We are human

in.vent DIAGNOSTICA GMBH

Status and new ideas of sepsis diagnostics

LIVE WEBINAR 20 AUGUST 2020

Prof. Dr. Jörg-M. Hollidt

- Overview: Sepsis as a diagnostic task
- Requirements in Point of Care Diagnostics and Laboratory measurements
- State of the art and new markers in Assay Development

The Mission

Targeted Procurement

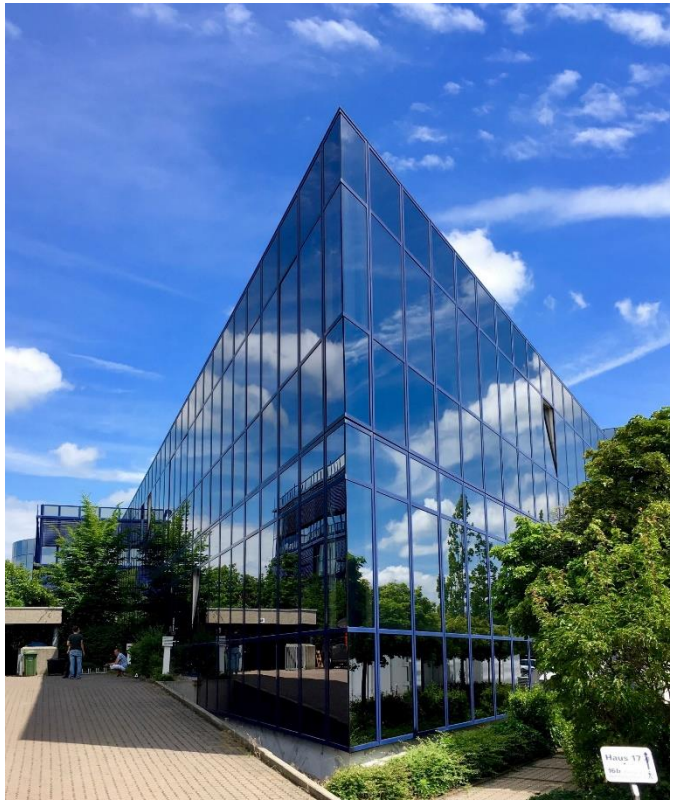
of

Human Bio-Materials

A precise assay needs precise samples.

in.vent – Competences

certified according to: **DIN EN ISO 9001** & **DIN EN ISO 13485**



- own **Donation Center** in Hennigsdorf for donors and patients
- own **manufacturing** site for controls and calibrators as OEM
- **ICS**: in.vent clinical services for IVD
- own **R & D Unit** for **IVD**
  ELISA, rapid tests, coated tubes: proof of principle / transfer to production
- extensive experience in procuring, handling, processing,
  storage and logistics of **human Bio-Materials**
- **50+** highly qualified employees

Meeting the demands

inquiry to in.vent

samples are in our biorepository

yes

picking of samples and shipment to customer

no

start of a procurement project

shipment of samples to customer

in.vent procures:

- any kind of human Bio-Material
- large volumes and pools
- normal/healthy and disease state
- clinically defined specimen
- cohorts and panels

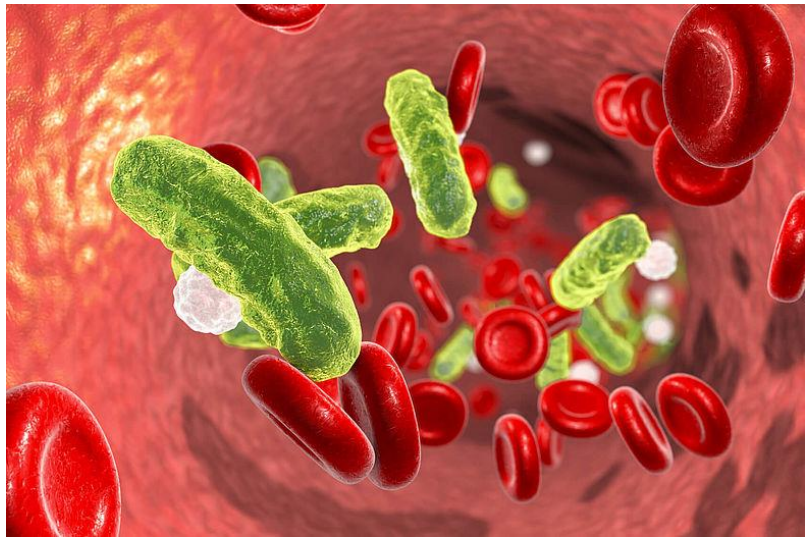
Overview:

