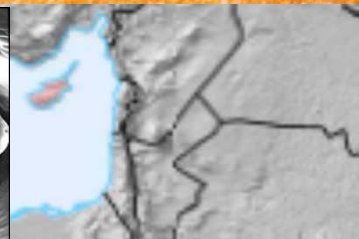
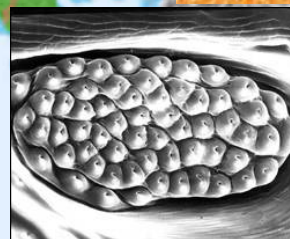


Barna Vásárhelyi

Lab tests to investigate inflammation



Melanophila acuminata

- A bug, a few inches sized
- Notifies the flame 4-5 m high from 10 kms
- If it does not detect the flame, vanishes



Homo sapiens & officinalis

- Size: 1,5 – 2 meter
- should detect the 'fire' in a few micro- or nanometers
- if not detected, the patient may die
- Laboratory diagnostic parameters



Diagnostic lab tests to detect the inflammation

WHAT IS THE
INFLAMMATION?

PROTECTIVE REACTION AGAINST INSULTS OF
DIFFERENT ORIGINS (biological, physical, chemical,
metabolic, immunological triggers)

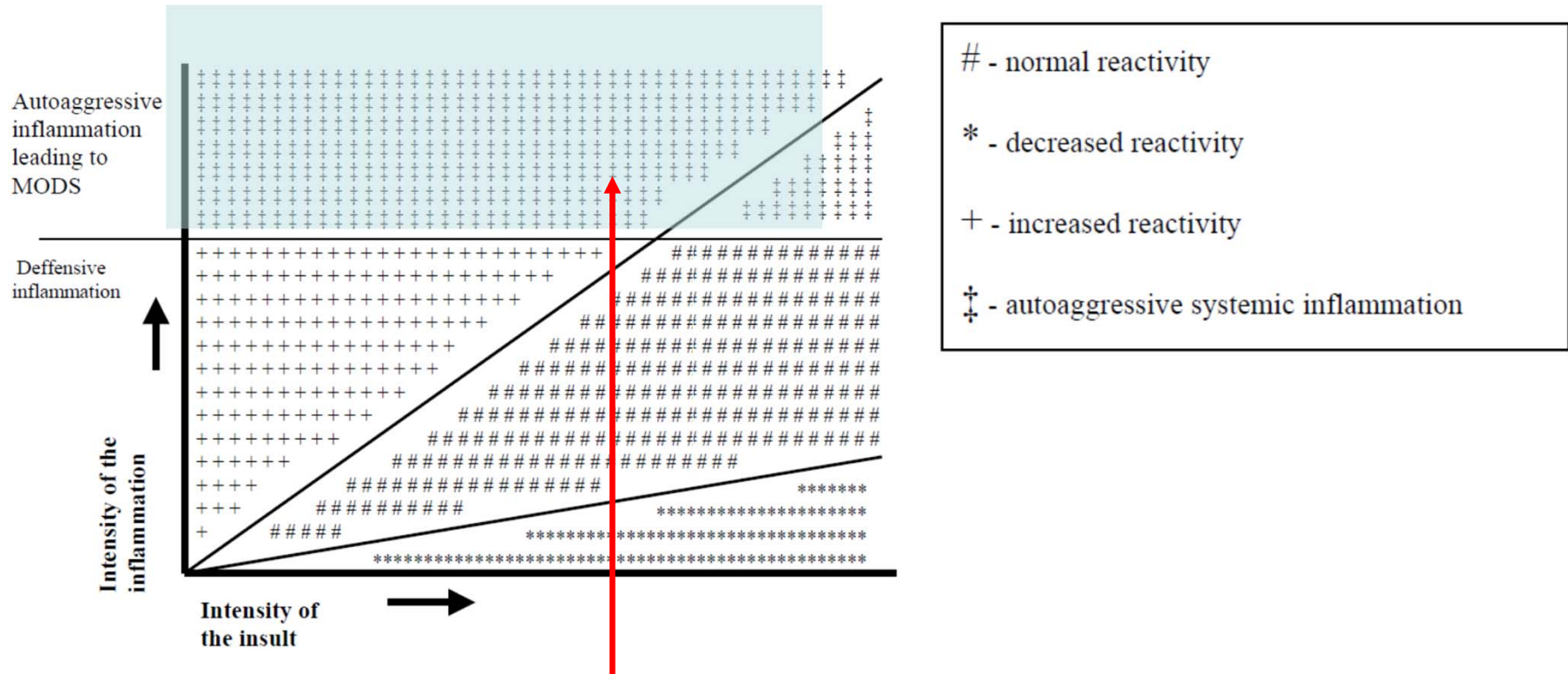
WHAT SHOULD BE
DETECTED?

CHARACTERIZATION THE INTENSITY
OF INFLAMMATION

ESTIMATION OF RESPONSE TO
THERAPY AND THE PROGRESSION

IDENTIFICATION THE POSSIBLE
CAUSE OF THE INFLAMMATION

There is a large variation of inflammations



The doctor's task: timely recognition of SIRS.
(or: sepsis)



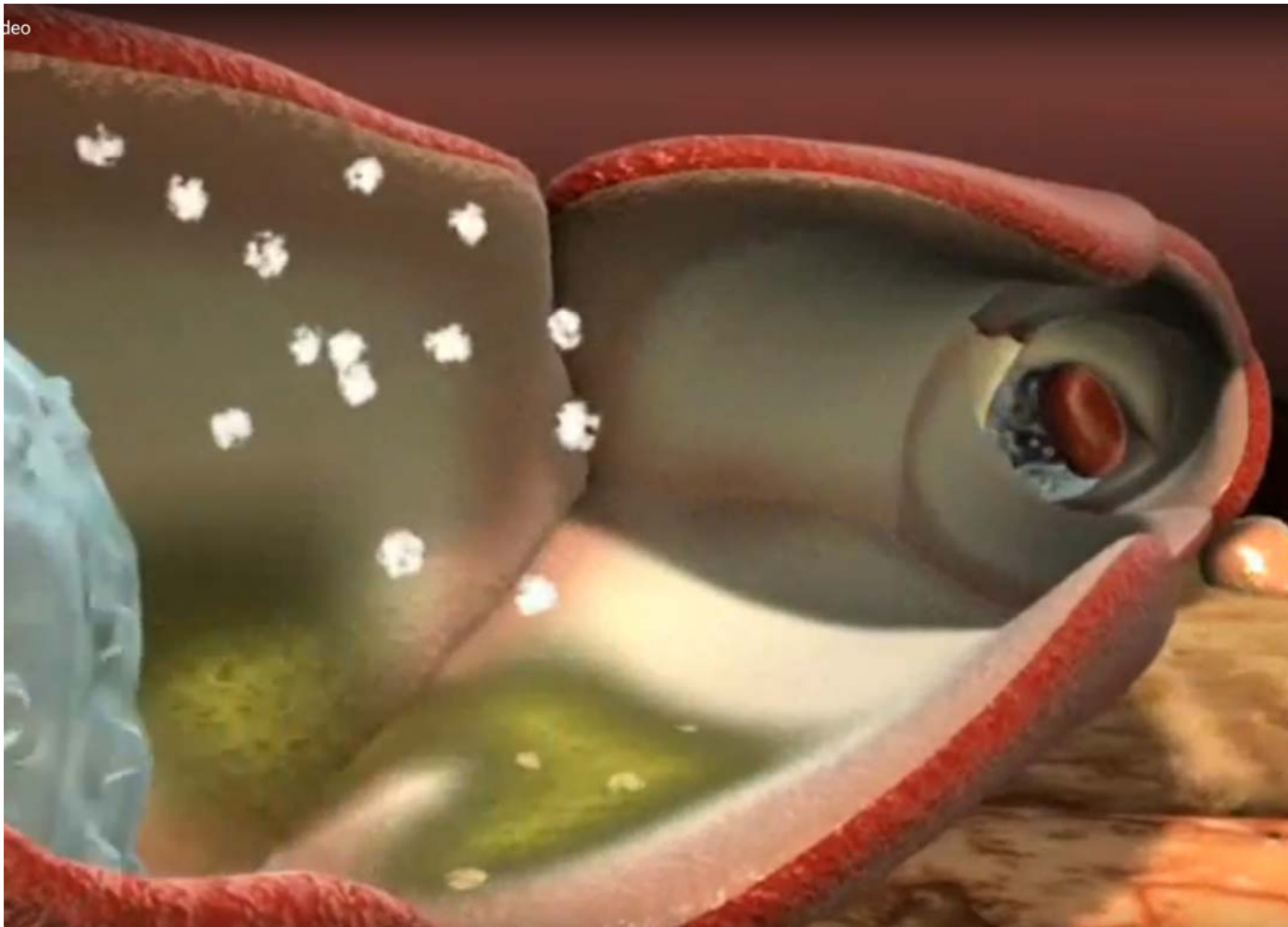
Sepsis

The condition or syndrome caused by the presence of microorganism or their toxins in the tissue /or bloodstroom



