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Method for the Selective Measurement of Amino-Terminal Variants of Procalcitonin

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BACKGROUND: Procalcitonin (PCT) is an established marker for diagnosing and monitoring bacterial infections. Full-length PCT [116 amino acids that make up procalcitonin (PCT1–116)] can be truncated, leading to *des*-Ala-Pro-PCT (*des*-Alanin-Prolin-Procalcitonin; PCT3–116). Current immunoassays for PCT ("total PCT") use antibodies directed against internal epitopes and are unable to distinguish amino-terminal PCT variants. Here we describe the development of monoclonal antibodies recognizing the amino-termini of PCT1–116 and PCT3–116 and their use in the selective measurement of these PCT species.

METHODS: With newly developed monoclonal antibodies against the amino-termini of PCT1–116 and PCT3–116, and an antibody against the katacalcin moiety of PCT, we developed and characterized immunoluminometric assays for the 2 PCT peptides. We comparatively assessed the kinetics of PCT variants in a human endotoxemia model.

RESULTS: Monoclonal antibodies against the amino-termini of PCT1–116 and PCT3–116 showed <1% cross-reactivity with other PCT-related peptides. The sandwich assays for PCT1–116 and PCT3–116 had functional assay sensitivities of 5 and 1.2 pmol/L, respectively, and exhibited recoveries within 20% of expected values. Plasma PCT1–116 was stable for 6 h at 22 °C and 24 h at 4 °C, and PCT3–116 was stable for at least 24 h at both temperatures. During experimental endotoxemia in healthy people, both PCT1–116 and PCT3–116 increased early in parallel with total PCT, but further increases in PCT1–116 were significantly slower than for PCT3–116 ($P = 0.0049$) and total PCT ($P = 0.0024$).

CONCLUSIONS: The new assays selectively measure PCT1–116 and PCT3–116. Both PCT species increase early during endotoxemia but differ in their kinetics thereafter. The selective measurement of PCT species with different in vivo kinetics may be useful in improving PCT-guided therapies.

For several years, procalcitonin (PCT)[4] has been known as a biomarker that reflects the presence and severity of local and systemic bacterial infections, i.e., sepsis *(1–10)*. With use of available assay technologies *(4, 11, 12)*, PCT also has been found to be increased in conditions such as major surgery and trauma not associated with bacterial infections, and such nonspecificity has limited the diagnostic utility of these assay results. Evidence from immunoneutralization experiments suggests that PCT acts as a proinflammatory hormokine *(13–15)*. As mechanistic basis for its action, it has recently been proposed that PCT is a CGRP (calcitonin gene–related peptide) receptor antagonist *(16)*. PCT is derived from the *CALCA* gene, which is expressed in numerous tissues under septic conditions *(17, 18)*. The primary translational product of *CALCA*, preprocalcitonin, is initially cleaved by the signal peptidase, resulting in the 116 amino acids that make up procalcitonin (PCT1–116). At a later time, the first 2 N-terminal amino acids of PCT1–116 can be cleaved off by dipeptidyl peptidase IV (DPPIV), leading to *des*-Ala-Pro-PCT (*des*-Alanin-Prolin-Procalcitonin; PCT3–116, which lacks the first 2 amino-terminal amino acids) *(19, 20)*. PCT1–116 and PCT3–116 might have different functional properties and different half-life times in vivo, which could potentially be translated into diagnostic applications superior to those available with present-day PCT assays. However, no assays have been available to selectively measure these PCT species. Here we describe the development of sandwich

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[4] Nonstandard abbreviations: PCT, procalcitonin; CGRP, calcitonin gene–related peptide; PCT1–116, 116 amino acids that make up procalcitonin; DPPIV, dipeptidyl peptidase IV; PCT3–116, *des*-Ala-Pro-PCT (*des*-Alanin-Prolin-Procalcitonin), which lacks the first 2 amino-terminal amino acids; MBS, m-maleimidobenzoyl-*N*-hydroxysuccinimid ester; NHS, N-hydroxysuccimimide; RLU, relative light units; TNF-α, tumor necrosis factor-α; ICU, intensive care unit; SIRS, systemic inflammatory response syndrome.

immunoassays that facilitate such selective measurement. The new assays employ new monoclonal antibodies, which recognize with high specificity the amino-termini of PCT1–116 and PCT3–116, respectively; we demonstrate their use during experimental human endotoxemia.

