rhinorrhea. Screening for CSF-specific components in nasal secretions has become a valuable component in our diagnostic work-up. Our studies suggest that measurement of βTP in serum and nasal secretions fulfills the criteria for such a screening procedure. The rationale for using βTP to detect CSF in nasal secretions is based on observations showing a 30- to 40-fold higher concentration in CSF than in serum (8). Compared with β2Tr testing, isof orm separation is not required. Hence, βTP testing can be automated, has a higher analytical sensitivity, and is less time-consuming. Contaminating blood or wound secretions that affect the analytical performance of β2Tr testing are of little concern for βTP testing because of the predilection step.

The high analytical performance of βTP measurements coupled with a large difference in βTP between CSF and serum/nasal secretions would allow detection of 1–2% CSF admixtures to nasal secretions as shown by our dilution experiments. However, interindividual variability of βTP in CSF, serum, and nasal secretions (Table 1) may affect the detection limit. Preanalytical variability in nasal secretion collection, as suggested by higher βTP values in nasal secretions in comparison with serum in some healthy individuals, also broadens the range of reference values in nasal secretions. Moreover, the decreased βTP concentration in CSF found in acute bacterial meningitis [our unpublished observations and Ref. (15)] must be taken in account. Thus, the detection limit for βTP testing in the clinical setting may increase from 1–2% CSF in nasal secretions to 5% or even higher in the case of acute bacterial meningitis.

The development of cutoff values is essential for the clinical utility of βTP as a screening procedure for CSF rhinorrhea. Clinicians expect yes-or-no answers for the presence of CSF in secretions. On the basis of our own experience in more than 200 patients with suspected CSF rhinorrhea, we propose a cutoff value of 1.31 mg/L in nasal secretions. Results between 1.31 mg/L (97.5 percentile) and ~1.7 mg/L (the highest value measured in controls) should be confirmed by testing of another sample because cutoff values are affected by preanalytical variability. Importantly, these criteria are valid only when βTP in serum is ≤1.27 mg/L (97.5 percentile). Thus, suspected CSF rhinorrhea in patients with impaired glomerular function requires additional considerations.

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References

Protein-bound Homocystamide Measured in Human Plasma by HPLC, Yashinori Uji,1 Yoshihiro Motomiya,2 Naohiro Hanyu,3 Fumio Ukaji,3 and Hiroaki Okabe1 (1 Department of Laboratory Medicine, Kumamoto University School of Medicine, 1-1-1 Honjo, Kumamoto 860-8556, Japan; 2 Suiyukai Clinic, 676-1 Kuzumocho, Kashiwara, Nara 634-0007, Japan; 3 Tsukuba Research Laboratories, Tokuyama Dental Corporation, 40 Wadai, Tsukuba City, Ibaraki 300-4247, Japan; *author for correspondence: fax 81-96-373-5282, e-mail uji@gpo.kumamoto-u.ac.jp)

Homocysteine (Hcy) is a sulfur-containing nonprotein amino acid derived from the metabolism of methionine. Plasma Hcy is an independent risk factor for atherosclerosis (1–5).

The cyclic internal lactone form of Hcy, homocysteine thiolactone, may be the critical molecular form in the pathogenesis of atherosclerosis. In cultured cells, homocysteine thiolactone is synthesized through the reaction of methionyl-tRNA synthase (6, 7). This enzyme reacts with free amino groups of proteins to produce protein-bound homocystamide (7–9). The toxicity of homocysteinylated to the cell has been demonstrated in both in vitro and in vivo experiments. Lysyl oxidase, which catalyzes the posttranslational modification essential to the pathogenesis of connective tissue matrices, is irreversibly inactivated by homocysteine thiolactone (10).

Homocysteinylated increases the internalization of LDL by macrophages (11). Furthermore, homocysteinylated LDL elicits an autoimmune response (12). Homocysteine thiolactone hydrolyse is present in human plasma and is identical to paraoxonase (13). Homocys-
teen thiolactone hydrolase may hydrolyze homocysteine thiolactone to Hcy and thereby prevent the homocysteinilation of proteins. In the absence of a suitable assay system for homocysteine thiolactone, however, no detailed study is available on homocysteine thiolactone or Hcy-related proteins. We have developed and evaluated an assay system for measuring protein-bound homocystamide in plasma and used it in samples from healthy adults and hemodialysis patients. Here we report the results of that study.

