PRESEPSIN: The Sepsis Biomarker
A short monograph

» Automated quantitative POC method
» From 100 μl whole blood or plasma in 15 minutes
» For early monitoring of effectiveness of antibiotic treatment
» Superior prognostic value for risk assessment of patients
» Excellent performance demonstrated in numerous studies
» Multi marker testing with other analytes possible

TIME IS SURVIVAL

LSI Medience Corporation

MITSUBISHI CHEMICAL EUROPE
Sepsis

Sepsis is a complex whole-body inflammatory state caused by severe infection by bacteria, fungi or other microorganisms. Severe sepsis is accompanied by single or multiple organ dysfunction or failure, often leading to death. Cytokines and other substances of the innate immune system are released into the blood to combat the infection. This results in a systemic inflammatory state with formation of thrombi, bleeding and leaky vessels. It proceeds finally in impaired blood flow which damages the organs by depriving them of nutrients and interferes with oxygen supply.

The severity of organ damage and sepsis is often estimated from clinical risk stratification scores such as the Sequential Organ Failure Assessment (SOFA) Score, APACHE II („Acute Physiology and Chronic Health Evaluation II“), or MEDS (Mortality in Emergency Department Sepsis).

Definition of different stages of sepsis

<table>
<thead>
<tr>
<th>Definition</th>
<th>Example</th>
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<tbody>
<tr>
<td>Sepsis</td>
<td>SIRS in response to a confirmed infectious process. Infection can be suspected or proven.</td>
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<tr>
<td>Severe sepsis</td>
<td>Sepsis with organ dysfunction, hypoperfusion, or hypotension.</td>
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<tr>
<td>Septic shock</td>
<td>Sepsis with arterial hypotension or hypoperfusion and abnormalities in spite of adequate fluid resuscitation.</td>
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</table>

Bone et al, Chest. 1992

Mortality and Morbidity

Death is common among sepsis patients, with a large proportion non surviving within the first month of diagnosis. Mortality in patients with severe sepsis can be up to more than 50%. Survivors are often strongly disabled and require long rehabilitation treatment. Especially elderly survivors of severe sepsis are up to three times as likely to develop persistent cognitive and functional impairments.
**Sepsis incidence and costs increase dramatically**

More than 18 million cases of severe sepsis are reported worldwide each year. Incidence increases strongly with a rate of 1.5%/year.\(^5\) Hospitalizations for sepsis have more than doubled over the last 10 years.\(^9\) Sepsis occurs in 1–2% of all hospitalizations and accounts for as much as 25% of intensive-care unit (ICU) bed utilization. Recent US data suggest the annual cost of hospital care for patients with septicemia is USD 14 billion.\(^10\) Incidence and cost aspects seem to be even worse in Europe.\(^11\)

**Diagnosis of sepsis: time is survival**

About 20-40% of sepsis patients develop sepsis already outside the hospital. The mainstay of sepsis treatment is a rapid diagnosis and early goal directed therapy.\(^12\) By the time physicians realize a patient is septic, it can be too late for starting therapy with broad-range antimicrobials, fluids, medication for stabilizing the circulation, and other steps. Clinical symptoms such as increased pulse or breathing rate, raised body temperature or laboratory parameters such as white blood cell count or lactate have only limited specificity. About one third of patients with severe sepsis do not show positive blood cultures\(^13\) and with current methods results would take too long. For every hour delay in the administration of appropriate antibiotic therapy there is an associated 7% rise in mortality.\(^14\) The problem of increased bacterial resistance is problematic, prolonging length of stay and duration of mechanical ventilation. Therefore monitoring any therapeutic measures that have been taken is highly important.