Materials and Methods

PEPTIDES

Nine peptides related to PCT were chemically synthesized and purified (>95%) by JPT GmbH (peptide name, sequence, positions of PCT): PAS10, APFRSALES + C-terminal cystein, 1–9; PFP10, FRSALESSP + C-terminal cystein, 3–11; PAD20, APFRSALESSPADPATLSED, 1–20; PPD19, PFRSAL ESSPADPATLSED, 2–20; PFD18, FRSALESSPAD PATLSED, 3–20; PRD17, RSALESSPADPATLSED, 4–20; PSD16, SALESSPADPATLSED, 5–20; PAN40, APFRSALESSPADPATLSEDMSSDLERDHRPHVSM PQNAN, 1–19 plus 96–116; and PFN38, FRSALESS PADPATLSEDMSSDLERDHRPHVSMPQNAN, 3–19 plus 96–116. We obtained recombinant PCT2–116 from InVivo GmbH and purchased calcitonin and katacalcin from Bachem.

ANTIBODIES

Monoclonal antibodies directed against the N-termini of peptides PAS10 and PFP10 were generated by standard procedures *(21, 22)*. Briefly, peptides were conjugated to BSA using sulfo-MBS (m-maleimidobenzoyl-*N*-hydroxysuccinimid ester). Balb/c mice were immunized and boostered with these conjugates, and spleen cells were fused with SP2/0 myeloma cells to generate hybridoma cell lines. We screened cell lines for their ability to secrete antibodies that would bind to immobilized peptide PAD20 but negligibly to PPD19 (in the case of PAS10 as immunogen), and to PFD18, but negligibly to either PPD19 or PRD17 (in the case of PFP10 as immunogen). With this approach, we generated cell lines secreting monoclonal antibodies 295/3H12 (against the N-terminus of PCT1–116) and 282/2E3 (against the N-terminus of PCT3–116). We quantitatively assessed cross-reactivities of the antibodies as follows. Purified antibodies (1 g/L) were labeled by incubation with MACN-acridinium-NHS(N-hydroxysuccimimide)-ester (1 g/L; InVent GmbH) in a 1:5 mol/L ratio for 30 min at room temperature. The reactions were stopped by addition of 1/10 volume of 1 mol/L Tris for 10 min at room temperature. We separated labeled antibodies from free label by size-exclusion chromatography on a NAP-5 column (GE Healthcare) and a Bio-Silect® SEC 400-5 column (Bio-Rad) for HPLC. Polystyrene tubes (Greiner) were coated with peptides PAD20, PPD19, PFD18, PRD17, PSD16, PFN38, PAN40, calcitonin, and katacalcin as follows. Peptides were diluted to a final concentration of 7 mg/L in 20 mmol/L Na-phosphate, pH 7.4, 50 mmol/L NaCl, and 300 μL were pipetted in tubes. After 20 h incubation at 4 °C, solutions were aspirated, and 300 μL 10 mmol/L Na-phosphate, 3% Karion FP, 0.5% BSA, pH 6.5, were added for another 2 h at 22 °C. The solution was aspirated. The chemiluminescence-labeled antibodies 295/3H12 and 282/2E3 were diluted in assay buffer (100 mmol/L Na-phosphate, 150 mmol/L NaCl, 0.5% BSA, 1 g/L unspecific mouse and bovine IgG, 0.1% Na-azide, pH 7.4) to a concentration of 0.9×10^6 relative light units (RLU)/200 μL. Per peptide tube, 200 μL of these tracer solutions were added and incubated for 2 h at 22 °C. Then we washed the tubes 4 times with 1 mL BRAHMS washing solution (BRAHMS AG) and measured bound chemiluminescence for 1 s per tube with a LB952T luminometer (Berthold).