Sepsis as a diagnostic task

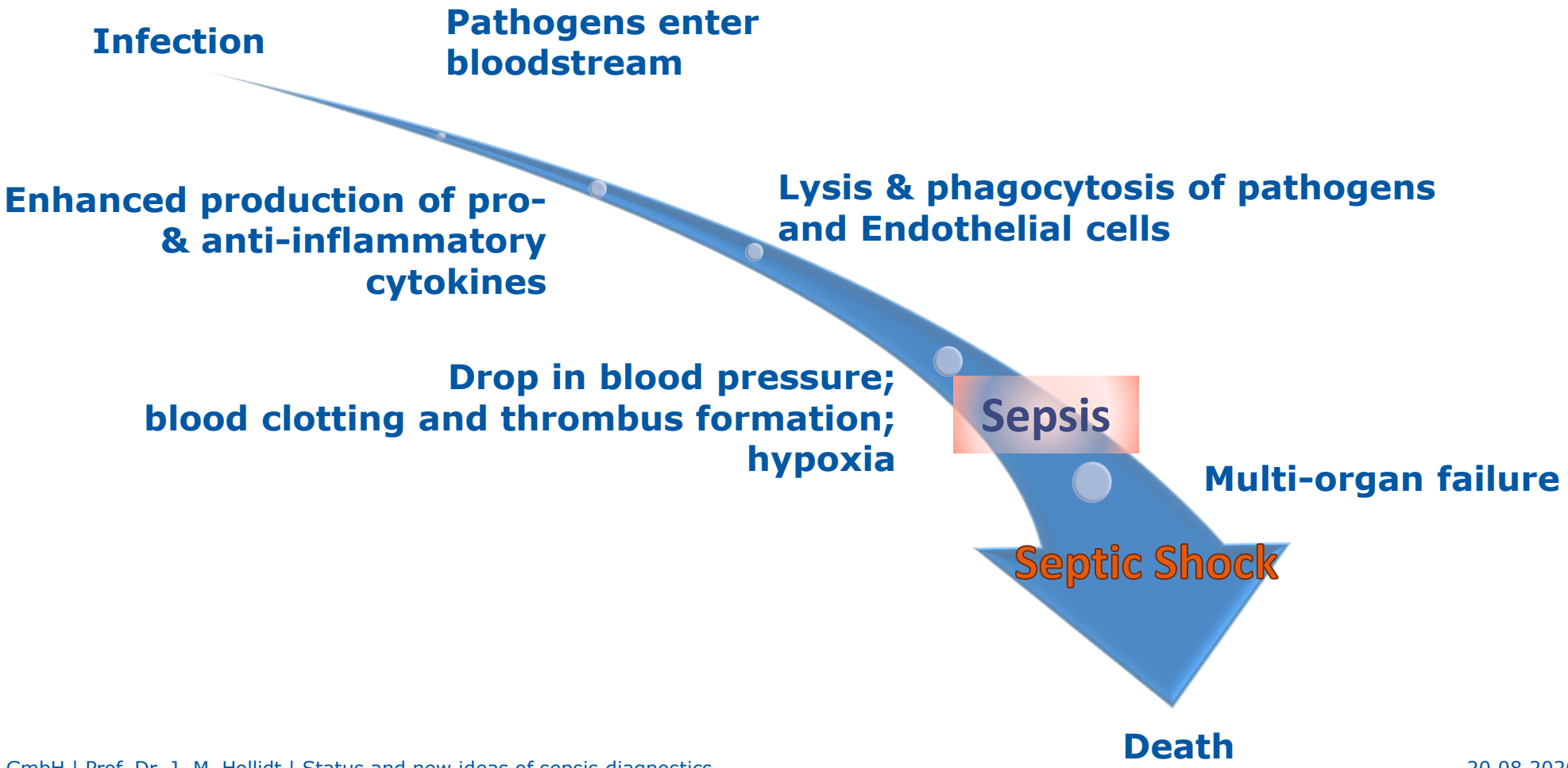
Background Sepsis

Sepsis = life-threatening organ dysfunction caused by dysregulated host response to infection

- third most common cause of death (6 mio. worldwide)
- increasing mortality
- **multi-organ failure**
- infection → sepsis → septic shock
  mortality risk increases 7 % every hour
- therapy: **antibiotics**



Background Sepsis



International guideline

3rd international consensus definitions for sepsis and septic shock:

- published 2016
- follows 2001 guideline
- definition through correlation of potential criteria with patient outcome using electronic health care date
- multidisciplinary consortium

Medical guidelines, Germany

Instruments of quality development in healthcare.

*Systematic developed statement to **support** decision making of doctors and patients for a appropriate care in specific health issues,* therefore „**scope for action and decision-making**".

Points of criticism:

- consensus process causes a reduced inclusion of relevant treatment steps into guidelines.
- conflicts of interests of authors and editors of guidelines
- inconsistency of different guidelines, disorientation (inflation of guidelines)
- bias of publications
- lentgh of time
  (development of guidelines: approx. 2-5 years)

Methodischer Hintergrund von Leitlinien: S-Klassifikation

S3	Evidenz- und konsensbasierte Leitlinie	Repräsentatives Gremium, Systematische Recherche, Auswahl, Bewertung der Literatur, Strukturierte Konsensfindung
S2e	Evidenzbasierte Leitlinie	Systematische Recherche, Auswahl, Bewertung der Literatur
S2k	Konsensbasierte Leitlinie	Repräsentatives Gremium, Strukturierte Konsensfindung
S1	Handlungs-empfehlungen von Expertengruppen	Konsensfindung in einem informellem Verfahren

SYSTEMATIK

Abb. 3 ▲ Stufenschema nach AWMF-Regelwerk

German S3-level medical guidelines for Sepsis

Consensus process completed on Dec 31st 2018 (scope of guideline 12/31/18 to 12/31/23)

Authors:

- German sepsis association (leading)
- + further 14 associations, including German Sepsis Aid support group as patient involvement
- Guideline committee consists of 34 authors (thereof 33 MDs)

Versions: short version (41 p.), extended version (124 p.), guideline review (46), evidence report (234 p.)

- Short version includes tabularly recommendations incl. level of consent, if not 100% then grading of recommendations and level of evidence
- Extended version includes additionally preamble, references and reasons for individual recommendations
- Evidence report describes reviewed studies/publications in tabular form with question, details as study design, patient number, risk of bias, outcome
- Guideline review contains additionally to recommendations also the description of methode like systematic literature research, consideration of other guidelines, research for aggregated evidence, wording of recommendation and consensus building

Sepsis-related Organ Failure Assessment (SOFA)

SOFA is used to track a person's status during the stay in an intensive care unit (ICU) to determine the extent of a person's organ function or rate of failure. The score is based on six different scores, one each for the respiratory, cardiovascular, hepatic, coagulation, renal and neurological systems.

1.	**Nervous system**:	Glasgow Coma Scale (state of consciousness: open eyes, motor function, speech)
2.	**Respiratory system:**	Horowitz index (ratio of partial pressure of oxygen in blood and the fraction of oxygen in the inhaled air)
X 3.	**Blood clotting**:	platelet concentration
X 4.	**Liver**:	Billirubin concentration
X 5.	**Kidney**:	creatinine concentration (in serum)
6.	**Cardiovascular system**:	mean arterial blood pressure

≥ 2

plus: microbiological testing to identify the causative pathogen

Quick SOFA (qSOFA)

qSOFA is used to identify patients outside the ICU setting in bedside situations with suspected infection likely to have poor outcomes typical to sepsis.