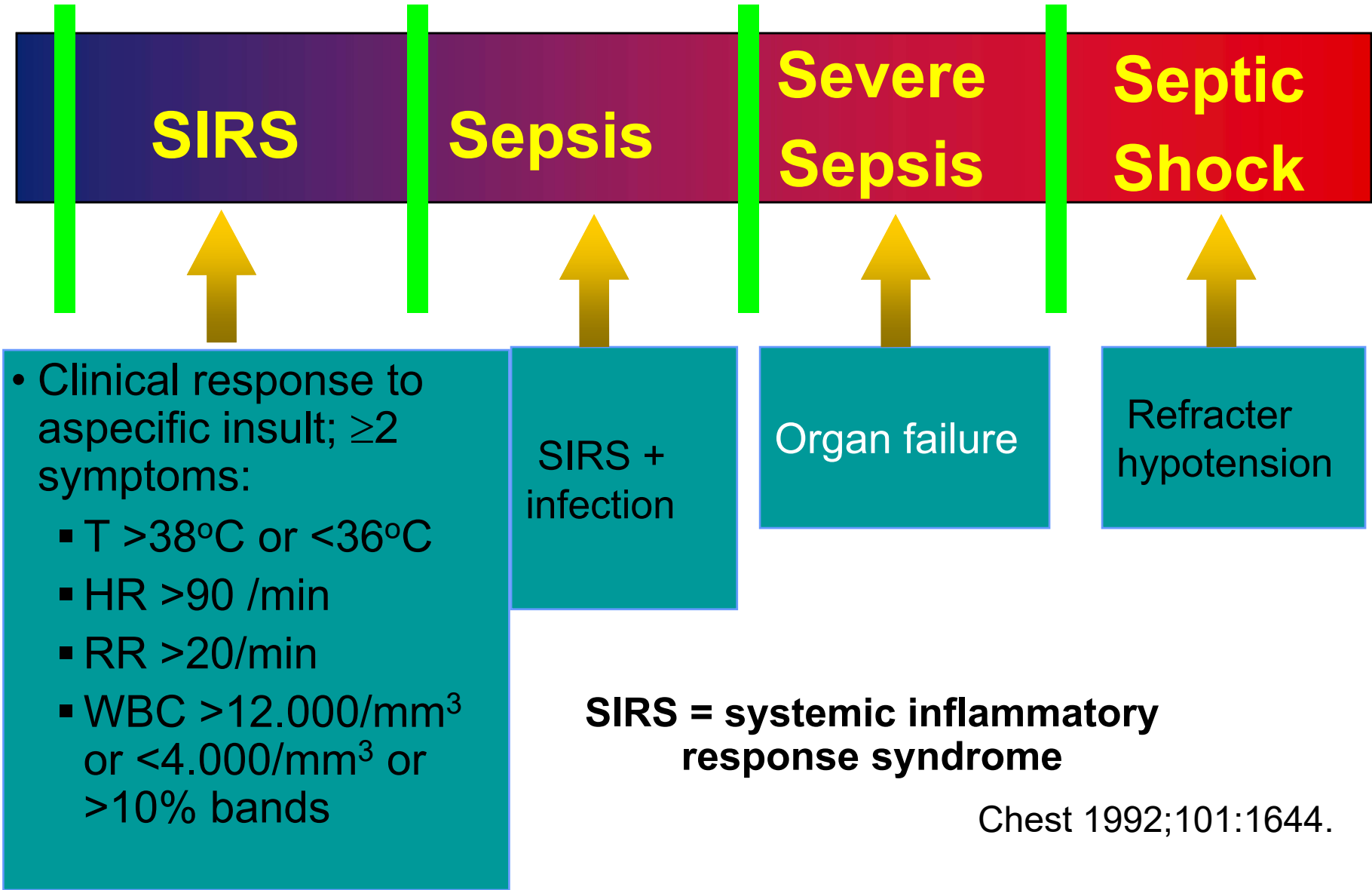
<https://www.youtube.com/watch?v=giwxnHgZ0Ig>

Pathophysiology of Sepsis

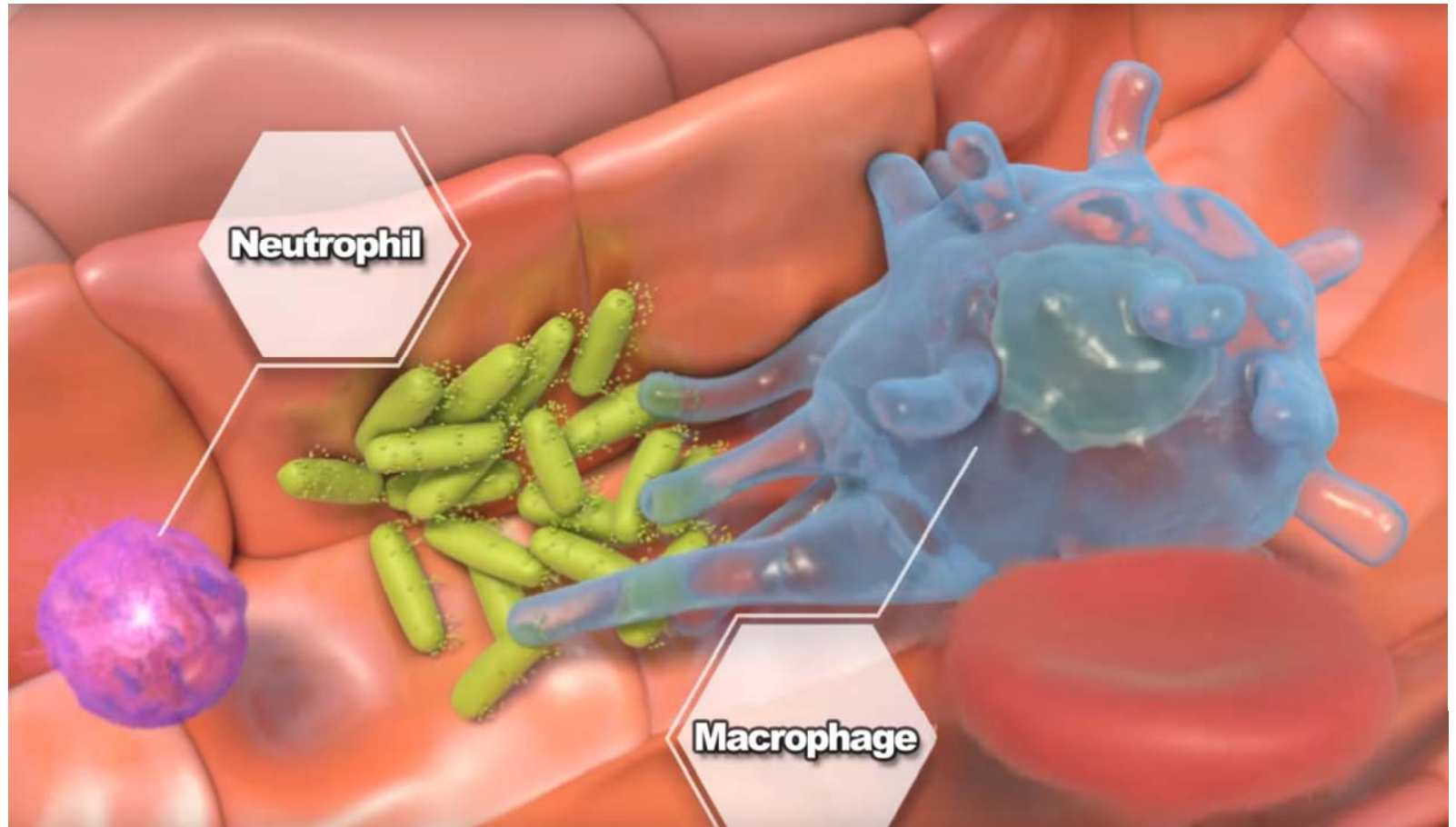


<https://www.youtube.com/watch?v=J-ahUBMkrdM>

The 'Sepsis Continuum'