IMMUNOASSAYS

We set up 2 chemiluminescence sandwich immunoassays in the coated tube format as follows. We labeled purified antibodies 295/3H12 and 282/2E3 (1 g/L) as described above. Tracers were produced by diluting the labeled antibodies into assay buffer [300 mmol/L K-phosphate; 100 mmol/L NaCl; 10 mmol/L sodium EDTA; 0.5% Triton X-100; 5 g/L BSA protease-free (Sigma); 1 g/L each of nonspecific sheep, bovine, and mouse IgG; 0.9 g/L Na-azide; pH 7.0]. Polystyrene tubes (Greiner) were coated with a monoclonal anti-katacalcin antibody (per tube, 2 μg antibody in 300 μL of 10 mmol/L Tris, 10 mmol/L NaCl, pH 7.8) *(23)* overnight at 22 °C. Tubes were then blocked with 10 mmol/L Na-phosphate (pH 6.5) containing 3% Karion FP (Merck) and 0.5% BSA protease-free and lyophilized. Dilutions of peptide PAN40 in normal horse serum (Sigma) served as calibrators for PCT1–116, and dilutions of peptide PFN38 in normal horse serum served as calibrators for PCT3–116.

We performed immunoassays by incubating 100 μL samples/calibrators and 200 μL tracer in coated tubes under agitation for 3 h at 22 °C. We washed the tubes 4 times with 1 mL BRAHMS washing solution and measured bound chemiluminescence for 1 s per tube with a LB952T luminometer (Berthold). The assay designs are schematically depicted in Fig. 1. Total PCT was measured with the BRAHMS PCT-sensitive luminescence immunoassay (BRAHMS AG) *(23)*, and the assay was run according to the manufacturer's instruction manual. For method comparisons, we used the BRAHMS PCT-sensitive Kryptor according to the manufacturer's instruction manual. We measured tumor necrosis factor-α (TNF-α) as described *(24)*.

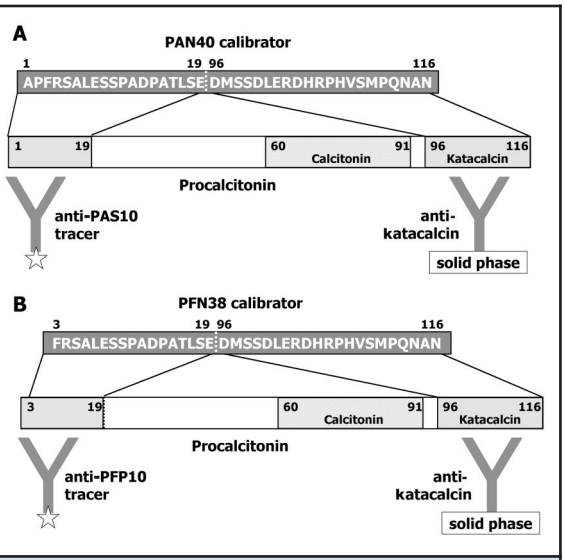

Fig. 1. Principle of the PCT1–116 and PCT3–116 assays.

(A), PCT is depicted schematically, and regions of interest are indicated; numbers indicate amino acid positions in PCT1–116. Top: Amino acid sequence and positions corresponding to PCT1–116 of the PAN40 calibrator. Bottom: Tracer antibody and solid-phase antibody. (B), PCT is depicted schematically, and regions of interest are indicated; numbers indicate amino acid positions in PCT3–116. Top: Amino acid sequence and positions corresponding to PCT3–116 of the PFN38 calibrator. Bottom: Tracer antibody and solid-phase antibody.

PATIENT SAMPLES

Serum and EDTA plasma samples used for the technical characterization of the assays were collected from intensive care unit (ICU) wards and healthy human volunteers following ethical guidelines and were stored at −80 °C until further use. We treated healthy human volunteers with endotoxin as follows; the study protocol was approved by the Ethics Committee of the Radboud University Nijmegen Medical Centre and complies with the Declaration of Helsinki including current revisions and the European Good Clinical Practice guidelines. Written informed consent was obtained from all study participants. All volunteers had normal physical examinations, electrocardiography, and routine laboratory studies before start of the experiment. Volunteers were not taking any prescription medications, and they were negative for hepatitis B surface antigen and HIV infection. We used US reference *E. coli* endotoxin (lot Ec-5, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD) in this study. Ec-5 endotoxin, supplied as a lyophilized powder, was reconstituted in 5 mL 0.9% saline for injection and vortex-mixed for at least 5 min after reconstitution. We administered the endotoxin solution as a single intravenous bolus injection during 1 min at a dose of 2 ng/kg. This dose of Ec-5 endotoxin was pyrogenic and elicited subjective side effects in healthy nontolerant male volunteers in previous studies. We obtained plasma samples at baseline ($t = 0$) and 15 min, 30 min, and 1, 2, 4, 6, and 22 h after challenge with endotoxin; the samples were stored at −80 °C until further use.