A rapid and reliable point of care test for detection of sepsis and its differentiation from SIRS that is applicable directly in the ICU or ER and that allows monitoring is a major step forward.
Presepsin: the innovative early biomarker for sepsis

Presepsin is a specific 13 kDa fragment derived from CD14, a 55 kDa membrane glycoprotein of monocytes, macrophages and polymorph nuclear neutrophils. CD14 serves as a receptor for complexes of bacterial lipopolysaccharides (LPS) and LPS binding protein (LBP). It can bind to peptidoglycan and other surface structures in both Gram-positive and Gram-negative bacteria. The toll-like receptor 4 (TLR4, CD 284) and/or toll-like receptor 2 specific pro-inflammatory signaling cascade is activated inducing a series of signal transduction pathways that result in a systemic inflammatory response.

The fragment soluble CD14 (sCD14) is shed from the cell membrane into the circulation where it is further fragmented by proteases such as cathepsins or during phagocytosis to sCD14 subtype (sCD14-ST) or Presepsin. In healthy persons, Presepsin is found in very low concentrations. However in patients with sepsis, direct involvement of bacterial LPS and probably phagocytosis, increased values of Presepsin are found already at a very early stage, even before IL-6 rises. The plasma half live has been reported to be 4-5h.

Measurement of Presepsin

Presepsin can easily be measured from whole blood or plasma with the compact PATHFAST™ analyzer at the point of care or in the lab. The PATHFAST™ Presepsin immunoassay is based on chemiluminescence and shows excellent precision. The fully automated procedure takes just 15 minutes and it requires only 100 µl of EDTA- or heparin-anticoagulated blood, or plasma. This makes the method ideal also for pediatric samples. In whole blood samples the effect of hematocrit is automatically corrected. There is excellent correlation between whole blood and plasma results.

Running a test on PATHFAST™ is very simple and does not require special operator skills. Reliable results at the point of care are a prerequisite for patient risk stratification and initiation of immediate targeted therapy.

In addition to Presepsin, PATHFAST™ offers several other STAT assays with relevance in sepsis such as D-Dimer, NT-proBNP, hs-cTnI, CK MB and hsCRP. All assays are provided in economical precalibrated unit-dose cartridges. Up to six samples can be tested in parallel in one run following in 3 simple steps.

Normal range and kinetics

The normal range of Presepsin in healthy adults is usually very low with values up to around 320 pg/ml (upper reference limit stated by the manufacturer). Similar values were found in several other studies. In a small study the mean Presepsin blood level in 26 preterm newborns was 643 pg/ml and a median value of 578 pg/ml. However, there is evidence that in elderly patients or patients with impaired renal function (which is frequently found in elderly patients) there is an increase of values, at least in patients that had no signs of infections. This may be related to bioaccumulation of Presepsin (13 kDa) due to reduced nephrotic mass, similar as described for Procalcitonin (PCT). Therefore interpretation of results should consider the actual renal function of the patient.

In a rabbit cecal ligation and puncture (CLP) model, along with occurrence of blood bacteria, Presepsin levels were elevated even earlier than IL-6, and much earlier than PCT, with a peak at about 3h after onset of the infection and a decline after 4-8 hours. In survivors, Presepsin declines after a few hours whereas it remains elevated in those who died.
Presepsin: a specific and early diagnostic biomarker for sepsis

The levels of Presepsin are significantly higher in septic patients than in patients with SIRS or apparently healthy individuals. This has been demonstrated in several recent studies in various countries and various groups of patients and in infections caused by either Gram-positive or Gram-negative infections or mixed infections. The following table summarizes recent studies in which Presepsin has been often compared with other biomarkers.