1. **Respiratory rate**: RR > 22
2. **Altered cognition (AMS):** GCS <15
3. **Hypotension**: SBP <100 mmHg

Septic shock

Definition of a septic shock provided the patient has a confirmed source of infection

1. **Requirement of vasopressors**: MAP > 65 mmHg (mean arterial pressure)
2. **Lactate:** > 2 mmol/L (18mg/dL)
3. **Absence of hypovolemia**

Requirements in Point of Care

Diagnostics and Laboratory measurements

Point-of-Care Testing (POCT) for Sepsis

Issue:

- delay of results due to sample transport and pre-analytical steps such as centrifugation → Interest in point-of-care tests (POCT)
- POCT: fast results and accelerated treatment start
- biomarkers that are currently included in suspected sepsis:
  - CRP, PCT, Presepsin and lactate
- topics with POCT:
  - appropriate clinical context
  - performance, limitations and cost of the assay

Point-of-Care Testing (POCT) for Sepsis

- lactate measurement:
  - blood gas analysis devices which are equipped with a lactate measuring electrode
  - POCT devices (handheld devices with similar measurement technology)

    → portable (more practical) and similar performance to blood gas analysers

- POCT-PCT:
  - POCT-PCT assays (Brahms PCT Direct)
    → good & comparable performance to automated monoclonal sandwich PCT assay (Brahms)
  - AQT90 Flex analyzer (radiometer)
    → good correlation with Brahms' original PCT assay
  - systems differ in their implementation, thus they are subject to different requirements / application areas

Point-of-Care Testing (POCT) for Sepsis

Areas of interest:

- microfluidic devices

  → miniaturization of analytical technologies
  → enabling automation of complex fluidic processes
  → hurdles: Cost, scalability of production and analytical quality

- integration of biosensors in biomarker measurement

  → successful for PCT, IL-6

- use of detection techniques
  such as resonator-amplified absorption spectroscopy (CEAS)

  → increasing of the sensitivity e.g. ELISA

Above all: quick identification of pathogen…

State of the art and new markers in Assay Development

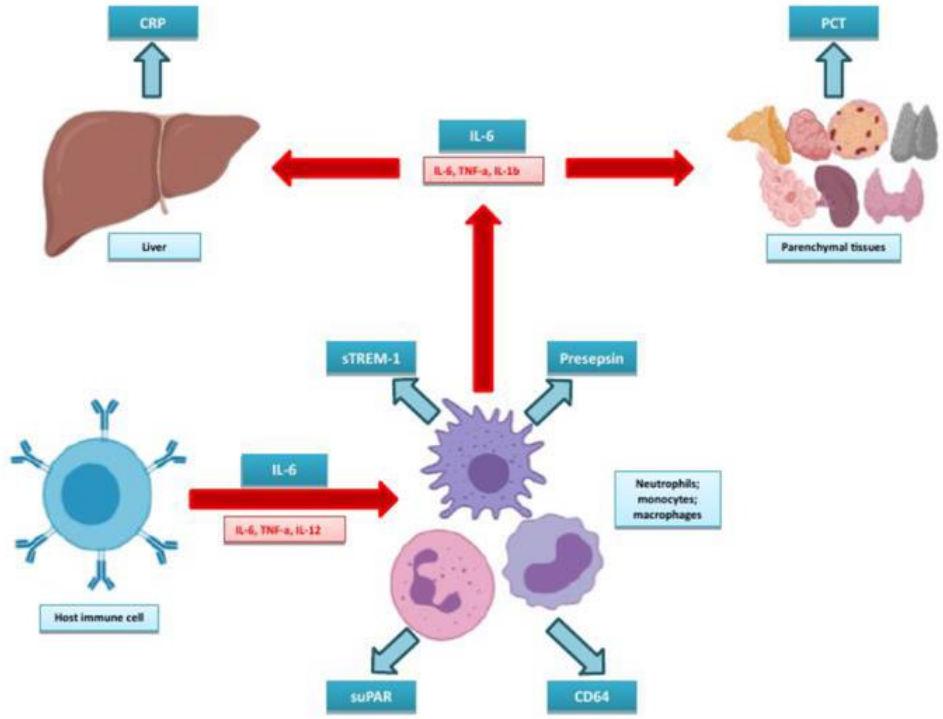
Sepsis biomarker

Why do we need biomarkers?

- problems with suspected sepsis: no fully validated biomarkers
  → help in critical time period, complicates initial treatment/monitoring
- gold standard for the diagnosis of blood-borne infections:
  → pathogen identification by blood cultures
  → problem: delayed result, not relevant for initial diagnosis

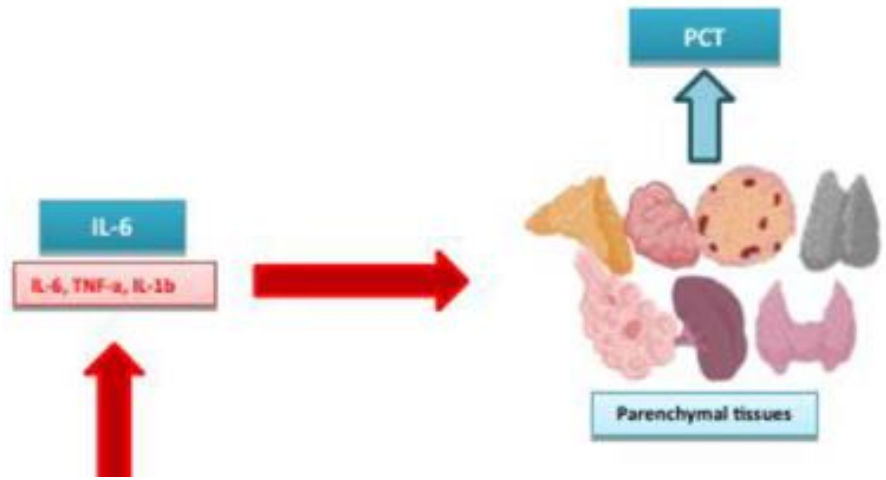
Solution: Sepsis biomarker
→ improvement of diagnostic accuracy helps optimize patient management with suspected sepsis

Which biomarkers are there in sepsis?