STATISTICAL ANALYSIS

We performed all statistical analysis with GraphPad Prism 5.00. We compared matched datasets using the nonparametric Friedman test. A P value <0.05 was considered significant.

Results

MONOCLONAL ANTIBODIES

We analyzed the epitope specificity of the 2 newly developed monoclonal antibodies (295/3H12 and 282/2E3, both IgG1κ) by quantifying their binding to several PCT-related peptides (Table 1). Antibody 295/3H12 showed maximal binding to a peptide representing the very N-terminus of PCT1–116. If only the first N-terminal amino acid was missing, binding dropped to <1% of maximal binding. This was true also for the calibrator peptide PFN38 later used in the PCT3–116 assay. Binding of antibody 282/2E3 was highly specific for a peptide representing the N-terminal region of PCT3–116: if only the first N-terminal amino acid was missing or if the peptide was extended by 1 or 2 amino acids, binding dropped to <1% of maximal binding. This was true also for the calibrator peptide PAN40 later used in the PCT1–116 assay. Binding of both antibodies to calcitonin and katacalcin was <1% of maximal binding (data not shown).

TECHNICAL CHARACTERISTICS OF THE PCT1–116 AND PCT3–116 ASSAYS

Measuring range and precision. As calibrators, we used chemically synthesized peptides covering the epitopes of the antibodies used in the assays. Typical standard curves are shown in Fig. 2. The lower limit of detection, as determined with horse serum (mean RLU of 10 determinations plus 2 SD), was 2 pmol/L for the PCT1–116 assay and 1.16 pmol/L for the PCT3–116 assay. For both assays, a high-dose hook effect was observed at analyte concentrations >20 000 pmol/L (data not shown). We determined the total assay imprecision by measuring in duplicate 12 human samples containing native PCT peptides in both assays. These data were

Table 1. Epitope specificity of monoclonal antibodies (mAbs).[a]

Peptide	Amino acid sequence	Position in PCT1–116	(B – NSB)/B_{max}, % mAb 295/3H12	(B – NSB)/B_{max}, % mAb 282/2E3
PAD20	APFRSALESSPADPATLSED	1–20	100	0.58
PPD19	PFRSALESSPADPATLSED	2–20	0.33	0.06
PFD18	FRSALESSPADPATLSED	3–20	0.10	100
PRD17	RSALESSPADPATLSED	4–20	0.01	0.00
PSD16	SALESSPADPATLSED	5–20	0.01	0.01

[a] The binding (B) of labeled antibodies on tubes coated with the indicated peptides was analyzed. Nonspecific binding (NSB) was assessed on control tubes, which were not coated with a peptide.

generated by 8 different operators for 12 assay runs using 1 lot and 2 luminometers according to protocol EP-5A2 recommended by CLSI. The CV was <20% for measured samples with a PCT1–116 concentration >5 pmol/L (Fig. 2C) and a PCT3–116 concentration >1.3 pmol/L (Fig. 2D), and the CV was <10% for measured samples with a PCT1–116 concentration >9 pmol/L (Fig. 2C) and a PCT3–116 concentration >3.5 pmol/L (Fig. 2D).

ACCURACY

Six PCT-containing plasma samples obtained from ICU patients were serially diluted (up to 1:32) with normal EDTA-plasma, which did not contain detect-