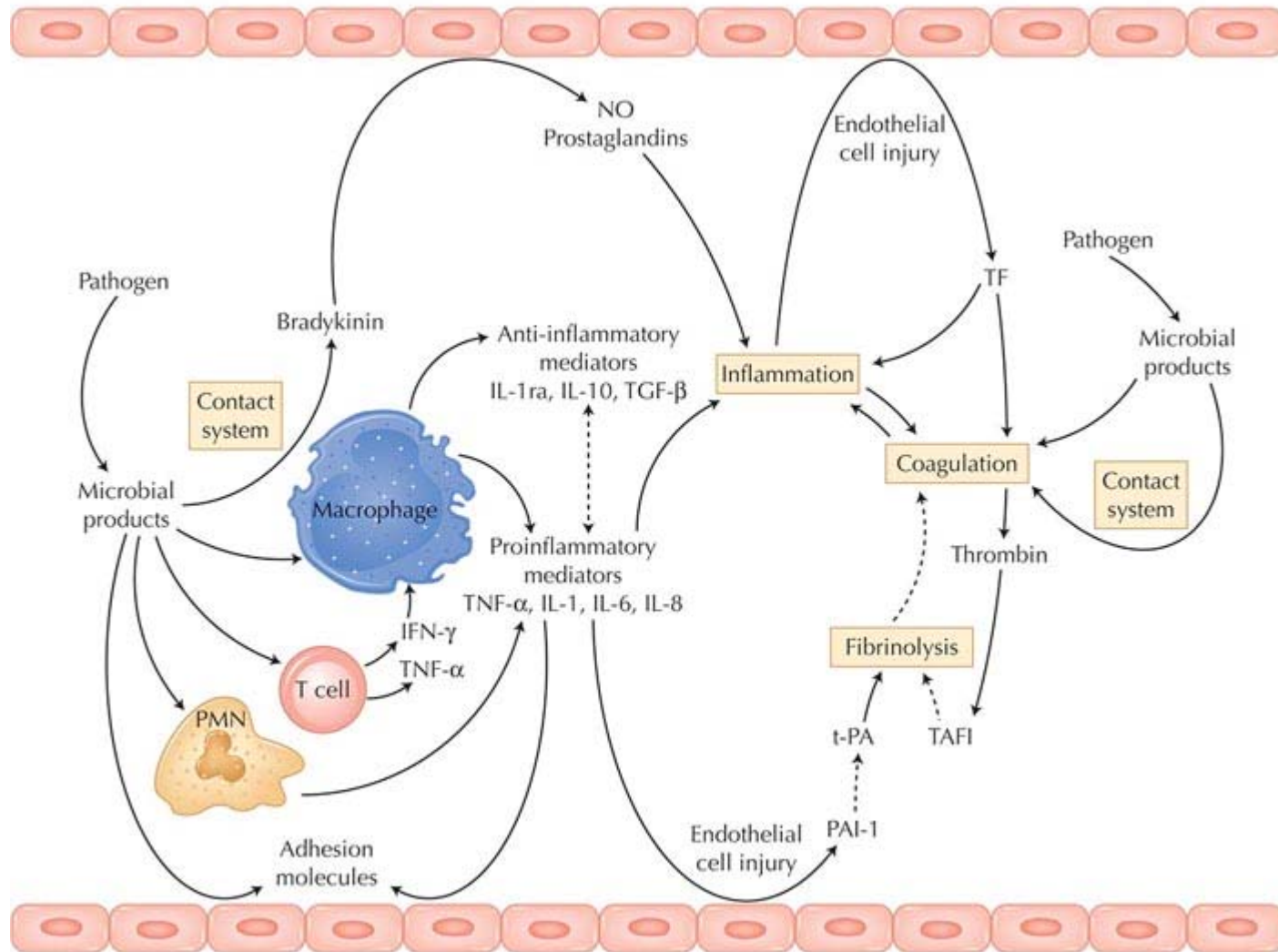


Endothelial barrier dysfunction in septic shock

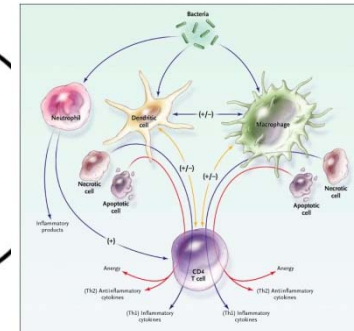
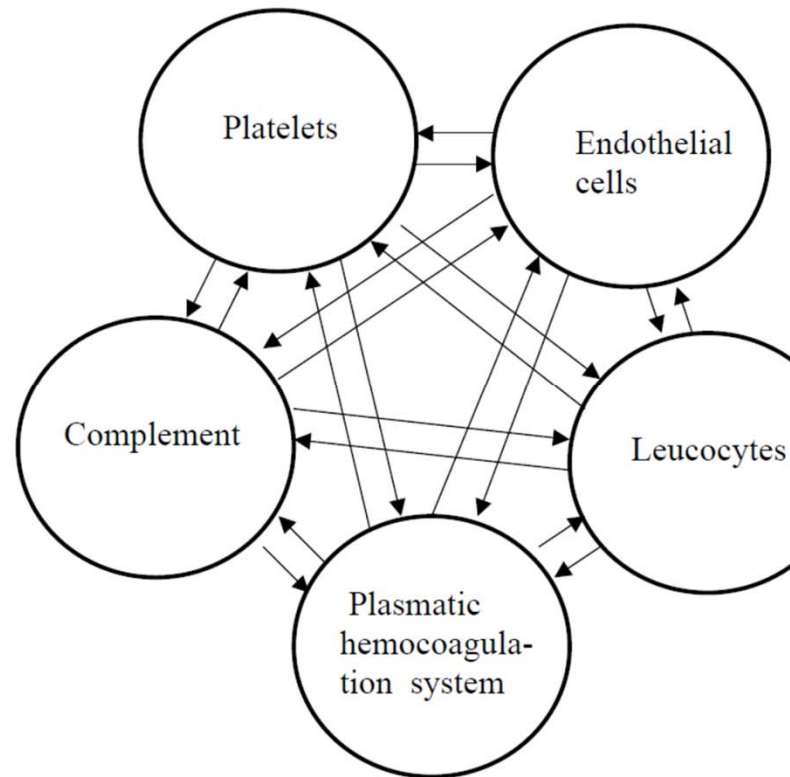


https://www.youtube.com/watch?v=yl6R_3Jrs_s

Systems affected in septic inflammation

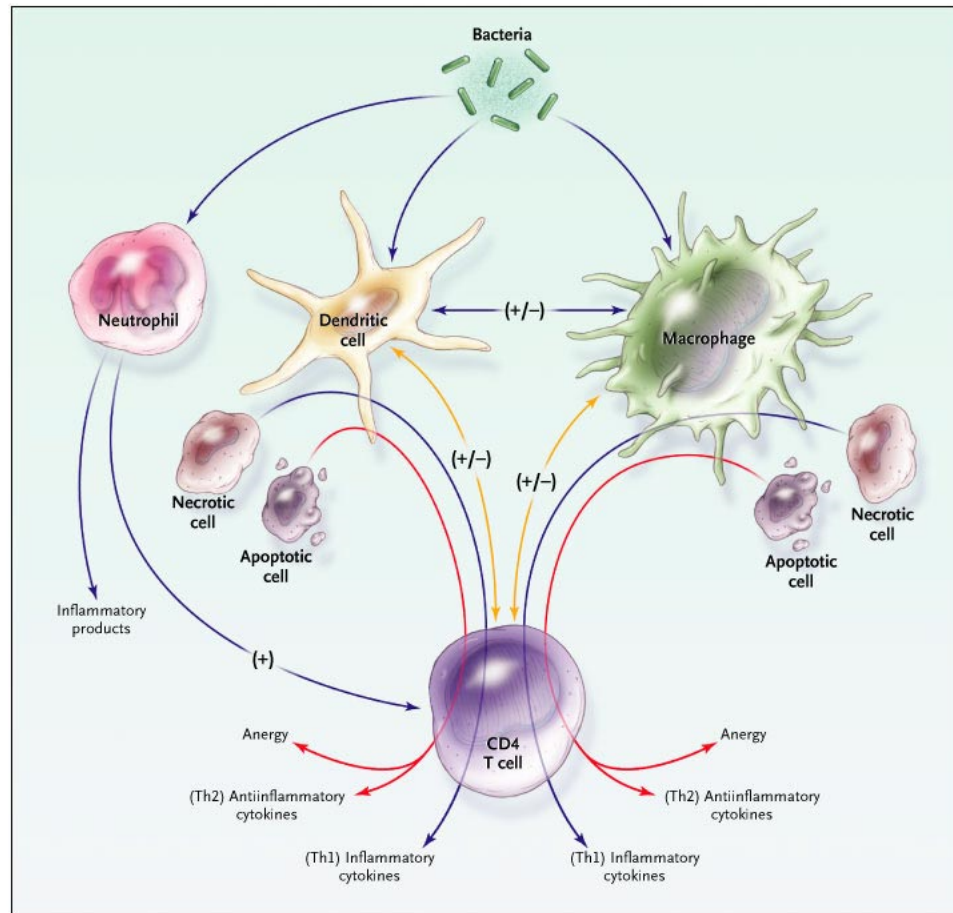


Systems participating in the inflammatory process



The communication between players is mediated via the blood = **these parameters can be measured from blood samples**

Systems participating in the inflammatory process



The communication between players is mediated via the blood = these parameters can be measured from blood samples

THE TOPIC OF THIS LECTURE

The definition of inflammation

The inflammation is an aspecific, complex stereotype response of the body induced by internal and external triggering factors that aims to resolve the cause and consequences of tissue injury

What are the clinical questions related to inflammations?

- Is there any inflammation?
- If the answer is 'YES', what is the cause of that (players' identification)?
- What injury is caused or followed by?

Problems:

1. We would like to characterize a local process through the analysis of a sample originated from blood. INFLAMMATION IS LOCATED DOMINANTLY IN THE TISSUES, NOT IN BLOOD (usually)
2. The systemic effects of local inflammatory processes vary (eg. due to blood-brain barrier, abscess or lymph nodes)
3. The intensity of inflammatory process alters in a dynamic manner
4. ASPECIFIC

Considering the previous points:

Is there any inflammation?

Requirements from inflammatory markers:

- sensitive and specific for the process
- detectable in the blood
- sufficiently stable (can be measured under routine lab conditions)

Acute phase reaction

Cytokines (IL-1, TNF- α and IL-6) and other inflammatory mediators are released at the site of tissue damage

Induce PGE₂ production

Affect ACTH & cortisol production through pituitary and adrenals

Acute phase reaction

- Lasts for a few days
- Affect the immune, CV and CNS systems
- IMPORTANT: altered hepatic functions



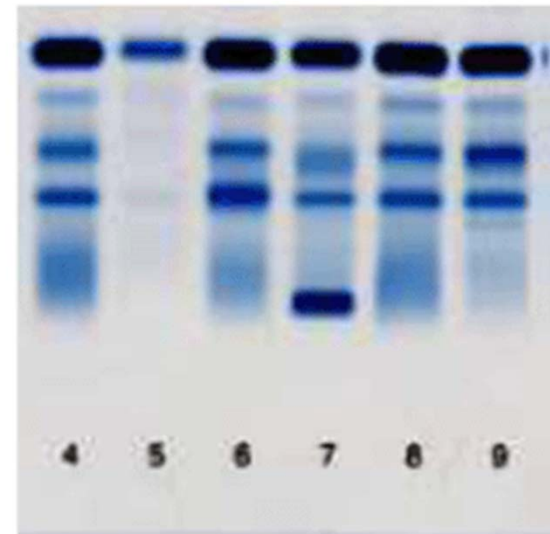
The quantity and quality of hepatic proteins also change

Acute phase reaction – HEPATIC FUNCTIONS

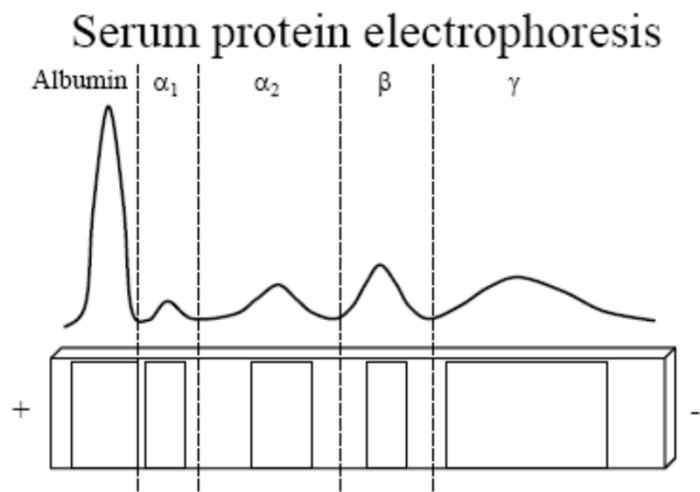
Serum protein ELFO

Separation of proteins

- Size and shape
- Charge
- Voltage
- Measurement conditions



Serum electrophoresis



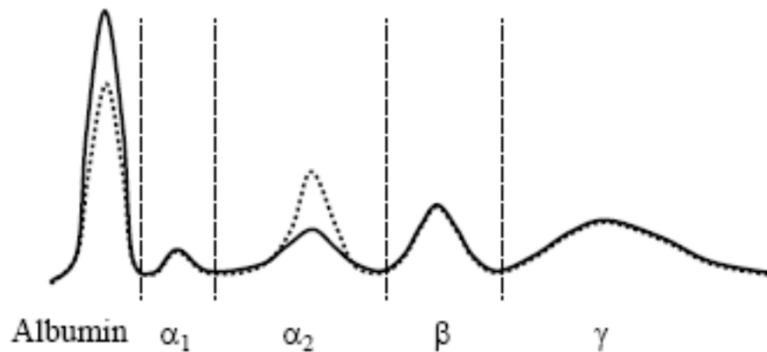
Protein fraction	Plasma protein component	Plasma level
<i>Albumin</i>	Albumin	35-50 g/l
<i>α_1-globulin</i>	α_1 -antitrypsin α_1 -acidic glycoprotein α_1 -lipoprotein (HDL)	2-4 g/l 0,8-1,2 g/l 0,5-0,6 g/l
<i>α_2-globulin</i>	Haptoglobins α_2 -macroglobulin Coeruloplasmin Tyroxin binding globulin	0,3-2,0 g/l 2-3 g/l 0,2-0,6 g/l 12-25 mg/l
<i>β-globulin</i>	Transferrin β -lipoprotein (LDL) Complement protein (C3) β_2 -microglobulin C-reactive protein (CRP) Fibrinogen (between band β and γ)	2-4 g/l 1,0-1,1 g/l 0,7-1,8 g/l 1-2 mg/l 1-5 mg/l 1,5-4 g/l
<i>γ-globulin</i>	IgA IgM IgG IgD IgE	1-4 g/l 0,7-2,5 g/l 8-16 g/l 0,1-0,4 g/l < 0,1 mg/l