- **PCT (Sepsis/bacterial infection)**
- **CRP, IL-6 (inflammation)**
- sTREM-1 (soluble triggering receptor expressed on myeloid cells-1)
- **Presepsin (Sepsis)**
- **CD64**
- suPAR (soluble urokinase-type plasminogen activator receptor)
- **Lactate (septic shock)**
- Neutrophil-lymphocyte ratio (WBC)
- Liposaccharide binding protein (LBP)
- Bilirubin (liver)
- Creatinine (kidney)
- D-Dimer (hemostatsis)

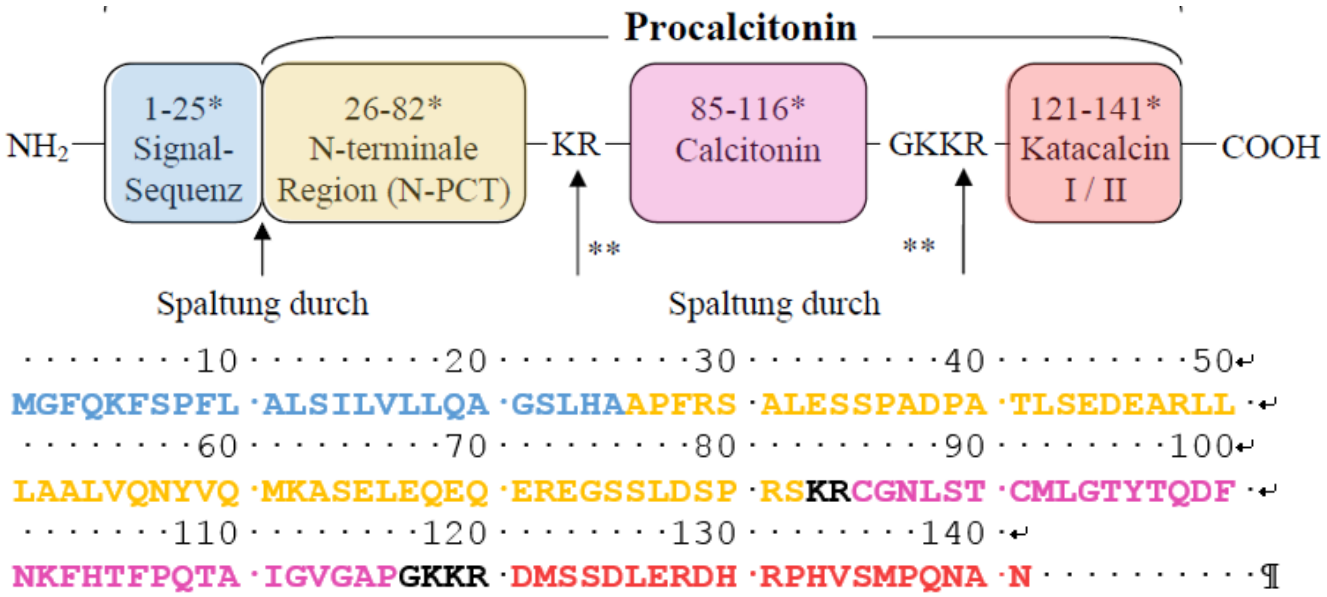
Procalcitonin (PCT)



- increased production in response to bacterial infection
  - → potential antimicrobial monitoring
  - → indication for antibiotic withdrawal

Procalcitonin

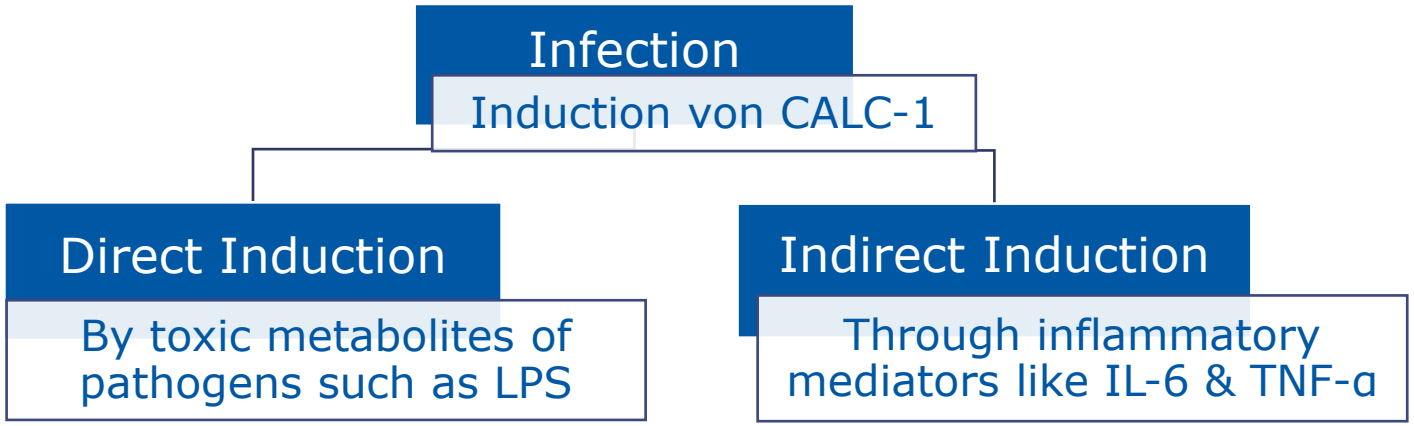
PCT concentration in human serum of healthy people < 0.1 ng/mL



Sequence of PCT (yellow to red sequence)

- 116 AS PCT from gene CALC-1 produced by thyroid C-cells
- Preprocalcitonin (blue to red sequence) is converted into procalcitonin (yellow to red) by splitting off the signal sequence (blue) and glycosylation to procalcitonin (yellow to red)
- Procalcitonin becomes calcitonin by splitting off N-PCT (yellow) and catacalcin sequence (red).