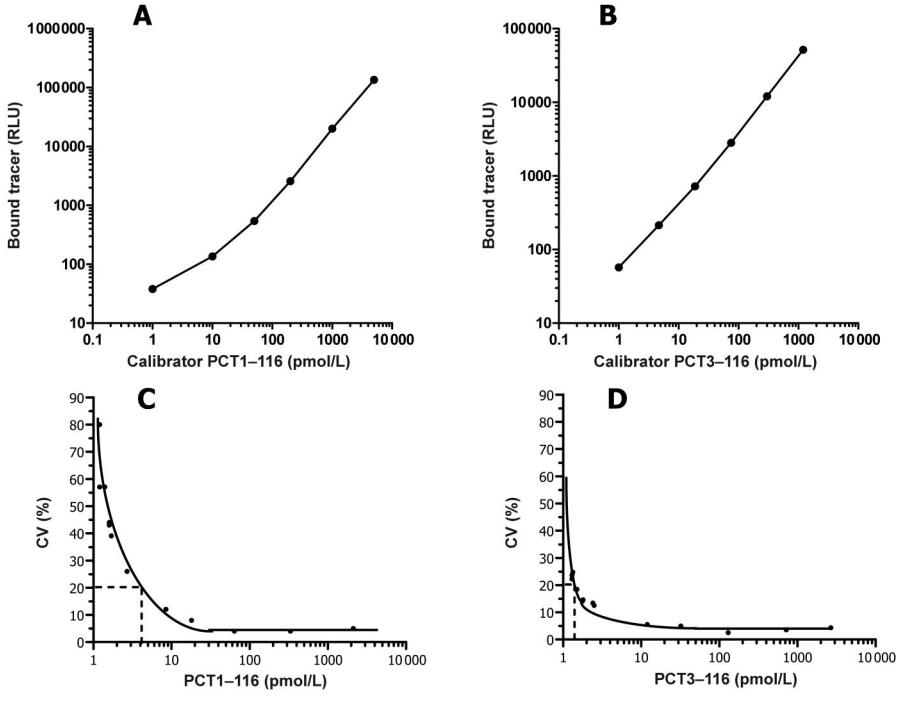

Fig. 2. Measurement range and precision of the assays.

Representative calibration curves of the PCT1–116 (A) and PCT3–116 (B) assays are shown. The signals of bound tracer are given in RLU. Precision profiles of the PCT1–116 (C) and PCT3–116 (D) assays are shown. The total assay imprecision was determined using 8 different operators to measure 12 human samples containing native PCT analytes in duplicate in both assays over 12 assay runs.

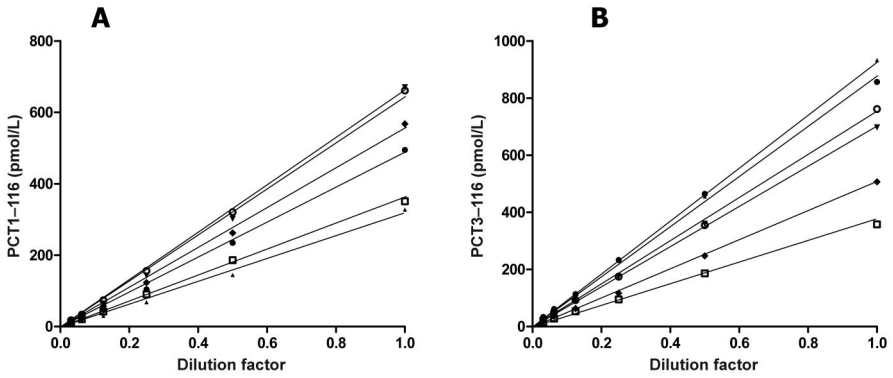

Fig. 3. Linearity and accuracy of the PCT1–116 assay (A) and the PCT3–116 assay (B).
Shown are results of serial dilutions (up to 1:32) of 6 native PCT-containing samples.

able PCT (Fig. 3). Measured concentrations were multiplied by the dilution factor and compared with the values for the undiluted samples. Over the entire dilution range tested, none of the 6 samples showed a deviation >20% of the original value.

Pooling of equal volumes of 8 different plasma samples containing low PCT1–116 concentrations (range 0–157 pmol/L) with 8 different plasma samples containing high PCT1–116 concentrations (range 76–735 pmol/L) gave a mean measured concentration that was 97.4% (SD 6.7%) of the expected concentration (range 87.3%–105.5%) (data not shown).

Pooling of equal volumes of 6 different plasma samples containing low PCT3–116 concentrations (range 2.95–270 pmol/L) with 6 different plasma samples containing high PCT3–116 concentrations (range 99–725 pmol/L) gave a mean measured concentration that was 100.6% (SD 5.4%) of the expected concentration (range 94.0%–108.2%) (data not shown).

