Ενδοθηλιακοί δείκτες: παρακολούθηση και παρέμβαση στη Σήψη

Στυλιανός Ορφανός

Α' & Β' Κλινικές Εντατικής Θεραπείας ΕΚΠΑ

Box 1. SIRS (Systemic Inflammatory Response Syndrome)

Two or more of:

Temperature >38°C or <36°C

Heart rate >90/min

Respiratory rate >20/min or $PaCO_2$ <32 mm Hg (4.3 kPa) White blood cell count >12 000/mm^3 or <4000/mm^3 or >10% immature bands

From Bone et al.[9]

JAMA. 2016 February 23; 315(8): 801–810.

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The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)

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Box 2. Key Concepts of Sepsis

- Sepsis is the primary cause of death from infection, especially if not recognized and treated promptly. Its recognition mandates urgent attention.
- Sepsis is a syndrome shaped by pathogen factors and host factors (eg, sex, race and other genetic determinants, age, comorbidities, environment) with characteristics that evolve over time. What differentiates sepsis from infection is an aberrant or dysregulated host response and the presence of organ dysfunction.
- Sepsis-induced organ dysfunction may be occult; therefore, its presence should be considered in any patient presenting with infection. Conversely, unrecognized infection may be the cause of new-onset organ dysfunction. Any unexplained organ dysfunction should thus raise the possibility of underlying infection.
- The clinical and biological phenotype of sepsis can be modified by preexisting acute illness, long-standing comorbidities, medication, and interventions.
- Specific infections may result in local organ dysfunction without generating a dysregulated systemic host response.

JAMA. 2016 February 23; 315(8): 801–810.

- In lay terms, sepsis is a life-threatening condition that arises when the body's response to an infection injures its own tissues and organs.
- Patients with suspected infection who are likely to have a prolonged ICU stay or to die in the hospital can be promptly identified at the bedside with qSOFA, ie, alteration in mental status, systolic blood pressure ≥100 mm Hg, or respiratory rate ≥22/min.
- Septic shock is a subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality.
- Patients with septic shock can be identified with a clinical construct of sepsis with persisting hypotension requiring vasopressors to maintain MAP ≥65 mm Hg and having a serum lactate level >2 mmol/L (18mg/dL) despite adequate volume resuscitation. With these criteria, hospital mortality is in excess of 40%.

JAMA. 2016 February 23; 315(8): 801–810.

Am J Physiol Lung Cell Mol Physiol 303: L355–L363, 2012.
First published June 15, 2012; doi:10.1152/ajplung.00069.2012.

Review

Inflection points in sepsis biology: from local defense to systemic organ injury

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Seeley EJ, Matthay MA, Wolters PJ. Inflection points in sepsis biology: from local defense to systemic organ injury. *Am J Physiol Lung Cell Mol Physiol* 303: L355–L363, 2012. First published June 15, 2012; doi:10.1152/ajplung.00069.2012.—Sepsis and septic shock lead to considerable morbidity and mortality in developed and developing countries. Despite advances in understanding the innate immune events that lead to septic shock, molecular therapies based on these advances have failed to improve sepsis mortality. The clinical failure of laboratory-derived therapies may be, in part, due to the pleiotropic consequences of the acute inflammatory response, which is the focus of this review. A brisk response to infecting organism is essential for pathogen containment and eradication. However, systemic spread of inflammation beyond a single focus leads to organ injury and higher mortality. The primary goal of this article is to discuss recent animal- and human-based scientific advances in understanding the host response to infection and to highlight how these defense mechanisms can be locally beneficial but systemically detrimental. There are other factors that determine the severity of sepsis that are beyond the scope of this review, including the virulence of the pathogen and regulation by Toll-like receptors. Specifically, this review focuses on how the effector mechanisms of platelets, mast cells, neutrophil extracellular traps (NETs), and the endothelium participate in combating local infections yet can induce organ injury during systemic infection.

innate immunity; neutrophil extracellular traps; mast cells; platelets; Toll-like receptor

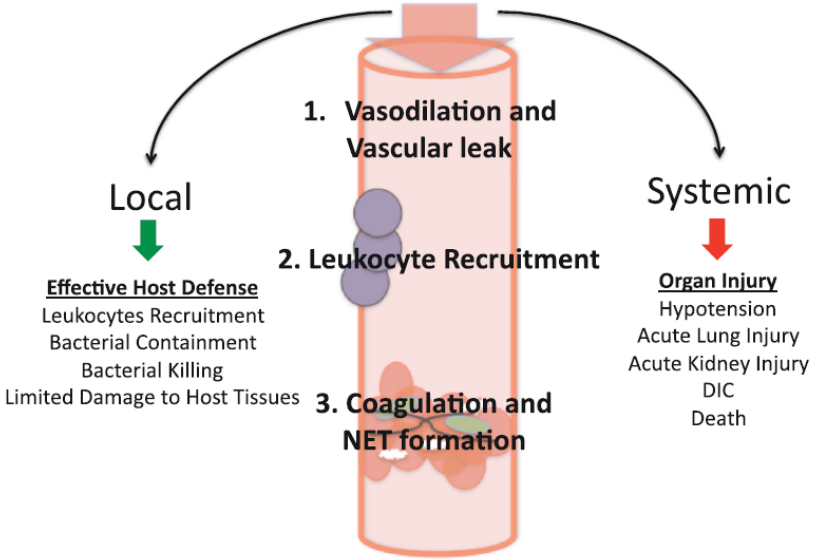


Fig. 1. Locally beneficial host defense mechanisms can become detrimental during the systemic spread of infection and inflammation. Local inflammatory mediators, including tumor necrosis factor (TNF) and interleukin (IL)-1β, lead to vasodilation, which recruits leukocytes to sites of infection and sets off a cascade of leukocyte activation, neutrophil extracellular trap (NET) formation, and coagulation. These mechanisms help contain and kill pathogens during localized infection. In contrast, the systemic spread of these same immune mechanisms leads to septic shock, acute organ injury, and potentially death. DIC, disseminated intravascular coagulation.

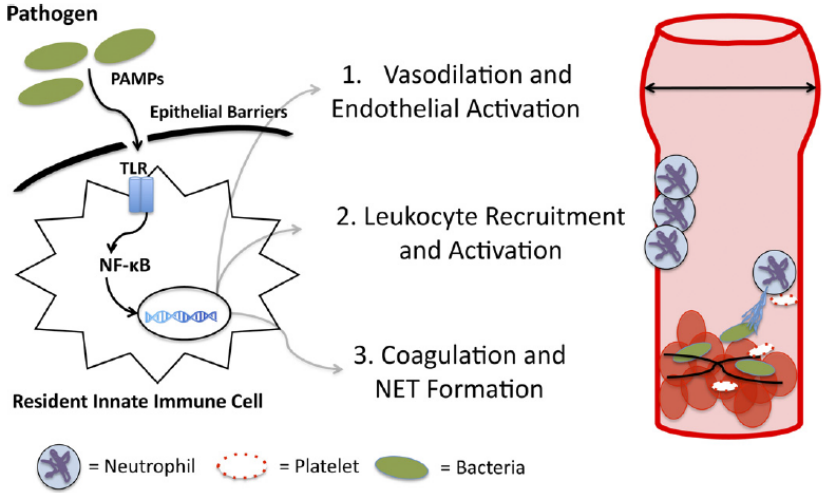


Fig. 2. Early cellular and molecular events during infection. Molecular motifs specific to pathogens (PAMPs) are sensed by resident innate immune cells, which express Toll-like receptors (TLRs). TLRs signal via the nuclear factor (NF)-κB signaling cascade, leading to the expression of nitric oxide, which induces vasodilation and increases blood flow to sites of infection. TLR signaling also leads to inflammatory cytokine (TNF, IL-1β, IL-6) and chemokine [monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1α] production, which recruits monocyte and neutrophil sites of infection. Local cytokine release and the effects of these cytokines on the endothelium increase the procoagulant properties of endothelial cells and induce platelet-neutrophil interactions that lead to the formation of NETs.

Am J Physiol Lung Cell Mol Physiol 303: L355–L363, 2012.

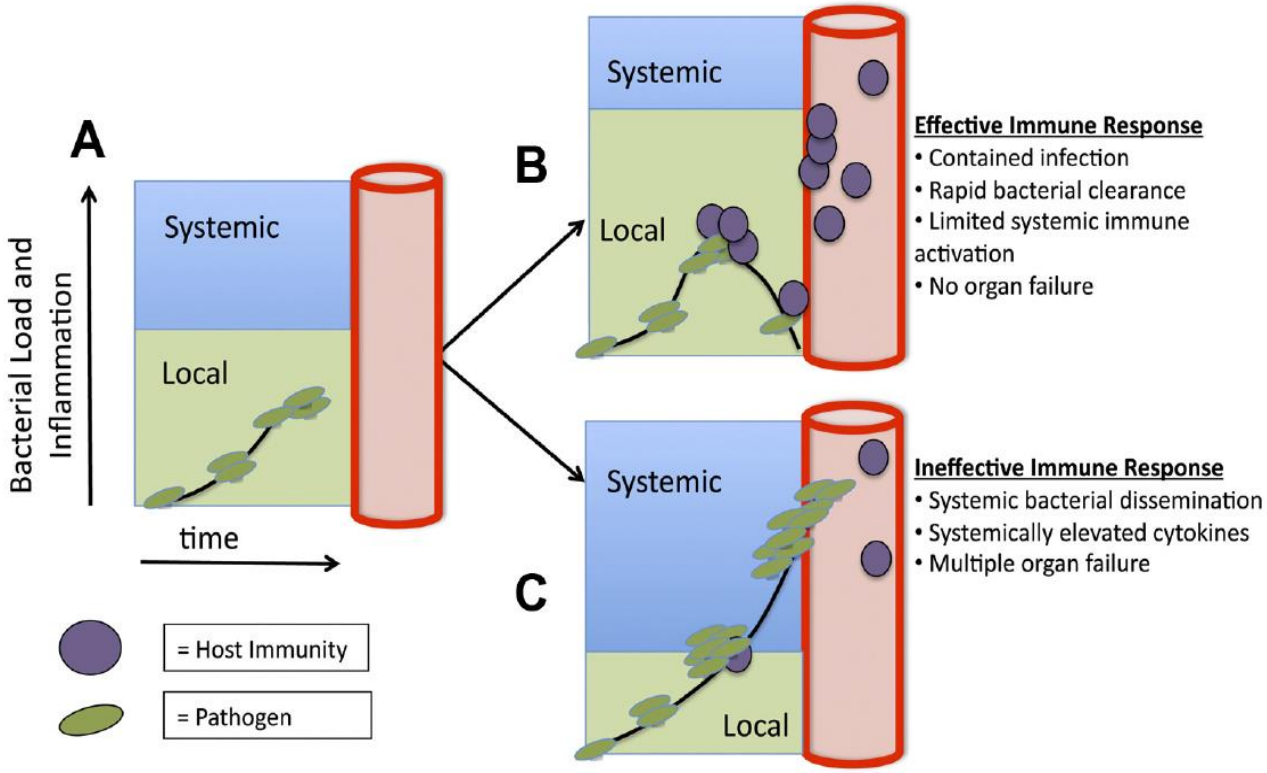
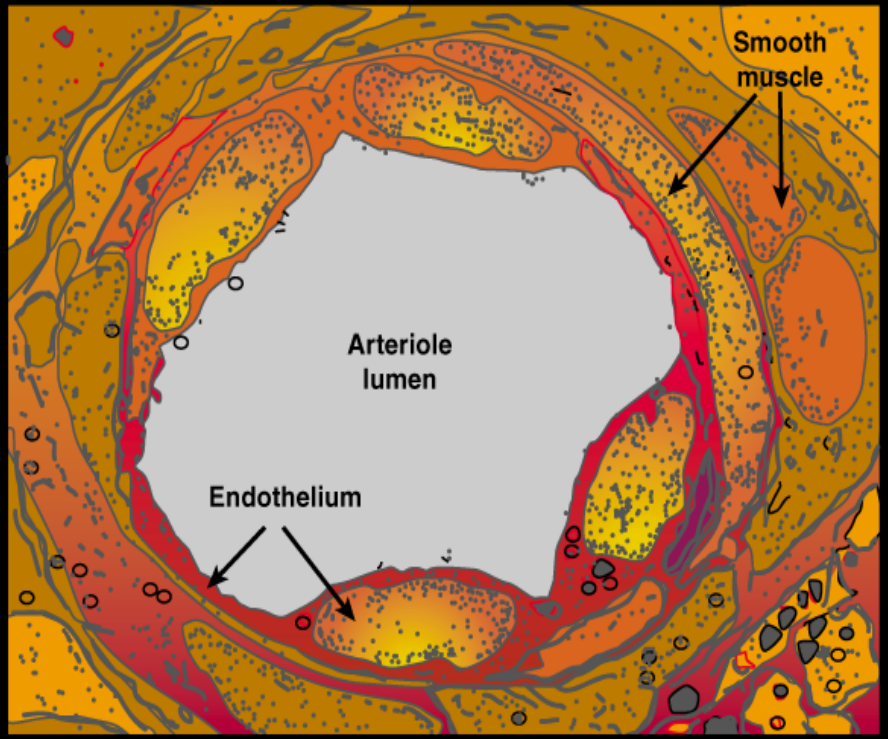


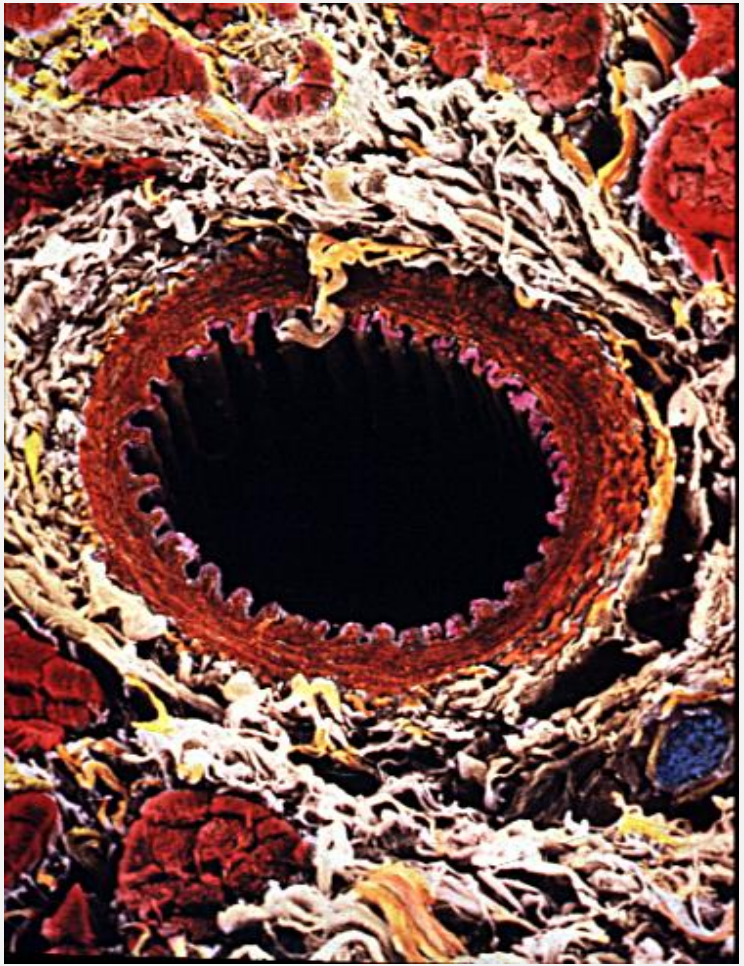
Fig. 3. Sepsis is a numeric and geographic race between bacterial growth and host defense. *A*: after the initial inoculum, bacteria or other pathogens begin to propagate within local compartments. *B*: if the immune response is sufficiently fast, then the spread of pathogens is limited by defense mechanisms, including NET formation, local thromboses, and neutrophil and monocyte recruitment. *C*: in contrast, if invading pathogens are able to spread outside a single compartment and the inflection point where specific host defense mechanisms shift from benefit to detriment is crossed, then both infection and the inflammatory response to the infection become systemic, resulting in diffuse organ injury and shock.

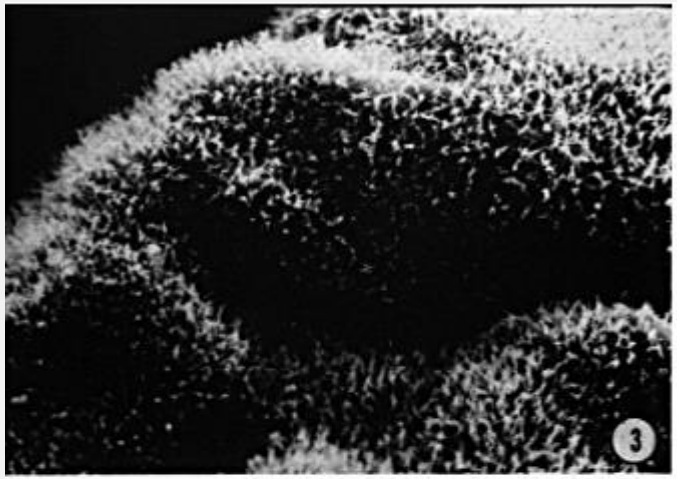
Am J Physiol Lung Cell Mol Physiol 303: L355–L363, 2012.

The endothelium: A living organ



Blood Vessel

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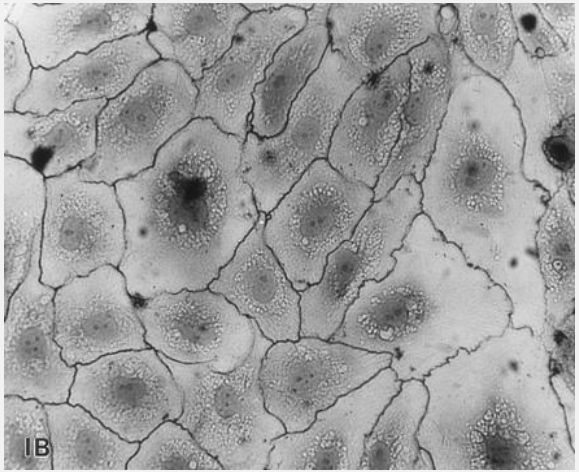
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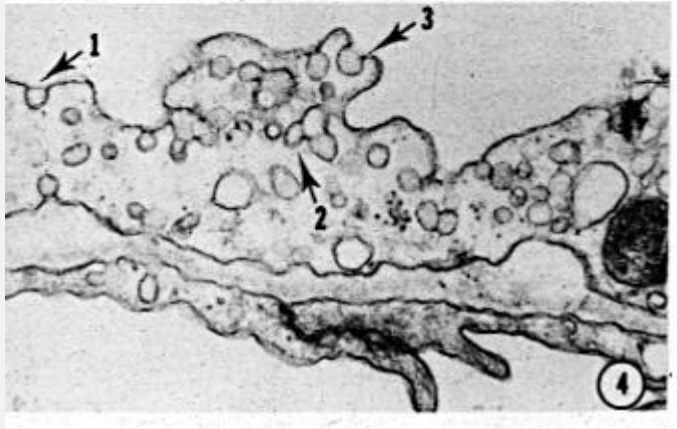
1
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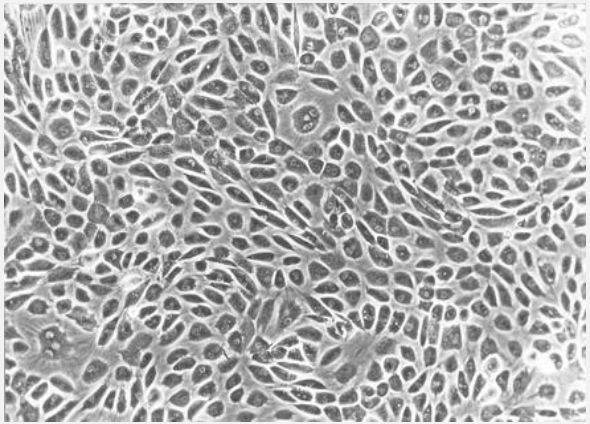


ENDOTHELIUM AT A GLANCE

In a human body:

- 10^{13} endothelial cells
- 1 kg weight
- 4,000-7,000 m^2 surface area

Wolinsky H., Circ. Res., 1980.

MAJOR ENDOTHELIAL CELL FUNCTIONS

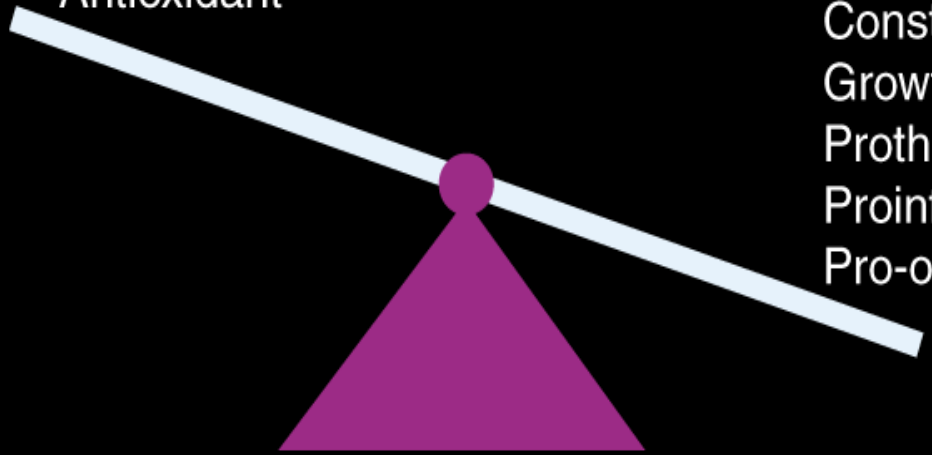
- Synthesis of vasoactive peptides (PGI_2, TxA_2, Angiotensin II, NO, endothelins)
- Expression of enzymes (ACE, NCTs, LPS)
- Expression of receptors & signal transduction molecules
- Removal & biotransformation of drugs
- Regulation of coagulation & thrombolysis
- Participation in immune reactions
- Interactions with bacteria (phagocytosis) and blood components (PMNs, PLTs)
- Expression of adhesion molecules
- Participation in local vasoregulation
- Induction of smooth muscle differentiation
- Production of growth promoting and inhibitory factors
- Barrier function

Mediators of Endothelial Injury

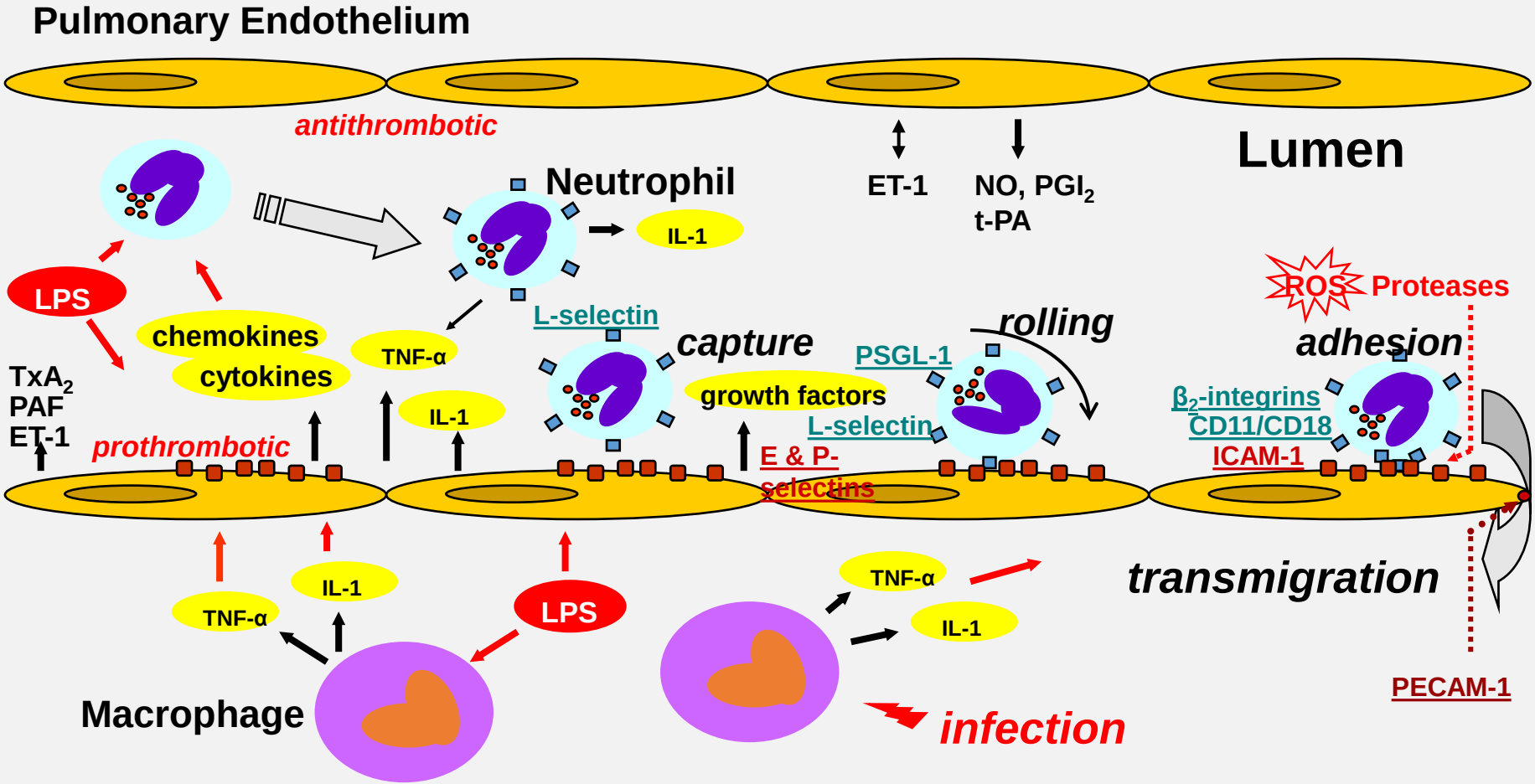
- Pro-inflammatory cytokines (IL-1, TNFα)
- Cellular response: *neutrophils, macrophage/monocytes, lymphocytes*
- Cellular events include: *adhesion, chemotaxis/chemokinesis & activation (expression of cell adhesion molecules)*
- Complement system
- Coagulation/fibrinolysis
- Kinin systems
- Cell-generated mediators: *cytokines, lipids, oxidants, proteases, vasoactive peptides, growth factors, neuropeptides*
- Induction of protein synthesis (NFκB activation)