Procalcitonin for sepsis

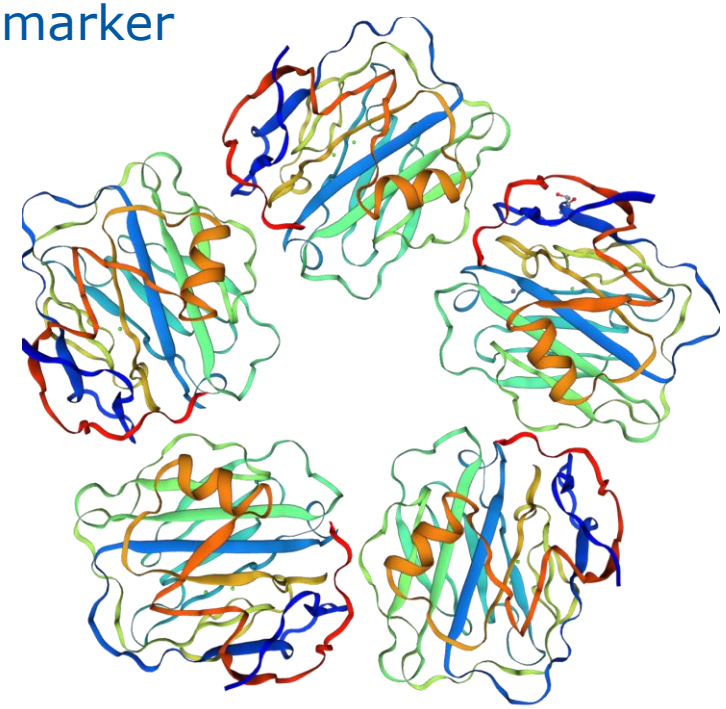


- PCT synthesis 1-6 h after infection
- half-life of 20-24 h
  → prognostic significance
- reliable early detection
- grading of severity
- differentiation of fungemia and bacteremia
- PCT ensures more accurate detection of sepsis than CRP

C-reactive Protein

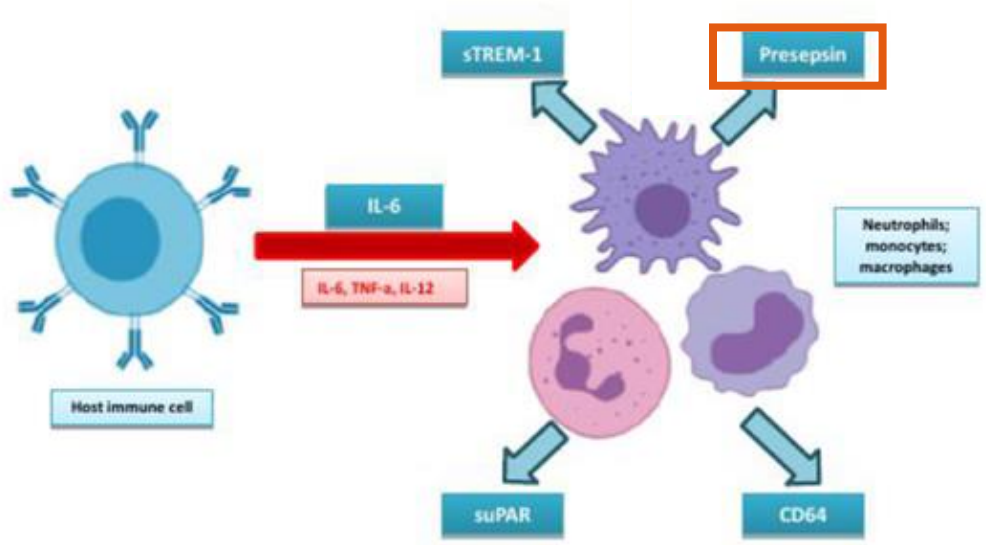
The acute phase protein CRP is the most widely used sepsis marker

- > 50 mg/L easily detectable
- synthesis 6-8 h after infection onset;
  → max. after 1-2 days;
  → 48 h half-life
  → slow marker
- no conclusion on septic origin on the inflammation



CRP as 3D Modell (25KDa)
https://swissmodel.expasy.org/repository/uniprot/P02741?csm=669228636A8544F6
(02.07.2020)

Presepsin



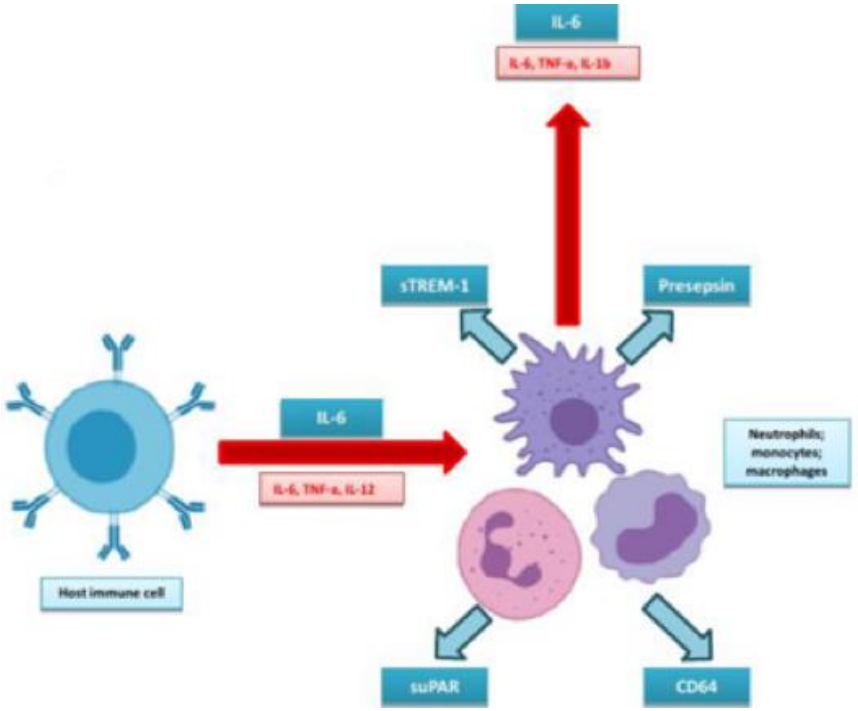
- increased production in response to bacterial infection

  → fragment of CD14

  → CD14 (Receptor):

