MD Maastricht, The Netherlands; 3 Department of Pathology, Atrium Medical Center Heerlen, 6401 CX Heerlen, The Netherlands; 7 address correspondence to this author at: Cardiovascular Research Institute Maastricht, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands; fax 31-43-3670916, e-mail w.hermens@carim.unimaas.nl)

In patients with acute myocardial infarction (AMI), the activity of creatine kinase isoenzyme MB (CKMB) in plasma consistently accounts for ~15% of the total CK activity (1, 2). By contrast, the CKMB content of cardiac tissue, although sometimes reported to be consistent with the 15% plasma activity of CKMB (2, 3), has also been reported to be negligible in healthy myocardium (4, 5). In these studies, the higher CKMB found in diseased hearts was thought to reflect cellular adaptation to disease. An alternative explanation for low CKMB is its limited thermostability and susceptibility to pH (6).

The objective of the present study was to examine whether inactivation of CKMB, either postmortem or during perimortal tissue acidosis, could explain the absence of CKMB in cardiac tissue at autopsy. The influence of tissue acidosis was studied by exposing heart tissue to pH values of 5.0–7.5. Finally, because myocardial ischemia and tissue acidosis attributable to AMI are located predominantly in the endocardium (7, 8), transmural differences in CKMB content were studied in the hearts of patients who died after AMI.

Slices (1 cm), midway between apex and base, were obtained from 20 hearts of patients (11 males and 9 females) who died from noncardiac causes and without history of cardiac complaints. Mean values for age, autops delay, and heart length and weight (± SD) were 71 ± 15 years, 29 ± 22 h, 167 ± 12 cm, and 467 ± 83 g, respectively. Similar heart slices were obtained from 6 patients who died within 6 h after AMI.

Tissue samples (133 ± 34 mg wet weight) were homogenized as described previously (9), and dry weight (dw) was determined by freeze drying the homogenate in a Leybold Heraeus GT2. CK, CKMBmass, CKMBact, and α-hydroxybutyrate dehydrogenase (HBD) in the supernatant were measured and expressed per milligram of dw of tissue. CK and CKMBact were determined at 37 °C, CK with a N-acetyl-cysteine-activated test from Merck Diagnostics and CKMBact with the Isomune assay of Roche Diagnostics. HBD was measured at 25 °C with the optimized HBD test from Roche Diagnostics. CKMBmass was...