Cardiac Troponin T in Serum as Marker for Myocardial Injury in Newborns, Mauro Panteghini,1∗ Gabriella Agnosletti,2 Franca Pagani,1 and Michele Spandrio3 (1 1st Lab. of Clin. Chem. and 3 Neonatal Intensive Care Unit, Spedali Civili, and 2 Div. of Cardiol., Umberto I Hosp., 25125 Brescia, Italy; * author for correspondence: fax 39-30-3995430)

In neonates, acute perinatal asphyxia may lead to ischemic myocardial damage [1]. In some cases, subendocardial infarction has been documented [2]. Generally, diagnosis of the myocardial injury (MI) is based on clinical findings, suggestive electrocardiographic and echocardiographic patterns [3], decrease in myocardial uptake of thallium [4], and classical creatine kinase (CK)-MB isoenzyme measurement [5]. However, CK-MB in serum cannot be regarded as a cardiac-specific marker in the neonate, and extreme caution should be used in the interpretation of increased CK-MB activity during this period [6]. Cardiac troponin T (cTnT), the structural protein that binds the troponin complex to the tropomyosin molecular strand, has recently been proposed as a more specific biochemical marker for diagnosis of myocardial infarction in the adult population [7]. Here we evaluated the use of cTnT measurement in serum in the diagnosis of MI in newborns, as well as that of the determination of CK-MB mass concentration by a sensitive and specific monoclonal anti-CK-MB antibody-based immunoassay.

Three groups of infants were studied. Group I consisted of 27 preterm infants (gestational age ranging from 28 to 36 weeks) without major respiratory and cardiovascular dysfunctions. Group II was 27 healthy full-term newborns (15 born by vaginal delivery and 12 by cesarean section) with a mean gestational age of 39.7 weeks. Group III was composed of seven infants (four preterm and three term) who demonstrated, during the first 3 days after birth, clinical, electrocardiographic, and echocardiographic signs of MI. In particular, in electrocardiogram (ECG) evaluation, MI was considered to be present when inversion of T waves or ST-segment depression ≥1 mm in more than two precordial leads was noted. Groups I and II underwent a clinical examination, ECG, echocardiogram, and blood collection for the measurement of total CK, CK-MB, and cTnT on day 2 after birth. Group III was evaluated with ECG and echocardiography at presentation and every 24 h until clinical recovery or death. Total CK, CK-MB, and cTnT were measured 12 and 48 h after presentation and, when possible, after 45 days. The protocol of the study was approved by the local Ethical Committee, and parental consent was obtained.

An ELISA (Boehringer Mannheim, Mannheim, Germany) was used to determine cTnT in serum (detection limit, 0.02 μg/L). Total CK activity was measured at 37 °C by the method recommended by the IFCC. CK-MB mass concentrations were determined with the Magic Lite CK-MB assay (Ciba Corning Diagnostic Corp., Medfield, MA). A relative index (RI), i.e., (CK-MB, μg/L/total CK, U/L) × 100, was also calculated. In adults, the upper reference limit (URL) is 0.10 μg/L for cTnT, 175 U/L for total CK, 6.0 μg/L for CK-MB, and 3% for RI.

M-mode, two-dimensional, and pulsed Doppler echocardiographic examinations were performed with an Acuson echocardiograph (Acuson, Mountain View, CA) with a 7.5-MHz transducer. Each patient was examined according to the standards of the American Society of Echocardiography [8], and echocardiograms from MI infants were compared with those of infants without MI of similar gestational age. Left ventricular fractional shortening was calculated by the following formula: [left ventricular-end diastolic diameter (LVDD) − left ventricular-end systolic diameter × 100]/LVDD. Cardiac output was measured by multiplying the aortic flow integral by the area of the aortic orifice. Mitral and tricuspid incompetence were graded as trivial, mild, moderate, and severe.

