DiaSino® IL-6, PCT and CRP ELISA

A biomarker panel to support early recognition and management of sepsis

diasino®

Sepsis represents a significant medical burden

Sepsis is a potentially life threatening complication of infection, trauma or burn injury. **It remains one of the leading causes of death around the world** and is a primary cause of mortality from infection.[1,2] More than 1 in 1,000 people in developed countries develop sepsis each year, representing a major burden on healthcare.[3]

The first indication of possible sepsis is systemic inflammatory response syndrome (SIRS), a condition defined by the presence of two or more clinical indicators (Figure 1). Sepsis is defined as SIRS with evidence or suspicion of infection and if one or more organs become dysfunctional it is defined as severe sepsis. The patient is said to be in septic shock when sepsis is accompanied by refractory hypotension/hypoperfusion.[4]

The mortality rate of sepsis is very high and increases depending on the stage and severity of the sepsis; 24% of patients with SIRS die, increasing to over 50% of patients with septic shock (Figure 2).[2]

I. Confirmation of infection
Diagnosis of an infection on the basis of microbiological evidence or clinical criteria

II. Systemic inflammatory host response (SIRS) (at least 2 criteria)
- Fever (≥38°C) or hypothermia (≤36°C) confirmed by rectal, intravascular or intravesical measurement
- Tachycardia: heart rate ≥90 bpm
- Tachypnea (frequency ≥20/min) or hyperventilation (PCO2 ≤4.3 kPa/≤33 mmHg)
- Leukocytosis (≥12,000/mm3) or leukopenia (≤4,000/mm3) or ≥10 % immature neutrophils in differential blood count

III. Acute organ dysfunction (at least 1 criterion)
- Acute encephalopathy: reduced alertness, disorientation, agitation, delirium
- Relative or absolute thrombocytopenia: decrease in platelet counts by more than 30 % within 24 hours or a platelet count of less than 100,000/mm3. Thrombocytopenia due to acute hemorrhage or immunological causes must be ruled out
- Arterial hypoxemia: PaO2 ≤10 kPa (≤75 mmHg) while breathing ambient air or a PaO2/FiO2 ratio of ≤33 kPa (≤250 mmHg) on oxygen administration. A clinically manifest heart or lung disease must be ruled out as a cause of hypoxemia
- Renal impairment: diuresis of ≤0.5 mL/kg/h for at least 2 hours despite adequate volume resuscitation and/or an increase in serum creatinine level to > twice the upper limit of normal (ULN)
- Metabolic acidosis: Base excess of ≤-5 mmol/L or lactate concentration of >1.5 x ULN

Sepsis: criteria I and II
Severe sepsis: criteria I, II and III
Septic shock: criteria I and II, as well as a systolic arterial blood pressure of ≤90 mmHg for at least 1 hour, or mean arterial pressure of ≤65 mmHg, or the necessity of vasopressor administration to maintain a target systolic arterial pressure of ≥90 mmHg or mean arterial pressure of ≥65 mmHg. Hypotension persists despite adequate volume resuscitation and cannot be explained by other causes

Figure 1: Clinical indicators of SIRS/sepsis and organ-specific markers (from the Guidelines of the German Sepsis Society).[4]