CROSS-REACTIVITY

In each assay, we measured the recovery of 2 peptides that should not be recognized. One peptide was recombinant PCT2–116, and the other was the calibrator peptide of the respective other assay—i.e., PFN38 was tested in the PCT1–116 assay, and PAN40 in the PCT3–116 assay. Various dilutions of the peptides in normal plasma were tested. In the PCT1–116 assay, the cross-reactivity of PFN38 was 0.5% and of PCT2–116, 0.8%; in the PCT3–116 assay, the cross-reactivity of PAN40 was 0.9% and of PCT2–116, 0.4% (data not shown).

STABILITY OF THE ANALYTES

We analyzed the ex vivo stability of native PCT1–116 and PCT3–116 immunoreactivity in EDTA plasma samples freshly obtained from 10 ICU/critically ill patients at 22 °C and 4 °C (Fig. 4). At 22 °C, PCT1–116 was stable (<10% loss of immunoreactivity) for 6 h, and mean recovery fell to 69.3% after 24 h. The stability of PCT1–116 in serum was lower than that (mean loss of immunoreactivity after 6 h at 22 °C: 12.7%, SD 11.6%). In contrast, plasma PCT3–116 was stable over the entire observation period (24 h) at both 4 °C and 22 °C.

We investigated the influence of repeated freeze/thaw cycles of EDTA plasma samples (n = 25) on the stability of the native analytes. For PCT1–116, the recovery after 2, 3, and 4 cycles was [mean (SD); range] 100.3% (5.2%); 92.8%–111.5%; 97.1% (4.3%); 88.9%–105.7%; and 94.7% (4.6%); 88.5%–106.8% of the initial values. For PCT3–116, the recovery after 2, 3, and 4 cycles was 98.4% (8.1%); 92.0%–108.3%; 98.3% (10.5%); 87.8%–105.6%; and 97.8% (13%); 84.4%–112.0% of the initial values.

PCT AND TNF-α KINETICS DURING EXPERIMENTAL ENDOTOXEMIA

We induced acute systemic inflammation in 22 healthy individuals by challenging them with a bolus injection of endotoxin and measured TNF-α, PCT1–116, PCT3–116, and total PCT in plasma samples obtained at baseline and various time points after the endotoxin infusion. TNF-α peaked 90 min after injection [median (range) 492 (147–1718) pg/mL], showing that systemic inflammation was present as expected. Up to 2 h after injection, none of the PCT species was detectable; thereafter all PCT species increased until the end of the observation period (22 h) (Fig. 5). We calculated the ratios of PCT1–116 to PCT3–116 and of PCT1–116 to total PCT at 4, 6, and 22 h after stimulation (Fig. 5). Between 4 and 6 h, the concentration of PCT1–116 increased at the same rate as PCT3–116 and total PCT,

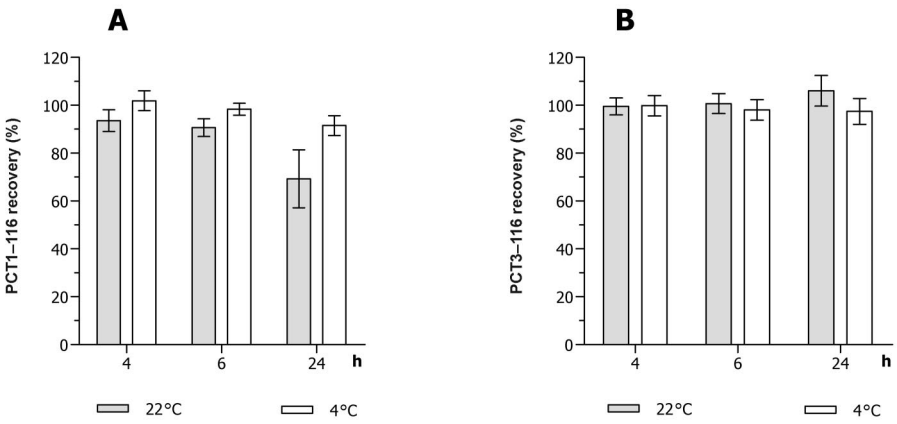

Fig. 4. Ex vivo stability.
The stability of native PCT1–116 (A) and PCT 3-116 (B) in 10 EDTA-plasma samples at 22 °C and 4 °C. Shown are mean (SD) measured PCT1–116 and PCT3–116 concentrations relative to baseline values.

but from 6 to 22 h, the median increase of PCT1–116 was 16% less than that of PCT3–116 and 15.6% less than that of total PCT. The rate of increase in PCT1–116 over the latter 3 time points was significantly different from the rates for PCT3–116 ($P = 0.0049$) and total PCT ($P = 0.0024$).

