Procalcitonin
New Findings Relating to Synthesis, Biochemistry and Function of Procalcitonin in Infection and Sepsis Diagnosis
Procalcitonin (PCT) is the precursor of Calcitonin. Site of formation is the CALC-1 gene on chromosome 11 of the human genome. After translation from CT-DNA into mRNA the first translation product is Pre-procalcitonin, which then changes by different modification steps into PCT.

**Structure of PCT** (adapted from Le Moullec et al. 1984)

- N-ProCT
- Calcitonin
- Katacalcin
- Cleavage of endopeptidases

**PAM** = Peptidyl-amidating mono-oxygenase
PCT is a peptide consisting of 116 amino acids. PCT is enzymatically degraded into lower molecular weight peptides. The final product consists of 32 amino acids and is named Calcitonin. All precursors including PCT and the mature hormone peptide can be detected in serum of healthy humans.

In septic patients only the 3-116 fragment is detectable, not the complete PCT molecule (Weglöhner et al. 2001).

Pattern of posttranslational modification of Calcitonin precursors (adapted from Nylen et al. 1996)

EP = Endopeptidase
PC = Prohormone Convertase
CP = Carboxypeptidase
AP = Aminopeptidase
PAM = Peptidyl Glycine Amidating Mono-oxygenase

G = Glycine
K = Lysine
R = Arginine
CT = Calcitonin
KAT = Katakalcin
The classic neuroendocrine pathway

Site of synthesis for PCT in healthy persons are the C-cells of the thyroid. Expression of CT-mRNA takes place only in the neuroendocrine cells. Release occurs in the form of the posttranslational processed hormone Calcitonin enclosed in Golgi vesicles. This hormone plays an important role in the pathway and regulation of calcium and phosphate in the bone metabolism.

Release of Calcitonin in the context of endocrine regulation (adapted from Linscheid et al. 2003)
The alternative pathway in sepsis and inflammation

Detection of PCT-mRNA-synthesis in different types of tissue after LPS-stimulation

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Calcitonin</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
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<tr>
<td>Thyroid</td>
<td></td>
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<td>White Blood Cells</td>
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<td>Perit. Macrophage</td>
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<tr>
<td>Spleen</td>
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<tr>
<td>Lung</td>
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<td>Liver</td>
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<tr>
<td>Kidney</td>
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<tr>
<td>Adrenal</td>
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<tr>
<td>Brain</td>
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<td>Spine</td>
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<tr>
<td>Pancreas</td>
<td></td>
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<tr>
<td>Stomach</td>
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<tr>
<td>Small Intestine</td>
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<tr>
<td>Colon</td>
<td></td>
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<tr>
<td>Heart</td>
<td></td>
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<tr>
<td>Muscle</td>
<td></td>
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<tr>
<td>Skin</td>
<td></td>
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<tr>
<td>Visceral Fat</td>
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<tr>
<td>Testes</td>
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</tbody>
</table>

In contrast to the role of PCT in the framework of the endocrine processes there are alternative possibilities of synthesis in connection with microbial infections.

Inductors for the synthesis are inflammatory cytokines like IL-1β and TNF-α but also elements of membranes or cell wall of the microbes like LPS or peptidoglycans.

This pathway was first described by Müller et al. 2001. After induction of sepsis it was possible to detect mRNA for PCT in all investigated tissues.
Linscheid et al. 2004 described the mechanism of the biochemical pathway and site of synthesis of PCT after bacterial infection. They investigated which factors affected the synthesis. Results show that in case of bacterial infection two mechanisms of synthesis are at work.

At first cytokine-stimulated adherent monocytes release PCT in low quantities (<2h). This synthesis is limited. But it plays an important role in the initiation of PCT synthesis in storage tissues of humans. This PCT burst is initiated in all storage tissues (>18h). Parenchymal tissue is the most common type of tissue in the human organism. This explains why extreme concentrations of PCT can be generated (100,000 fold increase in contrast to physiological concentrations). The PCT burst continues as long as the stimulus for synthesis exists.

In additional experiments it was clarified why PCT is only synthesized during bacterial but not in viral infections. Cells were incubated on one hand with IL-1β and on the other hand with IL-1β and INF-γ.

The results showed that cells treated with both cytokines do not synthesize PCT. The conclusion was that cells infected by viruses released INF-γ. This cytokine has a direct influence on the synthesis of PCT. These findings led to the explanation of the fact that PCT is a perfect tool to differentiate between viral and bacterial infections (e.g. Gendrel et al. 1999).

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**PCT concentrations of cells treated either with IL-1β or IL-1β and INF-γ** (adapted from Linscheid et al. 2003)
Available data led to a hypothesis effected model for the release of PCT in sepsis. Linscheid et al. 2003 and Christ-Crain et al. 2005 created a new class of biochemical substances. They believed that PCT is not a hormone or a cytokine but a hormokine.

The figure below has to be interpreted in the light of these findings.

**Alternative synthesis of PCT**
(adapted from Christ-Crain et al. 2005)
Function of PCT

Currently there is no evidenced explanation concerning the function of PCT, only speculations. In 1998 Nylen et al. showed that high levels of PCT increased in experimental induced sepsis in hamster (group 1) the mortality from 43% to 93%. After treatment with anti-PCT antibodies (group 2) the mortality decreased from 62% to 6%.

Possibly PCT has the same effect as jasmone acid in plants, a hormone for apoptosis (Parthier 1991). It is possible that PCT plays not only a role in signal transduction as biomarker but additionally an important biological role in the septic processes.