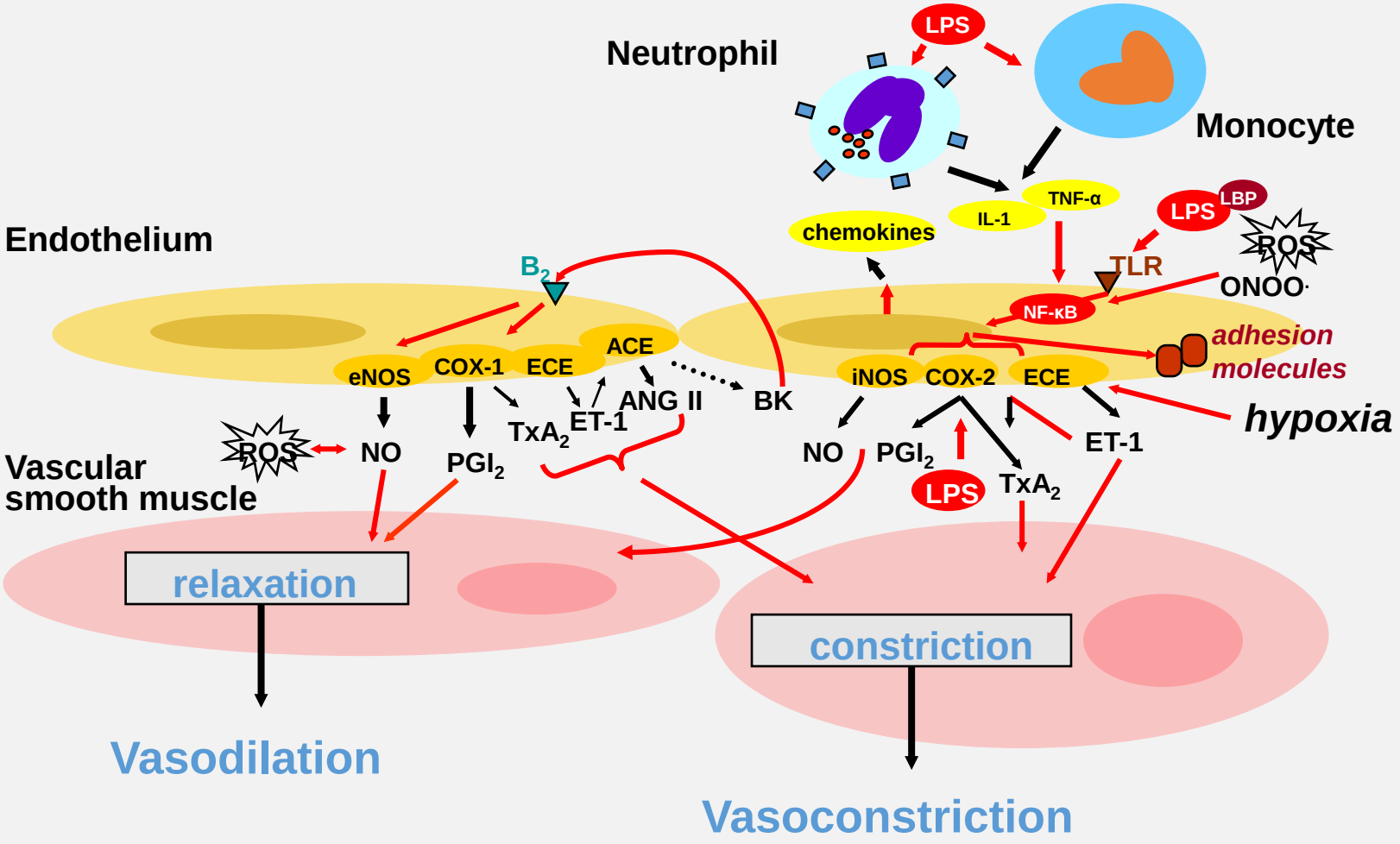
The endothelium maintains vascular health

Dilatation
Growth inhibition
Antithrombotic
Anti-inflammatory
Antioxidant

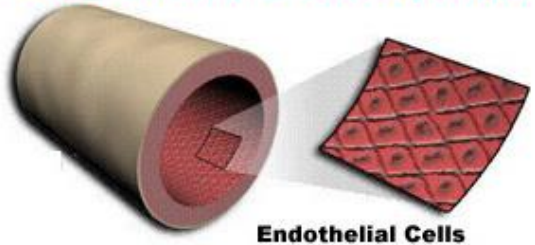


Constriction
Growth promotion
Prothrombotic
Proinflammatory
Pro-oxidant


Pulmonary Endothelium
antithrombotic
Lumen
ET-1
NO, PGI2
t-PA
Neutrophil
IL-1
LPS
chemokines
cytokines
TNF-α
L-selectin
capture
rolling
PSGL-1
growth factors
L-selectin
E & P-selectins
ROS
Proteases
adhesion
β2-integrins
CD11/CD18
ICAM-1
TxA2
PAF
ET-1
prothrombotic
IL-1
transmigration
PECAM-1
TNF-α
IL-1
LPS
Macrophage
TNF-α
IL-1
infection

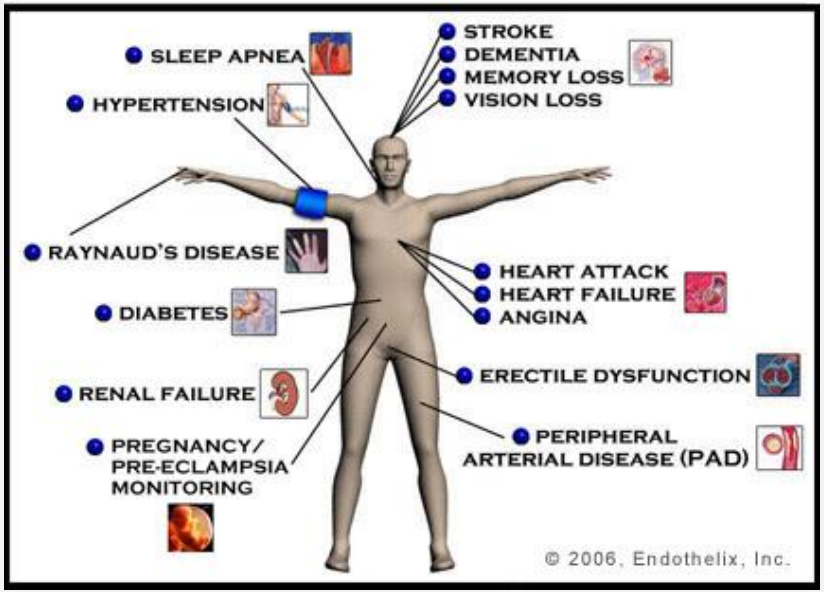

Neutrophil
LPS
Monocyte
TNF-α
IL-1
chemokines
LPS
LBP
ROS
ONOO·
Endothelium
TLR
NF-κB
adhesion molecules
B2
ACE
eNOS
COX-1
ECE
ANG II
BK
iNOS
COX-2
ECE
hypoxia
ROS
NO
PGI2
TxA2
ET-1
NO
PGI2
LPS
TxA2
ET-1
Vascular smooth muscle
relaxation
constriction
Vasodilation
Vasoconstriction

ENDOTHELIAL DYSFUNCTION

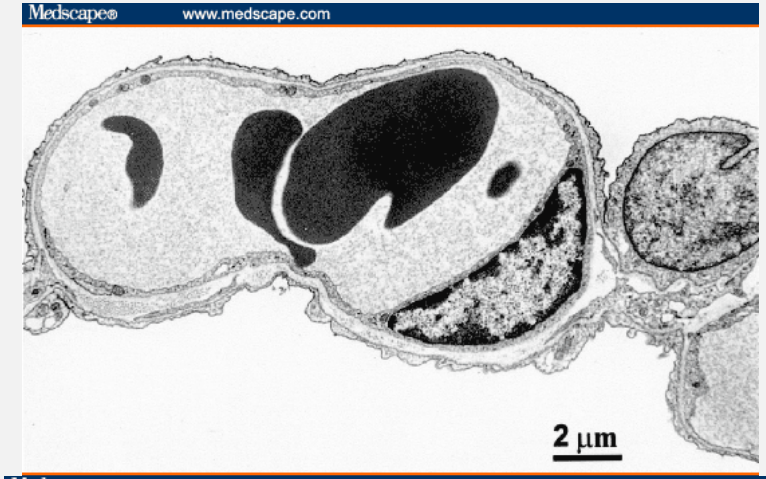


Endothelial Cells

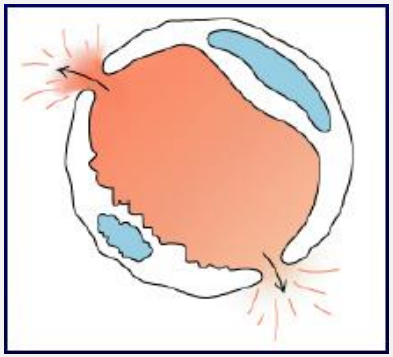
IS THE PRECURSOR OF:



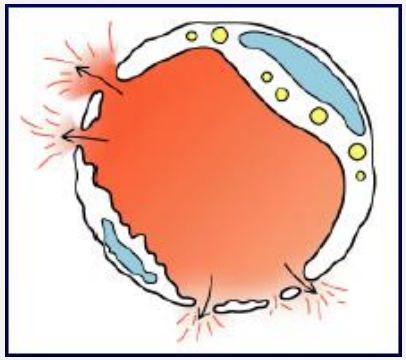
Normal capillary endothelium



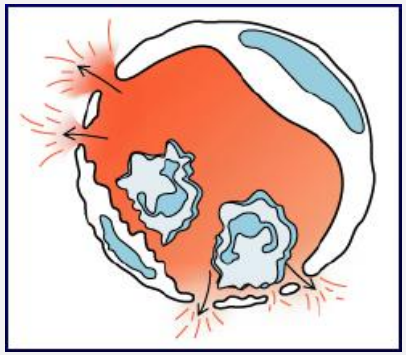
Mechanisms of EC hyper-permeability



Gaps due to EC contraction



Direct EC injury



Leukocyte mediated EC injury

Table 3. The Berlin Definition of Acute Respiratory Distress Syndrome

	Acute Respiratory Distress Syndrome
Timing	Within 1 week of a known clinical insult or new or worsening respiratory symptoms
Chest imaging[a]	Bilateral opacities—not fully explained by effusions, lobar/lung collapse, or nodules
Origin of edema	Respiratory failure not fully explained by cardiac failure or fluid overload Need objective assessment (eg, echocardiography) to exclude hydrostatic edema if no risk factor present
Oxygenation[b]	
Mild	200 mm Hg $<$ Pa_{O_2}/F_{IO_2} $\leq$ 300 mm Hg with PEEP or CPAP $\geq$5 cm H_2O[c]
Moderate	100 mm Hg $<$ Pa_{O_2}/F_{IO_2} $\leq$ 200 mm Hg with PEEP $\geq$5 cm H_2O
Severe	Pa_{O_2}/F_{IO_2} $\leq$ 100 mm Hg with PEEP $\geq$5 cm H_2O

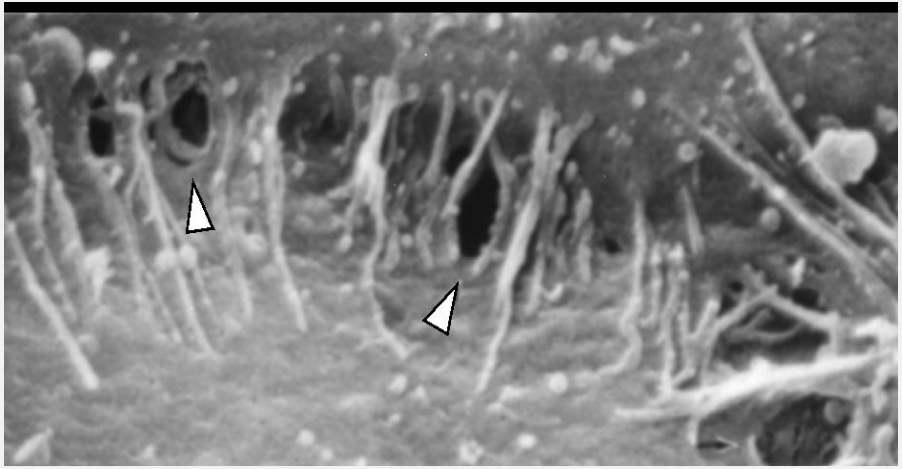
Abbreviations: CPAP, continuous positive airway pressure; F_{IO_2}, fraction of inspired oxygen; Pa_{O_2}, partial pressure of arterial oxygen; PEEP, positive end-expiratory pressure.

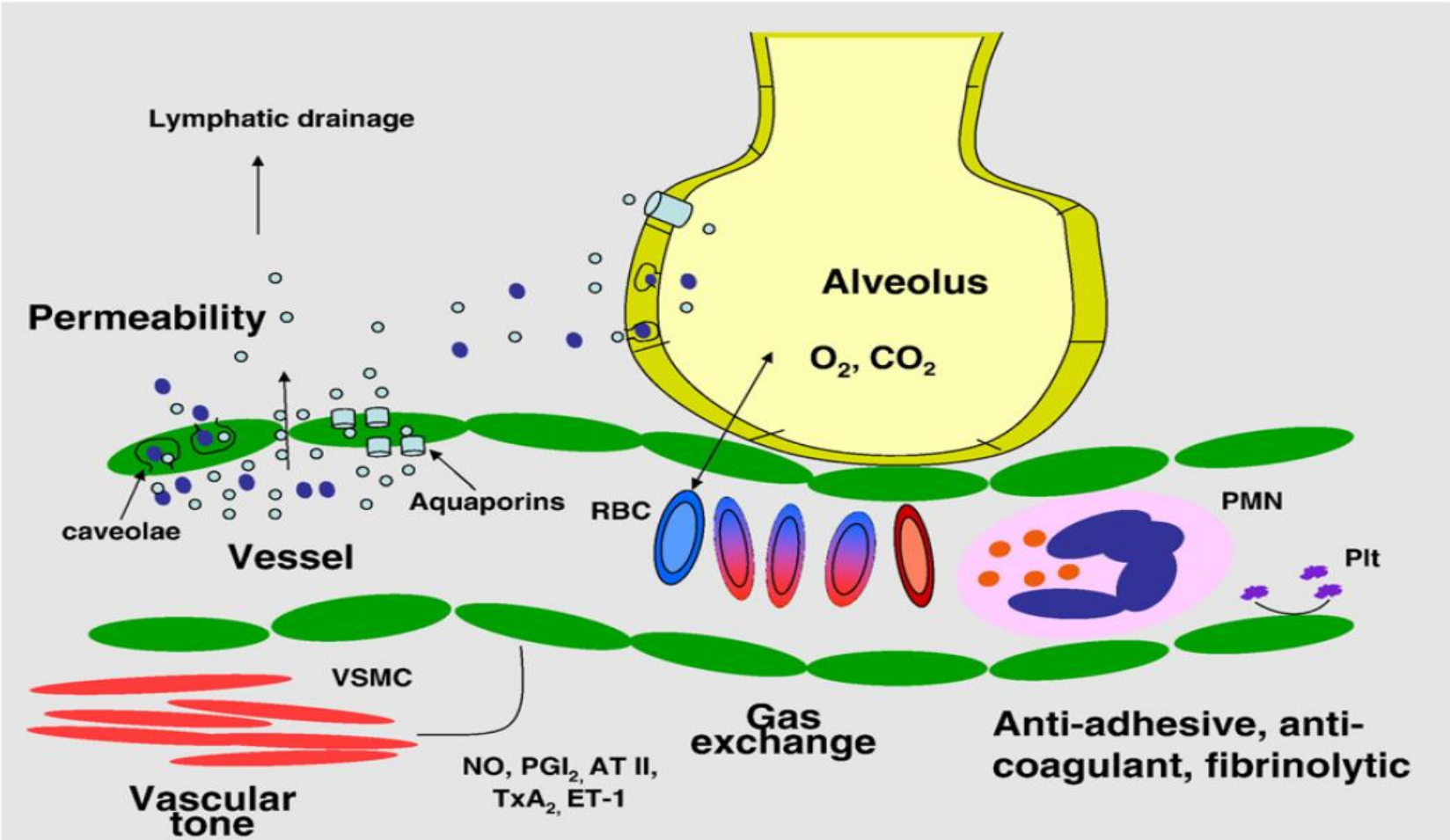
[a] Chest radiograph or computed tomography scan.

[b] If altitude is higher than 1000 m, the correction factor should be calculated as follows: [Pa_{O_2}/F_{IO_2} × (barometric pressure/760)].

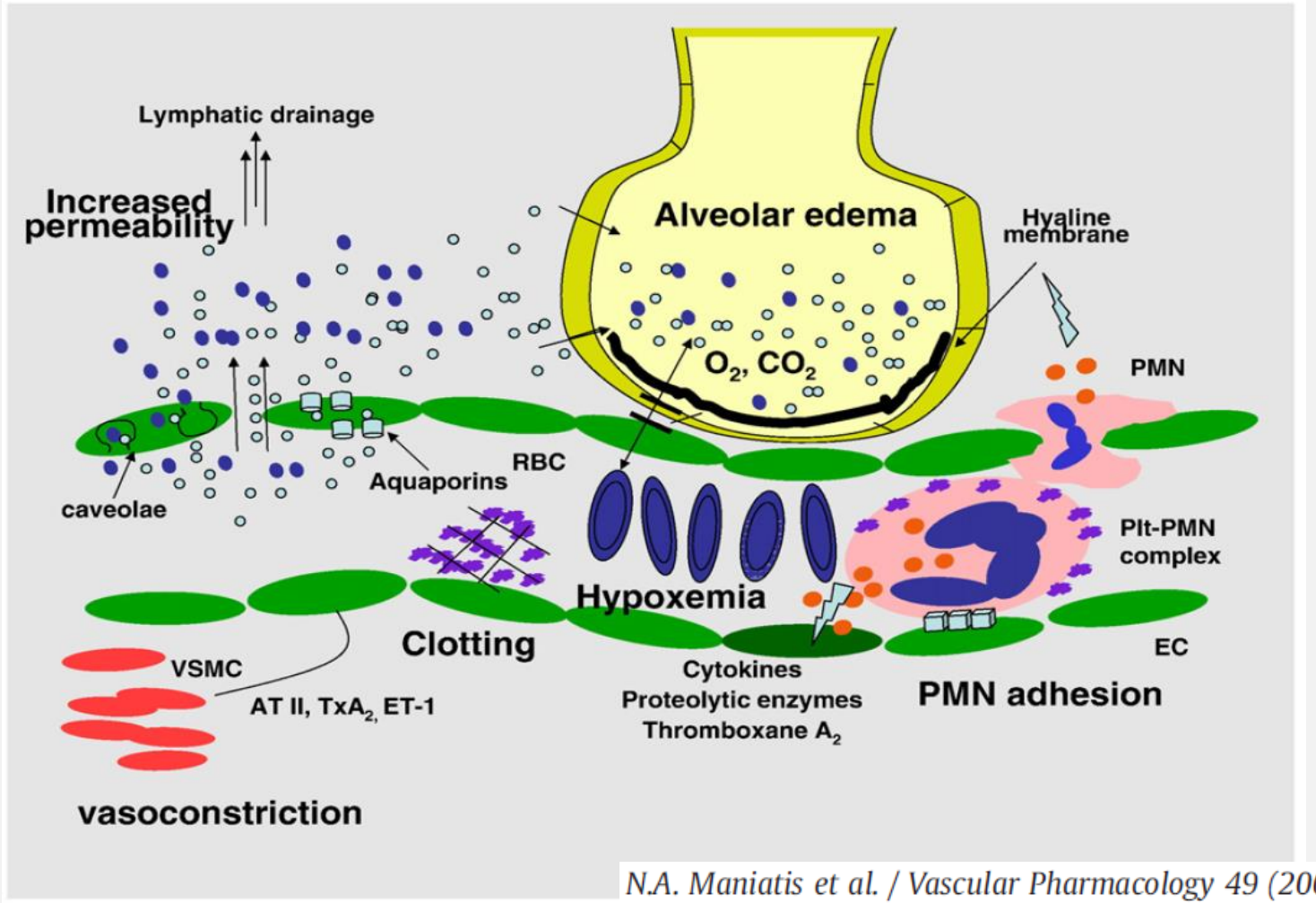
[c] This may be delivered noninvasively in the mild acute respiratory distress syndrome group.