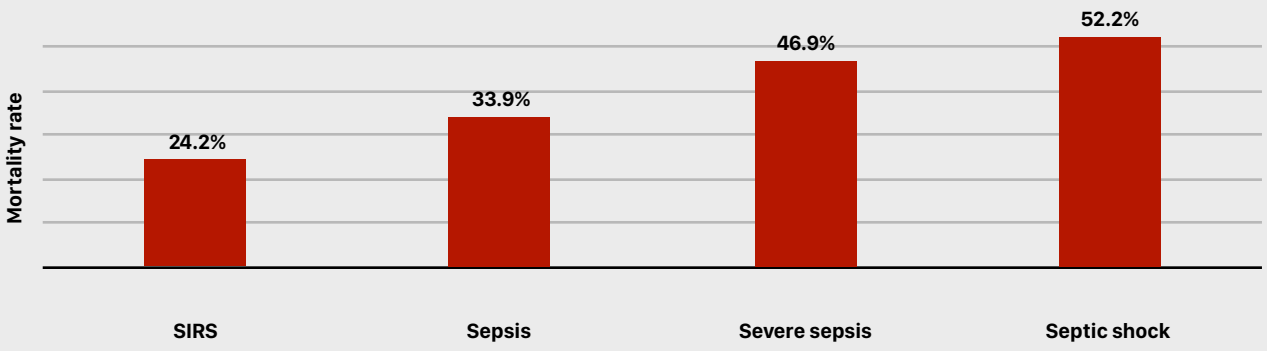

Figure 2: The four categories of inflammatory response to infection are associated with a significant increase in mortality.[2]

Timely diagnosis and treatment of sepsis to improve patient survival

Diagnosis of sepsis - Time matters

Clinical signs suggestive of sepsis are non-specific, making diagnosis of sepsis difficult.[5] Blood cultures remain the gold standard for diagnosis of patients with suspected sepsis, however, they have several limitations:[6, 7]

- Require up to 5 days to obtain the results
- Lack sensitivity
- Easy to contaminate
- Cannot assist in early sepsis management decisions

Timely diagnosis and initiation of effective antibiotic treatment and sepsis management has been shown to improve patient outcomes. Almost 80 % of patients with severe sepsis survive if treatment is initiated within 1 hour of diagnosis. Survival declines rapidly if treatment is delayed further, dropping to only 42 % if initiated 6 hours after diagnosis.[8]
In addition, it is desirable to prevent unnecessary antibiotic usage in order to reduce treatment costs, reduce the risk of adverse drug reactions and to slow the spread of antibiotic-resistant bacteria.[9, 10]

Biomarkers in sepsis

There is considerable unmet medical need in sepsis, and biomarkers may have an important clinical role to play (Figure 3). Biomarkers can indicate the presence of sepsis, differentiate bacterial from viral or fungal infection, differentiate local from systemic infection, stratify severity of sepsis, may help to guide antibiotic therapy, provide prognostic information, evaluate response to therapy, predict septic complications and predict the development of organ dysfunction. However, the exact role of biomarkers is yet to be defined.[5]

DiaSino clinical diagnostic solution to sepsis

DiaSino IL-6, PCT, in combination with CRP, deliver rapid, reliable information about the patient's immediate inflammatory status and likelihood of bacterial sepsis, which is important for antimicrobial therapy management (see Figure 4).

Diagnosis and differential diagnosis of infection

- Infection vs non-infection[5]
- Infection type (bacterial, viral, fungal)5 • Systemic or local[5]

Recognition and stratification of severity of sepsis[5]

Early and appropriate decision regarding antibiotic therapy decisions

- Initiation, and discontinuation of antibiotics[9, 11]

Figure 3: Unmet medical needs in the management of sepsis and suspected sepsis.

Acute inflammatory episode	Clinical indication of sepsis	Differential diagnosis	Severe sepsis/shock
	Suspicion/treatment	Characterization of infection*	Therapy stewardship
IL-6	Temperature Heart rate Breathing rate Leukocytes **CRP**	Blood culture **PCT** **IL-6** **CRP**	**PCT**

Figure 3: DiaSino clinical diagnostic solution to sepsis.
** Rapid identification of sepsis pathogens is possible with LightCycler® SeptiFast Test.*

Interleukin-6 (IL-6)

A key mediator for inflammation and an early alarm signal of infection

IL-6 is an early marker for inflammation in sepsis

IL-6, a key mediator for inflammation and an early alarm signal of infection that becomes elevated as part of the inflammatory response, has emerged as a valuable biomarker in the management of sepsis.[12]