ANALYSIS OF CLINICAL SAMPLES

We analyzed plasma samples from 100 healthy individuals using the PCT1–116 and PCT3–116 assays. In both assays, all measured concentrations were below the functional assay sensitivities, i.e., <5 pmol/L for PCT1–116 and <1.3 pmol/L for PCT3–116, except for 4 concentrations measured for PCT3–116 (range 1.35–5.7 pmol/L). For current PCT assays in routine use today, measured concentrations in healthy subjects are also below the functional assay sensitivity *(25, 26)*.

In a preliminary analysis, we compared the performance of both assays to a state-of-the-art PCT assay (BRAHMS PCT-sensitive Kryptor) using specimens from different patient groups [sepsis n = 35, systemic inflammatory response syndrome (SIRS) n = 35, cardiac surgery n = 39]. The Spearman correlation coefficients for the comparison of the BRAHMS PCT-sensitive Kryptor assay with the PCT1–116 assay were lower in all groups (sepsis, $r = 0.89$; SIRS, $r = 0.81$; cardiac surgery, $r = 0.88$) than for the comparison with the PCT3–116 assay (sepsis, $r = 0.98$; SIRS, $r = 0.97$; cardiac surgery, $r = 0.97$).

Discussion

In this study, we describe for the first time 2 new sandwich immunoassays for the selective, accurate, and sensitive measurement of amino-terminal variants of PCT (PCT1–116 and PCT3–116). The cross-reactivity of the assays for several very closely related PCT-derived peptides is <1%, and thus, practically negligible. Key to the achieved high selectivity has been the development and selection of new monoclonal antibodies by targeting specific epitopes. Precision and reproducibility of the assays are comparable to other PCT assays *(23, 25, 26)*. Whether the analytical sensitivity of the new PCT assays is sufficient for clinical use can only be fully answered after measurements are made in large sample cohorts; however, early data regarding detectability of PCT1–116 and PCT3–116 in human experimental endotoxemia indicate that the sensitivity might be suitable for most if not all indications that can be assessed by today's state-of-the-art PCT assays.

Despite the increasing use of PCT as a marker of bacterial infections, little is known about the structure of PCT and PCT-derived fragments, which circulate in the bloodstream in response to a bacterial infection. Initial analyses using size-exclusion chromatography indicated that circulating PCT in sepsis patients has the theoretically-expected size of 116 amino acids *(1)*, but there were also indications of the presence of breakdown products. A more detailed mass-spectrometric analysis of affinity-purified serum PCT of sepsis patients identified a molecule truncated at the N-terminus (PCT3–116) as the principal circulating PCT species *(19)*, and DPPIV was identified as the protease that converts PCT1–116 to PCT3–116 *(20)*. Up to now, however, the complexity of such analysis has precluded large-scale and routine measurement of samples. Hence, questions have gone unanswered that are