We obtained plasma samples from 20 healthy volunteers (12 males, 8 females; age range, 29–38 years) and 15 nondiabetic hemodialysis patients [7 males and 8 females; mean (SD) age, 49 (7) years] treated at the Suiyukai Clinic (Nara, Japan) after receiving their informed consent for this study. The patients were on hemodialysis using bicarbonate dialysate with systemic heparinization two or three times week a week. Blood was collected by venipuncture at the start of hemodialysis.

Human serum albumin (HSA) and d,L-Hcy were purchased from Sigma Chemical Co. Hydrochloric acid, trifluoroacetic acid (TFA), and HPLC-grade acetonitrile were purchased from Wako Pure Chemicals Co. Ltd. Triethylamine was purchased from Pierce Chemical Co, and 4-fluoro-7-sulfamoyl-benzofurazan (ABD-F) was purchased from Dojindo Laboratories. Microplate Enzyme Immunoassay reagent sets for total Hcy were purchased from Bio-Rad Laboratories. All other materials used were of analytical grade. The ABD-Hcy calibrator was prepared according to the method proposed by Toyo’oka and Imai (14) as follows: 3 mL of 100 μmol/L d,L-Hcy was mixed with 1 mL of 90 mmol/L ABD-F in acetonitrile, buffered to pH 8–9 with triethylamine, and incubated at 60 °C for 20 min. Synthesized Hcy-HSA was prepared according to Naruszewicz et al. (11). HSA (50 mg; 750 μmol/L) was reacted with 24 μmol/L Hcy in 5 mL of phosphate-buffered saline (pH 6.8), incubated at room temperature for 1 h, and filtered. Amino acid analysis was performed (data not shown), and the results confirmed that, under these conditions, ~1 g/L Hcy-HSA was synthesized, with nine molecules of Hcy combining with one molecule of HSA.

HPLC was performed using a 600E multisolvent delivery system equipped with a 470 scanning fluorescence detector (both from Waters Associates). The reversed-phase LiChrosorb 100 RP-18 column (4.6 × 250 mm) was from Merck Japan, and the Inertsil ODS-2 column (4.6 × 250 mm) was from GL Science Inc. A PD-10 gel-filtration column (Amersham Pharmacia Biotech), equilibrated with 20 mL of 100 mmol/L borate buffer (pH 8.2) containing 5 g/L SDS and 2 mmol/L EDTA (buffer A), was used to separate the protein-bound Hcy from the low-molecular weight forms. A 1-mL plasma sample was loaded on the column, and the column was then washed with 2 mL of buffer A to elute the protein-bound Hcy fraction. We then mixed 0.1 mL of this fraction with 0.8 mL of buffer A, followed by 0.1 mL of 40 mmol/L ABD-F in dimethylformamide and 0.01 mL of 500 mmol/L tri-n-butylphosphine in dimethylformamide, and incubated the mixture at 60 °C for 30 min according to the method proposed by Toyo’oka and Imai (14) and Treuheit and Kirley (15). With this procedure, it was possible to dissociate the S-S bond between Hcy and the protein by reduction and to label the -SH group simultaneously. In this step, S-S protein-bound Hcy was converted to its free form, which was then removed by a second gel-filtration step similar to the first step, and protein-bound homocystamide was collected.