IL-6 levels predict development of septic complications

In a study of 1,032 patients with severe trauma, patients who subsequently developed septic complications had the highest IL-6 levels on day 1 following injury.[13] Similarly, in a study of 50 patients following major surgery, IL-6 levels were correlated with the development of septic complications during the first 5 days following surgery (area under the curve [AUC] 0.82; 95 % CI: 0.66 – 0.98), with a sensitivity of 90 % and selectivity of 58 % . Furthermore, when IL-6 levels and clinical indicators were combined, sensitivity and selectivity increased to 100 % and 79 %, respectively.[14]

IL-6 levels predict severity of sepsis[15]

Early peak IL-6 levels correlate significantly with the development of SIRS and sepsis. The degree of elevation in IL-6 levels can be used to differentiate SIRS from severe sepsis and septic shock, with higher IL-6 levels correlating with increased severity (Figure 4).[15]

IL-6 levels are associated with patient outcome and organ dysfunction

As a marker for systemic inflammation, high IL-6 levels may be predictive of future organ dysfunction.[12] In addition, continually elevated IL-6 levels have been reported to be predictive of mortality in patients with sepsis.[15]

DiaSino Interleukin-6 (IL-6) ELISA assay

DiaSino® Interleukin-6 (IL-6) test characteristics	
Testing time	40 minutes
Test principle	Sandwich priciple
Calibrators	0, 10, 50, 250, 1000, 3000 pg/mL
Sample material	Serum, Li–heparin, K2–EDTA and K3–EDTA plasma
Sample volume	50 µL
Detection limit	2 pg/mL
Measuring range	2–3000 pg/mL
Traceability	WHO Standard NIBSC 1st IS 89/548
Expected values	≤ 7 pg/mL A testing study using the DiaSino assay on samples from 177 apparently healthy individuals, a reference range up to 7 pg/mL IL-6 (95th percentile) was determined. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Procalcitonin (PCT)

A clear signal for antibiotic starts and stops, and when managing critically ill patients with sepsis

Physiological role of PCT

PCT is a propeptide of calcitonin , produced by parafollicular cells of the thyroid and neuroendocrine cells of the lung and intestine.18 PCT levels are raised in response to bacterial endotoxins and proinflammatory stimuli, including IL-6, interleukin 1-b (IL-1b) and tumour necrosis factor-a (TNF-a).[11] PCT has been reported to be a sensitive marker for bacterial infection, with especially high levels being present following an infection of bacterial origin.19 Conversely, interferon-γ (IFN-γ) blocks the production of PCT, resulting in low PCT levels following viral infection.[11]

PCT can aid in the diagnosis and stratification of sepsis

Evidence supports a value for PCT in differentiating patients with sepsis caused by bacterial infection from those with SIRS due to a non-infectious cause. By combining PCT with clinical indicators, the accuracy of sepsis diagnosis may be improved further.[16] PCT levels increase as sepsis progresses and severity increases, allowing the differentiation of patients with SIRS, sepsis, severe sepsis or septic shock.[11] Furthermore, PCT levels correlate with increased organ dysfunction in patients with sepsis (as measured by the Sequential Organ Failure Assessment [SOFA] score)[13], and are increased in patients with poorer outcome.

PCT levels as a tool for antibiotic therapy guidance

Effective antibiotic treatment is reflected by declining PCT values. Consequently, serial determinations of PCT can be used to monitor the course of systemic bacterial infections and to tailor therapeutic interventions more efficiently.[11]
In multiple studies in various conditions, the use of PCT to guide antibiotic therapy not only reduced the mean number of days of antibiotic treatment, but also reduced the number of days within the intensive care unit (and thereby costs), with no detrimental effect on patient outcome.