Serum electrophoresis – acute phase reaction

Immediate response pattern

Decrease in albumin

Increase in haptoglobin and alpha 1-proteins



A / G rate: 1,5 – 2,5

Decreases (markedly if gamma globulin fraction is increased)

Acute phase reactants

‘positive’:

procalcitonin

C-reactive protein

complements

serum amyloid

coagulation proteins (fibrinogen, vWF)

proteinase inhibitors (α 1-antitrypsin, α 1-chymotrypsin, α 2-antiplasmin, PAI I)

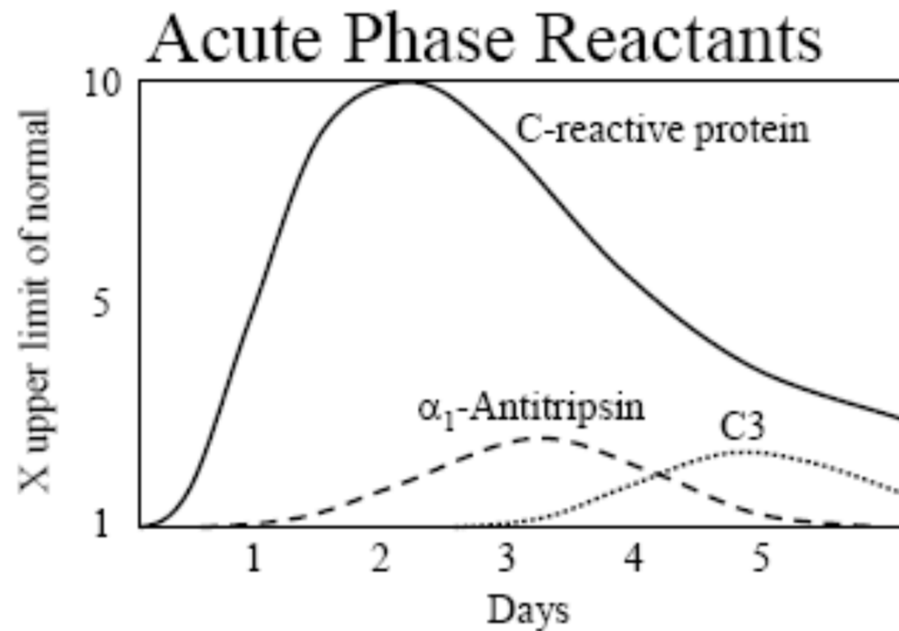
metal binding proteins (haptoglobin, hemopexin, ceruloplasmin, manganese SOD, ferritin, hepcidin)

Other proteins: α 1 acidic glycoprotein, CYTOKINES

‘negative’:

albumin, pre-albumin, transferrin, apoA1, apoA2, iron

Acute phase proteins



Diagnosis of inflammation

Most commonly measured:

Fever

Cell Blood Count

Erythrocyte Sedimentation Rate (ESR)

C-reactive protein

Procalcitonin

FEVER

Pirogens (from granulocytes and monocytes)

Release in an aspecific manner

Recurring fever: fluctuating temperature, 1-2 °C per day – purulent processes and tbc

Intermittant / septic fever: increase in temperature by 2–3 °C, chills. Pneumonia and cystitis

Continuous fever: daily fluctuation is within 1°C. Viral infection, bacterial endocarditis, tumour

Biphasic type: alternating days of febrile and afebrile days. Malaria, Hodgkin's disease

FUO: the cause is still unknown after 3 weeks (40%: infection, 20% tumour, 20% connecting tissue disease; 20% other)

CELL BLOOD COUNTS

Acute purulent bacterial infection:

Increased WBC (>15 G/L)

WBC: $>80\%$ granulocytes, increased prevalence of stabs (left-skewed CBC)

Tissue necrosis and sterile inflammation:

Slight increase in granulocyte count

chronic inflammation:

Normal WBC, often monocytosis

ESR: erythrocyte sedimentation rate

What is that?

Sedimentation of red cells is proportionally increased with the intensity of acute phase response.

The RBC gravity is 6-7% greater than that of plasma.

Influences: acute phase protein, alpha-globulin (27%), fibrinogen (55%), immunoglobulin (11%), albumin (7%)

ESR is increased after 24 hours following the initiation of inflammation.

Less sensitive to viral infection

ESR: erythrocyte sedimentation rate

Tubes to be used

Citrated tube ('black capped')

Blood 1.6; citric acid (3.8%) 0.4 ml

THE RATIO IS IMPORTANT:

more the citrate, increased the ESR

less the citrate, decreased the ESR

ESR: erythrocyte sedimentation rate

What should you note?

Test should be performed in 2 hours after sampling.

Appropriate time should be devoted for the test (1 hour);

Tube should be vertically positioned.

Room temperature.

<18 °C : no ESR

>24 °C : dramatically increased, doubled at 27°C

ESR: erythrocyte sedimentation rate

reference range:

Males: <10 mm/hour, females: < 15 mm/hour

Affects:

- Female periods (highest before menses), pregnancy
- RBC: may be high in anemia (less pronounced in microcytic anaemia)
- Red cell abnormalities (thalassemias)

Drug therapy

increased by: NSAID, cortisol, the Pills

CRP

What is that?

Protein produced by the liver as a response to cytokines; reacts with fraction C polysaccharide in pneumococcus wall.

Calcium binding protein of five subunits.

acute phase reactant: CRP is produced as up to 20% of hepatic proteins under acute phase conditions (up to 1 gramm/day)

Binds endogenous and exogenous ligands, facilitates their opsonisation.

Endogenous ligands: necrotized cells, cell fragments

Exogenous ligands: bacteria, fungi, parasites

Activates complement; enhances phagocytosis, binds to Fc receptors of lymphocytes

CRP

- Tubes to be used

Serum is measured (red capped tube); heparinized capillary may be also convenient. Immunoassay-based.

- What should you note?

Sensitivity of measurement methods differs ('hypersensitive CRP-test, hsCRP')

- reference range: < 5 mg/l

Extensively increased levels in bacterial infection; rapidly reacting parameter.

Procalcitonin (PCT)

What is that?

Propeptide of calcitonin; produced in C-cells of the thyroid gland; and also in the intestine and the lungs as response to infections.

Sample to be used

Serum (red-capped tube)

What should you note?

Earlier marker of inflammation than the CRP.

Measurement should be done within 4 hours after sampling.

reference range: $< 0,5 \mu\text{g/l}$

Its level excessively increased in bacterial infection; very expensive

Biomarkers: that have been already tested

Sepsis biomarker	Clinical study	Type of measurement	Outcome
1 aPTT** [25]	C	c	High negative predictive value
2 CD11b*** [33]	B	s	Higher values in neonates with sepsis than in those with possible infection
3 CD25 [87]	A	s	Distinguished between sepsis and SIRS
4 CD64*** [32,287]	C	s	Low sensitivity and specificity to distinguish between viral and bacterial infections
5 Complement (C3, C4, C5a) [219]	B	s	Distinguished between sepsis and SIRS
6 EA complex [230]	C	s	Diagnosis of sepsis, increased earlier than CRP
7 ELAM-1 (cellular and soluble) [129]	C(s)	c	Increased in trauma patients with sepsis compared with no sepsis
8 Endocan [127]	B	s	Distinguished between sepsis and SIRS
9 E-Selectin (cellular and soluble) [136]	B	s	Distinguished between sepsis and SIRS
10 Fibrin degradation products [36]	B	s	High negative predictive value
11 Gas6 [241]	B	s	Higher values in patients with severe sepsis compared with patients with organ failure but no sepsis
12 G-CSF [237]	C	s	Distinguished between sepsis and SIRS
13 Gelsolin [238]	B(s)	c	Higher in septic patients compared with patients without sepsis
14 IL-1 receptor antagonist [55]	C	s	Early diagnosis of sepsis before symptoms in newborns
15 IL-8* [61]	C	s	Higher in septic neutropenic patients compared with febrile neutropenic patients without sepsis
16 IL-10 [65]	A	s	Higher in septic shock compared with cardiogenic shock
17 IL-12*** [29]	C	s	Diagnosis of sepsis in pediatric patients
18 IL-18 [70]	B(s)	s	Distinguished between Gram-positive and Gram-negative sepsis. Higher in trauma patients with sepsis than in those without
19 IP-10*** [30]	C	s	Early diagnosis of sepsis in newborns
20 Laminin [38]	A	s	Distinguished between Candida sepsis and bacterial sepsis
21 LBP [204]	C	s	Distinguished between Gram-positive sepsis and Gram-negative
22 MCP-1 [61]	C	s	Distinguished between sepsis and SIRS in neutropenic pediatric patients
23 NO, nitrate, nitrite [161]	B	s	Higher in septic shock compared with cardiogenic shock
24 Osteopontin [75]	B	s	Distinguished between sepsis and SIRS
25 PAI-1 [118]	B	s	Higher in patients with sepsis and DIC compared with no-septic patients with DIC
26 Pentraxin 3 [207]	C	s	Distinguished between septic shock and SIRS
27 Peptidoglycan [262]	B(s)	c	Higher in postoperative patients with infection compared with no-infected postoperative patients
28 pFN [270]	B	s	Distinguished between sepsis and SIRS
29 PLA2-II (soluble)*** [31]	B	s	Distinguished between bacteremic and non-bacteremic infections
30 Serum lysozyme (enzyme activity) [258]	B	s	Distinguished between sepsis and organ rejection in transplanted patients
31 ST2 (soluble) [108]	A	s	Higher in septic patients compared with those with no sepsis
32 Surfactant protein (A, B, C, D) [192]	B	s	Early diagnosis of ARDS in septic patients
33 TREM-1 (soluble) [288,289]	C	s	Distinguished between sepsis and SIRS, diagnosed pneumonia
34 Troponin [193]	B	s	Diagnosis of myocardial dysfunction in septic patients

*sensitivity and specificity of less than 90%; **sensitivity of more than 90% but specificity of less than 90%; ***sensitivity and specificity more than 90%; A, Clinical study with less than 20 patients; B, Clinical study with 20 to 50 patients; C, Clinical study with more than 50 patients; (s), surgical patients only; (m), medical patients only; s, single value; c, values over time.

aPTT: activated partial thromboplastin time; ARDS: acute respiratory distress syndrome; CRP: C-reactive protein; DIC: disseminated intravascular coagulopathy; EA: elastase alpha 1-proteinase inhibitor; ELAM: endothelial leukocyte adhesion molecule; G-CSF: granulocyte colony-stimulating factor; IP: interferon-induced protein; LBP: lipopolysaccharide-binding protein; MCP: monocyte chemoattractant protein; NO: nitric oxide; PAI: plasminogen activator inhibitor; pFN: plasma fibronectin; PLA2: phospholipase A2; SIRS: systemic inflammatory response syndrome; TREM: triggering receptor expressed on myeloid cells.