In group I, four infants were small for gestational age, but none had positive cardiovascular examination and ECG. At echocardiography, LVDD (12.6 ± 1.6 mm) was within the previously reported age-related limits [9] and was positively related to weight (r = 0.71, P < 0.001) and gestational age (r = 0.65, P < 0.001). Fractional shortening (40.8% ± 7.2%) and cardiac output (0.81 ± 0.32 L/min) were also within normal limits, and no signs of pulmonary hypertension were pointed out (mean right ventricular pressure ± SD, 23.9 ± 9.4 mmHg). Trivial mitral incompetence was present in two infants, both born at 28 weeks’ gestation. The incidence of tricuspid incompetence (no more than mild), present in 20 infants, decreased with increase in gestational age.

All healthy full-term newborns (group II) also had negative clinical examination results and normal ECGs and echocardiograms. Furthermore, the method of delivery did not affect the concentrations of biochemical markers studied. Total CK and CK-MB concentrations were significantly (P <0.001) lower in group I than in group II (mean CK ± SE, 246 ± 57 U/L vs 751 ± 102 U/L; mean CK-MB ± SE, 9.8 ± 1.5 μg/L vs 29.1 ± 3.5 μg/L, respectively), even if RI did not change between the two groups (5.1% ± 0.5% vs 4.3% ± 0.3%, P = 0.34), suggesting that the difference in enzyme concentration reflected a difference in skeletal muscle mass. Indeed, total CK and CK-MB values were significantly correlated to birth weight (r = 0.50, P <0.001 for total CK, and r = 0.56, P <0.001 for CK-MB) and to gestational age (r = 0.56, P <0.001 for total CK, and r = 0.60, P <0.001 for CK-MB). cTnT did not change with gestational age (r = 0.20, P = 0.15), and no correlation was found between values for serum cTnT and total CK activity (r = 0.24, P = 0.08) or between cTnT and CK-MB mass concentration (r = 0.21, P = 0.12). However, virtually all healthy children (group I and II) had detectable cTnT (mean, 0.16 μg/L; range, 0.04–0.43 μg/L).

The 95th percentile values for these biochemical markers in healthy newborns were: total CK (preterm), 635 U/L; total CK (term), 2126 U/L; CK-MB (preterm), 26 μg/L; CK-MB (term), 72 μg/L; RI, 8.8%; cTnT, 0.33 μg/L.

Clinical, echocardiographic, electrocardiographic, and
biochemical findings of the newborns with MI are shown in Table 1. Evidence of MI occurred at 2.1 ± 0.7 days. Five infants (infants 1, 2, 4, 5, and 6) had signs of congestive heart failure and two (infants 1 and 2) suffered from cardiogenic shock. Infant 5 had bradyarrhythmias (sinus bradycardia and atrioventricular block with complete right bundle branch block) refractory to atropine and isoproterenol treatment. Patient 1 died on day 4 in consequence of cardiogenic shock. Patient 5 had cardiac arrest on day 2 with unsuccessful cardiac resuscitation; autopsy revealed a pale myocardium, with histologic signs compatible with recent hypoxic damage. Five babies survived. In these subjects, clinical recovery occurred 3–9 days after birth. All had complete normalization of contraction, left ventricular fractional shortening (from 26.0% ± 5.8% at admission to 41.6% ± 2.4% after 45 days), and cardiac output (from 0.63 ± 0.36 L/min at admission to 1.40 ± 0.33 L/min after 45 days).

None of the MI patients had increased CK-MB mass concentration in serum. One infant (infant 6) had a slight increase in total CK but normal CK-MB concentration, suggesting that the increase in CK activity was a result of MM isoenzyme, presumably released from skeletal muscle. By contrast, all infants with congestive heart failure had increased cTnT. In particular, four patients had extremely high values for cTnT (>0.70 μg/L), including the two babies who died. In the two babies of this subgroup who survived, cTnT measured after 45 days was within the reference range of adults.