DiaSino Procalcitonin (PCT) ELISA assay

DiaSino® Procalcitonin (PCT) test characteristics	
Testing time	40 minutes
Test principle	Sandwich principle
Calibrators	0, 0.5, 1.0, 2.5, 10, 25 ng/mL
Sample material	Serum, Li–heparin, K2–EDTA and K3–EDTA plasma.
Sample volume	25 µL
Detection limit	0.02 ng/mL
Measuring range	0.02-80 ng/mL
Traceability	Standardized against Roche Elecsys® PCT
Expected values	< 0.05 ng/mL A study performed with DiaSino PCT assay using 472 samples from apparently healthy males (237) and females (235) revealed the following normal value: 0.05 ng/mL (95th percentile). • < 0.5 ng/mL represent a low risk of severe sepsis and/or septic shock • 0.5-2.0 ng/mL, observe according to patient's medical history • > 2.0 ng/mL represent a high risk of severe sepsis and/or septic shock

C-Reactive Protein (CRP)[17-24]

The most sensitive of the acute phase reactants during inflammatory processes

CRP is the most sensitive of the acute phase reactants during inflammatory processes

C–reactive protein (CRP) is the classic acute phase protein in inflammatory reactions. It is synthesized by the liver and consists of five identical polypeptide chains that form a five–membered ring having a molecular weight of 105000 daltons.
CRP is the most sensitive of the acute phase reactants and its concentration increases rapidly during inflammatory processes. Complexed CRP activates the classical complement pathway. The CRP response frequently precedes clinical symptoms, including fever.

CRP levels as a tool for diagnosis of major trauma and severe infection (sepsis)

In normal healthy individuals CRP is a trace protein with a range up to 5 mg/L. After onset of an acute phase response the serum CRP concentration rises rapidly and extensively. The increase begins within 6 to 12 hours and the peak value is reached within 24 to 48 hours. Levels above 100 mg/L are associated with severe stimuli such as major trauma and severe infection (sepsis). CRP response may be less pronounced in patients suffering from liver disease.

Clinical applications of CRP testing

CRP assays are used to detect systemic inflammatory processes; to assess treatment of bacterial infections with antibiotics; to detect intrauterine infections with concomitant premature amniorrhexis; to differentiate between active and inactive forms of disease with concurrent infection, e.g. in patients suffering from SLE or Colitisulcerosa; to therapeutically monitor rheumatic disease and assess anti–inflammatory therapy; to determine the presence of post–operative complications at an early stage, such as infected wounds, thrombosis and pneumonia, and to distinguish between infection and bone marrow rejection. Postoperative monitoring of CRP levels of patients can aid in the recognition of unexpected complications (persisting high or increasing levels).

Measuring changes in the concentration of CRP provides useful diagnostic information about how acute and how serious a disease is. It also allows judgements about the disease genesis. Persistence of a high serum CRP concentration is usually a grave prognostic sign which generally indicates the presence of an uncontrolled infection.

DiaSino C-Reactive (CRP) ELISA assay

DiaSino® C-Reactive (CRP) test characteristics	
Testing time	40 minutes
Test principle	Sandwich principle
Calibrators	0, 5, 20, 50, 150, 300 mg/L
Sample material	Serum, Li–heparin, K2–EDTA and K3–EDTA plasma.
Sample volume	10 µL (diluted patient sample)
Detection limit	0.1 mg/L
Measuring range	0.1-300 mg/L
Traceability	Standardized against against IFCC CRM 470
Expected values	< 5.0 mg/L Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Use of biomarker panel

The use of a biomarker panel has the potential to provide greater information than measurement of single marker alone

IL-6, PCT and CRP are complementary biomarkers that provide different information throughout the course of sepsis (Figures 4 and 5). IL-6 levels increase rapidly following insult and peak at around 2–6 hours in surgical trauma patients.[13] Following insult, IL-6 stimulates expression of acute phase proteins such as CRP30 and induces PCT production through the immune response cascade.[11,25] PCT levels increase primarily in the presence of a bacterial infection, and raised PCT levels are highly suggestive of bacterial sepsis. CRP is a later marker of inflammation, with levels peaking 12–48 hours after infection.[13]

