to be no substantive objection to implementing the suggestion by Favus (11) with $^{45}$Ca as the tracer.

However, whether a stable or a radioactive tracer is used, there is now a suitable algorithm for both men and women, requiring only a single serum sample and providing results within 1 day.

This study was supported in part by an agreement with the University of Pittsburgh, Graduate School of Public Health, by contracts with Roots, Inc. and DepoMed, Inc., by a grant from Health Future Foundation, and by Creighton University funds.

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Sensitive Immunoluminometric Assay for the Detection of Procalcitonin, Nils G. Morgenhaler, Joachim Struck, Christina Fischer-Schulz, and Andreas Bergmann (Research Department, B-R-A-H-M-S AG, Biotechnology Centre, D-16761 Hennigsdorf/Berlin, Germany; * author for correspondence: fax 49-3302-883-451, e-mail n.morgenhaler@brahms.de)

Procalcitonin (PCT) and other calcitonin precursors are detectable in various conditions leading to systemic inflammatory response syndrome. Among them are pancreatitis (1, 2), burns (3), polytrauma (4), and most importantly, bacterial infection (5). PCT reflects the severity of bacterial infection and has been used as a marker for the diagnosis and therapeutic monitoring of sepsis, severe sepsis, and septic shock of bacterial origin (6–10). The usual two-sided chemiluminescence assay [immunoluminometric assay (ILMA)] for PCT has a functional assay sensitivity (FAS) of 300 ng/L. This FAS is sufficient for the monitoring of septic patients in intensive care units, but the usefulness of the present ILMA in the usual hospital or outpatient setting is limited. Furthermore, except for an initial report on PCT and other calcitonin precursors in a few controls (8), it has not been possible to define the range of PCT in healthy individuals or to determine whether increased PCT exerts a pathophysiologic role (11–13).

We developed a new PCT assay with a >30-fold lower FAS compared with the established ILMA and measured PCT values in 500 healthy controls.

Samples were obtained from healthy blood donors (age range, 18–62 years; 241 males, 259 females) with no history of acute or chronic disease and with no symptoms of the common cold for the last 7 days. Written consent was obtained from all donors.

For the PCT assay, tubes were coated with a monoclonal antibody specific for the kcatalcin part of PCT. This antibody binds to amino acids 102–111 of PCT (ERDHR-PHVSM). Coating of the antibody was done for 20 h on polystyrene tubes (2.0 µg/tube) in 0.3 mL of buffer (10 mmol/L Tris-HCl, pH 7.8, 10 mmol/L NaCl). Tubes were blocked with 10 mmol/L sodium phosphate buffer containing 30 q/L Karion FP, 5 g/L protease-free bovine serum albumin (Sigma), pH 6.8, and lyophilized. A polyclonal sheep antibody specific for the calcitonin part of PCT was used as tracer. This antibody was raised to peptide 69–79 (GTYTQDNLKHF) of PCT and was affinity-purified on a calcium-sulfolink column and subsequently labeled with acridinium ester as follows: 100 µg of antibody in 20 mmol/L sodium phosphate buffer, pH 8.0, was incubated for 20 min at room temperature with 10 µl of acridinium ester (1 g/L in acetonitrile; Hoechst AG). Labeled antibody was purified by HPLC using a Knauer hydroxyapatite column (buffer gradient, 1–500 mmol/L potassium phosphate, pH 6.8; flow rate, 0.8 mL/min).

PCT was measured in a coated-tube assay in which 100 µL of a patient sample or calibrator was added in duplicate to each antibody-coated tube and incubated for 30 min at room temperature; 200 µL of tracer containing acridinium ester-labeled anti-PCT antibody was then added, followed by a 2-h incubation at room temperature. Tubes were washed five times with 2 mL of standard LUMItest® washing buffer (B-R-A-H-M-S AG), and detection was performed in a luminometer (detection time per sample, 1 s). This assay system was named B-R-A-H-M-S ProCa-S® to distinguish it from the similar LUMItest PCT® (B-R-A-H-M-S AG). Relative light units for the chemiluminescence assay were expressed in ng/L PCT as calculated from a calibration curve that was included in every analytical run.
To prepare calibrators, human PCT (amino acids 1–115) was overexpressed in *Escherichia coli* and purified by anion-exchange and reversed-phase chromatography as described previously (14). For the highest calibrator (S6), 5000 ng/L human recombinant PCT was added to horse serum (Sigma). This was calibrated by use of the LUMItest PCT and diluted to prepare calibrators S2 to S5 with final concentrations of 20, 100, 500, 2000, and 5000 ng/L. The lowest calibrator, S1 (PCT-free horse serum), was defined as 5 ng/L PCT to allow logarithmic plotting of the calibration curve. As controls, horse sera containing 50 ng/L (control I) and 1000 ng/L (control II) were added at the beginning and end of each run.

The intraassay imprecision was determined by measuring 23 human serum samples covering the range of the calibration curve in 10 parallel determinations. The intraassay CV was <8% in samples containing 8–4000 ng/L PCT and <15% in samples containing <8 ng/L PCT. The interassay imprecision was determined by measuring the same samples on 10 different days (Fig. 1A). The functional assay sensitivity (interassay CV <20%) was <7 ng/L PCT.

To compare this new assay with the established LUMItest PCT, we measured 71 serum samples from patients with sepsis who had PCT values between 250 and 5000 ng/L in both assays. The mean difference (SD) was 11.6 (256.4) ng/L (15) (Fig. 1B).