We dissociated a 2-mL fraction of this protein-bound homocystamide by hydrolysis with 2 mL of 6 mol/L HCl, sealed under reduced pressure in a test tube, at 110 °C for 20 h, and then evaporated the mixture to dryness. This sample was dissolved in 2 mL of distilled water and then evaporated. This step was repeated two more times. The hydrolysis product was dissolved in 0.5 mL of 0.9 g/L TFA and filtered through a 0.22 μm centrifugal type filter. A 100-μL aliquot of the resulting sample was separated by reversed-phase HPLC using LiChrosorb 100 RP-18 (4.6 × 250 mm) and Inertsil ODS (4.6 × 250 mm) columns. The HPLC conditions for both the LiChrosorb 100 RP-18 and Inertsil ODS columns were as follows: eluent A, 0.9 g/L TFA; eluent B, 800 mL/L acetonitrile containing 0.75 g/L TFA. The flow rate was 1 mL/min, and elution was with a linear gradient from 0% to 28% B over 25 min. Detection was with a fluorescence detector (emission wavelength, 380 nm; excitation wavelength, 500 nm).

The tHcy (free Hcy and S–S protein-bound Hcy) concentration in plasma was determined using the Microplate Enzyme Immunoassay Homocysteine reagent set (Bio-Rad) according to the manufacturer’s instructions. The peak for the ABD-Hcy calibrator was detected at 29 min (Fig. 1A), but the ABD derivatives of the samples showed several fluorescent peaks eluting from the LiChrosorb 100 RP-18 column after hydrolysis (Fig. 1B). We therefore collected the peak fraction with a retention time of 29 min and introduced this fraction into an Inertsil ODS reversed-phase column (4.6 × 250 mm; Fig 1C), using similar HPLC conditions. As shown in Fig. 1C, the peak for ABD-labeled protein-bound homocystamide in the sample eluted at ~29 min. This peak was confirmed as ABD-Hcy by comparison with the retention time for the ABD-Hcy calibrator under various HPLC conditions (data not shown) and by amino acid analysis using the PICOTAG amino acid analysis method (Waters) performed according to the manufacturer’s instructions.

For the precise determination of protein-bound homocystamide by our proposed method, conventional Hcy (free Hcy and protein-bound Hcy via S–S bonds) needs to be removed before the acid hydrolysis step. The removal of the nonprotein-bound form of Hcy in the first gel-filtration step of our analytical procedure was assessed by the recovery of 0.1–500 nmol/L Hcy added to plasma. Less than 5% of the added Hcy was recovered. We then evaluated the elimination of nonprotein-bound ABD-Hcy, which was converted from S–S protein-bound Hcy in the second gel-filtration step. After the first gel-filtration step of the analytical procedure, ABD-Hcy calibrator at various concentrations (0.005–1.0 mmol/L) was added to
plasma samples, and recoveries were determined. The results showed that <8% of ABD-Hcy had been recovered.

Calibration curves with synthetic ABD-Hcy in pooled plasma were linear to 3 μmol/L. The within-run CV (pooled plasma) was 10% at 0.79 μmol/L. The recovery for 0.01–0.1 μmol/L synthetic Hcy-HSA added to plasma was 97–107%. The concentrations of tHcy and protein-bound homocystamide and the molar ratios of protein-bound homocystamide to tHcy in plasma are shown in Table 1.

In plasma samples from 20 healthy adults (ages, 29–38 years), the mean (SD) concentrations were 13.9 (5.8) μmol/L for tHcy and 0.51 (0.11) μmol/L for protein-bound homocystamide.

The mean (SD) molar ratio of protein-bound homocystamide to tHcy was 0.042 (0.015), or 4.2% of tHcy. In 15 hemodialysis patients, the mean (SD) concentrations were 47.2 (26.1) μmol/L for tHcy and 0.74 (0.20) μmol/L for protein-bound homocystamide.

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Table 1. Distribution (mean ± SD) of tHcy, protein-bound homocystamide, and molar ratio of protein-bound homocystamide to tHcy [(B)/(A)] in plasma of healthy adults and hemodialysis patients.