ARDS: Increased endothelial permeability





N.A. Maniatis et al. / Vascular Pharmacology 49 (2008) 119–133



N.A. Maniatis et al. / Vascular Pharmacology 49 (2008) 119–133

International Journal of
Molecular Sciences

MDPI

Review

Endothelial Damage in Acute Respiratory Distress Syndrome

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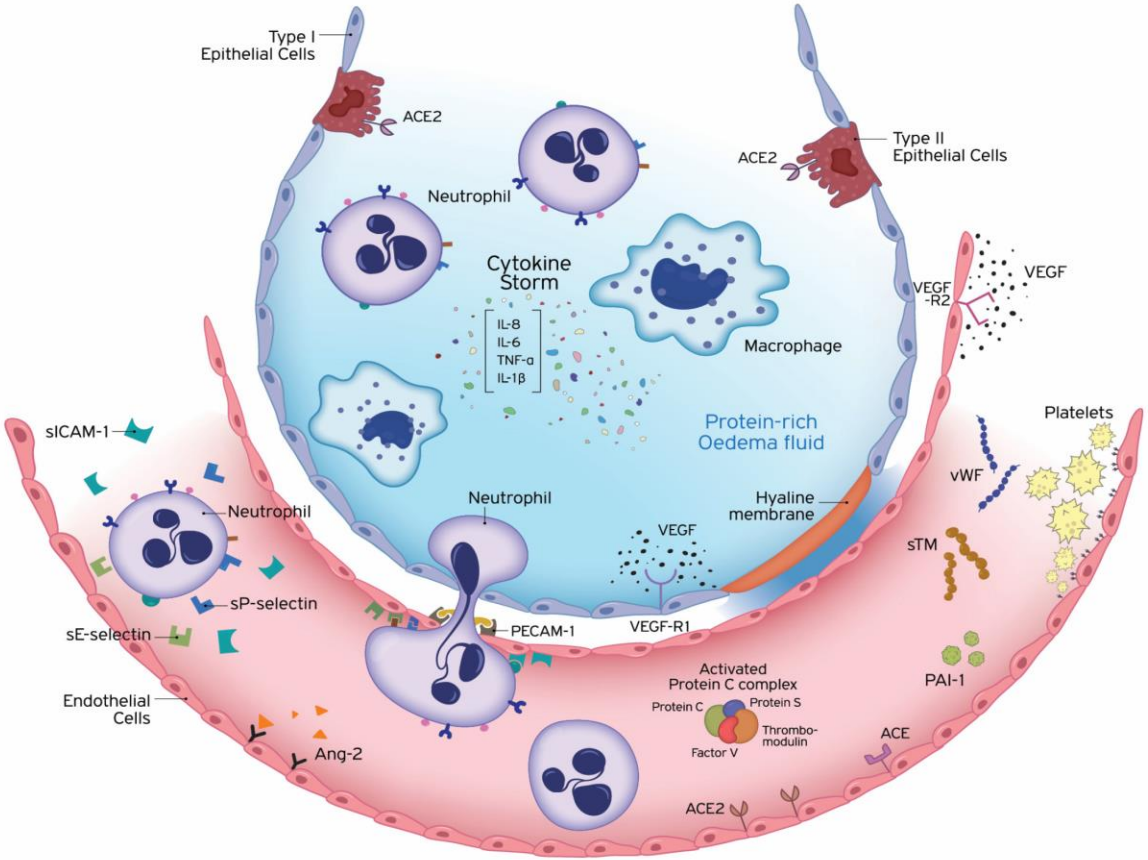
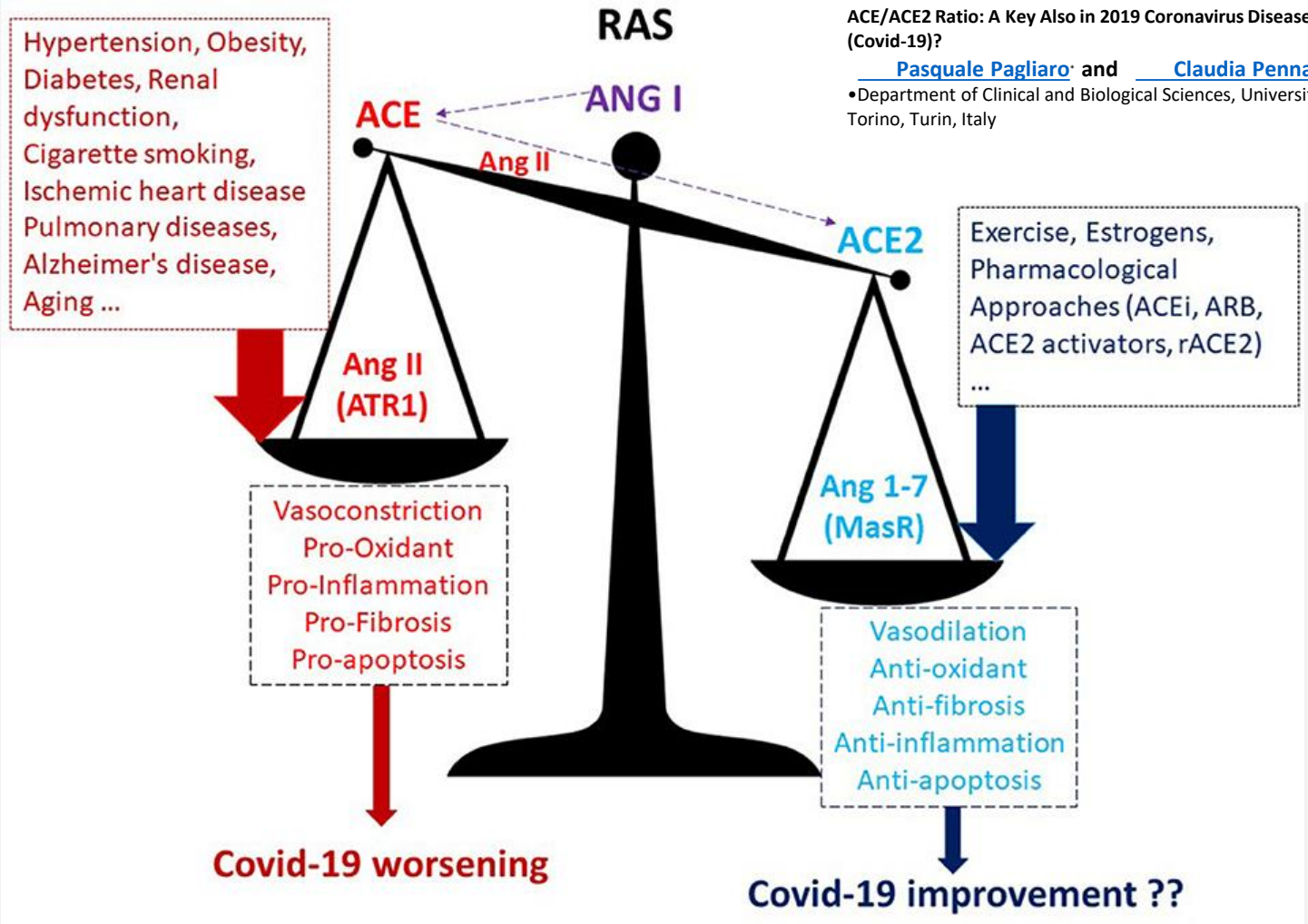
Abstract: The pulmonary endothelium is a metabolically active continuous monolayer of squamous endothelial cells that internally lines blood vessels and mediates key processes involved in lung homoeostasis. Many of these processes are disrupted in acute respiratory distress syndrome (ARDS), which is marked among others by diffuse endothelial injury, intense activation of the coagulation system and increased capillary permeability. Most commonly occurring in the setting of sepsis, ARDS is a devastating illness, associated with increased morbidity and mortality and no effective pharmacological treatment. Endothelial cell damage has an important role in the pathogenesis of ARDS and several biomarkers of endothelial damage have been tested in determining prognosis. By further understanding the endothelial pathobiology, development of endothelial-specific therapeutics might arise. In this review, we will discuss the underlying pathology of endothelial dysfunction leading to ARDS and emerging therapies. Furthermore, we will present a brief overview demonstrating that endotheliopathy is an important feature of hospitalised patients with coronavirus disease-19 (COVID-19).

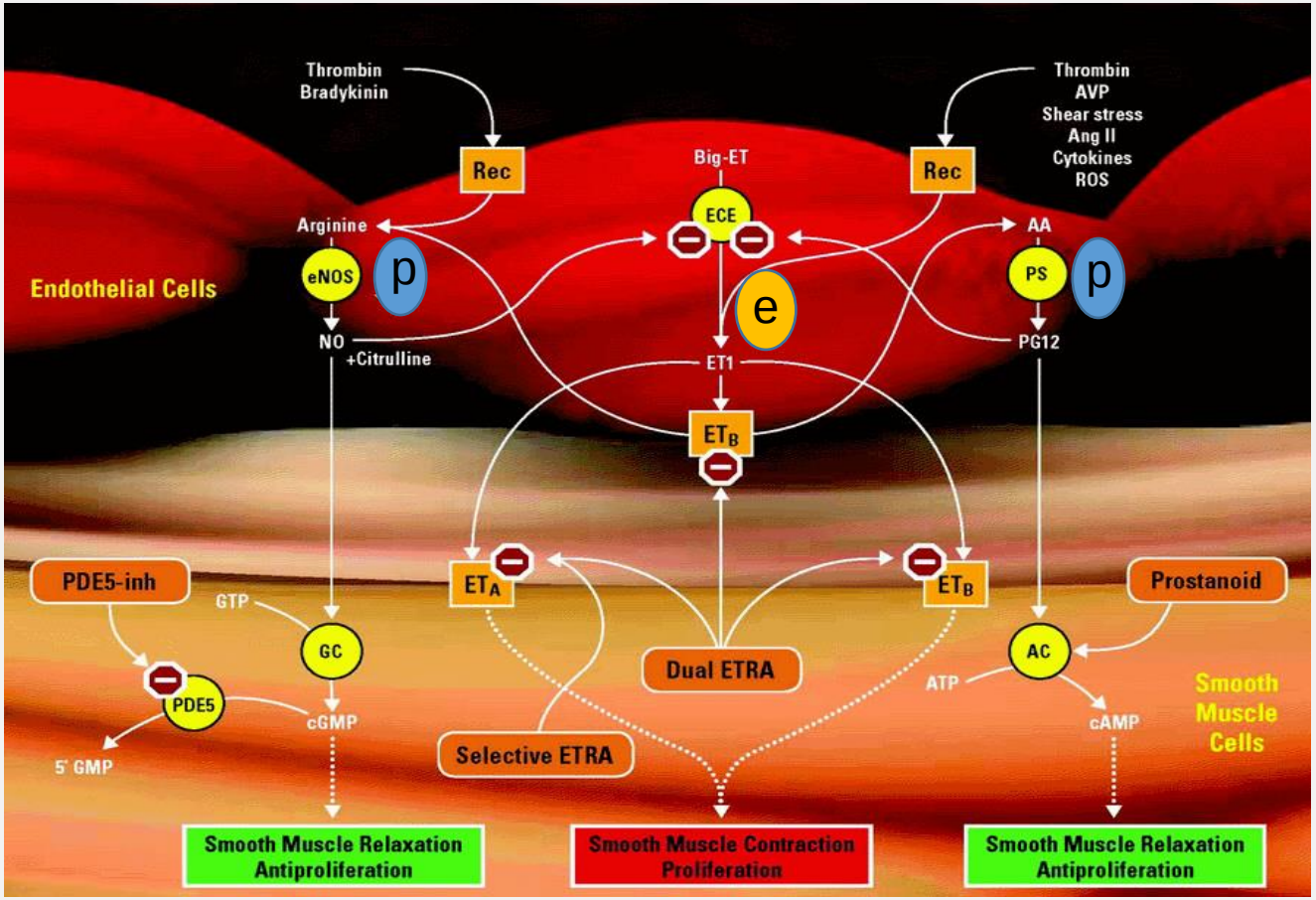
Keywords: ARDS; dysfunction; biomarkers; coagulation; inflammation


Type I
Epithelial Cells
ACE2
Neutrophil
Cytokine
Storm
IL-8
IL-6
TNF-α
IL-1β
Macrophage
ACE2
Type II
Epithelial Cells
VEGF
-R2
VEGF
Platelets
vWF
sTM
Protein-rich
Oedema fluid
Hyaline
membrane
sICAM-1
Neutrophil
sP-selectin
sE-selectin
Endothelial
Cells
Ang-2
Neutrophil
VEGF
VEGF-R1
PECAM-1
Activated
Protein C complex
Protein C
Protein S
Thrombo-
modulin
Factor V
PAI-1
ACE
ACE2

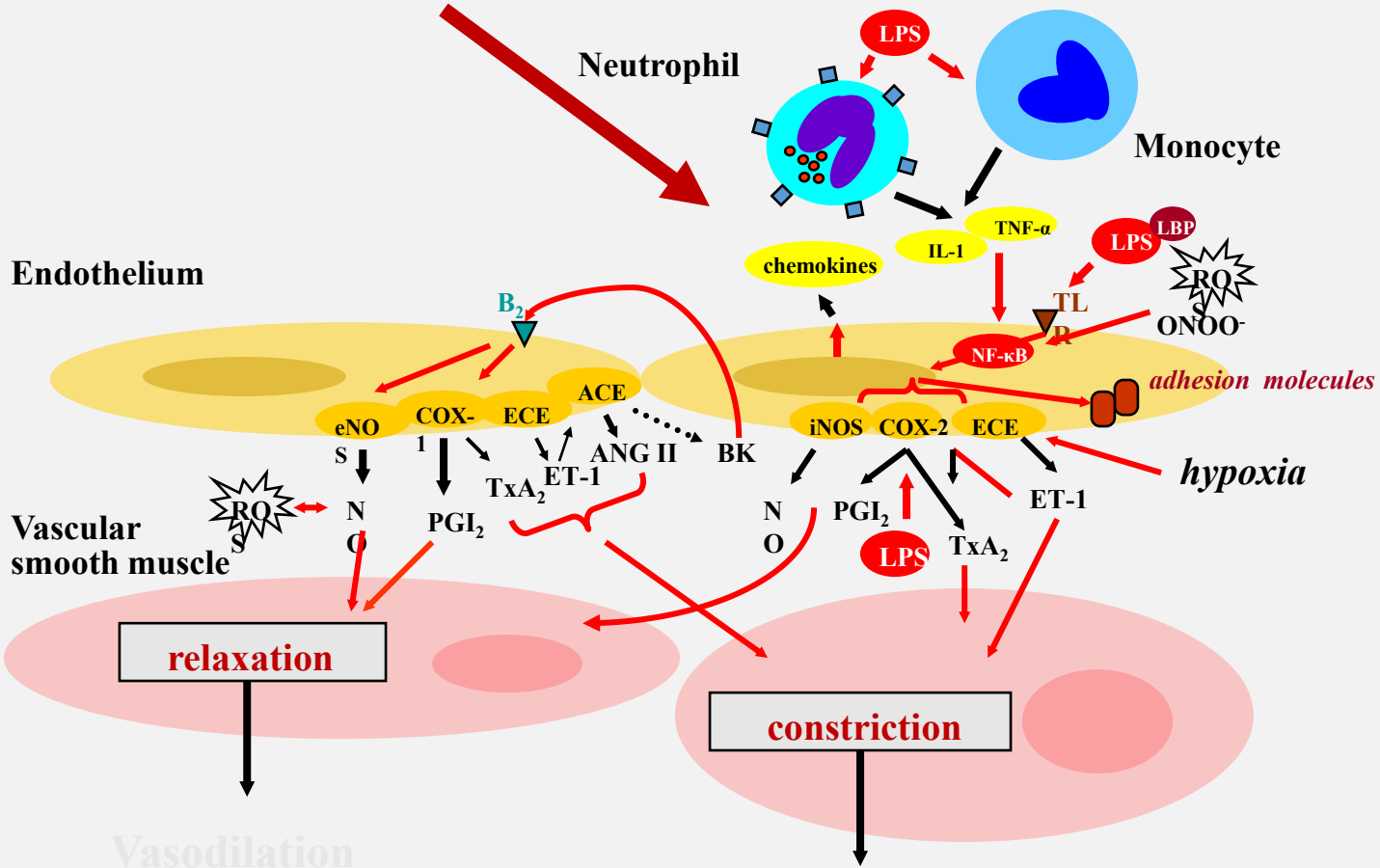
ACE/ACE2 Ratio: A Key Also in 2019 Coronavirus Disease (Covid-19)?

Pasquale Pagliaro· and Claudia Penna
•Department of Clinical and Biological Sciences, University of Torino, Turin, Italy

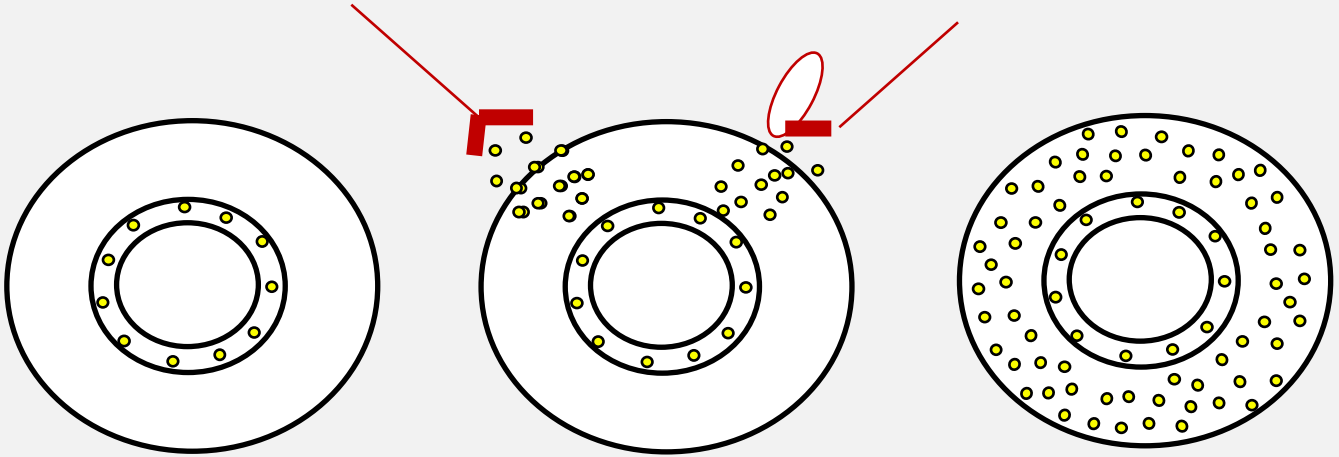




McLaughlin & McGoon *Circulation* 2006, 114:1417-1431

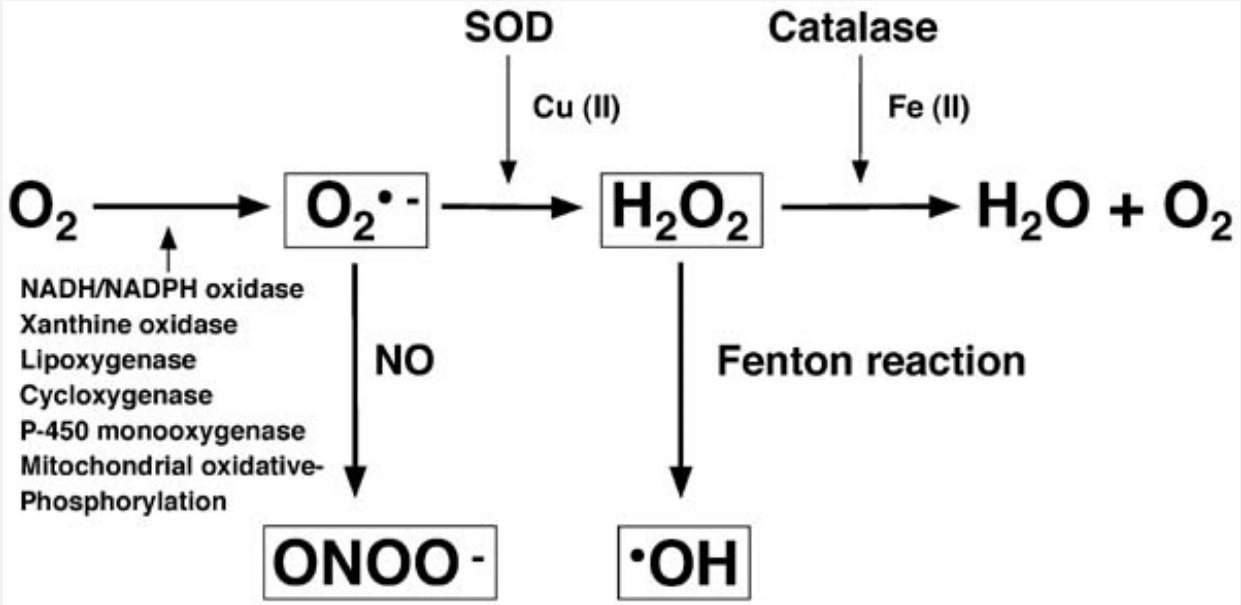

Neutrophil
LPS
Monocyte
Endothelium
TNF-α
IL-1
chemokines
LPS
LBP
ROS
ONOO-
TLR
B2
NF-κB
adhesion molecules
eNOS
COX-1
ECE
ACE
ANG II
BK
iNOS
COX-2
ECE
hypoxia
ROS
NO
PGI2
TxA2
ET-1
Vascular smooth muscle
NO
PGI2
LPS
TxA2
ET-1
relaxation
constriction
Vasodilation
Vasoconstriction

Three isoforms of NOS regulate vascular tone



eNOS nNOS iNOS

ROS and NO


SOD
Catalase
Cu (II)
Fe (II)
O_2
$O_2^{\bullet -}$
H_2O_2
$H_2O + O_2$
NADH/NADPH oxidase
Xanthine oxidase
Lipoxygenase
Cycloxygenase
P-450 monooxygenase
Mitochondrial oxidative-
Phosphorylation
NO
Fenton reaction
$ONOO^-$
$^{\bullet}OH$

Arch. Immunol. Ther. Exp. (2015) 63:41–52
DOI 10.1007/s00005-014-0310-1

REVIEW ARTICLE

The Role of Endothelin-1 and Endothelin Receptor Antagonists in Inflammatory Response and Sepsis

Agata Kowalczyk · Paulina Kleniewska · Michal Kolodziejczyk · Beata Skibska · Anna Goraca

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Abstract Endothelin-1 (ET-1) is a potent endogenous vasoconstrictor, mainly secreted by endothelial cells. It acts through two types of receptors: ETA and ETB. Apart from a vasoconstrictive action, ET-1 causes fibrosis of the vascular cells and stimulates production of reactive oxygen species. It is claimed that ET-1 induces proinflammatory mechanisms, increasing superoxide anion production and cytokine secretion. A recent study has shown that ET-1 is involved in the activation of transcription factors such as NF-κB and expression of proinflammatory cytokines including TNF-α, IL-1, and IL-6. It has been also indicated that during endotoxaemia, the plasma level of ET-1 is increased in various animal species. Some authors indicate a clear correlation between endothelin plasma level and morbidity/mortality rate in septic patients. These pathological effects of ET-1 may be abrogated at least partly by endothelin receptor blockade. ET-1 receptor antagonists may be useful for prevention of various vascular diseases. This review summarises the current knowledge regarding endothelin receptor antagonists and the role of ET-1 in sepsis and inflammation.

Keywords Endothelins · Sepsis · Inflammation · Reactive oxygen species · Endothelin receptor antagonists

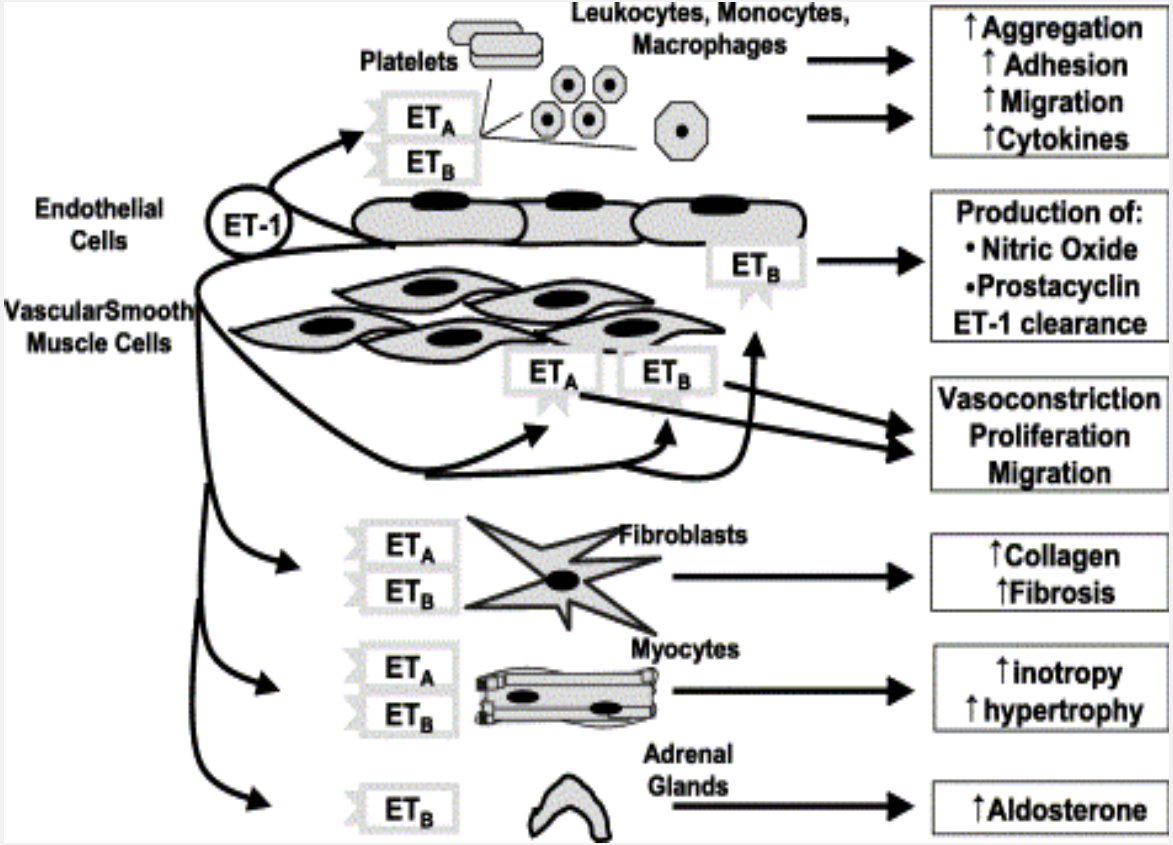
Pathogenesis of Sepsis

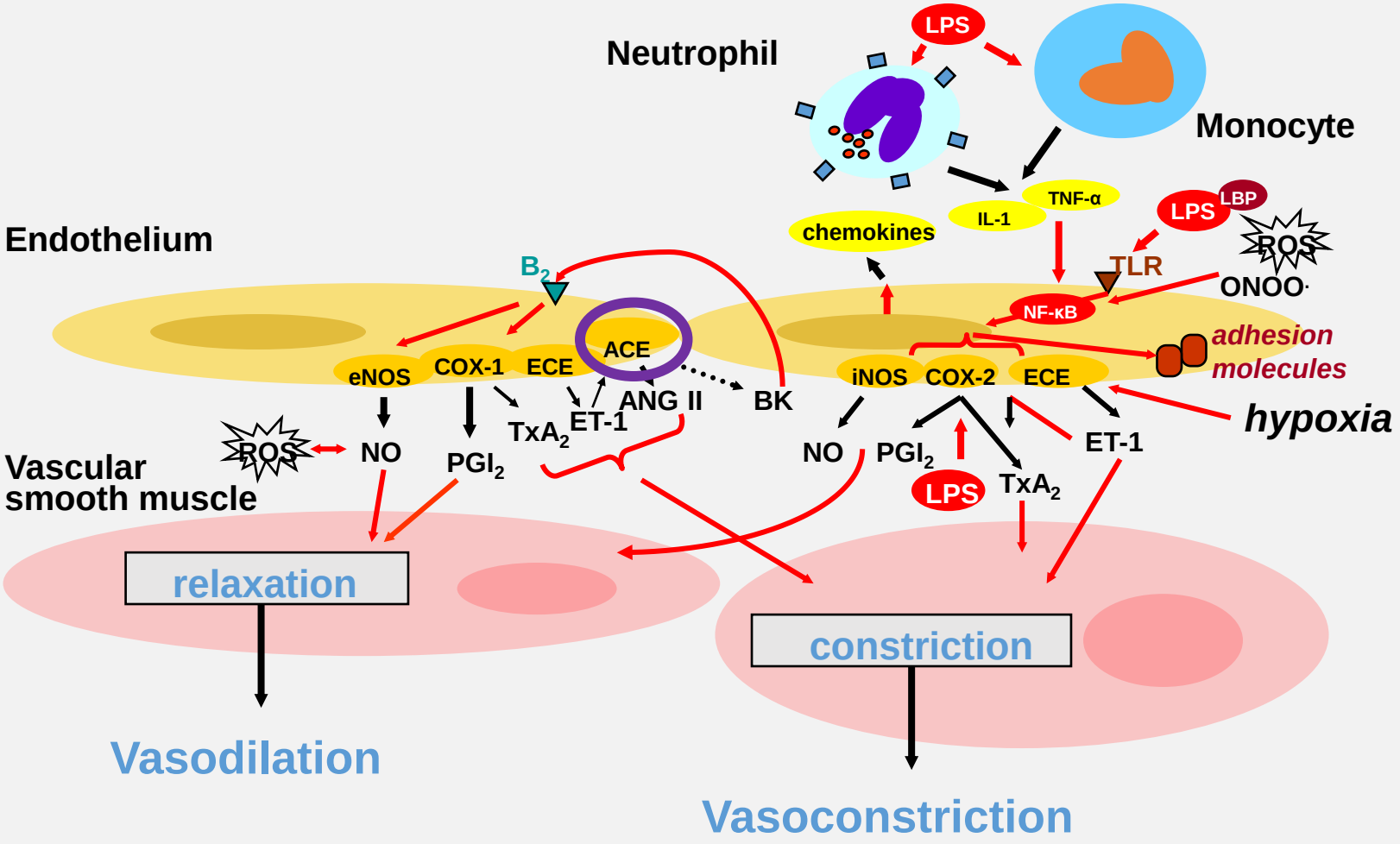
Sepsis is defined as a systemic inflammatory response syndrome, most commonly provoked by severe bacterial infection (Naito et al. 2014; Sagy et al. 2013; Zhang et al. 2014) This critical condition, with a mortality rate of about 50–80 %, is characterised by hyperthermia or hypothermia, tachypnea, tachycardia, leucocytosis or leucopenia, with immature neutrophils, and organ dysfunction due to impaired tissue perfusion (Sagy et al. 2013). Endotoxic shock is also associated with pulmonary hypertension, systemic hypotension and cardiac dysfunction (Forni et al. 2005). The mechanisms underlying the pathogenic effects of sepsis are still not completely understood.