In 500 healthy individuals, the range was <7 to 63 ng/L PCT. The median was 13.5 ng/L (95% confidence interval for the mean, 12.6–14.7 ng/L). The 97.5 percentile of the population studied was 42.5 ng/L (Fig. 1C). There were no significant differences in the range and median PCT values between males and females or among age groups. Similar low concentrations were reported by Snider et al. (8), who used HPLC-extracted calcitonin precursors from pooled serum of healthy males.

We conclude that the proposed assay can measure PCT in healthy individuals or patients without systemic inflammatory response syndrome/sepsis. PCT values in healthy individuals are more than 10-fold lower than the clinical cutoff used for the diagnosis of severe systemic bacterial infection or sepsis (500 ng/L). At present, PCT can not be used for the diagnosis or monitoring of local bacterial infections because the established ILMA does not detect PCT concentrations <300 ng/L. The proposed assay may be useful to evaluate whether local bacterial infections increase PCT above the reference intervals.

We thank Tao Chen, Uwe Zingler, and Elke Seidel-Müller for excellent technical assistance. We also acknowledge Dr. Barbara Thomas for helpful discussions.

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Fig. 1. Characteristics of the ProCa-S assay for PCT.

(A), interassay CVs for the same samples on 10 different days. (B), Bland–Altman plot comparing difference between the results obtained with the LUMItest and ProCa-S assays as a function of the mean values obtained with both assays (15). (C), frequency distribution of PCT values in 500 healthy controls.
Comparison of Cardiac Troponin I in Serum and Hepatitis Plasma with the Dimension RxL Assay, Alberto Cerutti,* Leonora Corsini, Roberto Finotto, and Carlo Perazzi (Clinical Laboratory, S. Biagio Hospital, 28845 Domodossola, Italy; * author for correspondence: fax 39-0324-4961247, e-mail labanalidisomo@asl14.it.)

Cardiac troponin [I (cTnI) and T (cTnT)] assays in blood have rapidly become alternatives to older methods for detecting myocardial damage (1, 2). Furthermore, the recently redefined criteria for myocardial infarction that are used to classify patients with acute coronary syndrome have been established on the basis of increased serum/plasma cTnI or cTnT (3). The National Academy of Clinical Biochemistry has recommended the use of plasma rather than serum as the specimen of choice (4), citing improved turnaround times and potentially avoiding incomplete serum separation that may influence some methods to produce falsely increased results (5). However, some studies have shown lower cTnI and cTnT concentrations in plasma than in serum (6, 7). Because heparin effects vary among different analytical methods, we performed a study to evaluate cTnI concentrations in plasma and serum specimens assayed on the Dimension RxL (Dade Behring).

We evaluated assay imprecision using lyophilized control sera with three different concentrations of cTnI (0.57, 5, and 15 μg/L) that were analyzed 10 times in one analytical run for the determination of within-run imprecision and 24 times on 24 different days for the determination of between-run imprecision. Our results confirmed the manufacturer’s claims that within- and between-run imprecisions (CVs) were 2.8–3.9% and 2.9–4.1%, respectively. The analytical sensitivity was 0.04 μg/L, defined as the concentration corresponding to a signal that was 2 SD above the signal detected for the 0 μg/L cTnI calibrator (n = 20).

To compare plasma and serum cTnI, 100 paired randomized blood samples were obtained from patients admitted to the Division of Cardiology (n = 64) or to the Emergency Room (n = 36) of our hospital for acute myocardial infarction (AMI) or suspected AMI. The paired samples were drawn in parallel into tubes without anticoagulant (cat. no. 367615; Becton Dickinson) and into tubes with lithium heparin (−65 IU of heparin/mL plasma considering an hematocrit of 50%; cat. no. 367684; Becton Dickinson). According to the consensus document of the European and American Cardiologists (3), blood was obtained from our patients on hospital admission, at 6 to 9 h and again at 12 to 24 h if the earlier samples were negative and the clinical index of suspicion was high. We used a cTnI cutoff for AMI at 0.6 μg/L as indicated by the manufacturer. The Dimension RxL assay, like all the other troponin assays (8), does not comply with the new consensus (3) of a ≤10% CV at the 99th percentile (0.07 μg/L) of a reference group. Within 10–15 min after venipuncture, both tubes were centrifuged at 3000 g for 10 min, and the serum and heparin-plasma samples were frozen at −20 °C until cTnI determination. Before assay, the specimens were thawed, gently mixed by inverting the tubes five to eight times, and recen trifuged at 3000 g for 10 min.

The cTnI concentration ranges for serum and plasma were 0.24–48.5 μg/L and 0.28–48.2 μg/L, respectively, well above the detection limit of the assay (0.04 μg/L as indicated above). No significant difference was found between serum and plasma cTnI concentrations (101.7 ± 2.4%; t-test for paired data; P = 0.90), with an excellent correlation (r = 0.993; P <0.001; cTnI_{plasma} = 1.00 × cTnI_{serum} − 0.02). Whereas the ratio between plasma and serum cTnI concentrations was rather wide (range, 53.8–125%), no significant correlation was found between this ratio and the mean plasma-serum cTnI concentration (P = 0.63). Interestingly, only one sample showed a high underestimate in plasma cTnI concentration compared with serum (−46%). This sample gave the same result when repeatedly analyzed (three times) to exclude sporadic error attributable to a small clot, bubble, or misidentification. For the other 99 samples, the plasma/serum cTnI ratio was between 0.76 and 1.25 (Fig. 1).

To clarify this problem, we also carried out heparin titration experiments by adding increasing volumes of heparin (5000 IU/mL) to serum aliquots of 10 samples (with cTnI between 0.24 and 20.2 μg/L) to final concen-