Our findings demonstrated that serum of apparently healthy infants has a high concentration of CK-MB protein when compared with adult reference limits, the highest values being found in term infants. Therefore, the adult URL for CK-MB is not useful for infants; furthermore, the relationship between enzyme concentration and gestational age suggests also that this finding should be considered when interpreting concentrations of this marker after birth. The reason is probably the known increased synthesis of the B subunit in skeletal muscle of the fetus [10]. This could also account for the increase of RI values over the adult URL and for the constant proportion that the CK-MB fraction represents of the total CK in term and preterm infants. In view of the high and scattered concentrations of CK-MB released from the skeletal muscle in serum of healthy neonates, this biochemical marker was unable to identify the cardiac damage in the neonates with MI, even with a highly sensitive and specific last-generation immunoassay.

Unlike the considered enzymes, the concentration of cTnT in serum on day 2 after birth was independent of gestational age, although the URL was substantially higher than in adults or older children [11]. cTnT is increased also in newborn animals [12]. The reported concentrations of cTnT could have been affected by cross-reactivity with skeletal troponin T in the first-generation cTnT assay that was used [13]. The lack of substantial correlation between CK, CK-MB, or gestational age and cTnT do not support this possibility. Anderson et al. [14] found small amounts of cTnT isoforms in skeletal muscle obtained from aborted fetuses after 14–15 weeks of gestation, and Mesnard et al. [15] showed that cTnT is indeed coexpressed in fetal skeletal muscle, its expression being down-regulated during further development. These developmental changes in troponin T gene regulation could partially explain the increased cTnT in the early neonatal period.

Although the number of patients was relatively small, our study raises the possibility that the serum concentration of cTnT might be of diagnostic value in young children with suspected myocardial damage. As recently discussed [16], great caution is, however, necessary before one can derive definitive conclusions on the value of new biochemical markers in this particular clinical setting.

<table>
<thead>
<tr>
<th>Case</th>
<th>Gestational age, weeks</th>
<th>Birth weight, g</th>
<th>RVP, %SP</th>
<th>CO, L/min</th>
<th>MI/TI</th>
<th>ECG</th>
<th>Total CK, U/L</th>
<th>CK-MB, μg/L</th>
<th>cTnT, μg/L</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>1240</td>
<td>100</td>
<td>0.3</td>
<td>Severe/severe</td>
<td>T inv (V1–V6); ST dep, 2 mm (V2–V5)</td>
<td>87</td>
<td>65</td>
<td>DEC</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>780</td>
<td>150</td>
<td>0.2</td>
<td>Moderate/severe</td>
<td>T inv (V1–V6); ST dep, 2 mm (V2–V5)</td>
<td>173</td>
<td>156</td>
<td>190</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>2050</td>
<td>50</td>
<td>0.8</td>
<td>Absent/moderate</td>
<td>T inv (V1–V6)</td>
<td>237</td>
<td>85</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>2210</td>
<td>43</td>
<td>0.5</td>
<td>Absent/severe</td>
<td>T inv (V1–V4); ST dep, 1 mm (V2–V4)</td>
<td>213</td>
<td>198</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>2685</td>
<td>70</td>
<td>0.7</td>
<td>Severe/severe</td>
<td>T inv (V1–V6); AVB HII; RBBB</td>
<td>940</td>
<td>DEC</td>
<td>66.7</td>
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</tr>
<tr>
<td>6</td>
<td>40</td>
<td>3010</td>
<td>63</td>
<td>0.6</td>
<td>Moderate/moderate</td>
<td>T inv (V1–V6); ST dep, 1 mm (V1–V3)</td>
<td>2260*</td>
<td>525</td>
<td>80</td>
<td></td>
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<tr>
<td>7</td>
<td>41</td>
<td>4050</td>
<td>47</td>
<td>1.3</td>
<td>Moderate/moderate</td>
<td>T inv (V1–V4)</td>
<td>729</td>
<td>227</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

RVP, right ventricular pressure; SP, systemic pressure; CO, left ventricular cardiac output; MI, mitral incompetence; TI, tricuspid incompetence; T inv, T-wave inversion; DEC, deceased; ST dep, ST-segment depression; ND, not done; AVB HII, atrioventricular block (first and second degree); RBBB, right bundle branch block.