Biomarkers: novel acute phase reactants

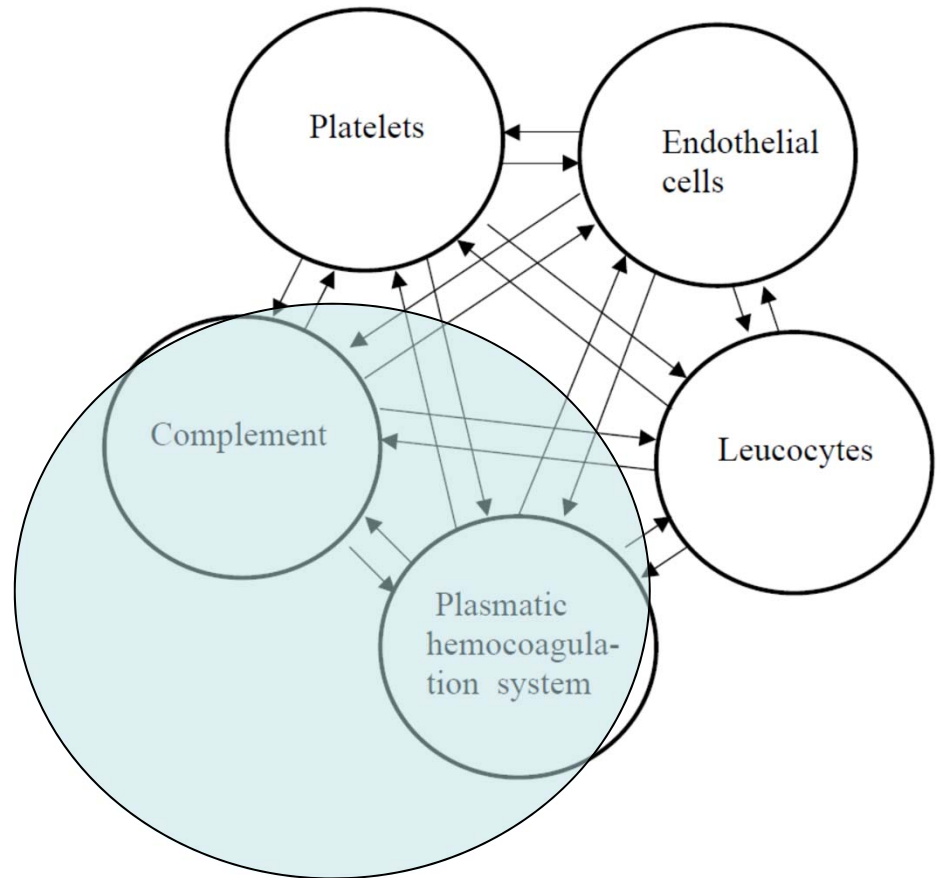
Complement system, coagulation factors,

Ferritin, caeruloplasmin

Alfa-1 antitrypsin

Lipoprotein-binding protein

(Pro)-hepcidin



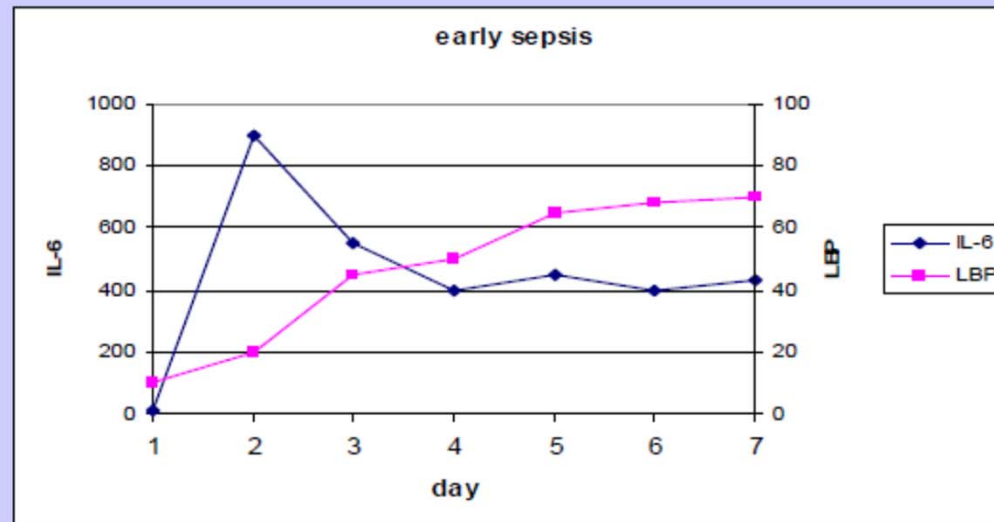
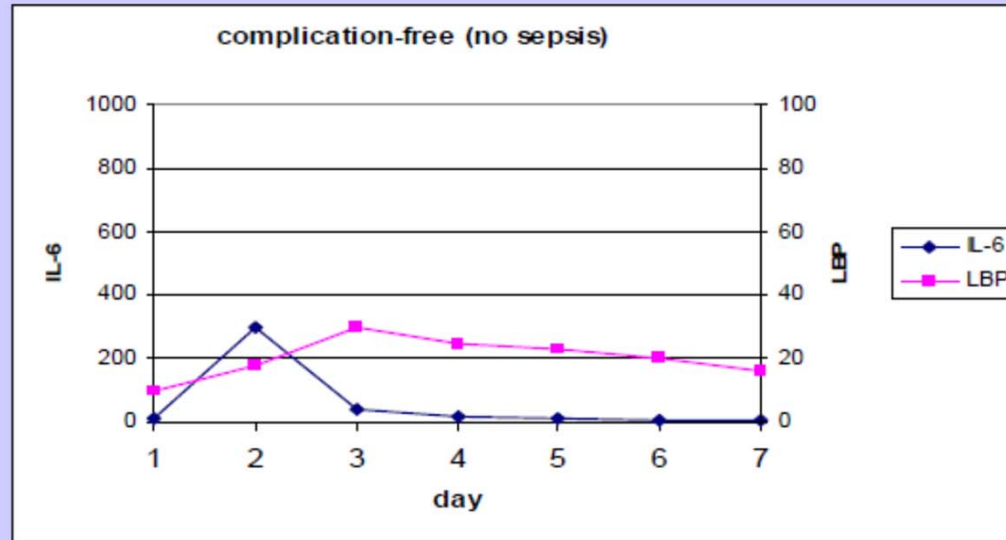
LPS-binding protein

- acute phase reactant
- CD14 macrophage activation and cytokine release triggers
- levels increased in sepsis
- outstanding diagnostic performance at newborns and infants

LPS-binding protein

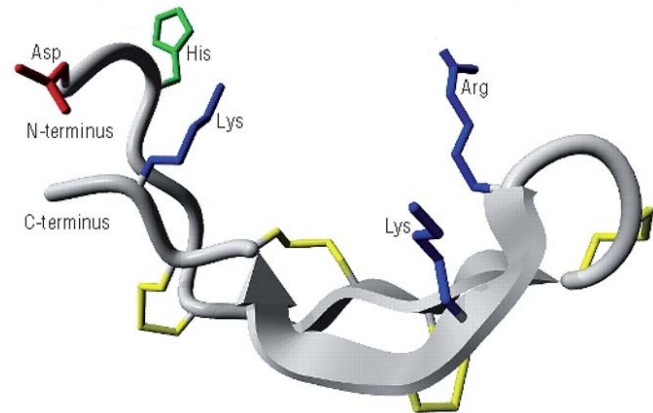
- act
- CD
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Hepcidin

- High levels in inflammation;
lowers plasma iron levels
- No exact method of measurement
- Marked daytime variability



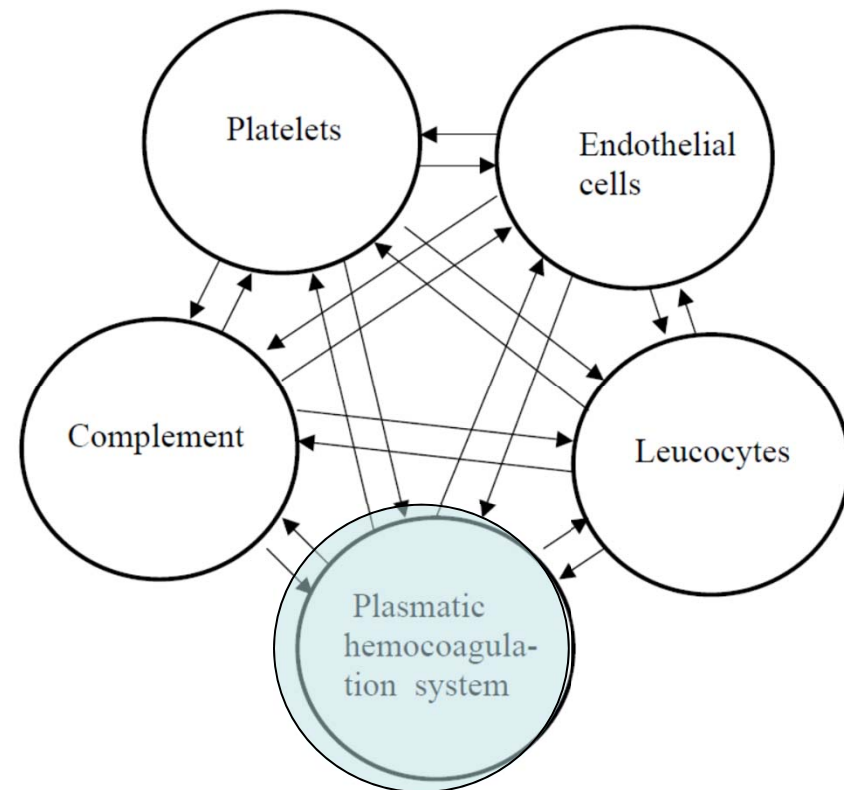
Biomarkers: blood coagulation tests

aPTT, thrombomodulin:
prognostic (MOF and DIC)

D-dimer, protein C & S:
predictive for survival

Fibrin:
Gram-negative sepsis

Importance of repeated measurements.