<table>
<thead>
<tr>
<th>Patients Description</th>
<th>Results (AUC- values of ROC curves)</th>
<th>Conclusions</th>
<th>Reference</th>
</tr>
</thead>
</table>
| 859 consecutive patients with at least two criteria for SIRS | Presepsin: 0.784  
PCT: 0.724  
Presepsin in combination with APACHE II score: 0.858  
Presepsin in combination with MEDS score: 0.875 | Presepsin is effective and superior over PCT for diagnosing sepsis, and predicting severe sepsis, septic shock and 28-day mortality in septic patients in the ED. Combination of Presepsin with clinical scores enhances the diagnostic efficacy. | Liu et al, 2014 |
| 226 patients with SIRS (37 with positive and 189 with negative blood culture) | Presepsin: 0.750  
PCT: 0.787  
CRP: 0.602 | Presepsin is significantly higher in patients with bacteremia and may be useful for ruling out bacteremia in patients with SIRS. | Romualdo et al, 2014 |
| 106 patients, with suspected sepsis or septic shock | Presepsin: 0.701  
PCT: 0.875 | Presepsin is useful in the early diagnosis of infection and showed a significant prognostic value. Mean Presepsin values were significantly higher in non survivors (60 day mortality) than in survivors. No correlation of PCT and survival. | Ulla et al, 2014 |
| 37 patients with burns | Presepsin: 0.834  
PCT: 0.847  
CRP: 0.819  
WBC: 0.508 | Presepsin had comparable performance as PCT in burn patients. No correlation of PCT and survival. | Madenci et al, 2014 |
| 30 patients with SIRS and 30 patients with sepsis | Presepsin: 0.996  
PCT: 0.912  
CRP: 0.857  
WBC: 0.777 | Discrimination of SIRS from sepsis: Presepsin values were significantly higher in patients with sepsis than the SIRS group. Presepsin was a significantly sensitive indicator of sepsis and useful marker for the rapid diagnosis of sepsis. | Vodnik et al, 2014 |
| 207 suspected sepsis patients, multicentric study | Presepsin: 0.908  
PCT: 0.905 | Presepsin and PCT showed similar diagnostic power by AUC analysis. | Endo et al, 2012 |
| 140 patients with suspected sepsis | Presepsin: 0.878  
APACHE II: 0.815  
PCT: 0.668 | Compared to PCT Presepsin is efficient in the diagnosis and risk stratification of sepsis. Presepsin values increased significantly in the first 72 hours in patients with poor outcome, while values decreased in survivors. | Spanuth et al, 2012 |
| 41 patients and 128 healthy subjects | Presepsin: 0.908  
PCT: 0.652  
CRP: 0.815  
IL-6: 0.672 | Presepsin is superior over PCT, CRP and IL-6. | Shoshuzima et al, 2011 |
| 231 SIRS and sepsis patients | Presepsin: 0.817  
PCT: 0.744 | The Presepsin concentration was a significantly more sensitive indicator of sepsis than the concentrations of other biomarkers tested. | Yaegashi et al, 2005 |
Presepsin and severity of the septic process

Shozushima et al found in a group of patients with signs of SIRS that the concentration of Presepsin was 333.5 pg/mL in the SIRS group, 721 pg/mL in the local infection group, 817.9 pg/mL in the sepsis group, and 1992.9 pg/mL in the severe sepsis group. The blood concentration of Presepsin among the groups increased sequentially.

**Staging the severity of sepsis with Presepsin**

<table>
<thead>
<tr>
<th>Presepsin (pg/mL)</th>
<th>Normal</th>
<th>SIRS</th>
<th>Local infection</th>
<th>Sepsis</th>
<th>Severe sepsis</th>
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<tr>
<td>100</td>
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**ROC curve analysis of 185 patients**

Though blood culture is not always positive in sepsis patients, it is still an important assay to compare the performance of sepsis biomarkers. In a study with patients with either Gram-positive or Gram-negative bacterial or with fungal infections with positive blood cultures, Presepsin levels were only significantly different between sepsis/infections and severe infections groups, respectively. Presepsin levels reflected the blood culture test and the severity of the clinical course more than other biomarkers such as IL-6 or PCT. A ROC-curve analysis showed superior performance of Presepsin over PCT and for both markers a clearly superior performance over IL-6.36

**Presepsin in the Emergency Room (ER)**

A large study in China with 859 consecutive patients admitted to the emergency unit with at least two criteria for a systemic inflammatory response investigated the relationship between the Presepsin levels at admission in the emergency department with the severity of disease.