* Values higher than respective upper reference limit at birth (see text).

Table 1. Clinical, echocardiographic, and electrocardiographic findings at presentation, and biochemical parameters at 12 h (a) and 48 h (b) after presentation, and after 45 days (c), in newborns with symptomatic myocardial injury.
We thank Francesca Stefani and Cristina Serena for technical assistance. We are also indebted to the nursing staff of the Neonatal Intensive Care Unit for the assistance in the collection of the blood samples.

References

Ferritin Is Not an Indicator of Available Hepatic Iron Stores in Anemia of Copper Deficiency in Rats, Meira Fields,1* Isabelle Bureau,2 and Charles G. Lewis3(1USDA, BHNRC, NRFL, Bldg. 307, Rm. 330, BARC-East, Beltsville, MD 20705-2350; 2visiting scientist, Université Joseph Fourier, La Tronche, France; *author for correspondence: fax 301-504-9062, e-mail fields@307.bhnrc.usda.gov)

Serum ferritin is a sensitive indicator of available iron stores [1], but in certain instances it cannot be used in diagnosis, e.g., in anemias of chronic disease, infections, inflammation, liver disease, and malignancies [2–7]. Iron stores may be normal or increased, though accompanied by increased serum ferritin, in anemias of chronic disorders, aplastic anemia, sideroblastic anemia, and chronic hemolytic anemia. Because ferritin is also a positive acute-phase reactant protein that is increased in inflammation [2], serum ferritin concentration is not a reliable index of available iron stores in individuals with chronic diseases. There is no information, however, on whether ferritin can be used as a marker of available iron stores in the anemia of copper deficiency.

Unlike iron-deficiency anemia, in which body iron stores are usually depleted as evidenced by diminished serum ferritin concentrations, anemia of copper deficiency [8–10] results from increased hepatic iron stores and impaired mobilization and delivery of iron from storage to bone marrow for hem synthesis, leading to iron-deficient erythropoiesis [11]. Can serum ferritin be utilized as a reliable tool to measure available iron stores in anemia of copper deficiency? We evaluated in experimental copper deficiency the potential usefulness of three different concentrations of dietary iron and their effects on iron availability and degrees of anemia. To measure accurately body iron stores, hepatic iron concentration was determined. The reliability of ferritin as an iron index was tested by comparison with hepatic iron concentration.

We fed weanling male Sprague–Dawley rats one of six diets [12] for 6 weeks. All rats were fed either a copper-deficient diet containing 0.6 μg Cu/g diet or a copper-adequate diet containing 6.0 μg Cu/g as analyzed by atomic absorption spectrophotometry. Cupric carbonate and ferric citrate were added to the copper- and iron-deficient diets. Analysis of the diets revealed that the dietary iron was either 19 μg Fe/g (low), 48 μg Fe/g (adequate), or 88 μg Fe/g (high). Added dietary iron was within the concentrations recommended for optimal growth of rodents. Rats were killed after an overnight fast. Livers were removed, rinsed in saline, and portions used for the quantitative analysis of copper and iron concentrations [13]. Blood was collected into heparinized test tubes. Ferritin was measured in plasma with rat ferritin test kit (cat. no. RF69; Ramco Labs., Houston, TX), a sandwich solid-phase enzyme immunoassay. Rat liver ferritin was used as a calibrator. Hematocrit and hemoglobin were measured by conventional procedures.

All data were expressed as mean ± SE and analyzed by ANOVA with two concentrations of copper and three concentrations of iron. The independent effects of copper and iron and the interaction between them were examined. Differences at P < 0.05 were considered statistically significant.