Biomarkers: cytokines, chemokines

Anti- and proinflammatory cytokine levels change in a variable manner.

Characteristic markers in sepsis:

GCSF, IL-1receptor alpha,

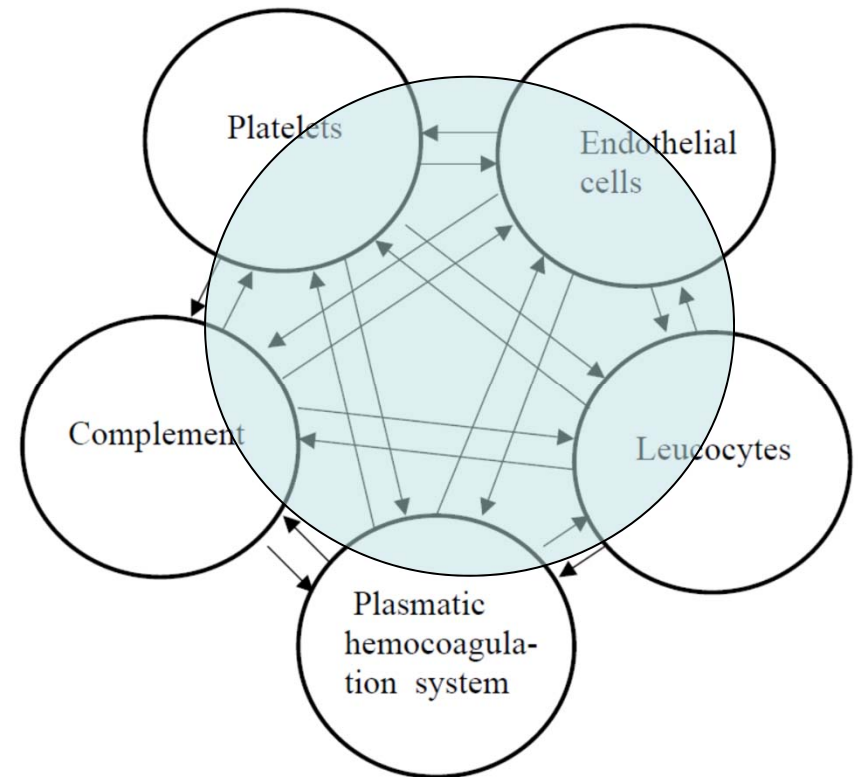
IL-4, IL-10, GRO- α

IL-8, IL-12, IP*-10

Predictive for survival:

IL-6, IL-12, IL-18, TNF

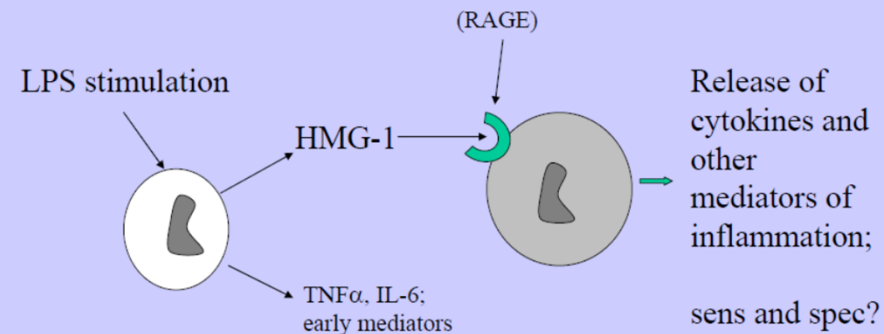
Predictive for complication
prediktív: IL-8



*IP: interferon-induced protein

High-mobility group 1 protein (HMG-1)

- Originates from the macrophages
- Produced >8 hours after stimulation with LPS, TNF-alpha and IL-1beta
- Late mediator of endotoxinaemia (triggers the release of proinflammatory cytokines)
- Increased levels in septic patients with severe condition
- Predicts mortality
- Low sensitivity and specificity



Macrophage migration inhibiting factor (MIF-1)

- Prevents inhibitory action of corticosteroids; pro-inflammatory
- Produced by T-cells and macrophages as a response to LPS
- Levels are increased in systemic infection
- Not suitable to differentiate between inflammations of infectious and non-infectious origin

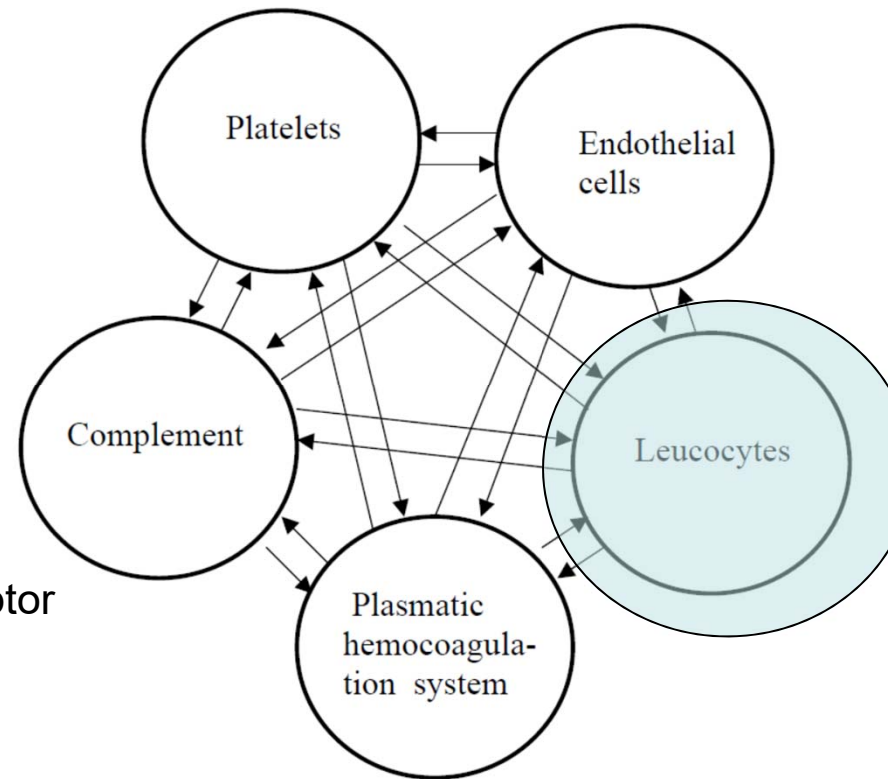
Biomarkers: cellular markers

CD14 ('presepsin'), CD64, CD163,
mHLA-DR, TREM-1*, suPAR**,
CCR CCR, CRTh2, CD25

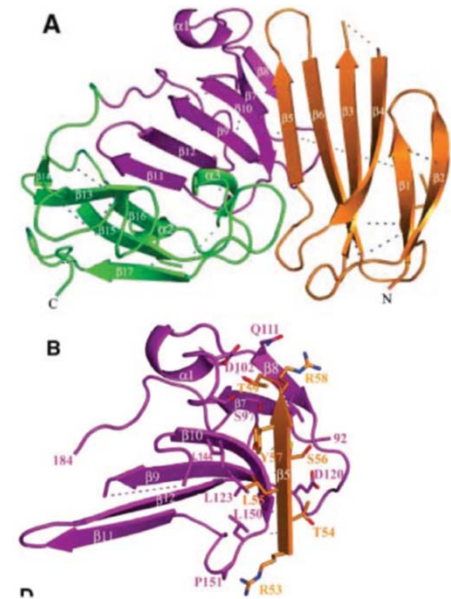
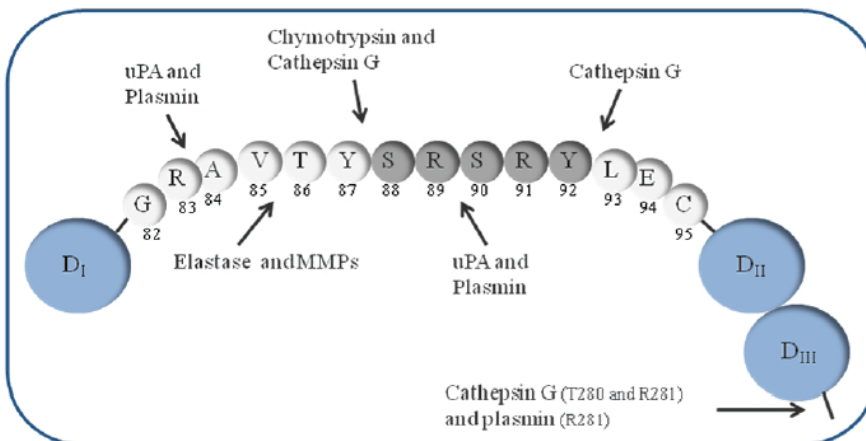
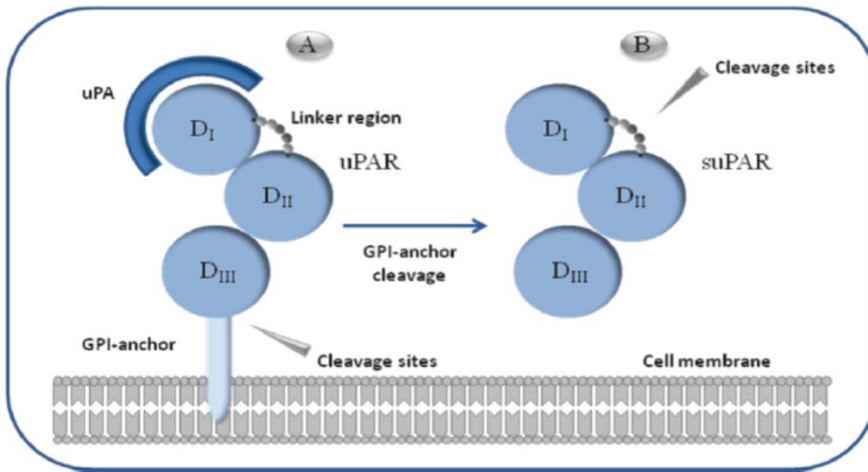
Surface markers that present in
soluble forms in the serum