**Comparison of Presepsin, PCT and IL-6 in patients with systemic infection, localized bacterial infections and non-infectious diseases**

[Graph showing comparison of Presepsin, PCT and IL-6 levels in different conditions]
A clear relationship between Presepsin levels and the different stages of sepsis severity was found while for PCT a major increase of concentration is only seen in the most severe form of septic shock. The ROC analysis showed a higher AUC value for Presepsin (0.84) as compared to PCT (0.741) or the clinical scores such as MEDS (0.818), or APACHE II (0.744). Combing of either score with Presepsin slightly enhanced the predictive power of Presepsin alone. Therefore Presepsin can be used for early risk stratification and for early decision for targeted therapy when required. A drop of Presepsin during the course of sepsis may show a response to successful therapy and hence the test may have potential as a monitoring tool.27

A Spanish study investigated the performance of Presepsin as a predictor of bacteremia for the early detection of blood-stream infections in 226 patients admitted to the emergency department with SIRS. A negative predictive value for Presepsin of 94.4% was found when using a cut-off value of 729 pg/ml.37

The relationship between sepsis biomarkers and clinical scores were compared and the clinical score values were tentatively multiplied by 100 in order to get a comparable scale, PCT is shown in pg/ml.

**Biomarkers and clinical scores**

**ROC-curves of biomarkers and clinical scores**
Presepsin as a predictor for infections in surgery

Novelli evaluated the analytical and clinical performance of the PATHFAST Presepsin assay system for early diagnosis of infection in 70 adult patients, including 35 cadaveric organ transplant recipients and 35 abdominal surgery patients with a mean age of 56.1 years. Presepsin was tested at 48 hours after surgery together with blood cultures and demonstrated 100% sensitivity to show the presence of infection, confirmed by positive blood cultures.28

Presepsin as a prognostic marker

In a study in Italy on 100 patients with sepsis (50 decedents and 50 survivors), the level on day 1 and the evolution of Presepsin levels over time was significantly higher in decedents than in survivors whereas PCT was not different in the two groups except from day 7. Presepsin was the only variable independently associated with ICU and 28-day mortality and showed better prognostic accuracy than PCT in the range of SOFA score (area under the curve (AUC) from 0.64 to 0.75 vs. AUC 0.53 to 0.65).38

A ROC curve analysis showed superior prognostic accuracy for Presepsin as compared to PCT, and addition of Presepsin to the clinical APACHE II score could increase the AUC-value from 0.815 to 0.905. Improvements of adding Presepsin also other clinical scores was shown as well, e.g. for MEDS from 0.819 to 0.936. The negative predictive value of Presepsin alone was 98.5% (PCT: 92.4%), showing a high potential to rule out sepsis with a single assay.

Presepsin or PCT, quartiles

Comparison between Presepsin (left) and PCT (right) in patients with severe sepsis or septic shock. Masson et al, 2014 (31)

Comparison between Presepsin (left) and PCT (right) in patients with severe sepsis or septic shock. Masson et al, 2014 (31)

Presepsin values and mortality of sepsis patients in ER (adapted from Spanuth et al, 2012)

Median values of survivors and non-survivors

Median values (Presepsin and PCT, both in pg/ml) of survivors and non-survivors (adapted from Spanuth et al, 2012)

A German study on 140 patients admitted to the emergency department with signs or suspicion of sepsis showed a clear and statistically highly significant (p<0.0001) relationship between Presepsin concentrations and mortality, while this relationship was not found for PCT. The median Presepsin values at admission for survivors was 823 pg/ml and 2124 pg/ml (p<0.0001) for non-survivors whereas the PCT values showed no significant difference (p=0.7452) with 1.84 ng/ml and 2.07 ng/ml.

A ROC curve analysis revealed an AUC of 0.878 for Presepsin for 30 days survival

Spanuth et al, 2012(25)
In a recent multicentric study Presepsin and other biomarkers used in sepsis were investigated in sepsis patients over the clinical course. All markers declined over time in patients with predicted favorable outcome according to SOFA or APACHE II score. Unlike other biomarkers, only Presepsin values showed a tendency to stay elevated in the group of patients with unfavorable outcome.26

A clear difference in the development of Presepsin and PCT values during the course of treatment could be demonstrated in 140 patients with sepsis who got antimicrobial treatment after diagnosis of sepsis. Presepsin showed a clear trend towards lower values in survivors over the period from 0 – 72 h observation time while non-survivors reached very high values. In contrast, PCT though also much higher in non-survivors showed only a marginal decline after 24 hours in the survivors.