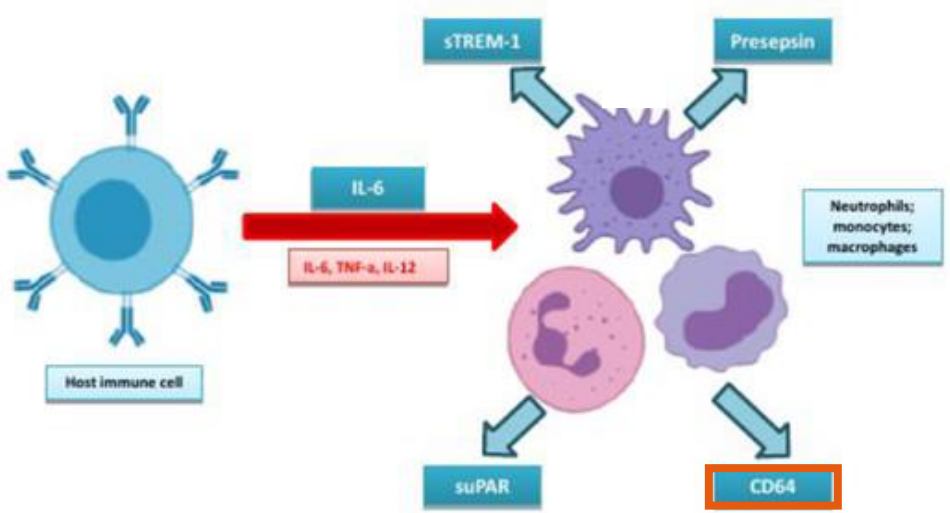
  interaction with TLRs (toll-like-receptors) and important in recognition of PAMPS (pathogen-associated patterns)

Interleukin-6 (IL-6)



- central role in inflammatory reaction
  → induces CRP production in liver
  → influences activity of B and T cells

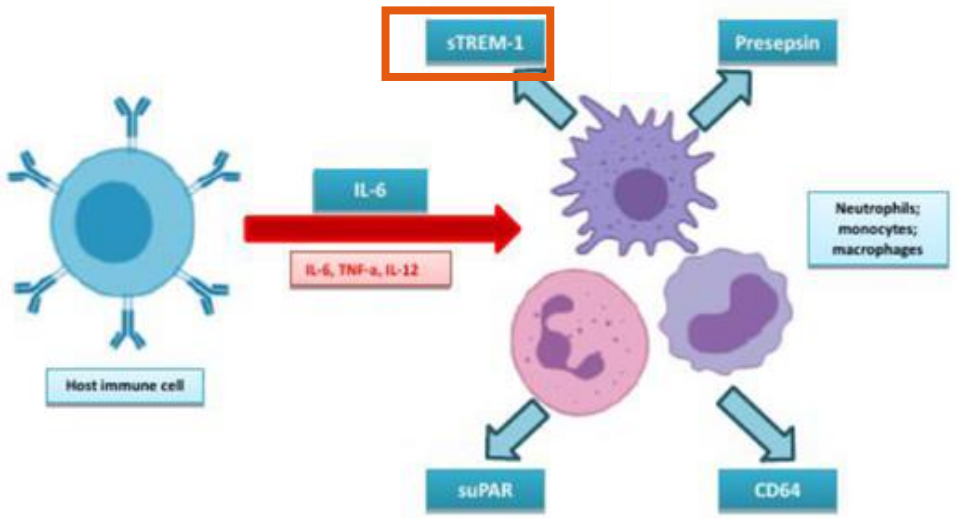
CD64



- IgG-binding receptor
- bacterial infection→ Release of cytokines by neutrophils, monocytes and macrophages

  → expressing cytokines CD64
- measurement method: flow cytometry
  → problematic, limited availability

sTREM-1



- increased expression on neutrophils, monocytes, macrophages in response to bacterial or fungal infection
- can bind exogenous and endogenous ligands

  → upregulation of cytokine release

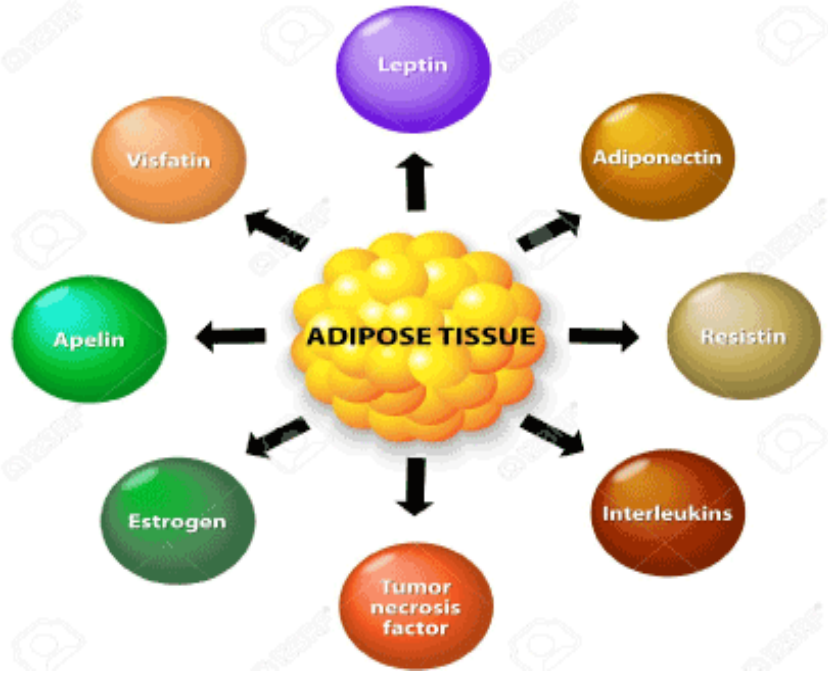
Overview

Biomarker	Newborns	Accuracy (AUC)	Adults	Accuracy (AUC)
CD64	Diagnosis of Sepsis	0.866 (low specificity, sensitivity)	Diagnosis of Sepsis	0.94 (very good sensitivity and specificity)
IL-6	Diagnosis late sepsis	0.959	Similar performance as PCT	no information
	Diagnosis early sepsis	0.751	/	/
sTREM-1	/	/	Diagnosis of Sepsis	0.82
	/	/	Differentiation between sepsis survivers and non-survivers	0.955 (Prognosis Outlook)
	/	/	Diagnosis of severe sepsis	no information
CRP	Diagnosis of Sepsis	0.96	Differentiation between sepsis survivers and non-survivers	0.791 (Prognosis Outlook)

Adipokine

Group of endocrine active proteins from fatty tissue.