* Triggering receptor expressed on myeloid cells
(TREM)

** soluble urokinase plasminogen activator receptor



suPAR



Low circadian variability

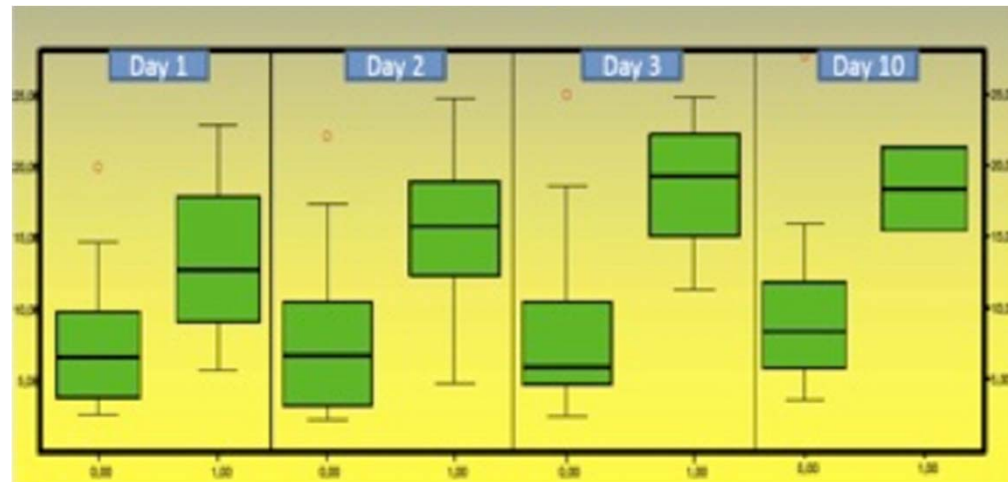
suPAR: survival in sepsis

17 surviving

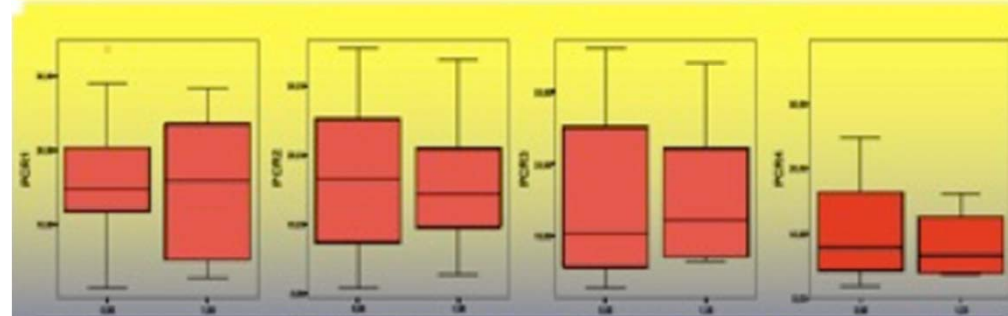
9 died

within 10 days

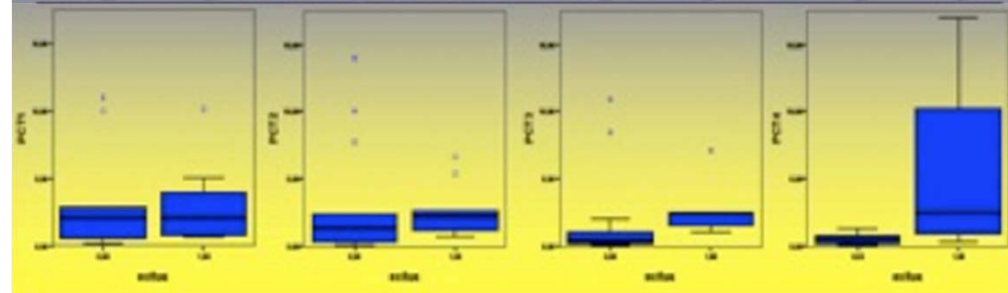
suPAR



CRP

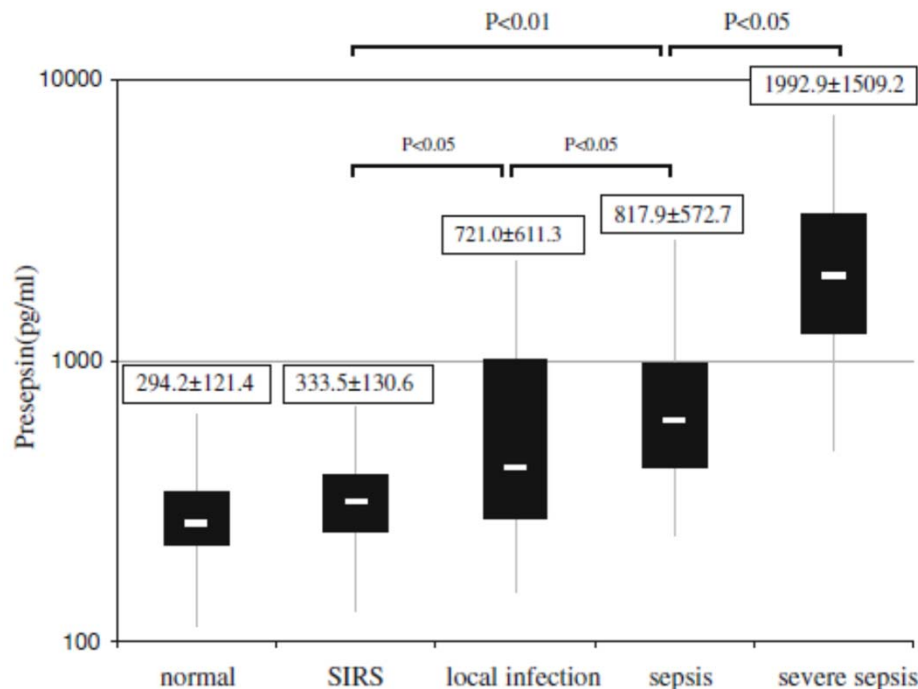


PCT



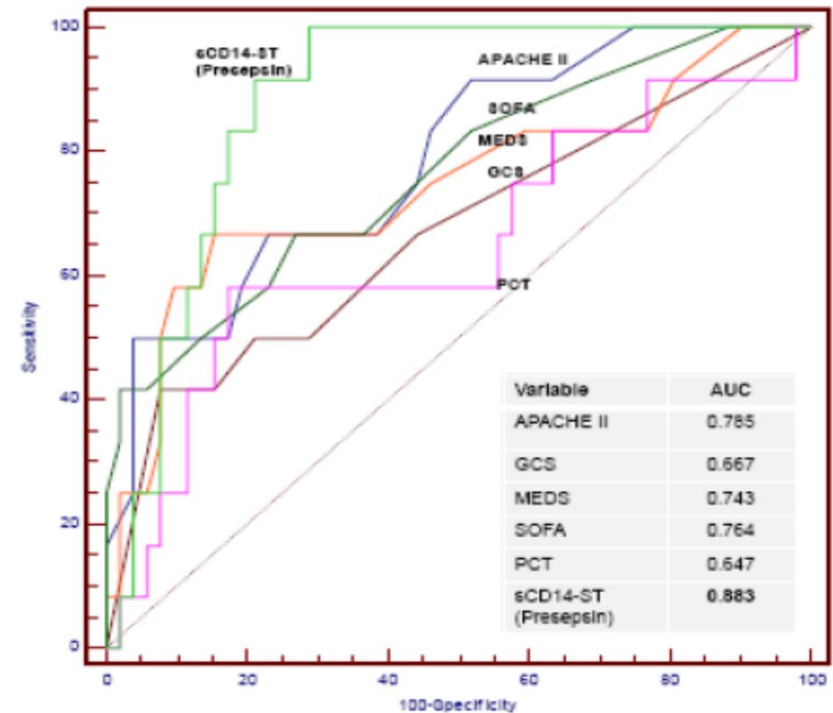
Presepsin (solubilis CD14)

- Differentiates between sepsis and severe sepsis
- Predicts 30-days mortality



J Infect Chemother

DOI 10.1007/s10156-011-0254-x

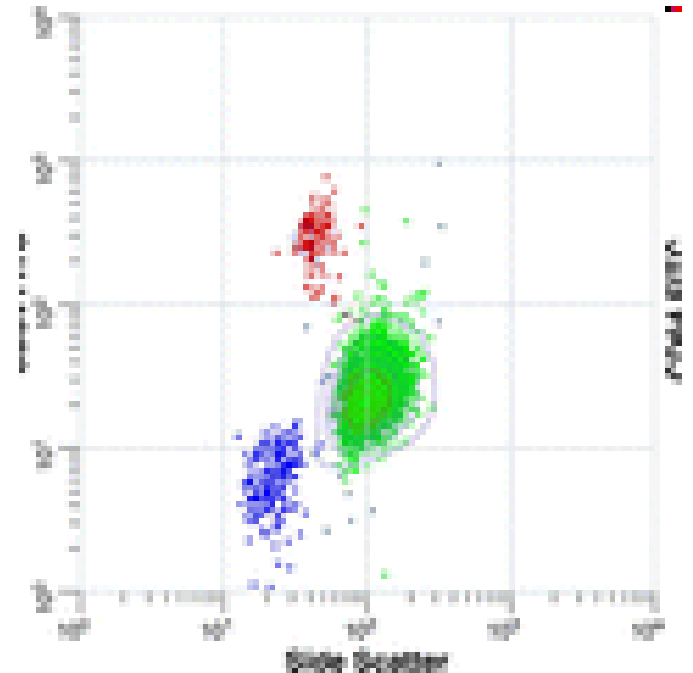


E. Spanuth

IFCC-WorldLab – EuroMedLab, Berlin, 15 -19 May 2011

Fc gamma receptor on neutrophil cells (CD64)