The primary cause of escalated inflammatory response in septic shock is the presence of bacterial toxins. These include the lipopolysaccharide (LPS) endotoxin, which is a compound of a Gram-negative bacterial cell wall and an exotoxin (superantigen) from Gram-positive bacteria. When released into the blood, these bacterial products induce macrophages to secrete large amounts of inflammatory cytokines like tumour necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and IL-8, by the activation of signalling cascades such as nuclear factor (NF)-κB and mitogen-activated protein kinase (MAPKs) pathways,

Table 2 Factors, which stimulate and inhibit release of ET-1

Factors stimulating release of ET-1	Factors inhibiting release of ET-1
Low shear stress[j]	High shear stress[f]
Adrenalin[i]	Nitric oxide[e]
Thrombin[e]	Prostacyclin[c]
Angiotensin II[5]	Heparin[e]
Hypoxia[e]	Prostaglandin[e]
Vasopressin[c]	Atrial natriuretic peptide[c]
Endotoxin (LPS)[g]	
IL-1[d]	
Transforming growth factor-β[c]	
TNF-α[c]	
Insulin[e]	
Free radicals[e]	
Cardiotrophin-1[e]	
Homocysteine[a]	
IL-6[h]	
Calcium ions[b]	

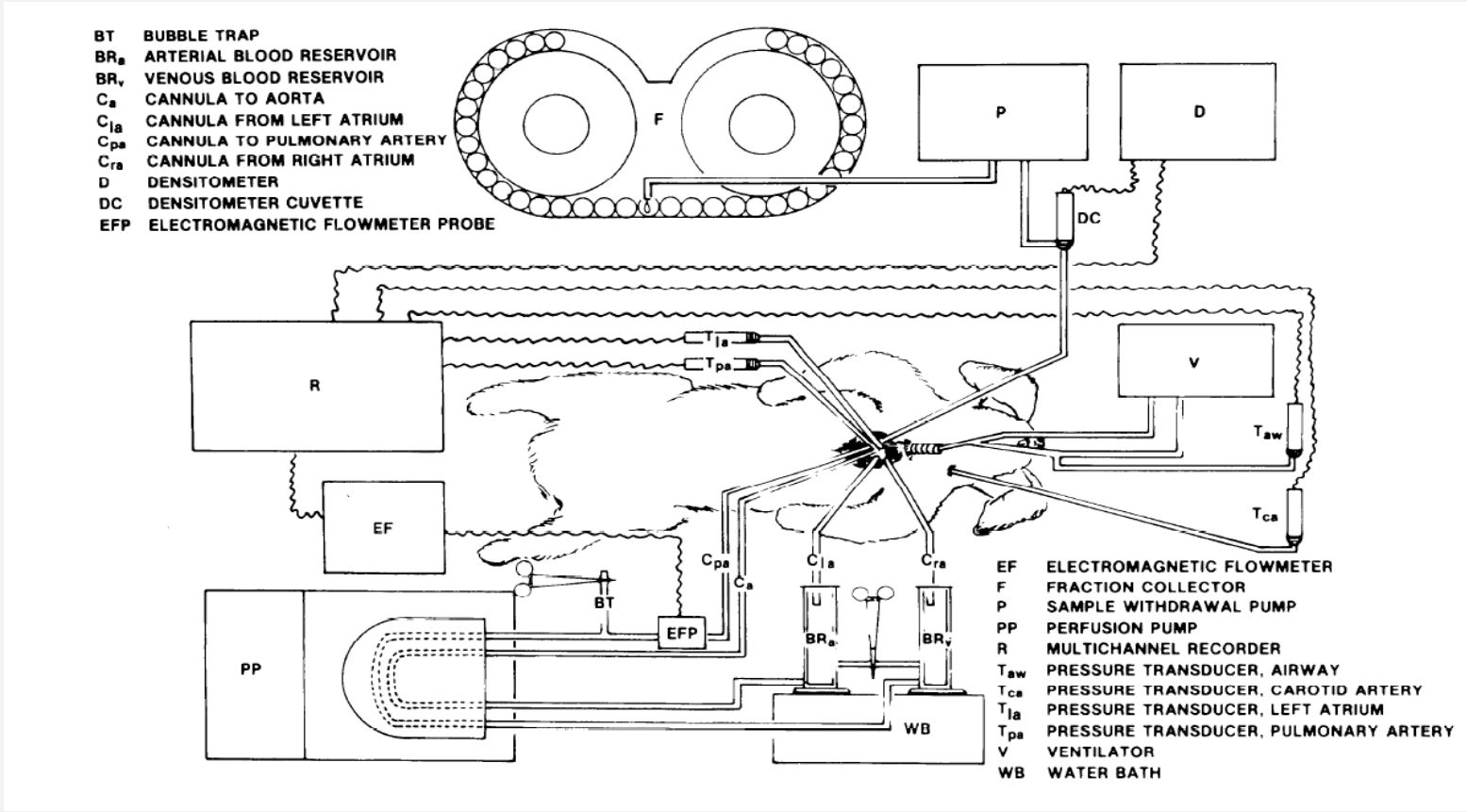

Leukocytes, Monocytes, Macrophages
Platelets
↑Aggregation
↑ Adhesion
↑Migration
↑Cytokines
ETA
ETB
Endothelial Cells
ET-1
ETB
Production of:
• Nitric Oxide
•Prostacyclin
ET-1 clearance
VascularSmooth Muscle Cells
ETA
ETB
Vasoconstriction
Proliferation
Migration
ETA
ETB
Fibroblasts
↑Collagen
↑Fibrosis
ETA
ETB
Myocytes
↑inotropy
↑hypertrophy
Adrenal Glands
ETB
↑Aldosterone

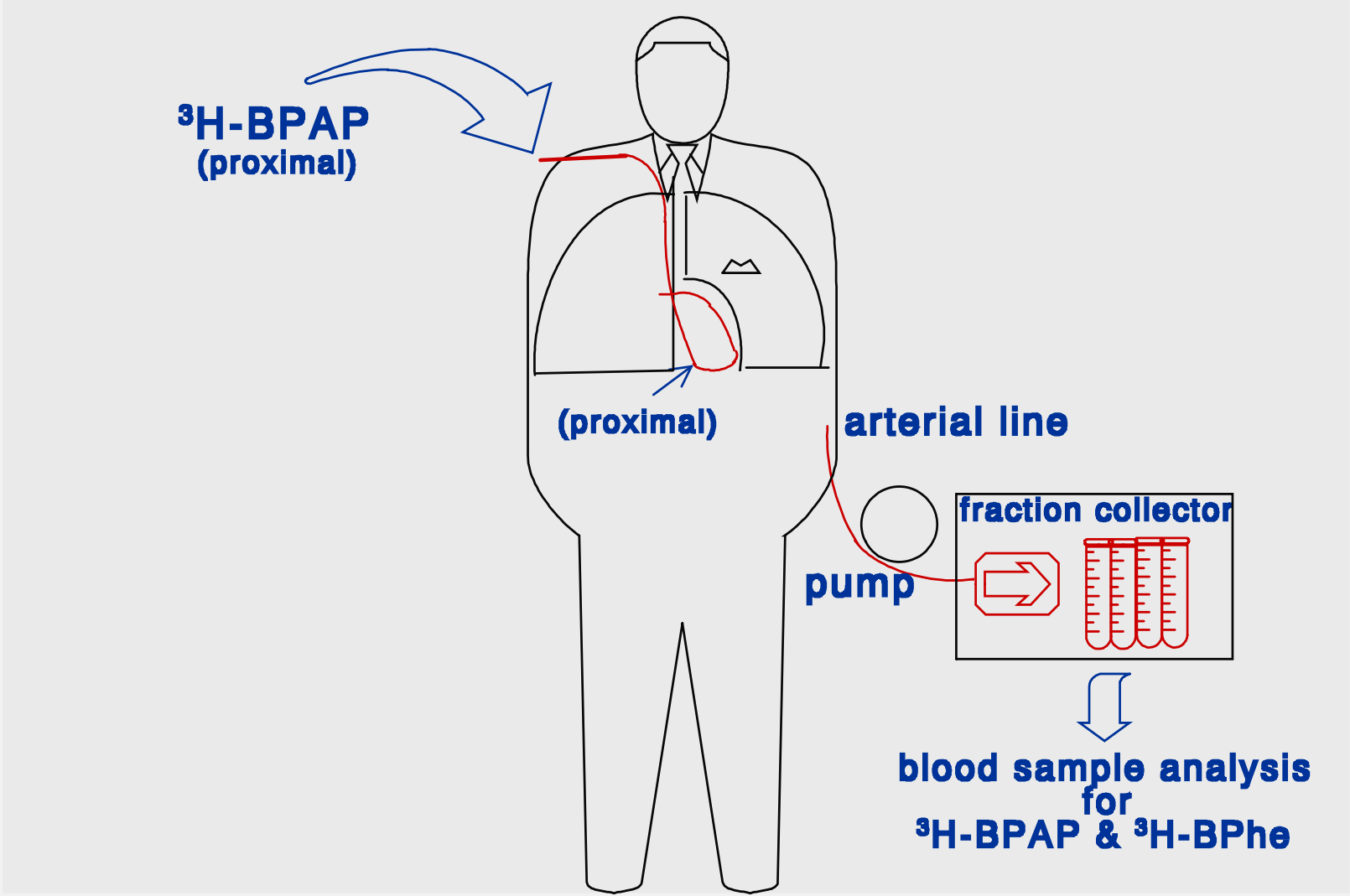

Neutrophil
LPS
Monocyte
TNF-α
IL-1
chemokines
LPS
LBP
ROS
ONOO·
Endothelium
TLR
NF-κB
adhesion molecules
B2
ACE
eNOS
COX-1
ECE
ANG II
BK
iNOS
COX-2
ECE
hypoxia
ROS
NO
PGI2
TxA2
ET-1
NO
PGI2
LPS
TxA2
ET-1
Vascular smooth muscle
relaxation
constriction
Vasodilation
Vasoconstriction

The indicator-dilution technique

Assessing Pulmonary Endothelial ACE Activity *In Vivo*

- **Pulmonary Capillary Endothelium Bound Angiotensin Converting Enzyme (PCEB-ACE) is homogeneously expressed on the luminal endothelial surface area (ectoenzyme)**
- **Due to its location, PCEB-ACE is directly accessible to blood-borne substrates and inhibitors; its activity may be assessed by means of indicator-dilution techniques**
- **PCEB-ACE activity has been shown to be a sensitive and quantifiable index of pulmonary endothelial function in both animals and humans, in health and disease**


BT BUBBLE TRAP
BR_a ARTERIAL BLOOD RESERVOIR
BR_v VENOUS BLOOD RESERVOIR
C_a CANNULA TO AORTA
C_{la} CANNULA FROM LEFT ATRIUM
C_{pa} CANNULA TO PULMONARY ARTERY
C_{ra} CANNULA FROM RIGHT ATRIUM
D DENSITOMETER
DC DENSITOMETER CUVETTE
EFP ELECTROMAGNETIC FLOWMETER PROBE
F
P
D
DC
R
T_{la}
T_{pa}
V
T_{aw}
T_{ca}
EF
C_{pa}
C_a
C_{la}
C_{ra}
BT
EFP
BR_a
BR_v
PP
WB
EF ELECTROMAGNETIC FLOWMETER
F FRACTION COLLECTOR
P SAMPLE WITHDRAWAL PUMP
PP PERFUSION PUMP
R MULTICHANNEL RECORDER
T_{aw} PRESSURE TRANSDUCER, AIRWAY
T_{ca} PRESSURE TRANSDUCER, CAROTID ARTERY
T_{la} PRESSURE TRANSDUCER, LEFT ATRIUM
T_{pa} PRESSURE TRANSDUCER, PULMONARY ARTERY
V VENTILATOR
WB WATER BATH

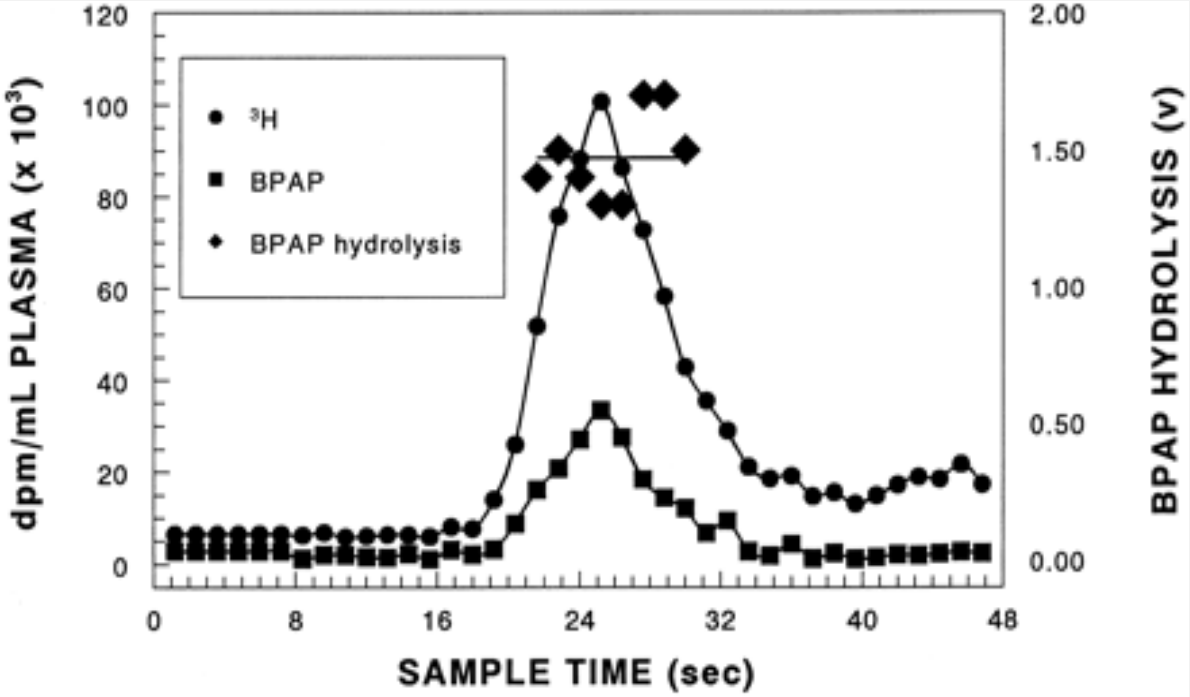

^{3}H-BPAP
(proximal)
(proximal)
arterial line
pump
fraction collector
blood sample analysis
for
^{3}H-BPAP & ^{3}H-BPhe

THE BASIC PRINCIPLE

^{3}H-Benzoyl-Phe-Ala-Pro

ACE

^{3}H-Benzoyl-Phe


dpm/mL PLASMA (x 10³)
120
100
80
60
40
20
0
³H
BPAP
BPAP hydrolysis
0
8
16
24
32
40
48
SAMPLE TIME (sec)
2.00
1.50
1.00
0.50
0.00
BPAP HYDROLYSIS (v)

$$\%M = (\text{product/substrate}) \cdot 100$$

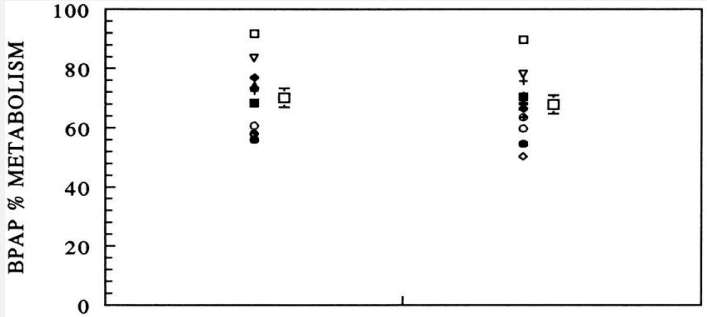
$$v = [E] \cdot t_c \cdot k_{cat}/K_m$$

v represents a reflection of enzyme activity per capillary

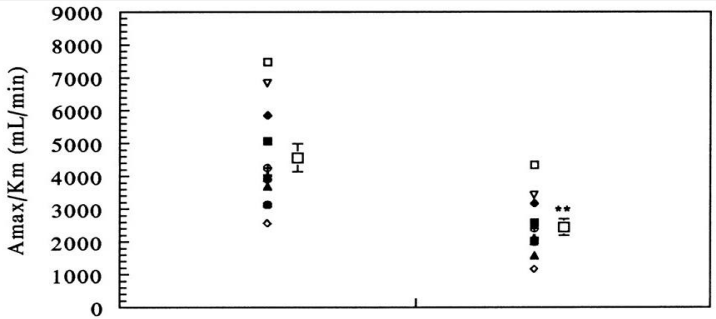
$$A_{max}/K_m = E \cdot k_{cat}/K_m$$

A_{max}/K_m, an index of FCSA, represents a reflection of enzyme activity per vascular bed

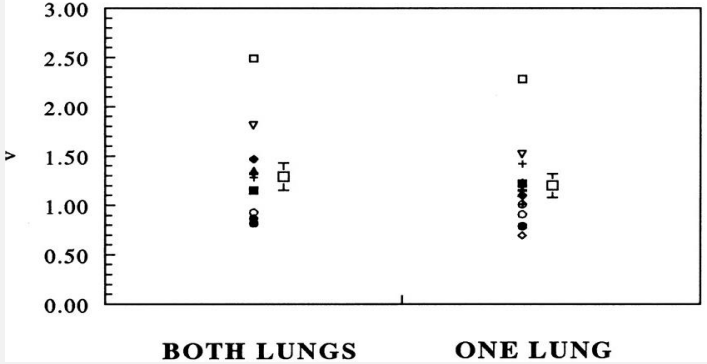
ACE METABOLISM IN NORMALS

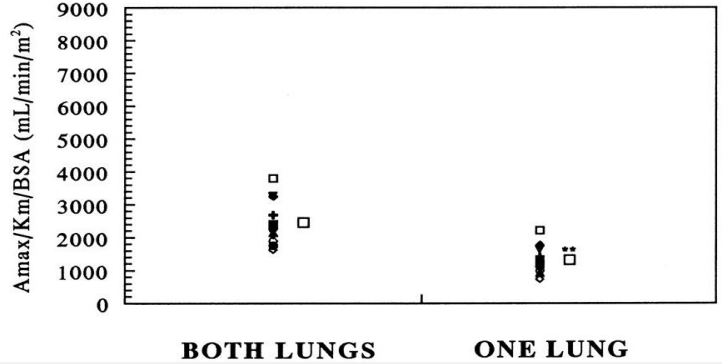


Substrate *%M* & *hydrolysis (v)* reflect endothelial function at the capillary level

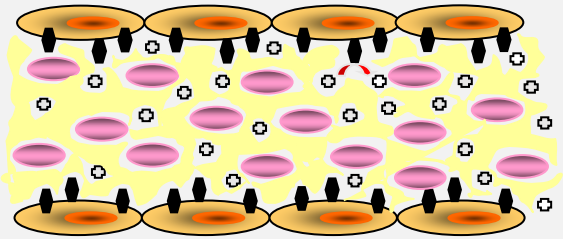


***Amax/Km* later renamed *FCSA* reflects perfused functional capillary surface**

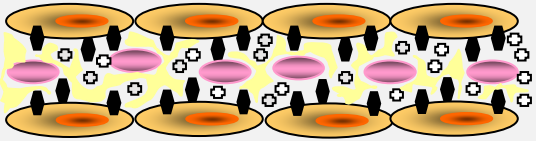




Orfanos, Langleben et al, Circulation 1999;99:1593-99

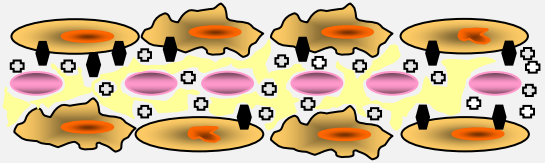


(I)



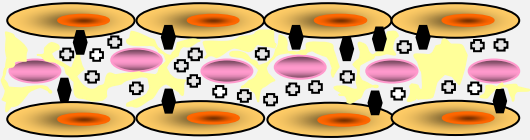
$v = [E] \bullet \bar{t} \bullet k_{cat} / K_m$

(II)



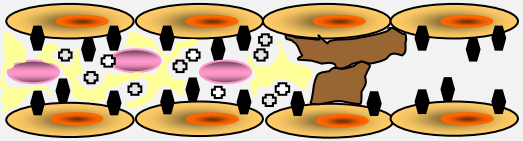
$[E]\downarrow,\ k_{cat} / K_m \downarrow$

(III)



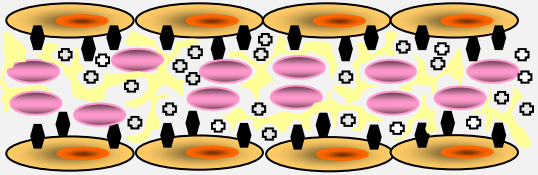
$[E]\downarrow$

(IV)