This function needs still to be clarified.
PCT kinetics can be used to assess the effectiveness of treatment

One major advantage of PCT compared to other parameters is its early and highly specific increase in response to severe systemic bacterial infections and sepsis (Harbarth et al. 2001; Müller et al. 2000). Thus, in septic conditions increased PCT levels can be observed 3-6 hours after infectious challenge.

As the septic infection resolves, PCT reliably returns to values below 0.5 ng/mL, with a half-life of 24 hours (Meisner 2000). Consequently, in vitro determinations of PCT can be used to monitor the course and prognosis of life-threatening systemic bacterial infections and to tailor the therapeutic interventions more effectively (see figure; Stüber 2001). This has for example been demonstrated for the monitoring of patients with ventilator-associated pneumonia (VAP) (Luyt et al. 2005).

Typical course of PCT serum level according to patient’s response to antibiotic treatment (n=109)
(adapted from Stüber 2001)
PCT as biomarker after criteria of evidence-based medicine

In 2006 the German Sepsis Society published the Guidelines for Diagnosis and Treatment of Sepsis. Based on a database of more than 500 trials, PCT obtained a valuation according to the criteria of evidence-based medicine.

Most important for this valuation were interventional trials, which showed the possibility of guiding antibiotic treatment with PCT in patients with infections of the lower respiratory tract (LRTI).

In 2004 a clinical trial was performed with a new sensitive assay for the determination of PCT. In this trial more than 200 patients with suspected infections of the lower respiratory tract were included (e.g. pneumonia, COPD, Bronchitis). Patients were randomized into a control group (antibiotic treatment according to the clinical practice) and a PCT guided group (antibiotic treatment dependent on the PCT value). This was the first interventional trial utilizing the biomarker PCT as decision maker whether the patient should receive antibiotic treatment or not. The outcome in both groups was the same. In the PCT group only half of the antibiotic prescriptions was necessary. These results led to a significant decrease of treatment costs for these patients.
A target directed therapy has direct influence on prevention of antibiotic resistances. The second interventional trial (Christ-Crain et al 2006) included also more than 200 patients with community acquired pneumonia (CAP). The trial consisted of two arms: A control group (antibiotic treatment according to clinical practice) and a PCT group (antibiotic treatment dependent on PCT value). In this trial the duration of antibiotic treatment could be shortened from 13 days (control group) to 5 days (PCT group).

Reduction of antibiotic treatment in patients with CAP under guidance of PCT (adapted from Christ-Crain et al. 2006)

- Control group (n=151)
- PCT group (n=151)

p < 0.001
Technologies for PCT determination

In the last years the methods of PCT determination have constantly advanced. The latest and highly sensitive technology for PCT determination is the so-called TRACE-technology (Time Resolved Amplified Cryptate Emission) on a B·R·A·H·M·S KRYPTOR system. With this method it is possible to detect locally confined bacterial infections like pneumonia.

From the findings of her trials and based on the broad spectrum of available PCT test formats Christ-Crain et al. 2005 derived a decision scale for a PCT guided antibiotic therapy with PCT as decision maker.

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### Potential use of the available PCT assays

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>PCT [ng/mL]</th>
<th>Antibiotic use</th>
<th>Assay</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ICU</td>
<td>Trauma</td>
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<tr>
<td>Septic shock</td>
<td>100</td>
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<td>Severe sepsis</td>
<td>10</td>
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<tr>
<td>Sepsis</td>
<td>2</td>
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</tr>
<tr>
<td>Pneumonia</td>
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</tr>
<tr>
<td>Bronchitis</td>
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</tr>
<tr>
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<td>0.25</td>
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</tr>
<tr>
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<td>0.1</td>
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</tr>
<tr>
<td></td>
<td>0.01</td>
<td>NO!</td>
<td></td>
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</tbody>
</table>

Consider non-bacterial differential diagnosis

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Summary

• In clinical relevant bacterial infections PCT is produced through an alternative pathway in all parenchymal tissues, and is excreted into the blood stream. This pathway is blocked in viral infections therefore PCT is a very specific marker for bacterial infections.

• Once the infection is under control, e.g. after surgical removal of the systemic focus or antibiotic therapy, the PCT production ceases. PCT values which are decreasing by 30-50% from day to day indicate, that the infection is under control and the patient recovering.

• With the availability of sensitive PCT measurements it is possible to detect slightly raised PCT values (e.g. in LRTI) early and to thus differentiate between bacterial and viral infections. Since the majority of respiratory tract infections are of viral origin, it is possible to reduce with the help of PCT the amount of antibiotic prescriptions. This will on the long run help to reduce the development of antibiotic resistances.


Austria
B·R·A·H·M·S Diagnostica GmbH
Schönbrunnerstr. 45/2/4
1050 Wien
Phone: +43-1- 585 66 67-0
Fax: +43-1- 585 66 67-9
E-Mail: office@brahms.at

Germany
B·R·A·H·M·S Aktiengesellschaft
Neuendorfstr. 25
16761 Hennigsdorf
Phone: +49-3302-883-0
Fax: +49-3302-883-100
E-Mail: brahms@brahms.de

France
B·R·A·H·M·S France SAS
78-80, rue du Docteur Bauer
93400 Saint Ouen
Phone: +33-1-49 18 90-00
Fax: +33-1-49 18 90-11
E-Mail: brahms@brahms-france.fr

Internet
www.brahms.de
www.procalcitonin.com
www.kryptor.net