- Exposed on polymorphonuclear cells during infection
- Responds within 4 – 6 hours after activation
- Sensitive and specific in systemic inflammation, infection and sepsis
- Predicts survival

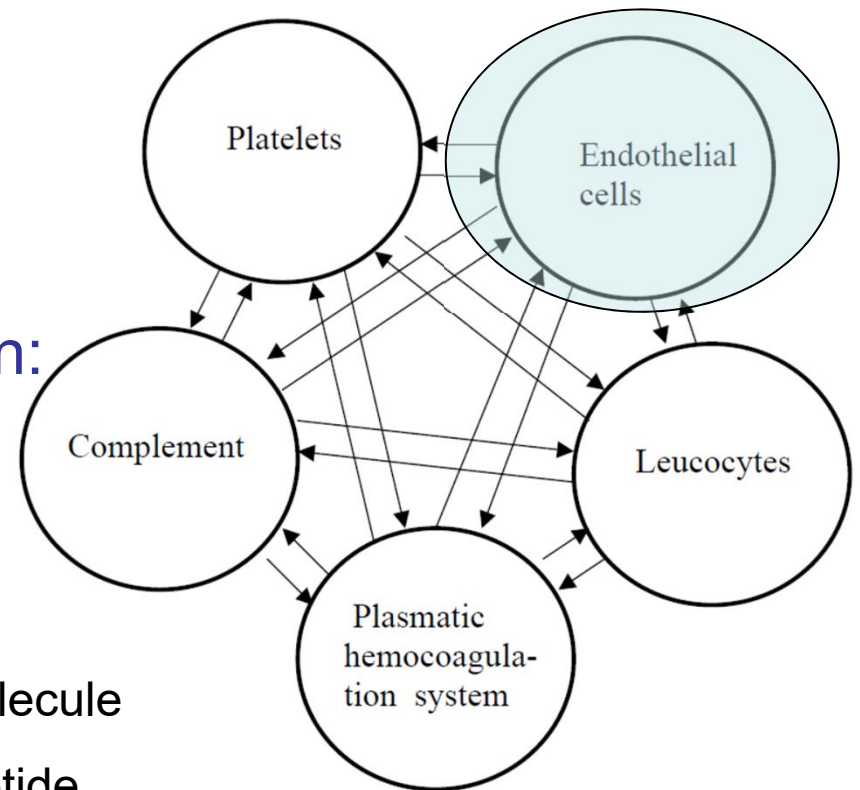


Biomarkers: endothelial cell injury

VCAM-1, E-selectin:
prognostic for MOF

ELAM*:
detection of sepsis

L-selectin, CGRP**, neopterin:
predicting survival



* Endothelial leukocyte adhesion molecule

** CGRP: calcitonin-gene related peptide

Neopterin

What is that?

- Produced in association with cellular immune response (viral infection); longer half-life than that of IFN-gamma
- Autoimmune and other inflammatory disorders
- Differentiates between bacterial and viral infections
- Indicates immune response (transplantation)
- Increased levels before extensive antibody production

What type of samples are used?

- Urine, serum, CSF (often with HPLC)

Neopterin

- What should you note?

First urine sample in the morning

Serum levels increase when renal function impairs

- reference range:

serum: <10 nmol/l

Liquor: <6 nmol/l

urine: <200 micromol/mol
creatinine

Serum amyloid A (SAA)

- What is that?

Apolipoprotein; produced in liver, induced by activated macrophages. Binds to HDL particles. Physiological role is unknown.

- What should you note?

Not a common test, the result depends on methods.

- Reference range:

<10 mg/l in serum

Identification of the trigger of inflammatory response

- Bacteria?
- Virus?
- Other?

Bacteria?

If YES, the infection can be controlled by antibiotics

Microbiological tests (culture, or, sometimes, PCR):

- Cave: preanalytics

DO NOT TAKE AFTER THE INITIATION OF
ANTIBIOTIC THERAPY

CONDITIONS FOR SAMPLING AND TRANSPORT
SPECIAL PATHOGEN, SPECIAL CONDITIONS...

see microbiology

Virus?

If yes, it determines the therapy

- Possibly antiviral agent
- prognosis
- Public health measures
- Antibody measurements

Cave: window period

See also: microbiology, internal medicine

- Increasingly: molecular biology

Serological tests: antibody titer

tests: bacterial agglutination (Widal-reaction),
complement fixation test, indirect
hemagglutination: does not differentiate between
IgG and IgM antibodies (may be positive for prior
infections)

Titer is increasing at two consecutive
measurements: acute infection.

continuously high titer: ongoing or resolving
infection

Decreasing titer: recent infection

Serological tests: antibody titer

tests: immunoassay, immune fluorescent test

Pathogen specific IgG, IgM, IgA antibodies are measured.

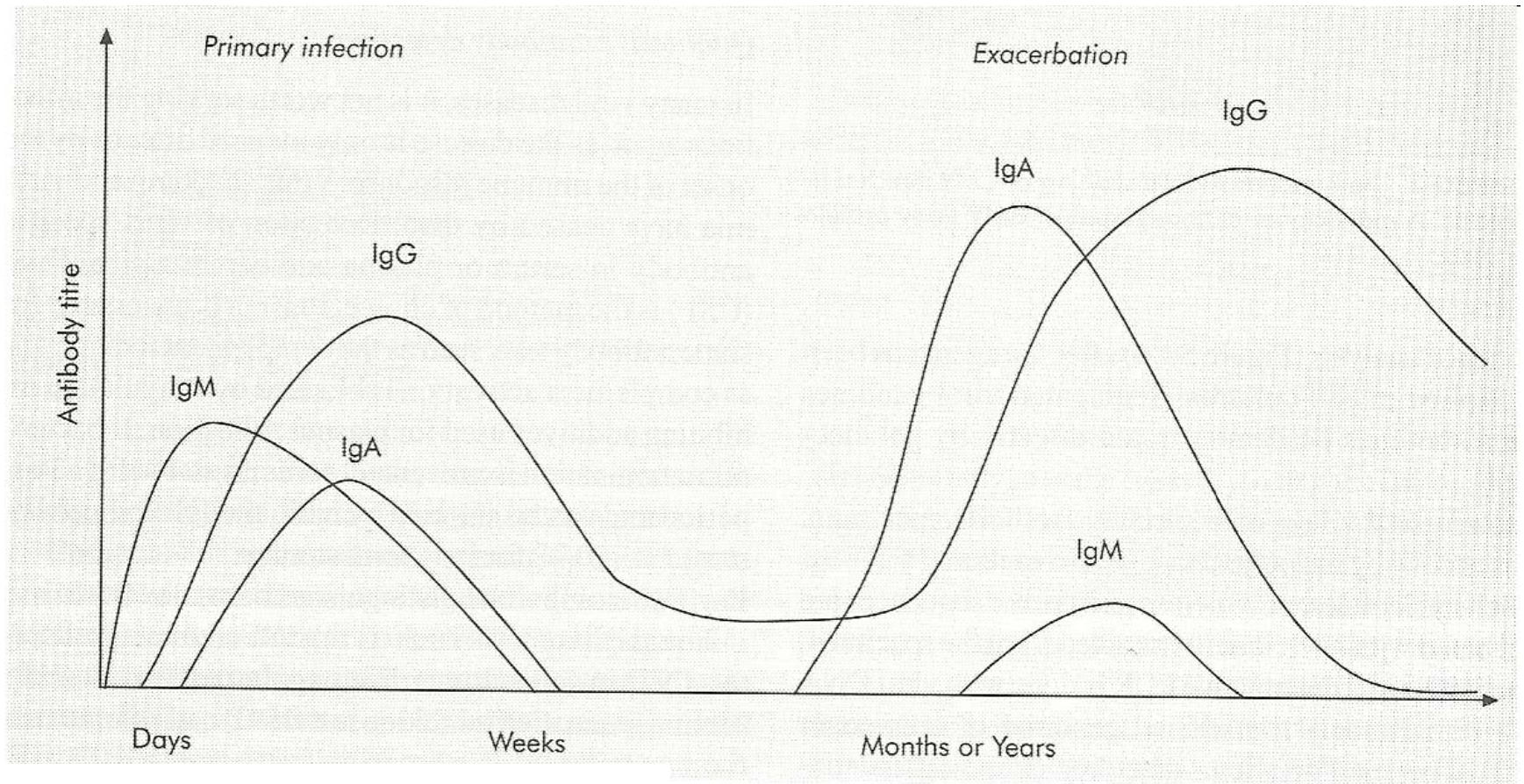
- negative result: the lack of a specified antibody – there is no infection (provided that the patient is not immune compromised). Window period: 5 – 7 days
- positive result: infection (currently or earlier).

Serological tests in suspected bacterial infections: antibody titer

- **IgM: primary immune response**
 - maximum level: in 1 week;
 - disappears: after 2 – 3 months
 - magzatban / újszülöttben: intrauterin infection
- **IgG: secondary immune response**
 - maximum level: at week 2 – 3.; decreases very slowly (detectable until the end of life)
 - Increasing IgG titers indicate acute infection or reinfections
 - Continuously high titer: earlier but still persisting infection

Important: IgG and IgM titers may increase after vaccination

Antibody-kinetics following infection



Titers frequently measured

- Streptococcus infection (GAS, group A streptococcus):
 - antistreptolysin titer (O reaction), ASO
 - antistreptococcal DNase B test
 - antistreptococcal hialurodinase reaction (AHy)

The goal of the test:

Detection of actual or earlier GAS-infection
(investigation of acute rheumatoid fever)

Antistreptolizin titer (O reaction), ASO

1.

Diluted serum + reagent.

Agglutination is read after 2 minutes

2.

Detection of haemolysis caused by streptolysin:
the test indicates the dilution (titer) of sample
that still inhibits the haemolysis.

In addition:

Lyme's disease, syphilis, borreliosis,
H.pylori, Campylobacter, Chlamydia,
Gonorrhea, Legionella, Leptospira,
Staphylococcus

TAKE HOME MESSAGE

- A local inflammatory response should be detected using a sample of systemic circulation
- Parameters change rapidly in time
- Parameters may indicate different aspects of inflammation
- Should be related to CLINICAL STATUS