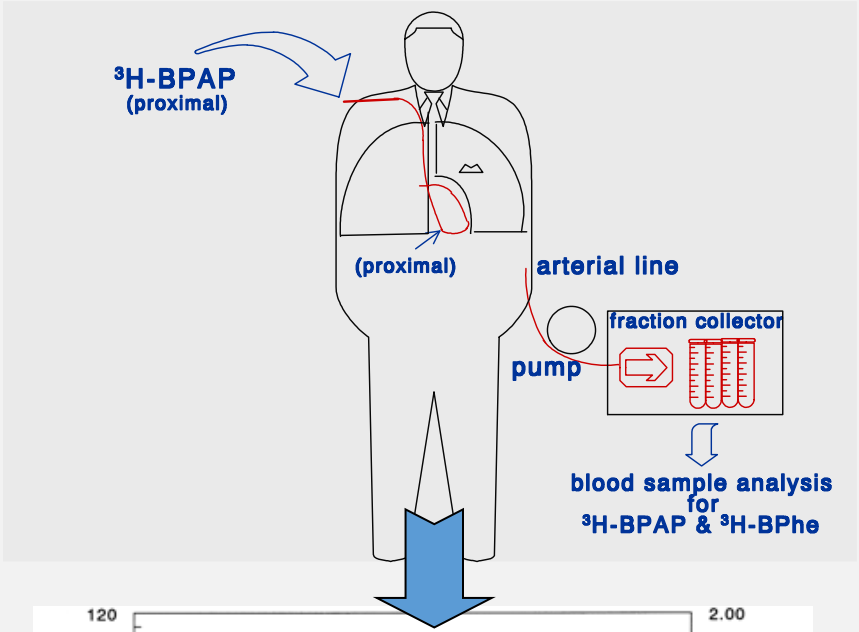


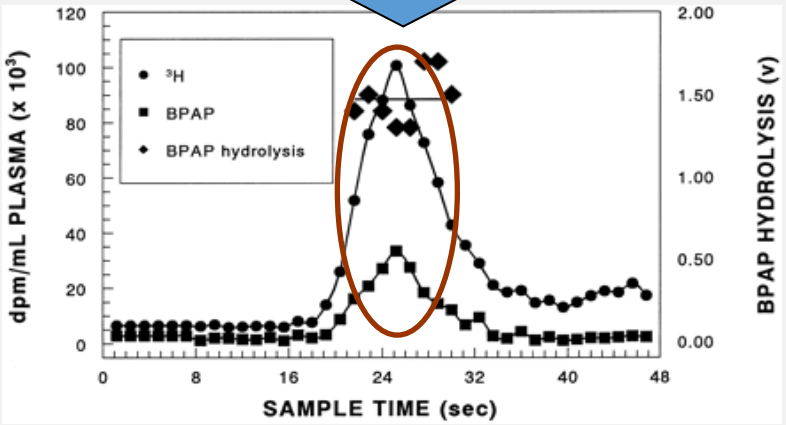
$\bar{t} = 0$

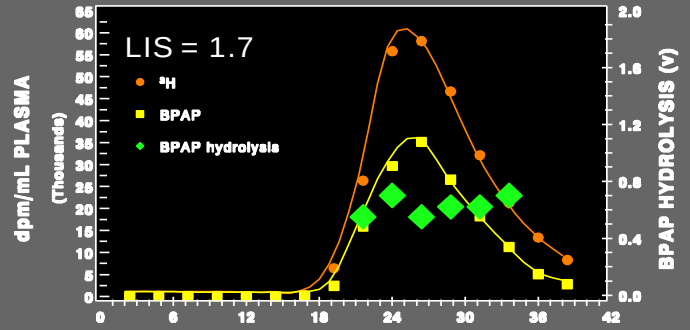
(V)

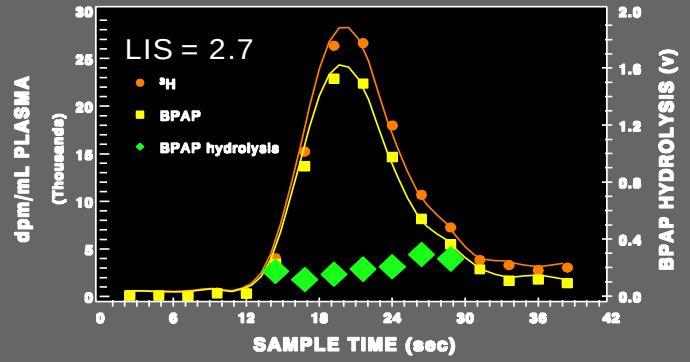


$\bar{t}\downarrow$

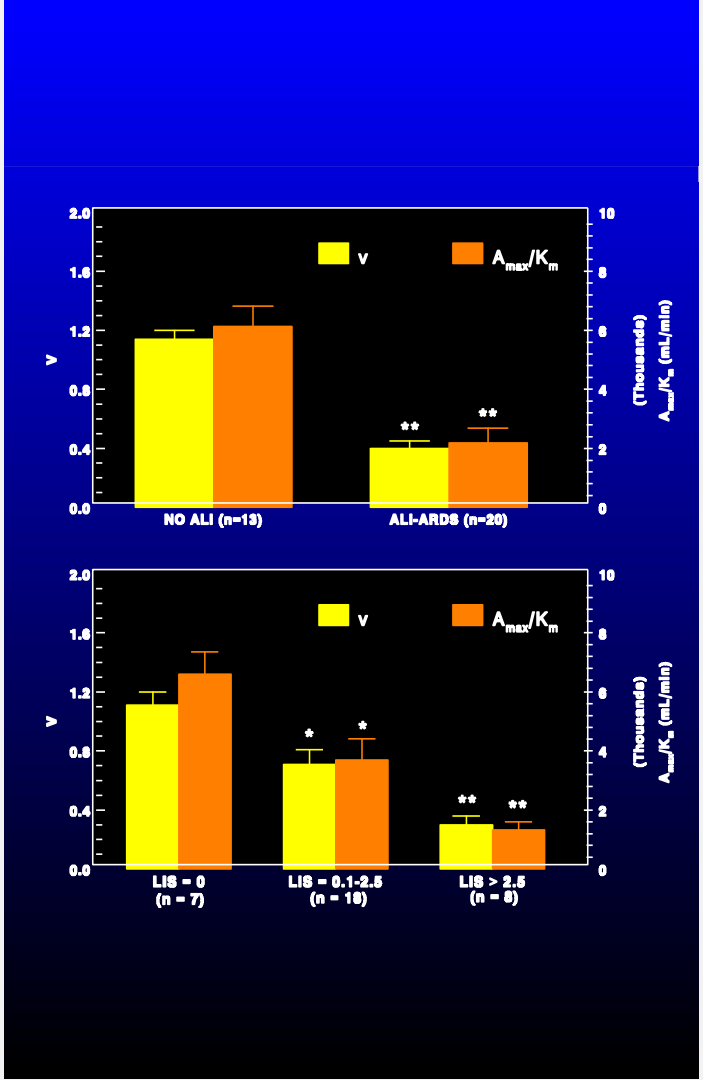




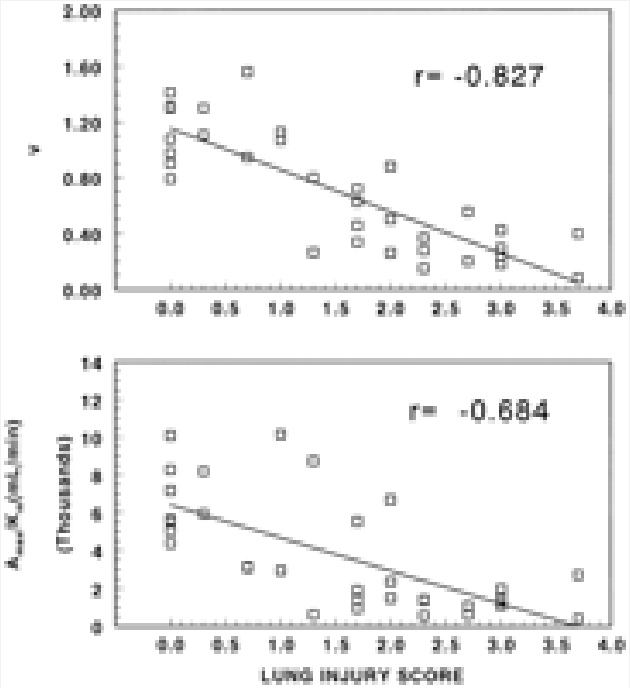


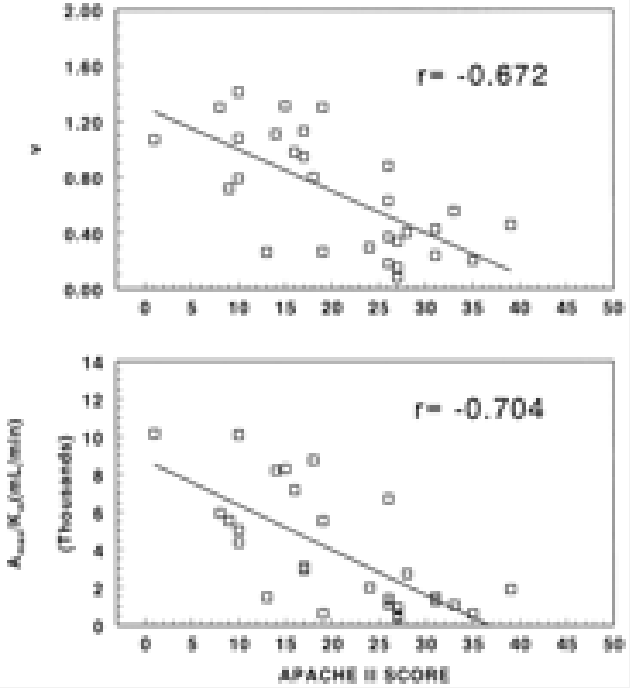


Orfanos et al. *Circulation 2000; 102:2011*

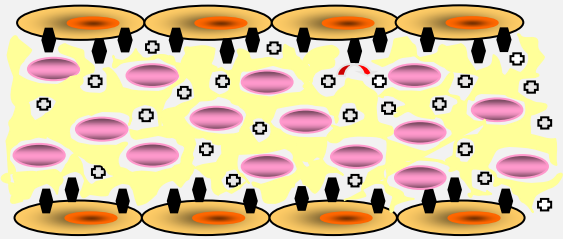


Orfanos et al.
Circulation 2000; 102:2011

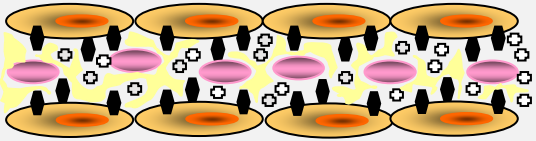




Circulation 2000; 102:2011

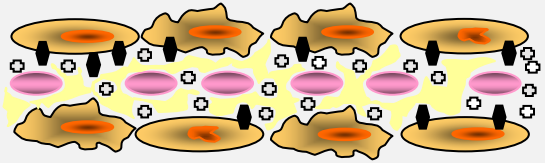


(I)



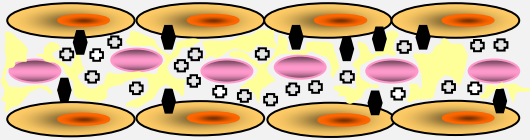
$v = [E] \bullet \bar{t} \bullet k_{cat} / K_m$

(II)



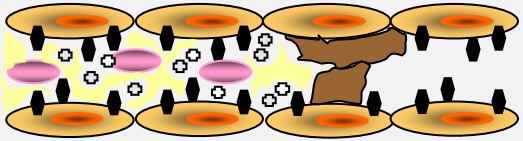
$[E]\downarrow,\ k_{cat} / K_m \downarrow$

(III)



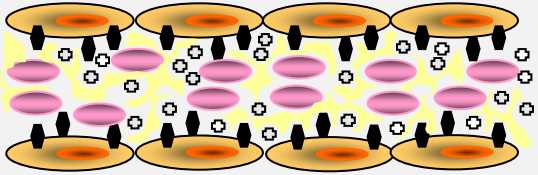
$[E]\downarrow$

(IV)



$\bar{t} = 0$

(V)



$\bar{t}\downarrow$

Biomarker(s)

- Should be:
- Easily measured
- Have adequate sensitivity and specificity
- Test results should not delay
- Not expensive!

Clinical Assays in Sepsis: Prognosis, Diagnosis, Outcomes, and the Genetic Basis of Sepsis

Alice Georgia Vassiliou, Stylianos E. Orfanos and Anastasia Kotanidou

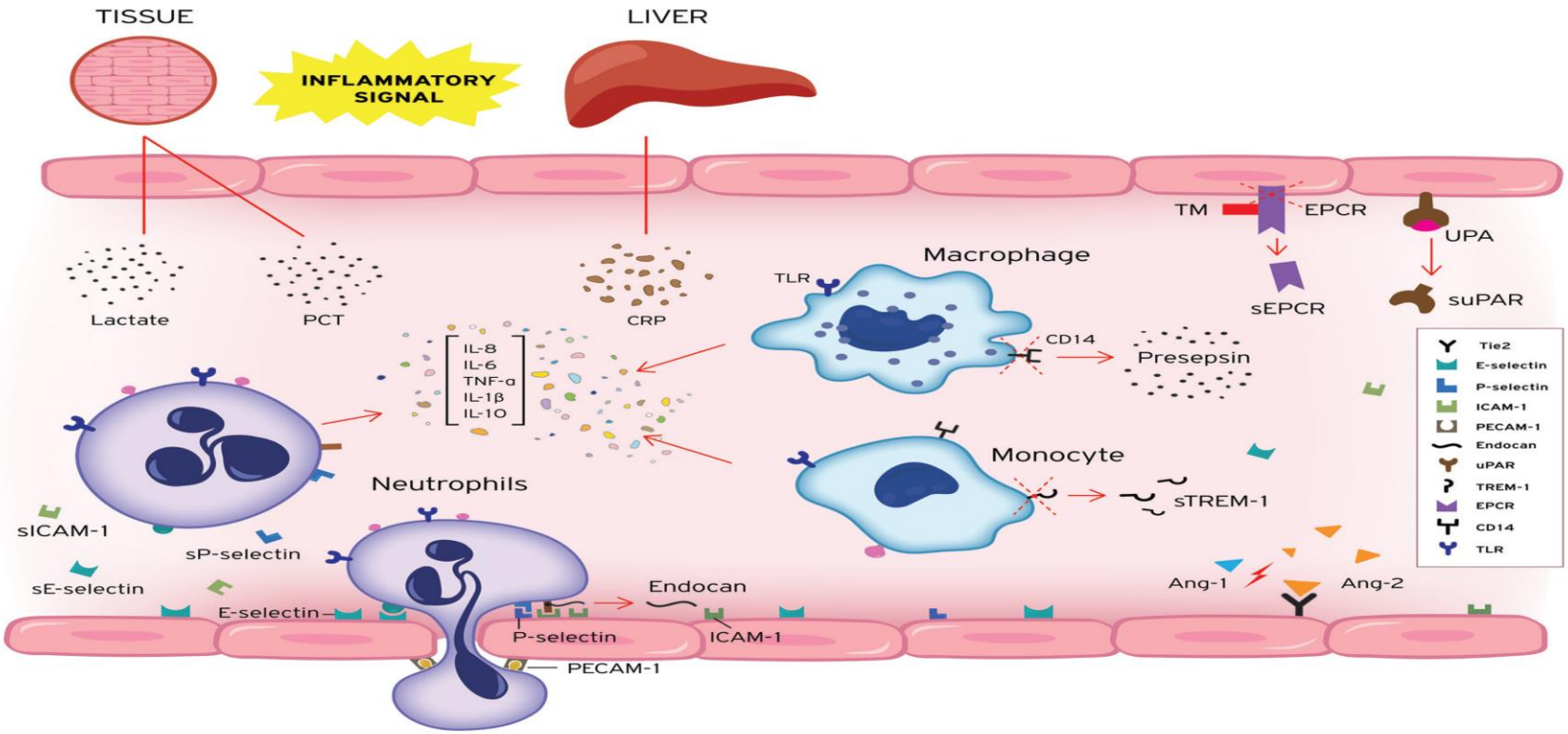
Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67985

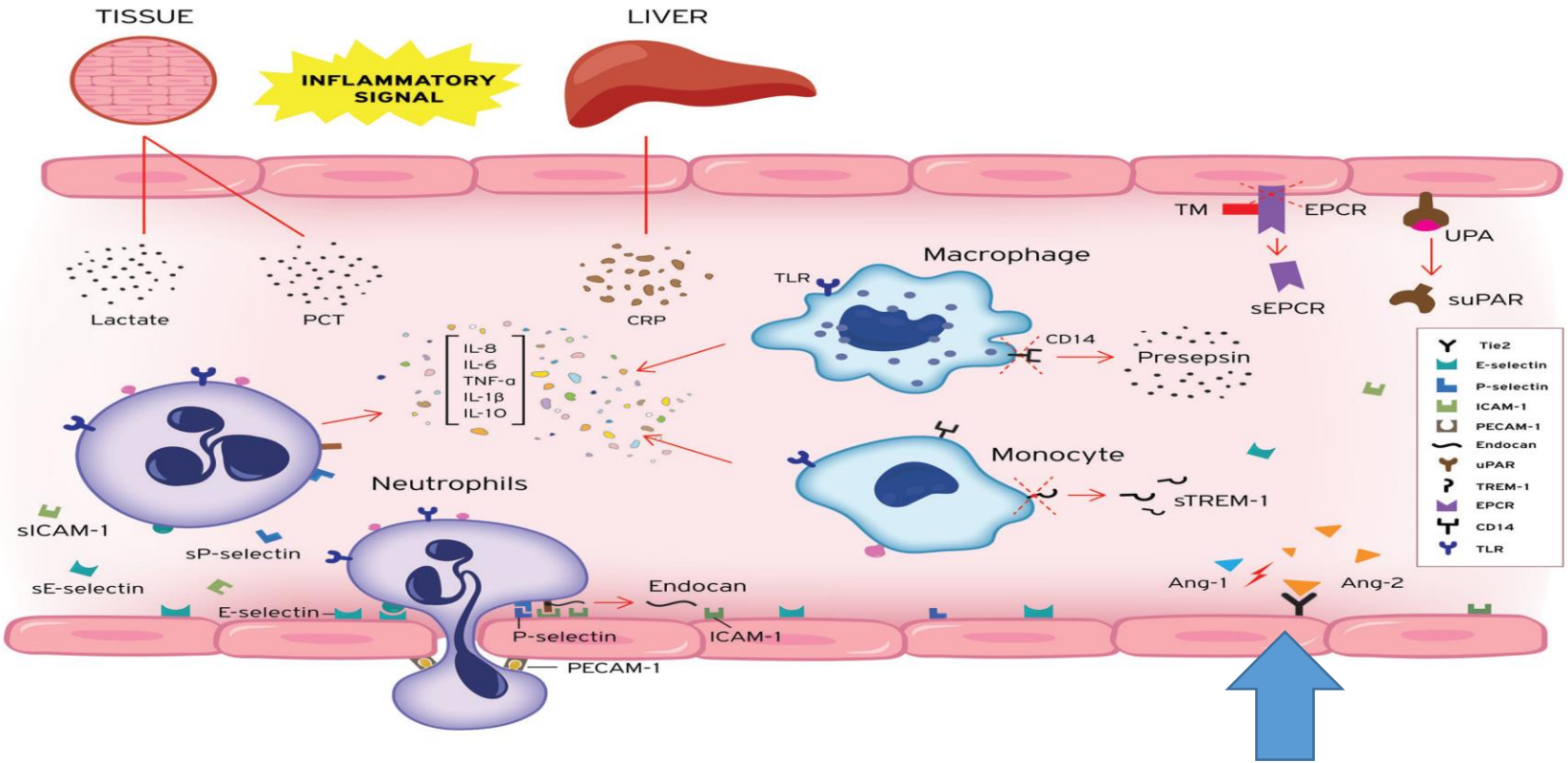
Abstract

Sepsis is the most widespread medical disorder of the intensive care unit (ICU) and the most common cause of death in hospitalized patients. Several endothelium-related molecules have been investigated as potential biomarkers for early diagnosis and/or prognosis of sepsis, providing different results depending on study designs. Therefore, it seems that we are still far from the right combination of sepsis markers to be used in clinical practice. It is more probable that a panel of diverse biomarkers will be more efficient in clinical practice. More recently, the potential use of genetic biomarkers for prognostic purposes started emerging for sepsis, in the form of genome-wide association studies. The successful use of modern molecular diagnostics could enable rapid identification of particularly susceptible or less susceptible individuals, leading to tailored therapeutic treatments.

Keywords: sepsis, biomarkers, polymorphisms



Clinical Assays in Sepsis: Prognosis, Diagnosis, Outcomes, and the Genetic Basis of Sepsis
http://dx.doi.org/10.5772/67985



Clinical Assays in Sepsis: Prognosis, Diagnosis, Outcomes, and the Genetic Basis of Sepsis
http://dx.doi.org/10.5772/67985

2.3.1. Angiopoietins

Angiopoietin-1 (Ang-1) and angiopoietin-2 are antagonistic factors that trigger endothelial cell (EC) activation; the role of angiopoietin-1 is to maintain vessel integrity and block vascular leakage, while angiopoietin-2 (Ang-2) counteracts the protective effects of Ang-1-Tie2 signaling [84, 85]. Ang-2 has been proposed as a biomarker in sepsis, since its release directly reflects vascular barrier breakdown [86–88]. More specifically, Ang-2 levels have been found to be elevated in patients with severe sepsis compared to patients with sepsis or not [89–91], higher Ang-2 levels have been reported in septic patients with worse clinical outcome [92–94], and increased Ang-2 levels have been demonstrated in nonsurvivors compared to survivors [95, 96]. Fewer studies have examined the role of Ang-1 in sepsis; those reports have shown either decreased levels of Ang-1 in critically ill patients compared to healthy controls, or have associated decreased levels at ICU admission with higher mortality [94, 97].

Clinical Assays in Sepsis: Prognosis, Diagnosis, Outcomes, and the Genetic Basis of Sepsis
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Angiopoietin-2 is increased in severe sepsis: Correlation with inflammatory mediators

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Objective: Angiopoietin (Ang)-2 is an endothelium-specific growth factor, regulated by proinflammatory stimuli, that destabilizes vascular endothelium and increases vascular leakage; consequently, Ang-2 may contribute to sepsis pathophysiology. We have studied 1) serum Ang-2 levels in critically-ill patients and investigated potential relationships with inflammatory mediators and indices of disease severity and 2) the effect of sepsis-related inflammatory mediators on Ang-2 production by lung endothelium *in vitro*.

Design: Prospective clinical study followed by cell culture studies.

Setting: General intensive care unit and research laboratory of a university hospital.

Subjects: Human and bovine lung microvascular endothelial cells and 61 patients (32 men). Patients were grouped according to their septic stage as having: no systemic inflammatory response syndrome (n = 6), systemic inflammatory response syndrome (n = 8), sepsis (n = 16), severe sepsis (n = 18), and septic shock (n = 13).

Interventions: Cells were exposed to lipopolysaccharide, tumor necrosis factor-α, and interleukin-6.

Measurements and Main Results: Patients' serum Ang-2 levels were significantly increased in severe sepsis as compared with patients with no systemic inflammatory response syndrome or sepsis ($p < .05$ by analysis of variance). Positive linear relationships were observed with: serum tumor necrosis factor-α ($r_s = 0.654$, $p < .001$), serum interleukin-6 ($r_s = 0.464$, $p < .001$), Acute Physiology and Chronic Health Evaluation II score ($r_s = 0.387$, $p < .001$), and Sequential Organ Failure Assessment score ($r_s = 0.428$, $p < .001$). Multiple regression analysis revealed that serum Ang-2 is mostly related to serum tumor necrosis factor-α and severe sepsis. Treatment of human lung microvascular endothelial cells with all mediators resulted in a concentration-dependent Ang-2 reduction. Treatment of bovine lung microvascular endothelial cells with lipopolysaccharide and tumor necrosis factor-α increased Ang-2 release, and interleukin-6 reduced basal Ang-2 levels.