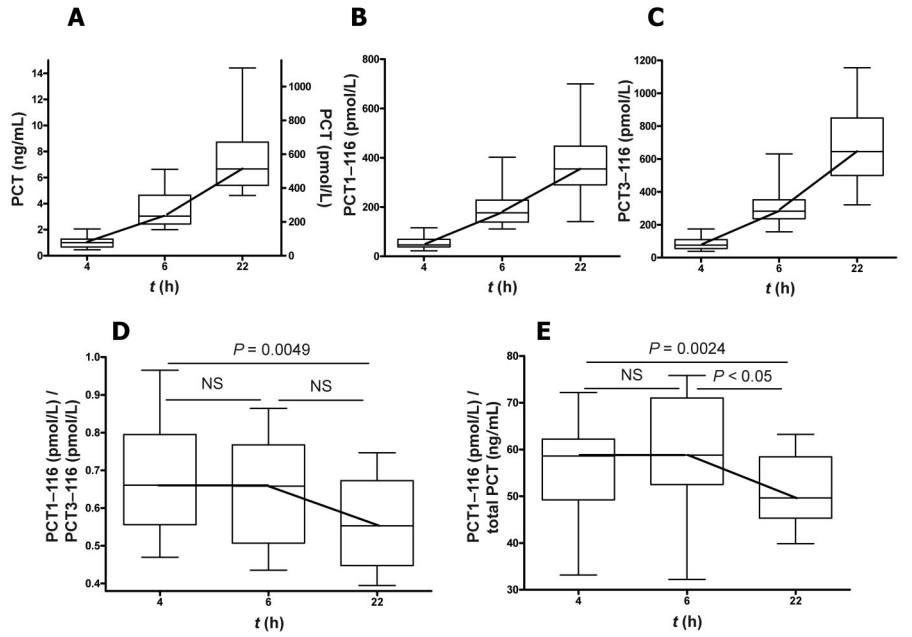

Fig. 5. PCT kinetics after endotoxin stimulation.

Twenty-two healthy human volunteers were challenged with an injection of *E. coli* endotoxin, and plasma samples were taken at the indicated time points. Shown are the median values (box-whisker plots) measured for total PCT (A), PCT1–116 (B), and PCT3–116 (C). Ratios for PCT1–116/PCT3–116 (D) and PCT1–116/total PCT (E) were calculated, and the median ratios are shown. NS, not significant.

relevant to our understanding of the biochemical pathway of PCT and its functional implications as well as to the diagnostic applications of PCT, e.g., Would the specific measurement of PCT1–116 offer a clinical advantage in certain settings, as PCT1–116 is rapidly truncated to PCT3–116? With the newly developed assays these questions can now be addressed.

As a first step, we chose a defined model system to assess the plasma kinetics of the different PCT species in response to a bacterial stimulus *(27)*. First, we could confirm that PCT3–116 is a major form of circulating PCT, especially late after the stimulation. Notably, we detected PCT1–116 in considerable concentrations in this setting as well. Relative to PCT3–116 or total measurable PCT, concentrations of PCT1–116 were highest early after the endotoxin stimulation. At the earliest time point that PCT was detectable (4 h after stimulation for all PCT species), the initially synthesized PCT1–116 already was partially converted to PCT3–116, indicating that N-terminal truncation of PCT1–116 starts quickly after its synthesis. Between 4 and 6 h after stimulation, de novo PCT synthesis and proteolytic conversion apparently occur at a comparable rate, leading to an unchanged ratio of PCT1–116/PCT3–116. But as the systemic inflammation fades out, the conversion rate exceeds the rate of synthesis, and concentrations of PCT1–116 increase at a lower rate than those of PCT3–116 or total PCT. Although unlikely, the possibility that PCT1–116 is cleared by an additional specific mechanism other than conversion cannot be excluded.

Our results indicate that in the course of a bacterial infection PCT1–116 has a more dynamic kinetic profile than total PCT, which is measured by all commercially available PCT assays so far. Along these lines, PCT1–116 was well correlated but not perfectly correlated with total PCT in various clinical samples. Clearly, future studies will be required to detail the variables affecting the rate of N-terminal truncation of PCT1–116 and to demonstrate its clinical relevance. Nevertheless, if this conversion occurs at a substantial rate in patients, then one can envisage numerous situations in which measurement of PCT1–116 alone or in conjunction with PCT3–116 or total PCT will be more informative than the total PCT assay alone. For example, a faster PCT decline might provide an earlier indication of the success of antibiotic therapy *(6, 9)*; likewise, a more rapid decrease of increased PCT concentrations often seen after major surgery or trauma *(11, 12)* might enable earlier detection/exclusion of complicating

bacterial infections. In both cases, these new assays may help to further improve therapeutic regimens.

In conclusion, the development of assays for the selective measurement of amino-terminal variants of PCT makes it possible to further investigate their kinetics in various clinical situations. Information derived from such investigations may potentially open new possibilities to further improve the PCT-guided therapy decision-making process in patients suffering from or suspected of having bacterial infections.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

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