Forty percent of rats fed the copper-deficient diet containing 88 μg Fe/g and 28% of copper-deficient rats fed 48 μg Fe/g died prematurely because of ruptured hearts in the apex. No mortality occurred in either copper-deficient rats fed the low, 19 μg Fe/g diet or any of the copper-adequate controls.

Liver copper and iron, hemoglobin, hematocrit, and ferritin are presented in Table 1. All copper-deficient rats exhibited reduced liver copper compared with copper-adequate rats. The lowest copper concentration was found in copper-deficient rats fed the added concentration of dietary iron. The highest liver iron stores were found in copper-deficient rats fed the fortified concentrations of dietary iron. The combination of copper defi-
ciency with added iron resulted in the most severe anemia, reflected in the lowest hematocrit and hemoglobin. The highest ferritin concentrations were found with the adequate-copper, added-iron diet and the lowest ferritin values with the adequate-copper, low-iron diet.

Correlations between concentrations of ferritin and hepatic iron and ferritin and hematocrit of copper-deficient and copper-adequate rats are shown in Fig. 1A. Plasma ferritin was significantly correlated with hepatic iron concentration ($r^2 = 0.860$) and ($r^2 = 0.738$) in copper-adequate and copper-deficient rats, respectively (Fig. 1). In copper-adequate rats plasma ferritin was significantly correlated with hematocrit ($r^2 = 0.861$) but not correlated with hematocrit ($r^2 = 0.044$) in copper-deficient rats (Fig. 1B).

The results of the present study clearly show that only in copper-adequate rats was there a direct relation between hematocrit and ferritin. The highest hepatic iron concentrations were correlated with the highest concentrations of plasma ferritin and hematocrit, and the lowest concentrations of plasma ferritin predicted the presence of the lowest hemoglobin, hematocrit, and hepatic iron. On the basis of data from copper-adequate rats it is clear that under normal conditions the liver is capable of mobilizing iron and making it available for utilization by hemopoietic tissues for heme synthesis, and therefore ferritin is a sensitive measure to assess available body iron stores. In contrast, there was no relation between degree of anemia, concentrations of hepatic iron, and ferritin in copper-deficient animals. Ferritin should not be used to assess functional liver iron stores in copper deficiency and as such may not provide a clue to potentially serious underlying disorders.

Unlike most iron-deficiency anemias, the anemia of copper deficiency reported herein was not due to depleted iron stores but to hepatic iron overload and an impaired release of iron from body iron stores. Unlike the anemia of iron

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**Table 1. Liver copper and iron, hemoglobin, hematocrit, and ferritin (mean ± SEM).**

<table>
<thead>
<tr>
<th></th>
<th>Copper adequate</th>
<th>Copper deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(High)Fe</td>
<td>(Adequate)Fe</td>
</tr>
<tr>
<td>Liver Cu, µg/g wet wt.</td>
<td>3.9 ± 0.2</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>Liver Fe, µg/g wet wt.</td>
<td>158 ± 17</td>
<td>117 ± 7</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>183 ± 9.0</td>
<td>181 ± 7.0</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>42.0 ± 0.5</td>
<td>38.9 ± 0.7</td>
</tr>
<tr>
<td>Ferritin, µg/L</td>
<td>309 ± 24</td>
<td>264 ± 18</td>
</tr>
</tbody>
</table>

ANOVA

<table>
<thead>
<tr>
<th></th>
<th>Cu</th>
<th>Fe</th>
<th>Cu × Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver Cu</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Liver Fe</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Ferritin</td>
<td>S</td>
<td>NS</td>
<td>S</td>
</tr>
</tbody>
</table>

S = significant ($P < 0.05$); NS = not significant.