Conclusions: First, patients' serum Ang-2 levels are increased during severe sepsis and associated with disease severity. The strong relationship of serum Ang-2 with serum tumor necrosis factor-α suggests that the latter may participate in the regulation of Ang-2 production in sepsis. Second, inflammatory mediators reduce Ang-2 release from human lung microvascular endothelial cells, implying that this vascular bed may not be the source of increased Ang-2 in human sepsis. (Crit Care Med 2007; 35:199–206)

KEY WORDS: lung; endothelium; sepsis; angiopoietin-2; tumor necrosis factor-α; interleukin-6

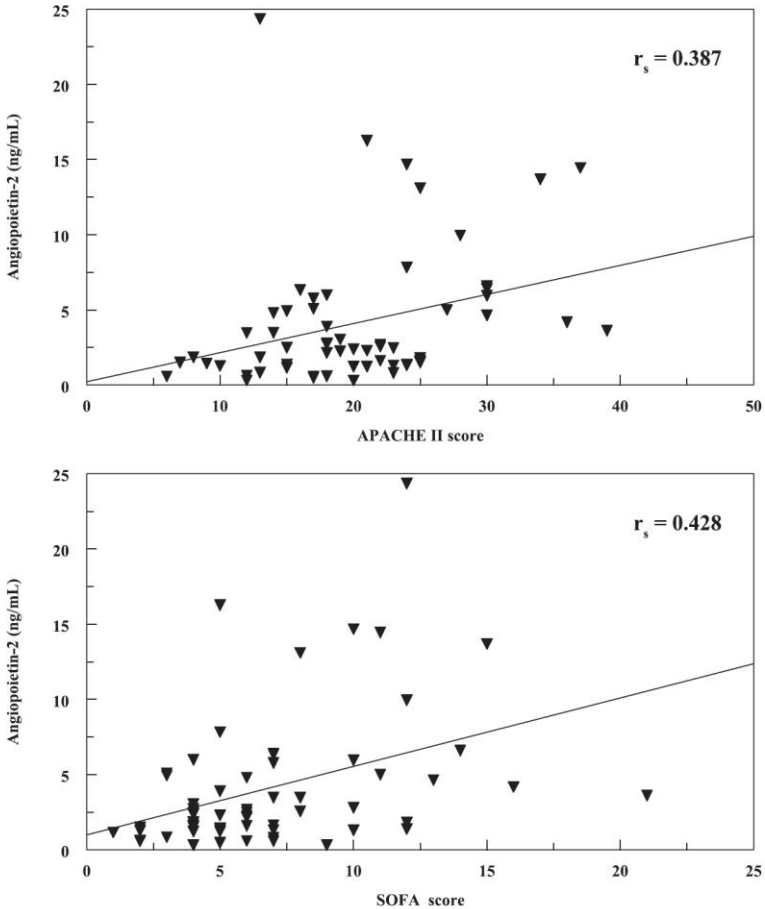


Figure 3. Serum angiopoietin-2 correlates with disease severity scores. *Top*, positive correlation of serum angiopoietin-2 with Acute Physiology and Chronic Health Evaluation (*APACHE*) II score (n = 61; Spearman's rank correlation coefficient [r_s] = 0.387, $p < .001$); *bottom*, positive correlation between serum angiopoietin-2 and Sequential Organ Failure Assessment (*SOFA*) score (n = 61; r_s = 0.428, $p < .001$). *Linear curves* represent least-squares lines.

Plasma angiopoietin-2 in clinical acute lung injury: Prognostic and pathogenetic significance*

Carolyn S. Calfee, MD, MAS; Diana Gallagher, MD, MMSc; Jason Abbott, BS; B. Taylor Thompson, MD; Michael A. Matthay, MD; the NHLBI ARDS Network

Background: Angiopoietin-2 is a proinflammatory mediator of endothelial injury in animal models, and increased plasma angiopoietin-2 levels are associated with poor outcomes in patients with sepsis-associated acute lung injury. Whether angiopoietin-2 levels are modified by treatment strategies in patients with acute lung injury is unknown.

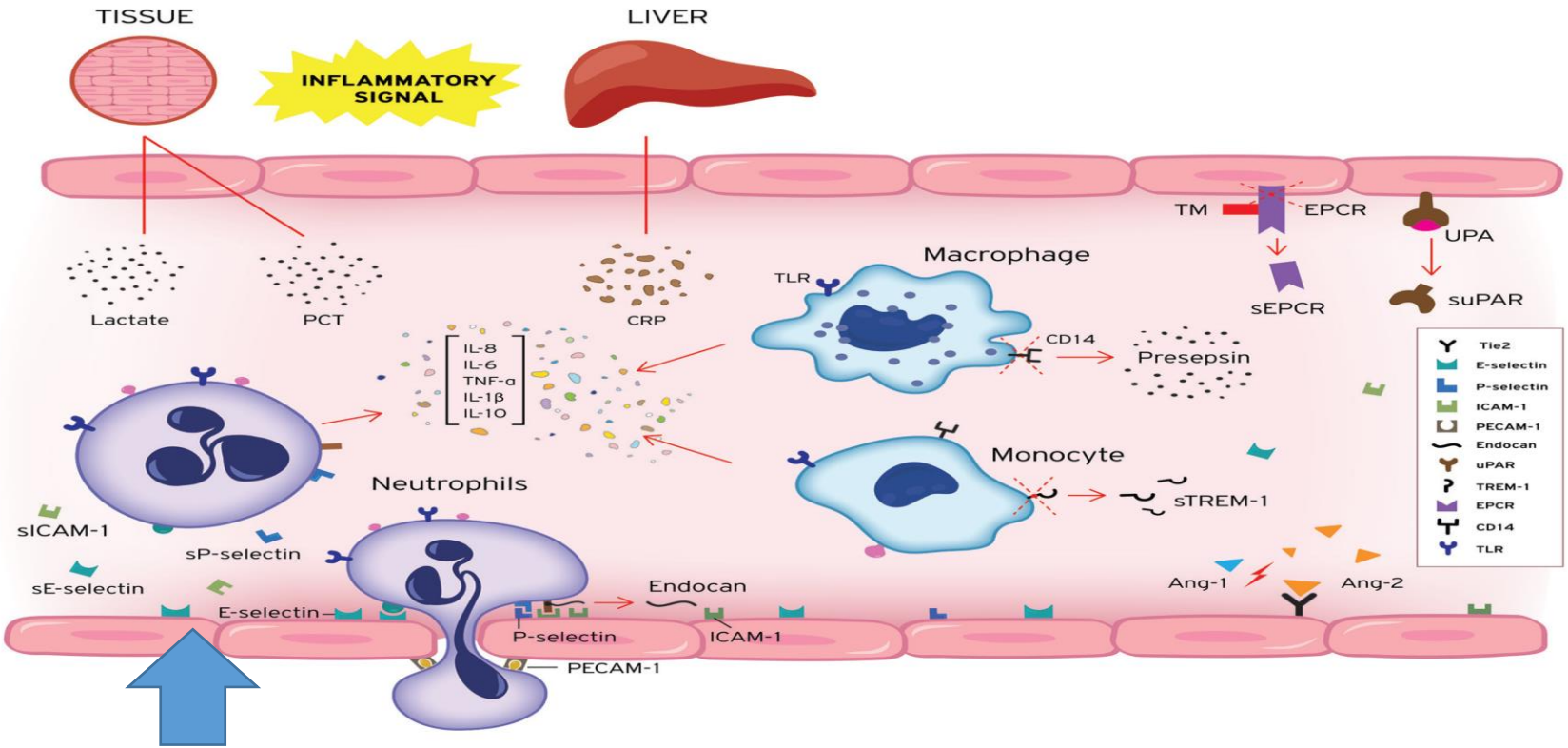
Objectives: To determine whether plasma angiopoietin-2 levels are associated with clinical outcomes and affected by fluid management strategy in a broad cohort of patients with acute lung injury.

Design, Setting, and Participants: Plasma levels of angiopoietin-2 and von Willebrand factor (a traditional marker of endothelial injury) were measured in 931 subjects with acute lung injury enrolled in a randomized trial of fluid liberal vs. fluid conservative management.

Measurements and Main Results: The presence of infection (sepsis or pneumonia) as the primary acute lung injury risk factor significantly modified the relationship between baseline angiopoietin-2 levels and mortality ($p = .01$ for interaction). In noninfection-related acute lung injury, higher baseline angiopoietin-2 levels were strongly associated with increased mortality (odds ratio, 2.43 per 1-log increase in angiopoietin-2; 95% confidence interval, 1.57-3.75; $p < .001$). In infection-related acute lung injury, baseline angiopoietin-2 levels were similarly elevated in survivors and nonsurvivors; however, patients whose plasma angiopoietin-2 levels increased from day 0 to day 3 had more than double the odds of death compared with patients whose angiopoietin-2 levels declined over the same period of time (odds ratio, 2.29; 95% confidence interval, 1.54-3.43; $p < .001$). Fluid-conservative therapy led to a 15% greater decline in angiopoietin-2 levels from day 0 to day 3 (95% confidence interval, 4.6-24.8%; $p = .006$) compared with fluid-liberal therapy in patients with infection-related acute lung injury. In contrast, plasma levels of von Willebrand factor were significantly associated with mortality in both infection-related and noninfection-related acute lung injury and were not affected by fluid therapy.

Conclusions: Unlike von Willebrand factor, plasma angiopoietin-2 has differential prognostic value for mortality depending on the presence or absence of infection as an acute lung injury risk factor. Fluid conservative therapy preferentially lowers plasma angiopoietin-2 levels over time and thus may be beneficial in part by decreasing endothelial inflammation. (Crit Care Med 2012; 40:1731–1737)

KEY WORDS: acute respiratory distress syndrome; angiopoietin-2; biomarkers; endothelial injury; pulmonary edema; von Willebrand factor



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2.3.2. *Selectins*

Prior to the firm adhesion of leukocytes to the vascular endothelium and their transmigration to the sites of injury and inflammation, capture and rolling of leukocytes along the endothelium occurs. This is mediated by a family of cell adhesion molecules (or CAMs), called the selectin family [98]. Levels of soluble (s)E-selectin are very low in healthy individuals, whereas increased concentrations have been reported in various inflammatory pathologies [99–102]; other investigations have shown higher levels in nonsurvivors than survivors [103, 104]. Recently, it was demonstrated that sE-selectin levels may be used as predictor of fatal outcome in patients with SIRS [105]. Moreover, sE-selectin has also been proposed as a predictor of bacteremia in severe sepsis patients [106]. P-selectin has a similar function, but is constitutively expressed in lung ECs, and correlates with lung endothelial injury [107]. A recent study by Wang *et al.* [108] demonstrated in patients hospitalized for infections that higher baseline levels of interleukin-6, sE-selectin, and soluble intercellular adhesion molecule-1 (sICAM-1) may differentiate those patients who will develop a mild response to infection from those who will develop full-blown sepsis. A most recent study showed that high levels of the circulating endothelial adhesion molecules sE- and sP-selectin, measured at ICU admission, appear to be associated with sepsis development in time [109].

2.3.3. *Soluble intercellular adhesion molecule-1 (sICAM-1)*

Both the innate and adaptive immune responses depend on the migration of leukocytes across endothelial cells [110]. Specific adhesion glycoproteins are required for the binding of leukocytes to ECs. One such glycoprotein, intercellular adhesion molecule-1 (ICAM-1), controls the firm adhesion of neutrophils on endothelium and consequently their transmigration to the sites of infection. ICAM-1 has been studied as a biomarker of sepsis severity and outcome. These studies have produced inconsistent and conflicting results, possibly reflecting the time point at which they were measured [105]. ICAM-1 production has been shown to be induced by endotoxins and has been associated with sepsis severity [102, 111] or mortality [111, 112], while sICAM-1 seems to be a reliable biomarker for distinguishing patients with sepsis from those with noninfectious SIRS [105].

Elevated biomarkers of endothelial dysfunction/activation at ICU admission are associated with sepsis development

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ABSTRACT

Widespread endothelial activation and dysfunction often precede clinical sepsis. Several endothelium-related molecules have been investigated as potential biomarkers for early diagnosis and/or prognosis of sepsis, providing different results depending on study designs. Such factors include endothelial adhesion molecules like E- and P-selectin, and the intercellular adhesion molecule-1, vascular endothelial cadherin, growth factors such as Angiopoietin-1 and -2 and vascular endothelial growth factor, as well as von Willebrand factor antigen. We sought to investigate whether circulating biomarkers of endothelial activation/dysfunction measured at ICU admission are associated with subsequent sepsis development.

Eighty-nine critically-ill patients admitted to a general ICU who met no sepsis criteria were studied. Plasma or serum levels of the above-mentioned endothelium-derived molecules were measured during the first 24 h post ICU; acute physiology and chronic health evaluation (APACHE) II and sequential organ failure assessment (SOFA) scores, age, sex, diagnostic category, and circulating procalcitonin (PCT) and C-reactive protein (CRP) levels were additionally measured or recorded.

Forty-five patients subsequently became septic and 44 did not. Soluble (s) E- and P-selectin levels, circulating PCT, SOFA score and diagnostic category were significantly different between the two groups. Multiple logistic regression analysis associated elevated sE- and sP-selectin levels and SOFA with an increased risk of developing sepsis, while multiple Cox regression analysis identified sE- and sP-selectin levels as the only parameters related to sepsis appearance with time [RR = 1.026, 95%CI = 1.008–1.045, $p = 0.005$; RR = 1.005 (by 10 units), 95%CI = 1.000–1.010, $p = 0.034$, respectively]. When trauma patients were independently analyzed, multiple Cox regression analysis revealed sE-selectin to be the only molecule associated with sepsis development with time (RR = 1.041, 95%CI: 1.019–1.065; $p < 0.001$).

In conclusion, in our cohort of initially non-septic critically-ill patients, high levels of the circulating endothelial adhesion molecules E- and P-selectin, measured at ICU admission, appear to be associated with sepsis development in time.

Table 1
Characteristics and circulating levels at ICU admission of endothelial adhesion molecules, growth factors and glycoproteins of the patients who developed sepsis (sepsis-positive) versus those who did not (sepsis-negative).

Parameters	Sepsis-positive patients	Sepsis-negative patients	*p*-Value
Number of patients (*N*)	45	44	
APACHE II score	13.87 ± 5.30	11.44 ± 4.31	0.063
SOFA score	6.53 ± 2.66	4.68 ± 2.20	<0.002*
Age (years)	42 ± 19	50 ± 20	0.056
Sex			
Male	34 (54.8%)	28 (45.2%)	
Female	11 (40.7%)	16 (59.3%)	0.221
Diagnosis			
Medical	5 (27.8%)	13 (72.2%)	
Surgical	11 (42.3%)	15 (57.7%)	
Trauma	29 (64.4%)	16 (35.6%)	0.019*
ICU mortality	5 (11.11%)	1 (2.27%)	0.203
Septic shock	13 (28.88%)	N/A	
ICU stay (days)	29.36 ± 16.22	7.30 ± 5.39	
Sepsis day	5.62 ± 2.92	N/A	
PaO_2/FiO_2 (mmHg)	304.00 ± 121.40	285.20 ± 136.28	0.455
White blood cell count (per μl)	12568.22 ± 4831.53	10623.26 ± 3424.37	0.072
SIRS	36 (50.00%)	36 (50.00%)	1.000
PCT (ng/ml)	0.55 (0.14–2.3)	0.215 (0.07–0.685)	0.015*
CRP (mg/dl)	4.80 (2.20–13.10)	5.00 (1.50–10.00)	0.45
sE-selectin (ng/ml)	35.72 (25.19–46.88)	19.54 (12.30–27.32)	$p < 0.001$*
sP-selectin (ng/ml)	142.40 (99.68–188.48)	83.28 (49.00–117.72)	$p < 0.001$*
sICAM (ng/ml)	161.72 (126.38–186.55)	160.75 (104.09–250.23)	0.749
Ang-1 (ng/ml)	15.15 (0.00–37.23)	13.23 (0.00–26.24)	0.308
Ang-2 (ng/ml)	5.50 (4.17–8.51)	5.43 (4.12–8.76)	0.815
sVE-cadherin (ng/ml)	1929.63 (1325.00–2620.37)	2293.52 (1630.09–2970.83)	0.074
VEGF (pg/ml)	82.00 (42.00–168.00)	97.50 (29.50–181.50)	0.679
vwF (mU/ml)	814.62 (662.83–1010.89)	832.35 (703.73–958.24)	0.812

Data are expressed as number of patients (*N*) and percentages of totals (%), except for age, duration of stay in ICU, sepsis day, PaO_2/FiO_2, APACHE and SOFA scores, and white blood cell count (mean ± SD), sE-selectin, sP-selectin, sICAM, Ang-1, Ang-2, sVE-cadherin, VEGF, vwF, PCT, and CRP [median (Q1–Q3)]. The *t*-test was used, except from diagnosis and sex (chi-square test), SIRS and mortality (Fisher's exact test) and sE-selectin, sP-selectin, sICAM, Ang-1, Ang-2, sVE-cadherin, VEGF, vwF, CRP, PCT (non-parametric analysis – Mann Whitney test). APACHE II and SOFA scores, circulating PCT, CRP, sE-selectin, sP-selectin, sICAM, Ang-1, Ang-2, sVE-cadherin, VEGF and vwF levels were estimated within the first 24 h post ICU admission. Sepsis day denotes the post-ICU admission day when sepsis developed. Ang = angiopoietin; APACHE = acute physiology and chronic health evaluation; CRP = C-reactive protein; sICAM = soluble intercellular adhesion molecule 1; ICU = intensive care unit; PCT = procalcitonin; SIRS = systemic inflammatory response syndrome; SOFA = sequential organ failure assessment; VEGF = vascular endothelial growth factor; vWF = von Willebrand factor.
* *p*-Value < 0.05.

Table 3
Detection source (body fluid) and type of infectious microorganisms identified in the patients who developed sepsis (sepsis-positive).

Parameters	Sepsis-positive patients
Detection source (Body fluid) (N)	
Bronchial secretions	33 (73.33%)
Cerebrospinal fluid	2 (4.45%)
Blood	10 (22.22%)
Patients with Gram-negative infections	39 (86.67%)
Acinetobacter baumannii	24 (53.33%)
Klebsiella pneumoniae	7 (15.56%)
Enterobacter cloacae	1 (2.22%)
Enterobacter aerogenes	1 (2.22%)
Serratia marcescens	2 (4.45%)
Acinetobacter baumannii + *Klebsiella pneumoniae*	3 (6.67%)
Pseudomonas aureginosa + *Serratia marcescens* + *Proteus mirabilis*	1 (2.22%)
Patients with Gram-positive infections (*N*)	6 (13.33%)
Staphylococcus species	6 (13.33%)

Data are expressed as number of patients (*N*) and percentages of totals (%). CNS = central nervous system.

Table 5
Cox regression models.

Parameters	Relative risk	95% CI	*p*-Value
sE-selectin (ng/ml)	1.035	1.019–1.052	<0.001*
sP-selectin (ng/ml) (by 10 units)	1.085	1.036–1.136	0.001*
sICAM (ng/ml) (by 10 units)	0.989	0.958–1.020	0.485
Ang-1 (ng/ml)	1.004	0.991–1.018	0.514
Ang-2 (ng/ml)	0.995	0.949–1.043	0.826
sVE-cadherin (ng/ml) (by 10 units)	0.998	0.994–1.002	0.294
VEGF (pg/ml) (by 10 units)	0.986	0.961–1.011	0.267
vWF (mU/ml) (by 10 units)	0.993	0.977–1.009	0.404
PCT (ng/ml)	1.059	0.954–1.176	0.284
CRP (mg/dl)	1.006	0.965–1.048	0.789
Diagnosis			
Trauma	1.000[a]	–	–
Surgical	0.725	0.362–1.454	0.366
Medical	0.424	0.164–1.095	0.076
Sex			
Male	1.000[a]	–	–
Female	0.672	0.339–1.330	0.254
APACHE II score	1.054	0.993–1.119	0.086
SOFA score	1.130	1.003–1.272	0.044*
Age	0.987	0.971–1.004	0.125
PaO_2/FiO_2 (mmHg)	1.001	0.998–1.003	0.603
White blood cell count (per μl)	1.000	1.000–1.000	0.237
SIRS	0.923	0.443–1.924	0.837

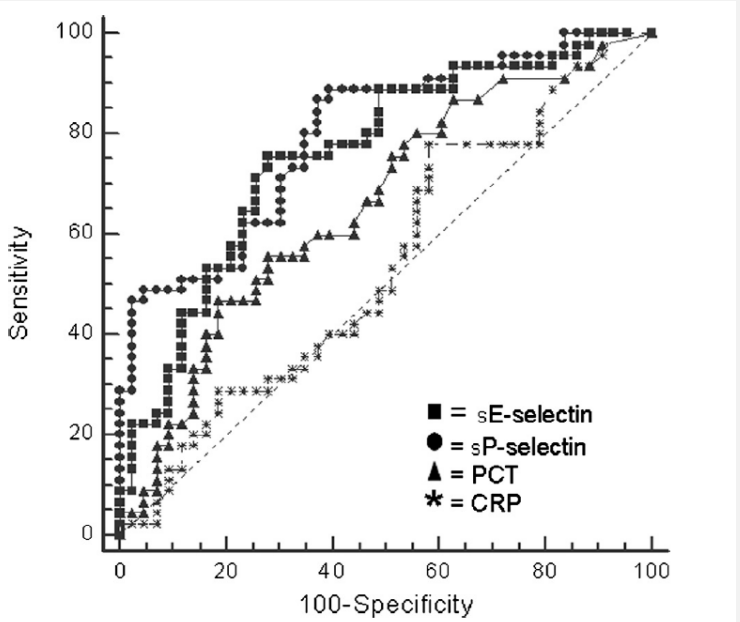
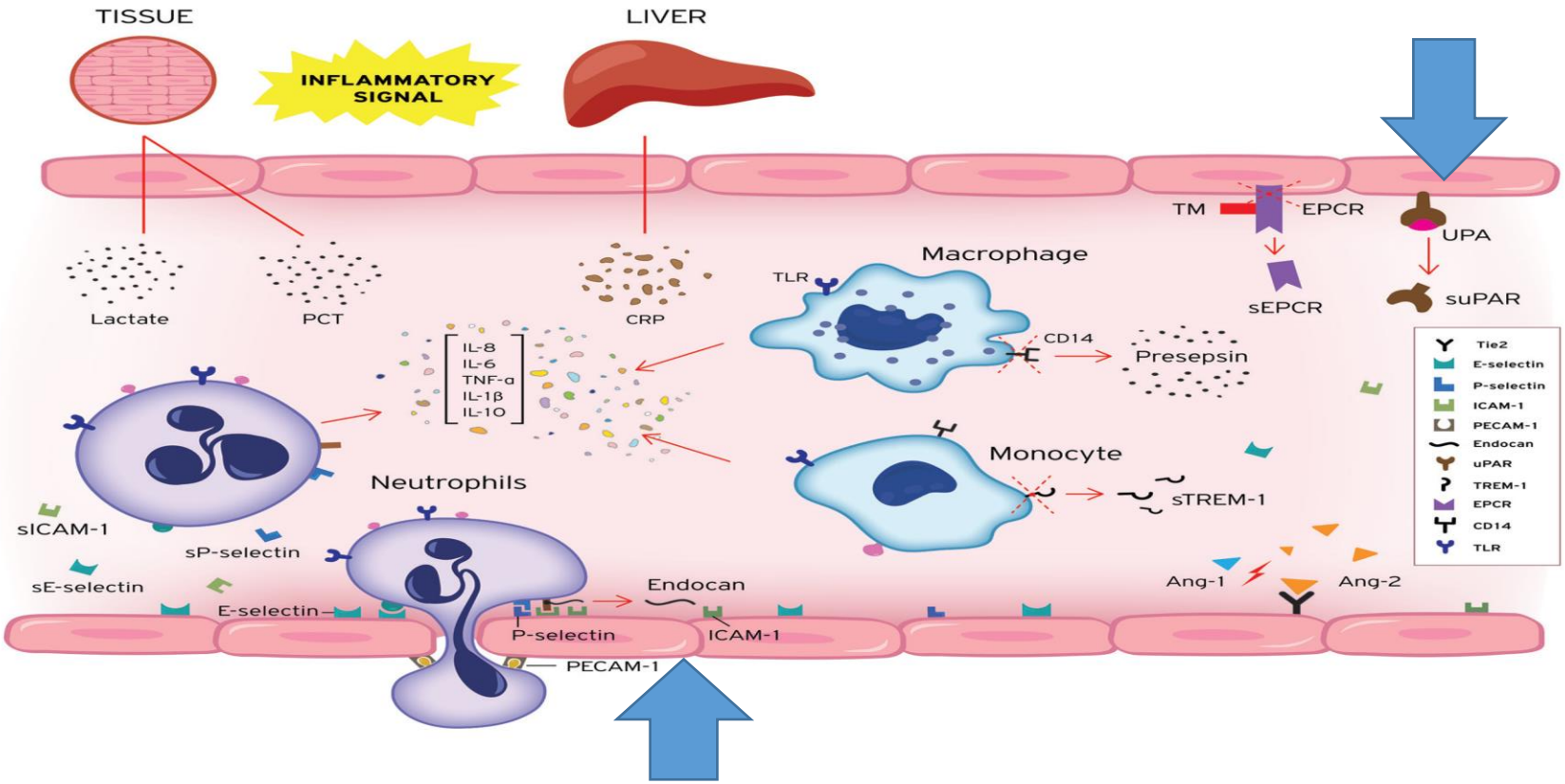


Fig. 1. Receiver operating characteristic curve analysis. ROC curves were generated to determine the prognostic accuracy of sE-selectin, sP-selectin, PCT and CRP; the corresponding areas under the curve (AUC) and 95% confidence intervals (CI) were estimated at: 0.761 (0.659–0.845; $p < 0.0001$), 0.789 (0.689–0.868; $p < 0.0001$), 0.659 (0.551–0.757; $p = 0.0065$) and 0.539 (0.430–0.645; $p = 0.5311$), respectively. Levels of all four molecules were estimated within the first 24 h post ICU in initially non-septic patients. PCT = procalcitonin, CRP = C-reactive protein.