The combination of PCT and IL-6 has been shown to have greater sensitivity and specificity for the detection of early-onset neonatal sepsis than either marker alone.[26,27] The use of a biomarker panel has the potential to provide greater information than measurement of single markers alone and should be investigated further.[5,16,28]

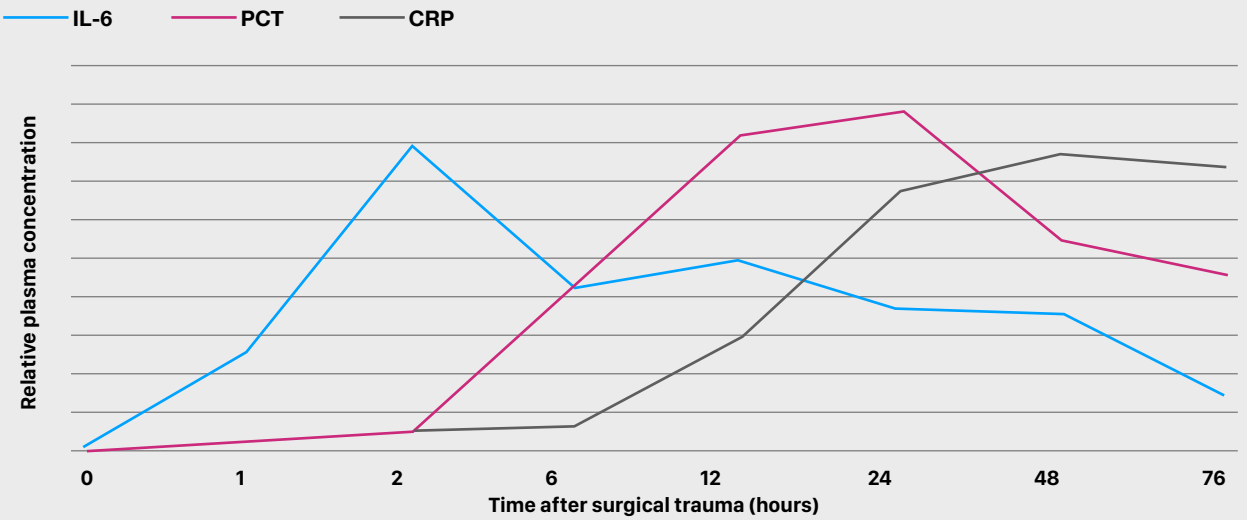

Figure 4: IL-6, PCT and CRP have distinct kinetic profiles following bacterial infection in patients who have undergone thoracic surgery.

IL-6
- Early mediator and indicator of inflammatory response, infection and sepsis
- Indicative of inflammation and infection earlier than blood cultures
  – IL-6 levels elevated within 2 hours of insult
- Increased levels correlate with increased sepsis severity and decreased survival

PCT
- Acute phase protein primarily raised during bacterial infection
- PCT levels can drive treatment algorithm for initiation, modification and discontinuation of antibiotics
  – Elevated within 6 hours of insult, and highly suggestive of bacterial sepsis
- Indicative of infection earlier than blood cultures
- Can differentiate between sepsis and severe sepsis

CRP
- Late marker for inflammation, infection and sepsis
- Confirms increases in IL-6 and PCT to aid diagnosis of sepsis

Figure 5: Complementary role of IL-6, PCT and CRP in the management of sepsis.

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DiaSino Headquarters
DiaSino Laboratories Co., Ltd.
No.58, 2nd Avenue
National Eco & Tech Development Area
450000 Zhengzhou, China
Phone: +86 371 5569 5727
www.diasino.com

DiaSino USA Office
DiaSino Laboratories, Inc.
iPOCT Research Center
228 Park Avenue S #45956
NY 10003
New York
USA

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