The specific advantage of Presepsin over other biomarkers becomes also visible from time courses of individual patients. Therefore Presepsin is a parameter that helps to guide therapy in sepsis as shown in a time course of a burn patient who developed a sepsis after hospitalization. Effectiveness of antibiotic treatment is shown at day 13.
Sepsis and disseminated intravascular coagulation

A large proportion of sepsis patients develops severe, sometimes lethal coagulation problems. In a study in which 11 biomarkers were tested in 82 patients with suspected sepsis admitted to the emergency unit, an optimal panel of markers for the detection of disseminated coagulation (DIC) and sepsis was the combination of Presepsin and protein C with an AUC-value of 0.913 for sepsis and 0.88 for DIC.39

Presepsin in children and neonates

In neonates, early diagnosis of sepsis increases the chances for an early and specific treatment and hence for survival. There is initial evidence that presepsin may be useful in this condition.

A study of 188 newborns with suspected infections, Presepsin and other biomarkers were measured on three subsequent days. Presepsin and PCT were able to differentiate between bacterial infection (n=102) and SIRS or other non-bacterial infection (n=64) while CRP was clearly less effective.

ROC analysis demonstrated that Presepsin showed advantages over PCT. It increased earlier, was more sensitive and more specific than PCT (see figure). The cut-off value for Presepsin (781 pg/ml) was consistent on all three days. PCT showed a dynamic cut-off (day 1:0.5ng/ml, day 2 and 3: 1.0 ng/ml) that aggravates diagnosis.

In a different study, the mean presepsin concentration in early onset of septic newborns was 1772 ± 1009 pg/ml while it was 556 ±158 pg/ml in healthy newborns. The value found in healthy newborns in this study is very similar to the value found in a previous study on preterm newborns.20

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**AUC values of various biomarkers in neonats with and without infections**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Day 3</th>
<th>Day 2</th>
<th>Day 1</th>
</tr>
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<tbody>
<tr>
<td>CRP</td>
<td></td>
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<tr>
<td>PCT</td>
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<tr>
<td>PSP</td>
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**Presepsin concentration in septic newborns and newborns without infection**

<table>
<thead>
<tr>
<th>Presepsin (pg/mL)</th>
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<tbody>
<tr>
<td>2400</td>
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<td>2200</td>
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<td>600</td>
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<td>400</td>
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**Kwiatkowska-Gruca M et al, 2013(41)**
Conclusions

» Presepsin is a reliable, specific and sensitive biomarker for sepsis and is a valuable tool for the very early diagnosis of sepsis by Gram-positive and Gram-negative bacteria and fungi.

» Presepsin rises earlier than other biomarkers and does not show unspecific increases.

» Presepsin values help to stratify the severity of the septic disease with excellent correlation to APACHE II and SOFA scores.

» Presepsin exceeds the prognostic power of other sepsis biomarkers and is specifically useful when combined with clinical risk scores.

» The time course of Presepsin can be used for monitoring: a decline demonstrates response to therapy and predicts a favorable outcome.
Literature


9. World Sepsis day 2013 (http://www.world-sepsis-day.org)


32 Popov DA, Pliushch MG, Ovseenko ST, Abramian MV, Podshchekoldina OO, Larusovskii MB. [SCD14-ST (presepsin) level monitoring in cardiac surgical patients during perioperative period]. Anesteziol Reanimatol. 2013 May-Jun;3(3):30-5


40 AbdElazizz H. Diagnosis of Neonatal Sepsis using different sepsis markers. 4th International Conference on Biomarkers Clinical Research. Philadelphia 2013, (Abstract)