- influence on energy metabolism and insulin sensitivity
- regulation of diseases, associated with systemic inflammatory response
- upregulated in patients with obesity
  → promotion of systemic inflammation
- known examples: leptin, TNF, IL-6



Evidence: https://www.oatext.com/clinical-significance-of-the-resistin-in-clinical-practice.php#gsc.tab=0

Added value of serial bio-adrenomedullin measurement in addition to lactate for the prognosis of septic patients admitted to ICU

28 days measurements

→ selection criteria: lactate level and bio-ADM

high lactate level: > 2mmol/L

normal lactate level: < 2mmol/L

keypoint of the paper:

relationship between lactate plasma level and bio-ADM

Bio-ADM: bio-adrenomedullin

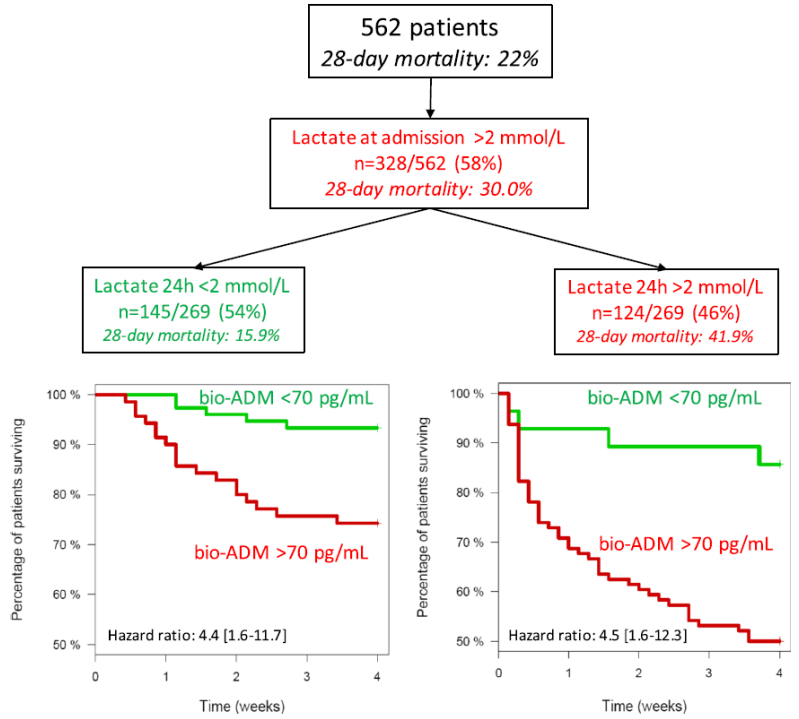


Fig. 1 Impact of 24 h lactate and bio-ADM values in patients with elevated lactate level at admission. The green curve in the left KM-plot illustrates data from 75 patients with 5 events, the red curve 70 patients with 18 events. The green curve in the right KM-plot illustrates data from 28 patients with 4 events, the red curve 96 patients with 48 events. Of note, differences in numbers between admission (n = 328) and 24 h (n = 269) is related to initial mortality

AMEOS

Jede Sekunde zählt – SEPSIS erkennen und behandeln!

SEPSIS:
Lebensbedrohliche Organdysfunktion, die durch eine fehlregulierte Wirtsantwort auf eine Infektion hervorgerufen wird.
Akute Organdysfunktion (mind. ein Organsystem):

- Enzephalopathie (Vigilanz ↓, Delir, Unruhe)
- Thrombozytopenie
- Arterielle Hypotonie
- Arterielle Hypoxämie
- Renale Dysfunktion
- Metabolische Azidose

SEPTISCHER SCHOCK

- Vasopressorengabe erforderlich, um bei persistierender Hypotonie einen mittleren arteriellen Druck ≥ 65 mmHg aufrechtzuerhalten
- Serum-Laktat > 2 mmol/l trotz adäquater Volumensubstitution
- Krankenhaussterblichkeit übersteigt 40%!

→

SOFORT:

- Blutkulturen (mind. 2 Pärchen)*
- Breitspektrum-Antibiotikum
- Volumengabe
- Laktatmessung

Innerhalb von 3 Stunden

- Weitere Volumengabe (bis zu 30 ml/kg Kristalloide)
- Arterielle Kanüle
- Laktatmessung im Verlauf
- Noradrenalin, falls MAD < 65 mmHg
- PCT-Messung
- Fokussuche
- Herdsanierung

* Die Abnahme von Blutkulturen darf die Antibiotikagabe nicht wesentlich verzögern.

How are **routine labs** coping with the situation?

How are **routine labs** supporting the treatment of Sepsis?

- guidelines / algorithms
- informations towards physicians
- considerations towards pre-analytics
- permanent education

courtesy by:

https://www.ameos.eu

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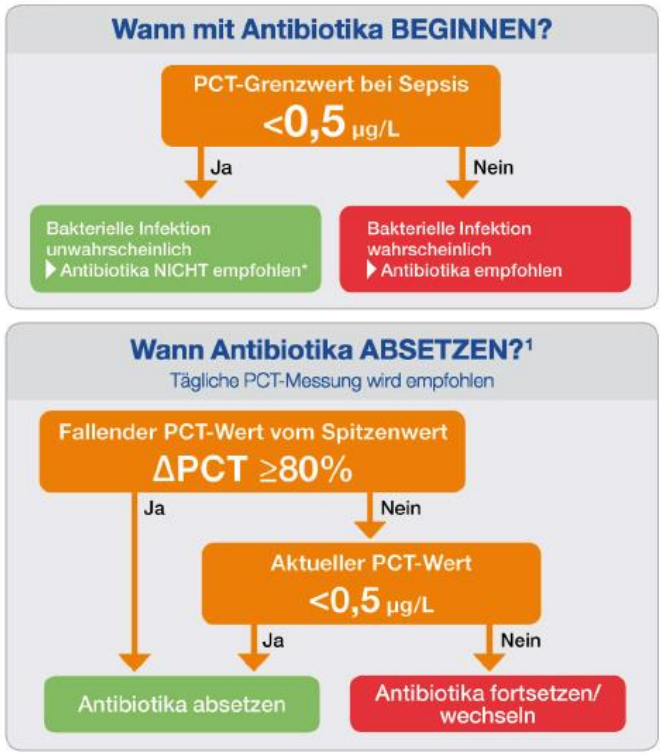
AMEOS