High Fe = 88 µg/g diet; adequate Fe = 48 µg/g; low Fe = 19 µg/g.
deficiency that responds to iron supplementation [14], the anemia of copper deficiency should not be treated by iron supplementation but should be treated by either lowering the intake of dietary iron or by chelation therapy [15–17]. As can be seen in the present study, the less severe anemia of copper deficiency was caused by the consumption of a low-iron diet. In contrast, the most severe anemia in copper-deficient rats was induced by consumption of additional concentrations of dietary iron and was associated with the highest concentrations of liver iron. This hepatic iron retention, however, could be toxic [18, 19]. Plasma ferritin did not reflect the magnitude of these abnormalities. This is the first report, however, that demonstrates that serum ferritin, a key conventional laboratory test, is inadequate in identifying anemia and assessing functional iron stores in copper deficiency. This finding may have practical significance to clinicians dealing with cases presenting as anemias of iron deficiency.

References

Electrochemical Enzyme Immunoassay for Serum Prostate-Specific Antigen at Low Concentrations, Sung-Fang Chen, Yan Xu,* and Michael Po-Cher Ip* (Dept. of Chem., Cleveland State Univ., Cleveland, OH 44115 and 1 Dept. of Pathol., MetroHealth Med. Center, Cleveland, OH 44109; *author for correspondence: fax 216-687-9298, e-mail y.xu@popmail.csuohio.edu)

Serum prostate-specific antigen (PSA) has been recognized as a sensitive indicator of recurrent prostate cancer after radical prostatectomy [1–5]. In the past 5 years, numerous PSA assays with improved limits of detection [6–15] have been developed by both clinical researchers and diagnostic assay manufacturers. The rationale behind the development of more sensitive PSA assays (e.g., lower limits of detection) is that the relapse of prostate cancer or the tumor-doubling time after radical prostatectomy can be detected much earlier if patients are monitored with more sensitive assays [16, 17].

Here we report a rapid enzyme immunoassay (EIA) for serum PSA at low concentrations by flow-injection electrochemical detection, which is based on the modification of the Tandem-E®-E PSA assay (Hybritech, San Diego, CA). EIA coupled with electrochemical detection offers low limits of detection with high specificity [18–21]. Electrochemical EIA is usually based on the conversion of an electroinactive substrate to an electroactive product by the enzyme label. In this work, the enzyme was alkaline phosphatase (EC 3.1.3.1), which converted p-aminophenyl phosphate to p-aminophenol [22]. The concentration of p-aminophenol was then determined amperometrically in the flow-injection system.

We had illustrated the flow-injection electrochemical detection system used for this work elsewhere [21]. Basically, it was a BAS chromatograph (Bioanalytical Systems, West Lafayette, IN) without the separation column. The thin-layer electrochemical cell had dual glassy carbon working electrodes (in the parallel mode), a Ag/AgCl (3 mol/L NaCl) reference electrode, and a stainless-steel auxiliary electrode. The detection voltage between the working and reference electrode was set at +500 mV. The system had a custom-built injector with 1-μL sample loop. The carrier fluid (0.1 mol/L Tris, 1 mmol/L MgCl₂, 65 mmol/L oxalic acid, and 0.2 g/L NaN₃ at pH 3.2) was pumped at 0.2 mL/min.

In our assay procedure, Tandem-E® PSA assay kit was used. A set of low-concentration PSA calibrators (0.02, 0.05, 0.1, 0.2, 0.5, and 1.0 μg/L) was prepared through serial dilutions of the PSA calibrators (2, 10, and 50 μg/L) with the zero diluent. The assay was carried out in duplicate as follows: (a) Pipette 100 μL of the zero diluent, calibrators, controls, and serum specimens, as well as 100 μL of anti-PSA antibody—alkaline phosphatase conjugate, into each appropriately labeled test tube; (b) add one anti-PSA antibody-coated bead to each tube and vortex-mix the tubes; (c) shake the test tube rack gently in the water bath of the shaking incubator at room temperature (~23 °C) for 2 h; (d) aspirate the liquid from the tubes and wash the beads with the wash solution (4 × 1 mL); (e)
place each bead into a fresh test tube containing 200 μL of 4 mmol/L p-aminophenyl phosphate (in 100 mmol/L Tris and 1 mmol/L MgCl₂, pH 9.0) and incubate for 5 min at room temperature; (f) pipette 30 μL of 0.5 mol/L oxalic acid into each tube to stop the enzyme reaction (oxalic acid lowers the pH from 9.0 to 3.2) and vortex-mix; and (g) draw the solution from each tube with a syringe and inject the sample into the flow-injection system, where the enzyme product, p-aminophenol, is detected by an amperometric detector.