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2.3.4. *Soluble platelet/endothelial cell adhesion molecule-1 (sPECAM-1)*

Platelet/endothelial cell adhesion molecule-1 (PECAM-1, CD31) is a 130-kDa cell adhesion molecule that is expressed on the surfaces of leukocytes, such as monocytes, neutrophils, and some T-cell subsets, as well as on platelets and the intercellular junctions of endothelial cells [113]. Serum levels of sPECAM-1 have been demonstrated to be higher in septic patients compared with nonseptic patients at admission and are also higher compared to healthy controls [114, 115].

2.3.5. *Endocan*

Endocan is a proteoglycan expressed and secreted by the vascular endothelium in the lung and kidney, in response to pro-inflammatory cytokines and pro-angiogenic factors, which inhibits leukocyte migration [116]. The molecule is cleaved through the activity by cathepsin G generating a novel endocan peptide fragment of 14 kDa, named p14, which exhibits higher concentrations in septic patients compared to healthy volunteers [117]. Several studies have shown that this glycoprotein can be used as a strong and significant predictor of sepsis severity and outcome [118–122].

2.4.1. Soluble urokinase-type plasminogen activator receptor (suPAR)

The soluble urokinase-type plasminogen activator receptor (suPAR) was first identified in 1985 as a cellular binding site for urokinase [123]. Since then suPAR has been investigated as a potential prognostic marker in the ICU. In critically ill patients, several studies have reported elevated suPAR in SIRS, bacteremia, sepsis, and septic shock, in which high circulating suPAR levels indicated a poor prognosis, including organ dysfunction and mortality [124–127].

Systematic reviews have concluded, however, that the diagnostic value of suPAR in sepsis is limited [128] and suPAR does not appear to be better in diagnosing sepsis compared to other biomarkers, like CRP and PCT [129, 130]. Plasma suPAR levels are, however, a sensitive and specific independent prognostic biomarker in patients with bacteremia. This plasma protein may be used to identify patients who are severely ill with pneumococcal bacteremia, and predict mortality [131–133].

CRITICAL CARE

RESEARCH Open Access

Risk assessment in sepsis: a new prognostication rule by APACHE II score and serum soluble urokinase plasminogen activator receptor

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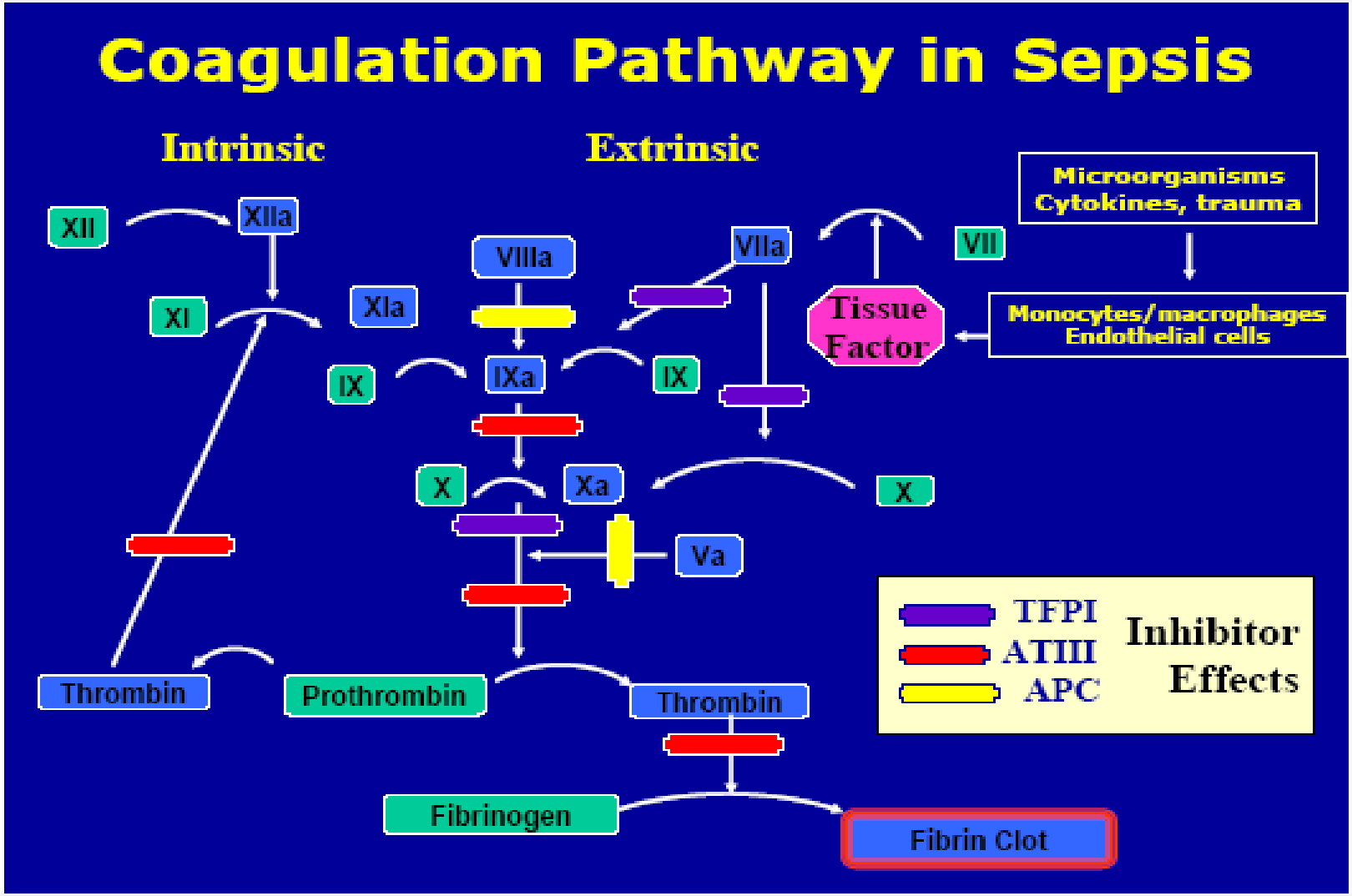
Abstract

Introduction: Early risk assessment is the mainstay of management of patients with sepsis. APACHE II is the gold standard prognostic stratification system. A prediction rule that aimed to improve prognostication by APACHE II with the application of serum suPAR (soluble urokinase plasminogen activator receptor) is developed.

Methods: A prospective study cohort enrolled 1914 patients with sepsis including 62.2% with sepsis and 37.8% with severe sepsis/septic shock. Serum suPAR was measured in samples drawn after diagnosis by an enzyme-immunoabsorbent assay; in 367 patients sequential measurements were performed. After ROC analysis and multivariate logistic regression analysis a prediction rule for risk was developed. The rule was validated in a double-blind fashion by an independent confirmation cohort of 196 sepsis patients, predominantly severe sepsis/septic shock patients, from Sweden.

Results: Serum suPAR remained stable within survivors and non-survivors for 10 days. Regression analysis showed that APACHE II ≥17 and suPAR ≥12 ng/ml were independently associated with unfavorable outcome. Four strata of risk were identified: i) APACHE II <17 and suPAR <12 ng/ml with mortality 5.5%; ii) APACHE II < 17 and suPAR ≥12 ng/ml with mortality 17.4%; iii) APACHE II ≥ 17 and suPAR <12 ng/ml with mortality 37.4%; and iv) APACHE II ≥17 and suPAR ≥12 ng/ml with mortality 51.7%. This prediction rule was confirmed by the Swedish cohort.

Conclusions: A novel prediction rule with four levels of risk in sepsis based on APACHE II score and serum suPAR is proposed. Prognostication by this rule is confirmed by an independent cohort.


Coagulation Pathway in Sepsis
Intrinsic
Extrinsic
Microorganisms
Cytokines, trauma
XII
XIIa
XI
XIa
VIIIa
VIIa
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Tissue Factor
Monocytes/macrophages
Endothelial cells
IX
IXa
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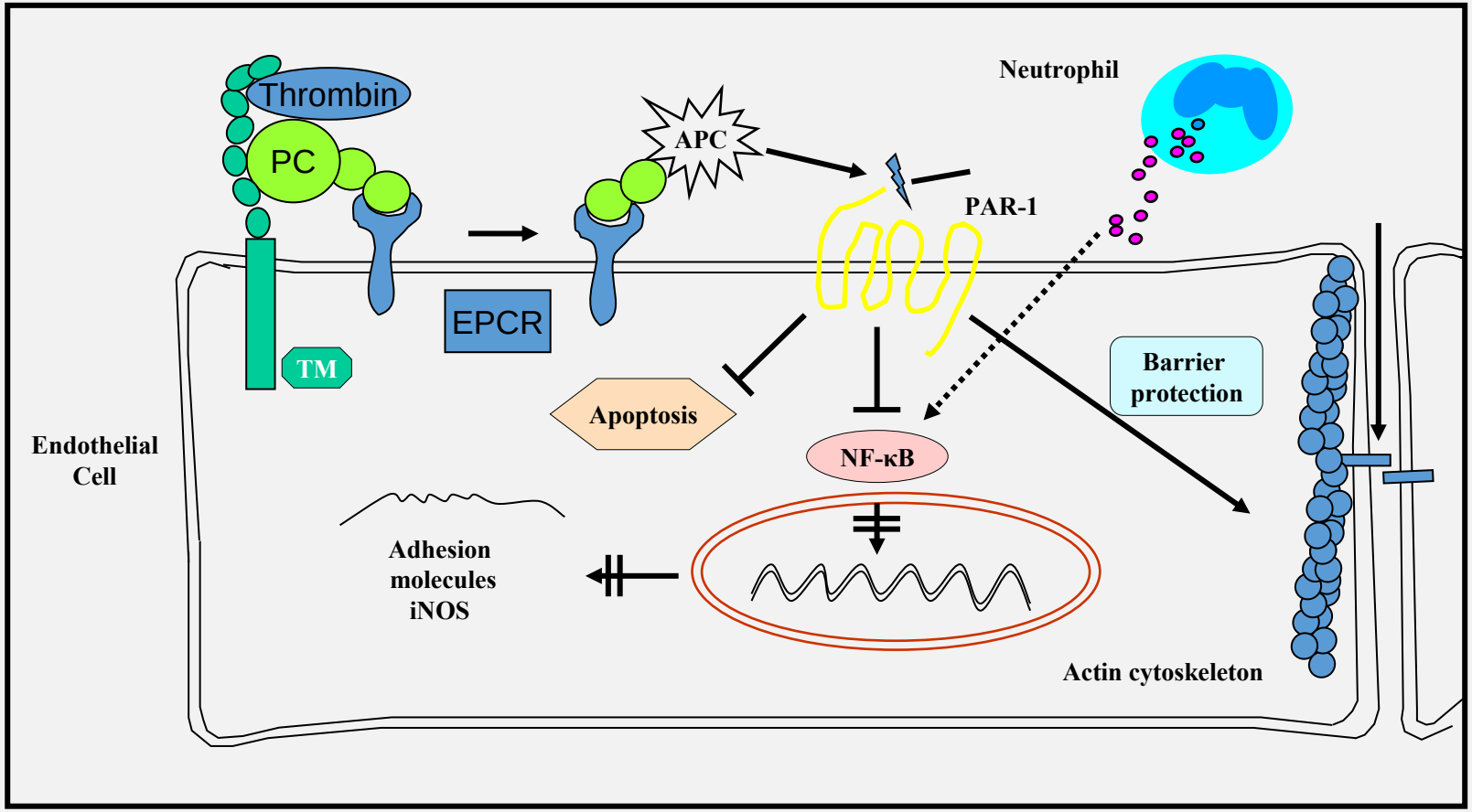

Orfanos et al. 2008 Yearbook Intensive Care & Emergency Med

2.4.3. *Soluble endothelial protein C receptor (sEPCR)*

The protein C (PC) anticoagulant system provides important control of both blood coagulation and inflammatory pathways [144]. This system also involves protein S (PS), and the endothelial receptors thrombomodulin (TM) and endothelial protein C receptor (EPCR). Conversion of PC to activated PC (APC) is generated by TM-bound thrombin and is drastically augmented by the presence of EPCR [145]. The presence of a soluble form of EPCR (sEPCR) that exists under normal conditions and which is elevated in conditions marked by enhanced inflammation [146], supports the notion of EPCR shedding. While the role of membrane EPCR is clearly antithrombotic and anti-inflammatory, the physiological significance of circulating sEPCR *in vivo* is as yet not fully understood and it is still unknown whether soluble EPCR levels may have a predictive value in the appearance of sepsis.

In previous studies, sEPCR levels in septic patients were found to be significantly higher [146, 147], unchanged [148], or even lower [149] than in healthy volunteers. A study by Kager *et al.* [150] showed that increased plasma sEPCR levels correlate with accelerated mortality in patients with melioidosis, while overexpression of EPCR in transgenic animals aggravates outcome during Gram-negative pneumonia-derived sepsis. In another recent report, early kinetics of sEPCR levels in severe sepsis was correlated with outcome [151], by a proposed mechanism of counteracting the anticoagulant action of membrane EPCR. The authors suggested that sEPCR could provide an early biological marker of outcome in severe sepsis. Vassiliou *et al.* [152] showed that levels of soluble EPCR at ICU admission are higher in originally nonseptic patients who subsequently become septic compared to those who will not.

Elevated soluble endothelial protein C receptor levels at ICU admission are associated with sepsis development

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ABSTRACT

Background. The endothelial protein C receptor (EPCR) is a protein that regulates the protein C anticoagulant and anti-inflammatory pathways. A soluble form of EPCR (sEPCR) circulates in plasma and inhibits activated protein C (APC) activities. The clinical impact of sEPCR and its involvement in the septic process is under investigation. In this study, we assessed the role of sEPCR levels as an early indicator of sepsis development.

Methods. Plasma sEPCR levels were measured in 59 critically-ill non-septic patients at the time of admission to the intensive care unit (ICU). Multiple logistic regression analysis was performed to identify potential risk factors for sepsis development and Cox-Regression models were fitted for variables to examine their relationship with time to sepsis development.

Results. Thirty patients subsequently developed sepsis and 29 did not. At ICU admission, sequential organ failure assessment (SOFA) scores were significantly higher in the subsequent sepsis group as compared to the non sepsis group (mean ± SD: 6.4±2.7 and 5±2.3, respectively, P=0.037). sEPCR levels were also higher in the patients who subsequently developed sepsis compared to the patients who did not (median and interquartile range: 173.4 [104.5-223.5] ng/mL *vs.* 98.3 [69.8-147.7] ng/mL, respectively; P=0.004). Cox regression analysis identified sEPCR as the only parameter related to sepsis development with time (relative risk: 1.078, 95% confidence interval: 1.016-1.144, by 10 units; P=0.013).

Conclusion. Upon ICU admission, sEPCR levels in initially non-septic critically-ill patients appear elevated in the subjects who will subsequently become septic. *(Minerva Anestesiol 2015;81:125-34)*

Key words: Sepsis - Critical illness - Prognosis.

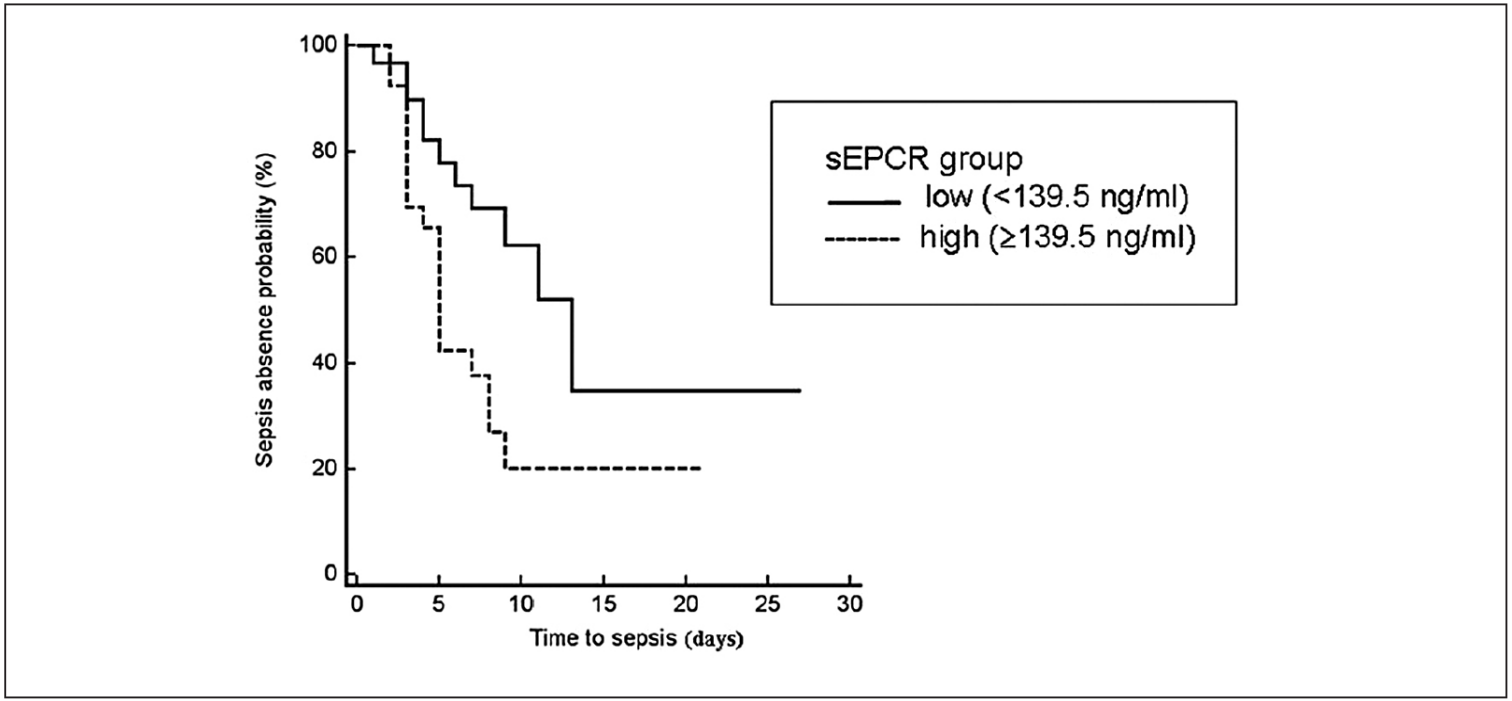
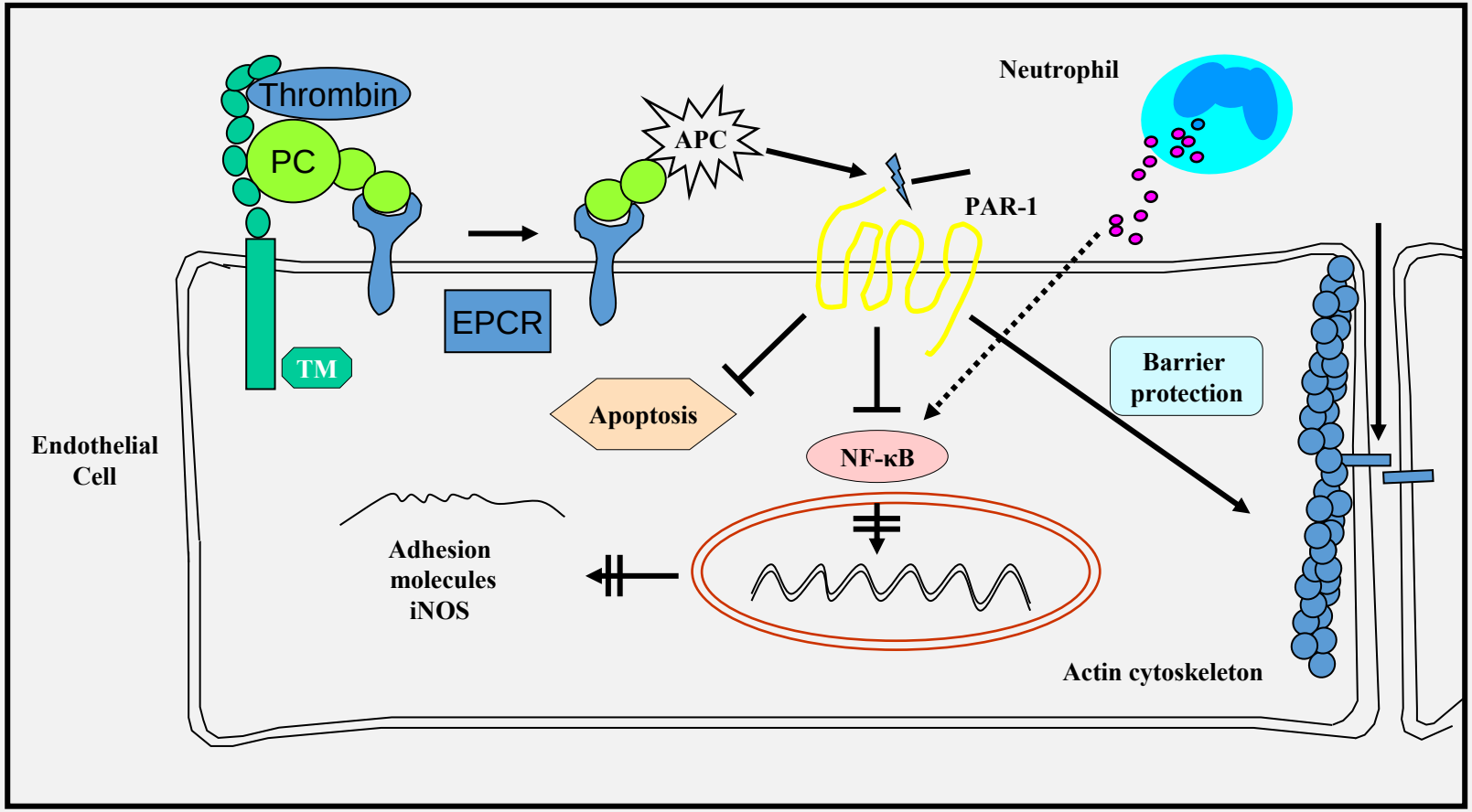


Figure 2.—Probability for sepsis development based on ICU admission (within the first 24 hours) sEPCR levels. The patient cohort was dichotomized above and below the soluble endothelial protein C receptor (sEPCR) median value. Dashed line: ≥139.5 ng/mL; solid line: <139.5 ng/mL. The Kaplan-Meier method was used for sepsis absence probability estimation and the log-rank test for two groups comparison. The respective median time to sepsis for the two groups was 13 days (95% CI: 8.29-17.71) for the low sEPCR group and 5 days (95% CI: 4.16-5.84) for the high sEPCR group (Log-Rank test, P=0.028).



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Inhaled activated protein C attenuates lung injury induced by aerosolized endotoxin in mice

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Abstract

The serine protease activated protein C (APC) possesses prominent anticoagulant and anti-inflammatory actions. In this study, we investigated the effect of inhaled recombinant human (rh) APC in a murine lung injury model. Animals inhaled 10 mg of *Pseudomonas* lipopolysaccharide (LPS) in 3 mL normal saline (NS); 30 min prior to LPS, mice were pretreated with inhaled rhAPC (4 mg/3 mL NS; APC+LPS group) or NS (LPS group). A control animal group inhaled vehicle (NS) twice. 24 h later, total cells and cell-types, protein content, and the cytokines tumor necrosis factor-α, interleukin (IL)-6, macrophage inflammatory protein-1α, and mouse keratinocyte-derived chemokine (a homolog of human IL-8) were estimated in bronchoalveolar lavage fluid (BALF). Lung pathology given as total histology score (THS), wet/dry lung weight ratios, and lung vascular cell adhesion molecule (VCAM)-1 expression were additionally assessed. rhAPC inhalation attenuated the aerosolized LPS-induced increases of: total cells, neutrophils and macrophages in BALF, lung tissue VCAM-1 protein levels, and THS. Total protein levels and cytokines in BALF, and wet/dry weight ratios were increased in the LPS group, but rhAPC pretreatment did not significantly alter the LPS-induced responses. In conclusion, in this murine septic model of lung injury, inhaled rhAPC appears to attenuate lung inflammation, without reversing the observed increases in lung permeability and BALF cytokines. This effect may be associated with leukocyte trafficking modifications, related, at least in part, to VCAM-1 reduction.