<table>
<thead>
<tr>
<th></th>
<th>Healthy adults (n = 20)</th>
<th>Hemodialysis patients (n = 15)</th>
</tr>
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<tbody>
<tr>
<td>tHcy (A)</td>
<td>13.9 ± 5.8</td>
<td>47.2 ± 26.1</td>
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<tr>
<td>Protein-bound</td>
<td></td>
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<tr>
<td>homocystamide (B)</td>
<td></td>
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<tr>
<td>(B)/(A)</td>
<td>0.042 ± 0.015</td>
<td>0.020 ± 0.010</td>
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</table>

*a,b* Significantly different from values (mean ± SD) obtained in healthy adults: *a* P < 0.0001; *b* P < 0.0003.
Additional information on the proposed method is available on the Clinical Chemistry Online web site (http://www.iclinchem.org/content/vol48/issue6).

References

Clinical Evaluation of the Elecsys Total Prostate-specific Antigen Assay on the Elecsys 1010 and 1010 Systems, Alexander Haese,1 Robert T. Dworschack,2 Steven P. Piccoli,2 Lori J. Sokoll,2 Alan W. Partin,3 and Daniel W. Chan2 (1 The James Buchanan Brady Urological Institute and 2 The Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD 21287; 3 Roche Diagnostics, Indianapolis, IN 46256; 4 Diagnostic Oncology, Seymour, CT 06483; * address correspondence to this author at: The Johns Hopkins University School of Medicine, James Buchanan Brady Urological Institute, 600 N. Wolfe St., Baltimore, MD 21287; fax 410-614-8096, e-mail apartin@jhmi.edu)

Prostate cancer (PCa) is a leading cause of cancer-related deaths. The American Cancer Society expects >198 000 newly diagnosed cases and 31 500 deaths related to PCa in the US in 2001 (1).

The indolent clinical course of PCa makes its early detection a challenge. Symptoms typically do not occur until PCa is locally advanced or metastasized, at which time cure is not usually possible. Early detection is therefore mandatory to reduce the mortality of PCa. Prostate-specific antigen (PSA) is a serum protease synthesized in prostatic epithelium. Introduced into clinical use in the late 1980s (2), PSA today is recognized as the most useful tumor marker in urologic oncology and as indispensable for early detection, staging, and monitoring of PCa (3).

Digital rectal examination (DRE) alone has a poor sensitivity of 2.2–25% (4, 5) and specificity of 39% (6) in detecting PCa. Measurement of serum PSA concentrations in combination with DRE markedly improves the detection of PCa and increases the lead time for diagnosis (4, 5).

This clinical study was designed to demonstrate the clinical utility of the newly developed Roche Diagnostics Elecsys® total PSA Assay on the Roche/Hitachi Elecsys 2010 and 1010 systems. This assay is a quantitative in vitro diagnostic test for detection of total PSA (tPSA) and is indicated for measurement of tPSA in combination with DRE to aid in the diagnosis of PCa.

A total of 1121 serum samples for PSA analysis were prospectively taken from men referred for urologic evaluation (mean age, 66 years; range, 50–91 years). DRE was performed by urologists. Symptoms leading to referral were, e.g., voiding dysfunction, lower back pain, or perineal discomfort. No previous PSA values were available, nor had any patient undergone a previous biopsy. No DRE was performed <15 days and no medical treatment for benign prostatic hyperplasia was administered <90 days before serum collection.

All patients underwent DRE and transrectal ultrasound-guided biopsy of the prostate. A total of 1097 (97.7%) men received six or more biopsies. DRE and biopsy results were classified as either benign or cancerous.

The Elecsys PSA assay is a dual monoclonal antibody sandwich assay that recognizes free PSA and PSA complexed with α1-antichymotrypsin on an equimolar basis. The method is based on an electrochemiluminescent immunoassay technique in which a bixinylated anti-PSA monoclonal antibody and second anti-PSA monoclonal antibody labeled with a ruthenium-based reporter molecule react with PSA-α1-antichymotrypsin and free PSA to form a sandwich. This complex is bound to streptavidin-coated microparticles via the biotin-streptavidin interaction. The microparticles are magnetically captured on the surface of an electrode, whereas unbound reactant is removed from the measuring cell. Application of a voltage to the electrode induces a chemiluminescent emission, proportional to the amount of tPSA in the sample, that is measured by a photomultiplier. Quantification is achieved via a two-point calibration curve generated on the Elecsys immunoanalyzer and compared with a master