With our electrochemical EIA procedure, a six-point calibration curve for serum PSA was constructed with oxidative currents of p-aminophenol vs PSA concentrations (Fig. 1A). The calibration curve had a linear dynamic range from 0.02 to 1.0 μg/L with a correlation coefficient of 1.00 and CVs <5.5% over its range. The limit of detection (LOD) of the method was calculated to be 0.008 μg/L, which was defined as the mean signal (n = 19) of the zero diluent + 2 SD. Compared with the Tandem-E PSA assay, which has a LOD of 0.3 μg/L, our assay lowered the LOD by >37-fold.

For serum sample analysis, two PSA calibrators and two PSA controls (which were prepared by serial dilutions of the high-concentration PSA controls provided in the assay kit with the zero diluent) were used. Because there is no internationally accepted PSA reference standard available, we could not study the accuracy of our assay procedure. However, comparing the results of our assay with the Tandem-E PSA assay performed on the Photon ERA® automated immunoassay analyzer (Hybritech) was informative. We carried out a comparison study with serum samples containing PSA ranging from 0.235 to 4.80 μg/L. Because a lower LOD (0.008 μg/L) was obtained by our assay procedure, a 10-fold dilution of sample with the zero diluent was performed before the analysis. The results of our electrochemical EIA were plotted against the results of the Tandem-E PSA assay by a factor of 0.1 (Fig. 1B). On 16 patients’ samples, a good correlation (r = 0.999) was obtained between these two assays, and no bias was observed (m = 1.00).

In conclusion, the electrochemical EIA of PSA offers a much lower LOD (0.008 μg/L) and requires shorter enzyme reaction time (5 min) than those of the Tandem-E PSA assay (LOD 0.3 μg/L, enzyme reaction time 30 min). It has a potential for use in small-volume analysis (only 1 μL sample was injected for final detection), and its LOD may be further improved by increasing the enzyme reaction time.

This work was supported by a research grant from the American Association for Clinical Chemistry.

References

Although previously thought to be produced almost exclusively by the epithelial cells of the prostate [1], prostate-specific antigen (PSA) is produced and secreted by several extraprostatic sources [2, 3]. Considering the high degree of homology of PSA with the human glandular kallikrein (hKGKI) [4, 5] and the activation of the kallikrein–kinin system in peritoneal effusions [6, 7], we undertook the study of PSA distribution and expression in ascitic fluids. Between May 1996 and January 1997 we collected ascitic fluids from 44 consecutive women of ages 29–61 years (mean 49 ± 7) undergoing ultrasound examination and a diagnostic paracentesis. After collection (~10 mL), ascitic fluids were centrifuged at 20 120 g for 20 min at +4 °C and the supernatants stored at −30 °C until processed. Blood samples were also taken, and after clotting were centrifuged at 360 g for 5 min at +4 °C and stored at −30 °C until assay. In 24 patients (ages 28–81 years), the ascites was associated with malignancies (ovarian, pancreatic, breast, gastrointestinal, and lung). In the other 20 patients (18–75 years), ascites arose from chronic liver diseases, bacterial peritonitis, congestive heart failure, thrombosis, and other nonmalignant diseases. Albumin, serum–ascites albumin concentration gradients, total protein, lactate dehydrogenase, and cholesterol concentrations were also measured in the fluids (data not shown) [8]. PSA was measured by two methods [9–11]: a solid-phase two-site IRMA (PSA-RIACT™) from CIS Bio International (Gif-sur-Yvette, France) and a microparticle capture enzyme immunoassay (MELA) (IMX®) from Abbott Labs, Abbott Park, IL. Patients with malignant ascites had not yet received cytotoxic drugs and (or) chemotherapeutic agents before sample collection. Results are expressed as means ± SE. Statistical analyses were performed through the StatView v.4.1 package (Abacus Concepts, Berkeley, CA) on Macintosh Power PC (Apple Computer, Cupertino, CA).