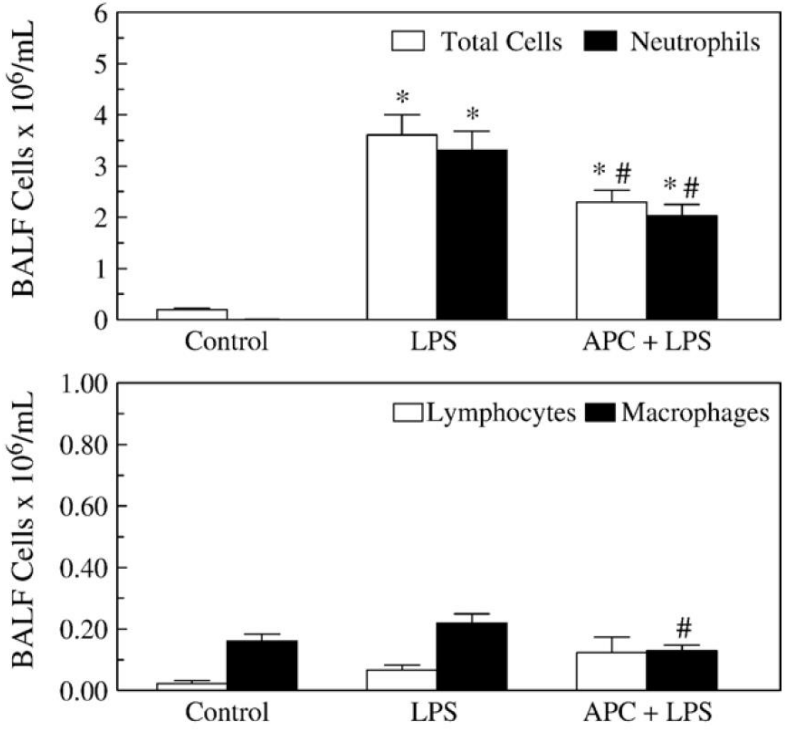
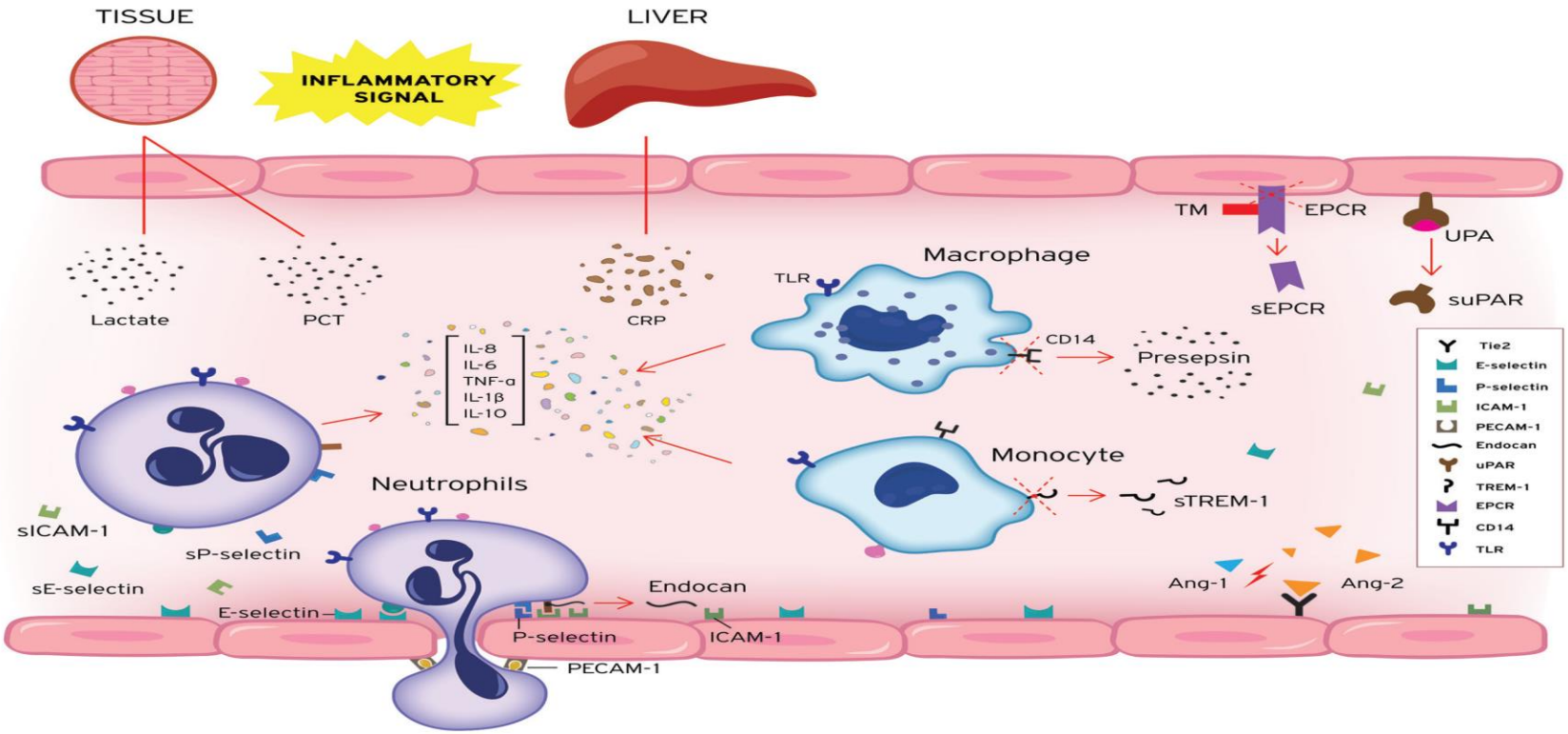


Fig. 1. Total cell and neutrophil counts (top), along with lymphocyte and macrophage counts (bottom) in bronchoalveolar lavage fluid (BALF) from control animals (*Control*, $n=8$) and animals treated with either endotoxin (*LPS*; $n=14$), or recombinant human activated protein C plus endotoxin (*APC+LPS*; $n=14$). Data are means±SEM. *: $p<0.05$ by ANOVA and Newman–Keuls test from the *control group*; #: $p<0.05$ by ANOVA and Newman–Keuls test from the *LPS group*.



Clinical Assays in Sepsis: Prognosis, Diagnosis, Outcomes, and the Genetic Basis of Sepsis
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Table 1 lists the role of major circulating biomarkers and genetic polymorphisms in the prognosis and diagnosis of sepsis.

Biomarker	Diagnostic significance	Prognostic significance
C-reactive protein (CRP)	- Discriminates bacterial and viral infections [14]	- CRP is a valuable marker for the disease severity [15, 20]
	- Measurement of CRP is an indicator of sepsis [15–19]	- Elevated concentrations of serum CRP on admission have been associated with increased risk of organ failure and mortality [21, 22]
Procalcitonin (PCT)	- PCT is important in the detection and differential diagnosis of inflammatory states [26]. The highest levels of PCT are achieved in acute bacterial infections and sepsis	- PCT nonclearance is a prognostic factor of death in patients with sepsis [33]
	- PCT is a good biological diagnostic marker for sepsis, severe sepsis, or septic shock [34]	- Significant difference between PCT levels as early as day 1 between survivors and nonsurvivors among septic patients [37]
	- PCT is a helpful biomarker for early diagnosis of sepsis in critically ill patients [36]	
	- Serial PCT concentrations may have value in monitoring sepsis outcomes [28, 29]	
Tumor necrosis factor-α (TNF-α)	- Levels of TNF-α are frequently increased in sepsis [49, 56]	- High concentrations of TNF-α are predictive of organ failure and increased mortality in septic patients [55]
Interleukin-1β (IL-1β)	- Levels of IL-1β are frequently increased in sepsis [56]	
Interleukin-6 (IL-6)	- Levels of IL-6 are frequently increased in sepsis [50, 54, 56]	- High concentrations of IL-6 are predictive of organ failure and increased mortality in septic patients [57, 59]
	- IL-6 levels are increased in patients with infectious complications and have been used to differentiate systemic inflammatory response syndrome (SIRS) from sepsis [58]	

Biomarker	Diagnostic significance	Prognostic significance
Interleukin-8 (IL-8)	- Levels of IL-8 are frequently increased in sepsis [50, 56]	- IL-8 has been used to predict the severity of sepsis in pediatric patients, although the use of IL-8 has not been confirmed in adults [60, 61]
Interleukin-27 (IL-27)	- Useful biomarker in estimating risk of bacterial infection among critically ill pediatric and adult patients [67–70]	
	- In combination with PCT, IL-27 may improve classification of critically ill adults with sepsis [68, 71]	
Interleukin-10 (IL-10)		- Poor patient outcome has been associated with increased blood levels of the anti-inflammatory cytokine IL-10 [74]
Angiopoietin-1 (Ang-1)	- Decreased levels in critically ill septic or nonseptic patients compared to healthy controls [94]	- Decreased levels of Ang-1 at ICU admission are correlated with higher mortality [97]
Angiopoietin-2 (Ang-2)	- Ang-2 levels are higher in patients with severe sepsis compared to patients with or without SIRS or sepsis [89–91]	- Increased Ang-2 plasma levels have been associated with worst clinical outcome in patients with major trauma and severe sepsis or shock [92–94]
		- Increased Ang-2 plasma in nonsurvivors compared to survivors [95, 96]
Selectins	- Soluble E-selectin concentration increases in various inflammatory pathologies [99–102]	- Higher sE-selectin levels in nonsurvivors than survivors [103, 104]
		- sE-selectin levels may be used as predictor of fatal outcome in patients with SIRS [105]
		- sE-selectin has also been proposed as a predictor of bacteremia in severe sepsis patients [106]
		High levels of sE- and sP-selectin at ICU admission, are associated with sepsis development in time [109]
Soluble in tercellular adhesion molecule-1 (sICAM-1)	-s ICAM-1 production has been shown to be related to increased sepsis severity [102, 111]	- sICAM-1 concentration has been shown to be related to increased mortality [111, 112]
	- sICAM-1 appears to be a reliable biomarker for classifying patients with infectious SIRS, i.e., sepsis, from those with noninfectious SIRS [105]	
Soluble platelet/endothelial cell adhesion molecule-1 (sPECAM-1)	- sPECAM-1 is higher at admission in septic patients compared with nonseptic patients and healthy controls [114, 115]	
Endocan	- Exhibits higher concentrations in septic patients compared to healthy volunteers [117]	- A strong and significant predictor of sepsis severity and outcome [118–122]

Biomarker	Diagnostic significance	Prognostic significance
Soluble urokinase-type plasminogen activator receptor (suPAR)	- Elevated suPAR in conditions of SIRS, bacteremia, sepsis, and septic shock [124–127]	- High circulating suPAR levels indicate an unfavorable prognosis, including organ dysfunction and mortality [124–127]
	- Diagnostic value of suPAR for identifying sepsis is limited [128]	- In patients with bacteremia, suPAR may be used to identify severely ill patients and predict mortality [131, 133]
Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1)	- Moderate diagnostic performance in differentiating sepsis from SIRS [140]	- Moderate prognostic significance in assessing the mortality of infection in adult patients and sTREM-1 alone is insufficient to predict mortality as a biomarker [142]
	- sTREM-1 represents a reliable biological marker of bacterial infection [143]	
Soluble endothelial protein C receptor (sEPCR)	- sEPCR levels in septic patients have been found to be significantly higher [146, 147], unchanged [148], or lower [149] than in healthy volunteers	- sEPCR levels correlated with worst outcomes; has been suggested that it may act as a biological marker of outcome in severe sepsis [150–152]
		- Levels of sEPCR at ICU admission are higher in originally nonseptic patients who subsequently become septic compared to those who will not [152]
Presepsin	- Discriminates between bacterial and nonbacterial infections [155]	- Initial values significantly correlated with in-hospital mortality of patients affected by sepsis, severe sepsis, or septic shock [156]
	- Early diagnosis of infection in a population of patients with SIRS, sepsis, severe sepsis, and septic shock [153]	- Presepsin reveals prognostic value with respect to 30 days and 6 months all-cause mortality throughout the first week of ICU treatment [153]
	- In patients with suspected severe sepsis and septic shock, presepsin reveals valuable diagnostic capacity to differentiate sepsis severity [156]	- Presepsin measured on the first day in ICU in patients with severe sepsis or septic shock was higher in nonsurvivors compared to survivors [157]
	- Presepsin can differentiate between septic and nonseptic patients with comparable accuracy to CRP and PCT [161, 162]	
Lactate	- Elevated serum lactate levels in sepsis [177, 178]	- Elevated serum lactate levels are associated with poor outcomes in diverse populations of critically ill patients, such as multiple organ failure, morbidity, and mortality [172–175]
	- Early serum lactate levels can diagnose sepsis in undifferentiated patients with suspected sepsis [176, 179]	- Serum lactate is a potentially useful biomarker to risk-stratify patients with severe sepsis presenting to the emergency department [170, 174, 175]

in vivo 29: 305-308 (2015)

Does Serum Lactate Combined with Soluble Endothelial Selectins at ICU Admission Predict Sepsis Development?

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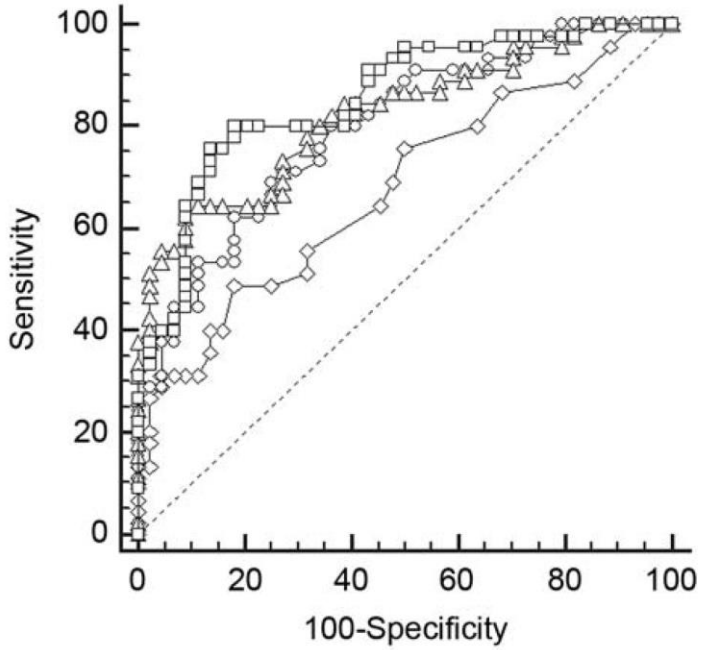

Figure 1. *Receiver operating characteristic (ROC) curve analysis. ROC curves were generated to determine the prognostic accuracy of either lactate alone or lactate combined with sE- and/or sP-selectin; the corresponding areas under the curve (AUC) and 95% confidence intervals (CI) were estimated as follows: lactate at 0.677 (0.566-0.788, p= 0.0018; open diamond); lactate+sE-selectin at 0.795 (0.704-0.886, p<0.001; open circle), lactate+sP-selectin at 0.823 (0.737-0.909, p<0.001; open triangle) and lactate+sE-selectin+sP-selectin at 0.854 (0.775-0.932, p<0.001; open box). Levels of all molecules were estimated on the day of ICU admission in initially non-septic patients.*

Genetic polymorphisms	- Distinguish patients with sepsis from patients with sterile inflammation	- Predict long-term outcomes and identify patients who will be at risk for developing adverse clinical outcomes
Toll-like receptors (TLRs)		- D299G allele of *TLR4* gene associated with increased susceptibility to severe bacterial infections and Gram-negative sepsis [192, 193]
IL-8		- The 251A/T allele has been associated with survival [194], protection against sepsis [195], and also increased risk of sepsis [196]
		- The heterozygote AT genotype has been associated with increased risk of developing sepsis [197]
		- Male T allele carriers are more susceptible to sepsis [198]
TNF-α	- G-to-A polymorphism associated with sepsis [201]	- G-to-A polymorphism associated with adverse outcomes in patients with severe sepsis and septic shock [199, 200]
	- No association of G-to-A polymorphism and development of sepsis [202, 203]	- G-to-A polymorphism not associated with sepsis mortality [201]
IL-6	- 174 G/C polymorphism showed modest association with neonatal sepsis [209]	- 174 G/C polymorphism was associated with improved survival rates in patients with sepsis [207]
		- 174 G/C polymorphism was not associated with a difference in survival [208]
Angiotensin-converting enzyme (*ACE*)		- Insertion/deletion (I/D) polymorphism does not have an effect on the incidence or outcome of sepsis in ventilated low birth infants [210, 211]
		- Carriers of the I allele at increased sepsis risk [212–214]
		- I polymorphism not associated with outcome in critically ill septic patients [214–216]
		- The presence of the D allele is associated with ARDS in patients with severe sepsis [217]

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Biomarker	Diagnostic significance	Prognostic significance
Endothelial protein C receptor (*EPCR*)	- The rare 23-bp insertion is significantly more common among patients with severe sepsis [218]	- Influences the risk of severe sepsis in children and adults [218, 219] - Simultaneous carriers of minor alleles belonging to both the H1 and H3 haplotypes may be at reduced risk of developing severe sepsis and/or septic shock among critically ill patients [219]

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ORIGINAL

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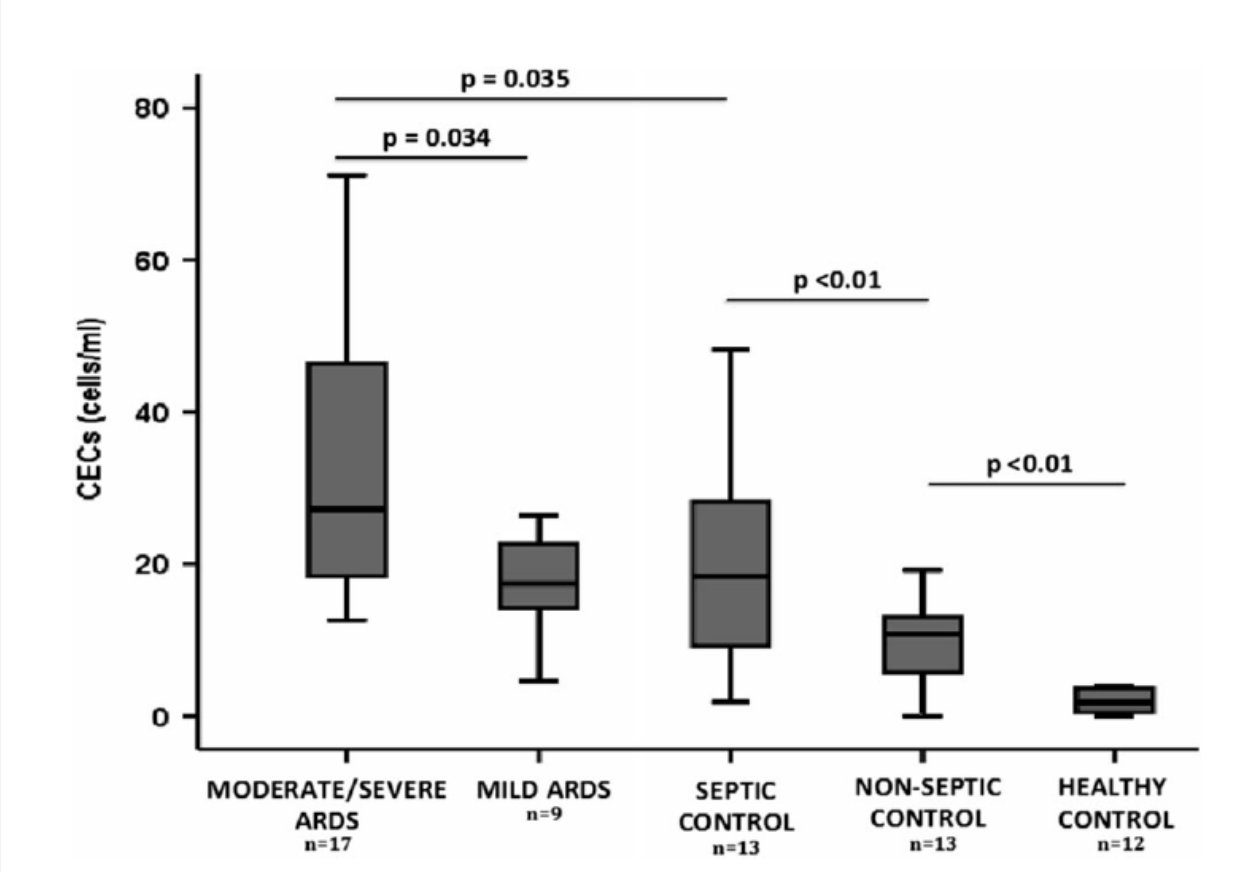
Evaluation of endothelial damage in sepsis-related ARDS using circulating endothelial cells

Circulating endothelial cells (CECs) are markers of endothelial damage that can be easily enumerated in peripheral blood using standardized methodology [15,



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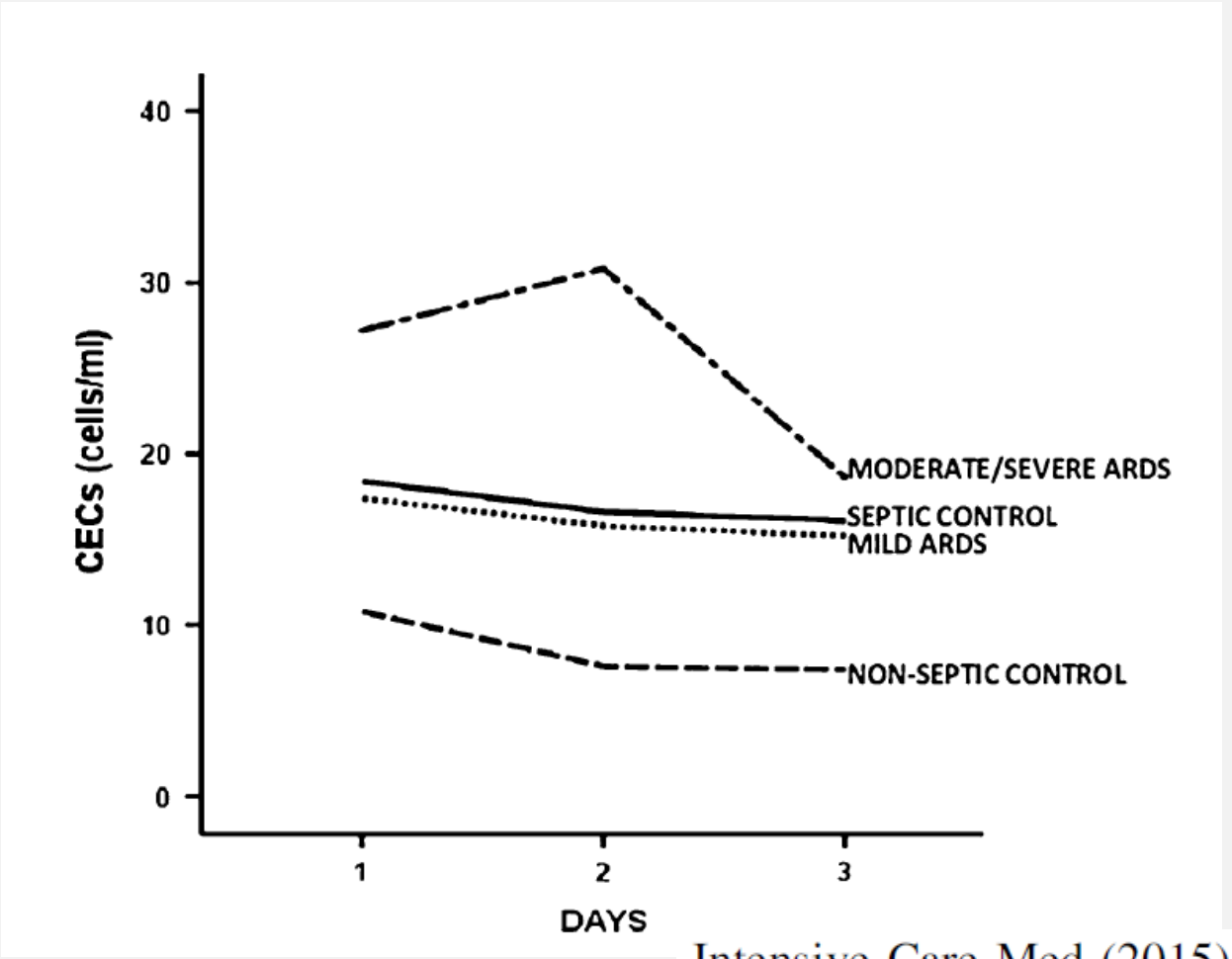

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Summary Points

- Early treatment of sepsis is associated with improved outcomes so that rapid diagnosis is important.
- The diagnosis of sepsis in critically ill patients is challenging, because it can be complicated by the presence of inflammation as a result of other underlying disease processes and prior use of antibiotics making cultures negative.
- Culture-dependent diagnosis of infection is slow, and biomarkers may provide a more rapid means of ruling in or out infection.
- Given the complexities of the sepsis response, no one biomarker will be sufficient to diagnose sepsis. Combinations of biomarkers are needed, and new technology is helping to speed the development of such panels.
- However, such tools cannot be used alone, and they must be seen as complementary to a careful clinical assessment and other laboratory signs.

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In Conclusion

- Sepsis is the result of a generalized not controlled inflammatory process
- Sepsis is still a major universal problem with severe health and economical implications
- Sepsis in the lung is associated with a spectrum of less to very severe pathologies - pneumonia AND ARDS)
- Treatment modalities focusing on the inflammatory pathways may change the disease course to the patients’ benefit