Procalcitonin (PCT) bei Sepsis

PCT-Algorithmen zur antibiotischen Steuerung



* Bei Verdacht auf eine Infektion sollte die Antibiotikabehandlung eingeleitet/fortgesetzt werden, besonders bei Patienten mit hohem Risiko.

$$\Delta PCT = \frac{\text{PCT-Spitzenwert} - \text{Aktueller PCT-Wert}}{\text{PCT-Spitzenwert}} \times 100\%$$

AMEOS

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AMEOS

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$$\Delta PCT = \frac{\text{PCT-Spitzenwert} - \text{Aktueller PCT-Wer}}{\text{PCT-Spitzenwert}}$$

AMEOS

PCT-Referenzbereiche zur Diagnose von systemischer bakterieller Infektion/Sepsis[2]

PCT <0,05 µg/L	Gesunde Personen
PCT <0,5 µg/L	Systemische Infektion (Sepsis) unwahrscheinlich. Lokale bakterielle Infektion möglich.

Geringes Risiko für Entwicklung einer schweren systemischen Infektion (schwere Sepsis). **Vorsicht:** PCT-Spiegel unterhalb von 0,5 µg/L schließen eine Infektion nicht aus, da lokalisierte Infektionen (ohne systemische Anzeichen) mit solch niedrigen Werten einhergehen können. Auch ist zu beachten, dass PCT-Werte noch niedrig ausfallen können, wenn die PCT-Messung sehr früh nach einer bakteriellen Belastung (normalerweise < 6 Stunden) durchgeführt wurde. In diesem Fall sollte PCT nach 6-24 Stunden erneut gemessen werden.

PCT ≥0,5–<2 µg/L	Systemische Infektion (Sepsis) möglich, jedoch kann der PCT-Spiegel aufgrund verschiedener anderer bekannter Ursachen erhöht sein*

Moderates Risiko für Entwicklung einer schweren systemischen Infektion (schwere Sepsis). Der Patient sollte streng überwacht und der PCT-Wert innerhalb 6-24 Stunden erneut gemessen werden.

PCT ≥2–<10 µg/L	Systemische Infektion (Sepsis) wahrscheinlich, sofern keine anderen Ursachen bekannt sind*

Hohes Risiko für Entwicklung einer schweren systemischen Infektion (schwere Sepsis)

PCT ≥10 µg/L	Ausgeprägte systemische Entzündungsreaktion, nahezu ausschließlich infolge einer schweren bakteriellen Sepsis oder eines septischen Schocks*

Hohe Wahrscheinlichkeit einer schweren Sepsis oder eines septischen Schocks.

* **PCT-Werte können unter bestimmten Bedingungen unabhängig von einer bakteriellen Infektion erhöht sein. Zu diesen Situationen gehören unter anderem:**
- Verletzungen einschließlich schweres Trauma, Verbrennungen und Hitzschlag
- Akute Erkrankungen wie biliäre Pankreatitis, chemische Lungenentzündung, virale Hepatitis und/oder dekompensierte schwere Zirrhose (Child-Pugh-Klasse 3), andauernder oder schwerer kardiogener Schock, andauernde schwere Organperfusionsanomalien und Postreanimationserkrankung
- Aktives medulläres Schilddrüsenkarzinom, kleinzelliges Lungenkarzinom oder Bronchialkarzinoid
- Ungewöhnliche Infektionskrankheiten einschließlich invasiver Pilzinfektionen und akuter Anfälle von Plasmodium falciparum-Malaria
- Nach Eingriffen wie einer Operation mit extrakorporaler Zirkulation, Behandlung mit Medikamenten, die die Freisetzung proinflammatorischer Zytokine stimulieren oder zu Anaphylaxie, Peritoneal- oder Hämodialyse führen
- Neugeborene während der ersten drei Lebenstage. Die PCT-Werte sollten in den ersten 72 Stunden nach der Geburt mit einem spezifischen Nomogramm interpretiert werden (Stocker et al., Neonatology 2010; 97: 165-174.)

Die PCT-Referenzbereiche sind wertvolle Richtlinien für den Arzt, sollten aber immer im Zusammenhang mit dem klinischen Zustand des Patienten betrachtet werden. PCT-Serumkonzentrationen sind bei klinisch relevanten bakteriellen Infektionen erhöht und steigen an, wenn sich die Infektion verschlimmert. Jedoch kann der gleiche Infektionsherd aufgrund von individuell verschiedenen Immunreaktionen und unterschiedlichen klinischen Situationen mit variierenden einzelnen Erhöhungen der PCT-Konzentrationen einhergehen. Bei Verdacht auf eine Infektion sollte die Antibiotikabehandlung eingeleitet/fortgesetzt werden, besonders bei Patienten mit hohem Risiko.

Literatur: 1 de Jong et al., Lancet Infect Dis 2016; 3099: 1-9. **2** Meisner M. Procalcitonin – Biochemistry and Clinical Diagnosis. Bremen 2010.

Further Discussion

Do we really need new markers in sepsis diagnosis?

- strenghten established markers
- reliable diagnostics in new designs
- updates and combinations of proven technologies
- discover challenges in daily routine in lab and hospital

  (panels of: biomarker postive specimen

  clinical samples, e.g. sepsis cohorts ready to use)

- find solutions and new insights and discuss with our expert:

  Ms. Julia Ettlinger, j.ettlinger@inventdiagnostica.de

Further Discussion

Do we really need new markers in sepsis diagnosis?

- time and effort of new developments
- best occasion and timing for great improvements
- end up in routine and as standard diagnostics

  (prospective analytical and clinical studies needed)

- find out and discuss with our experts of **ICS** about diagnostic & clinical trials:

  Mr. Lewin Günther, PhD, L.guenther@inventdiagnostica.de

  ICS: in.vent clinical services, www.ics.bio

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Thanks for your attention!

Take care of yourself

and stay healthy!