The work was carried out in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Among the 44 patients examined, 41% of ascitic fluids contained detectable amounts of PSA, tested with both assay methods (mean ± SE 0.278 ± 0.045 μg/L, range 0.06–0.78 μg/L, n = 18). Matrix effects of ascitic fluid constituents in the PSA assays were excluded by performing dilutions of samples having high PSA content. A good linearity (r = 0.973) between PSA content and dilution was obtained with the IMx method. The PSA-RIACT (y) and IMx-PSA (x) agreed (n = 15, y = 0.019 ± 0.863x, r = 0.953, P < 0.0001). In agreement with others [12, 13], we found a plasma PSA content ≤0.05 μg/L in ~91% (40 of 44) of the women examined. The PSA mean concentration in our series of ascitic fluid samples did not show a significant difference between malignant-related and nonmalignant ascitic fluids (0.262 ± 0.074 μg/L, 0.297 ± 0.047 μg/L, respectively; t = −0.317271, P = 0.761). The total PSA content in ascitic fluids was significantly greater than in plasma (0.278 ± 0.045 μg/L and 0.032 ± 0.011 μg/L, respectively; n = 18, P < 0.0001). PSA was not statistically significantly correlated with patient’s age.

A major 33-kDa immunoreactive band (due to the free form of this serine protease) was seen on Western blots with an anti-human PSA monoclonal antibody (Dako, Milan, Italy). The 100-kDa immuno-reactive protein due to the α1-antichymotrypsin-bound form was not detected, nor were other bands (Fig. 1).

Several sources could be suggested for PSA expression in ascitic fluids: (a) plasma ultrafiltration and accumulation, at an increased rate in the peritoneal space through a vascular hyperpermeability of the inflamed peritoneal tissue. Previous data have shown the liberation, accumulation, and activation of the kallikrein–kinin system in the pathogenesis of ascitic fluid accumulation [14], (b) Local secretion mainly due to the enhanced protease synthesis by the neoplastic ascitic cells. Several reports have documented the activity of proteolytic enzymes in peritoneal fluid [6, 15, 16].
animal model [17]. (c) Enhanced PSA expression modulated throughout the steroid receptors. Previous studies have revealed the presence of steroid hormones and their receptors in peritoneal fluid and mesothelium [18, 19].

The present report is the first evidence of PSA in ascitic fluids at measurable concentrations with commercial methods, even though it does not contribute to the discrimination of malignant-related and nonmalignant ascites. The detectable amounts of PSA in peritoneal effusions give further evidence of the distinctiveness of this widespread serine protease, even though the biological effects and the mechanism causing its increase still remain unexplained. Several hypotheses have been previously suggested for the new functions of PSA in nonprostatic sources [2, 3, 20]: The presence in ascitic fluids of several mitogens and growth factors could be related to the enhanced expression of PSA in peritoneal effusions [21–24].

We are currently investigating the potential role of PSA in nonprostatic tissues and in other biological fluids as a possible sensitive molecular marker implicated in hormone responsiveness and (or) in the inflammatory/neo-plastic processes, which could, in part, be responsible for ascitic fluid proteolytic activities [15, 16].

References


