

Βιοδείκτες ταξινόμησης στη Σήψη

George Dimopoulos MD, PhD, FCCP, FECMM

Prof Critical Care Medicine

Critical Care Department, University Hospital ATTIKON

Medical School, University of Athens, Greece

gdimop@med.uoa.gr



Biomarkers to be discussed in this lecture

- PCT : Procalcitonin
 - Pro-ADM : Proadrenomedullin
 - Neopterin
 - Presepsin
 - sTREM-1 : soluble Triggering Receptor expressed on Myeloid cells-1
 - sUPAR : soluble urokinase Plasminogen Activator Receptor
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PCT in bacterial infections

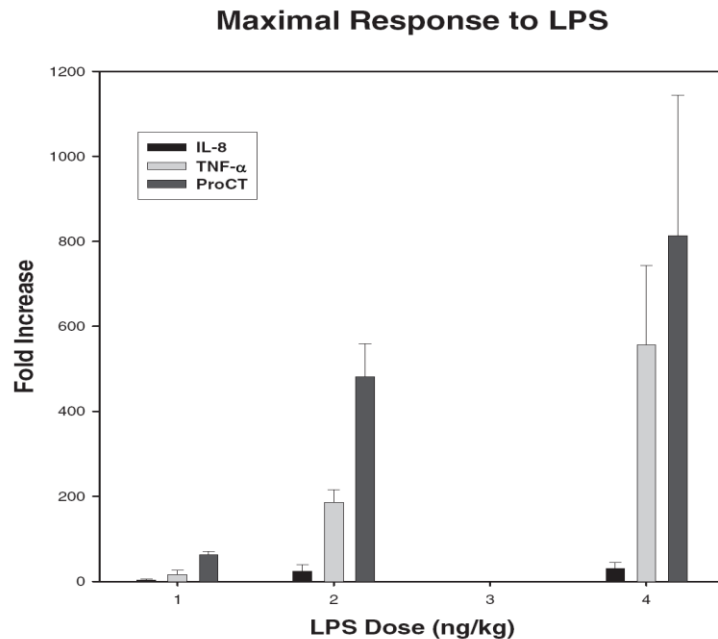


Figure 1. Peak (*fold*) increase of interleukin (*IL*)-8, tumor necrosis factor (*TNF*)- α , and procalcitonin (*ProCT*) in four healthy volunteers after increasing doses of endotoxin (lipopolysaccharide [*LPS*]; 1, 2, and 4 ng/kg). *IL*-8 reached peak levels at 4 hrs; *TNF*- α peaked at 1.5 hrs, and *ProCT* peaked at 24 hrs (unpublished data from Suffredini et al (164)).

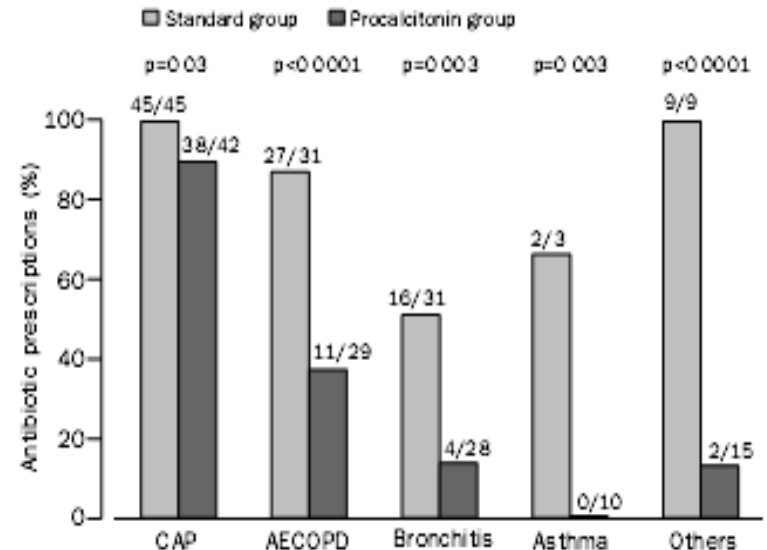
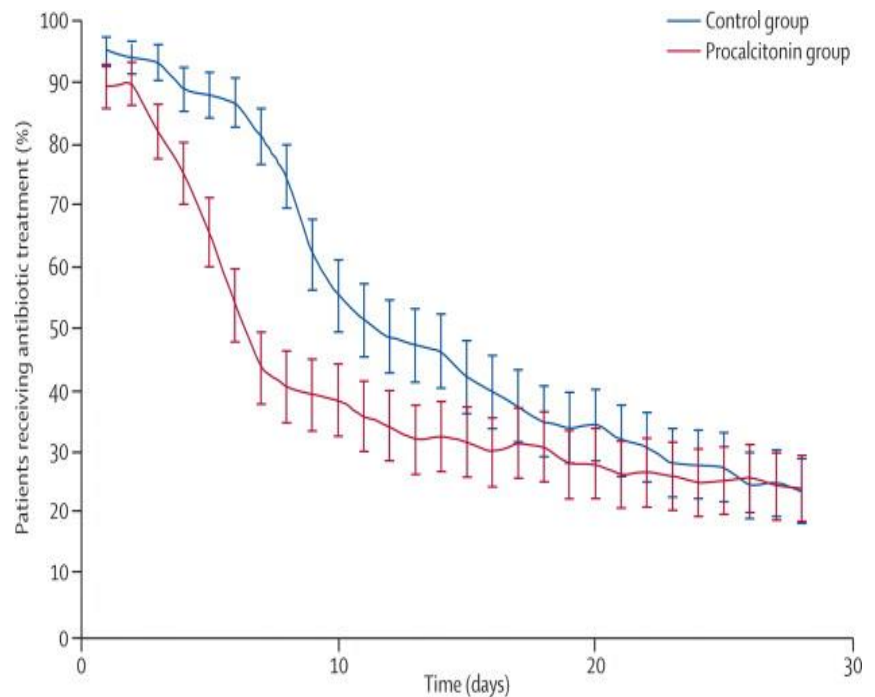
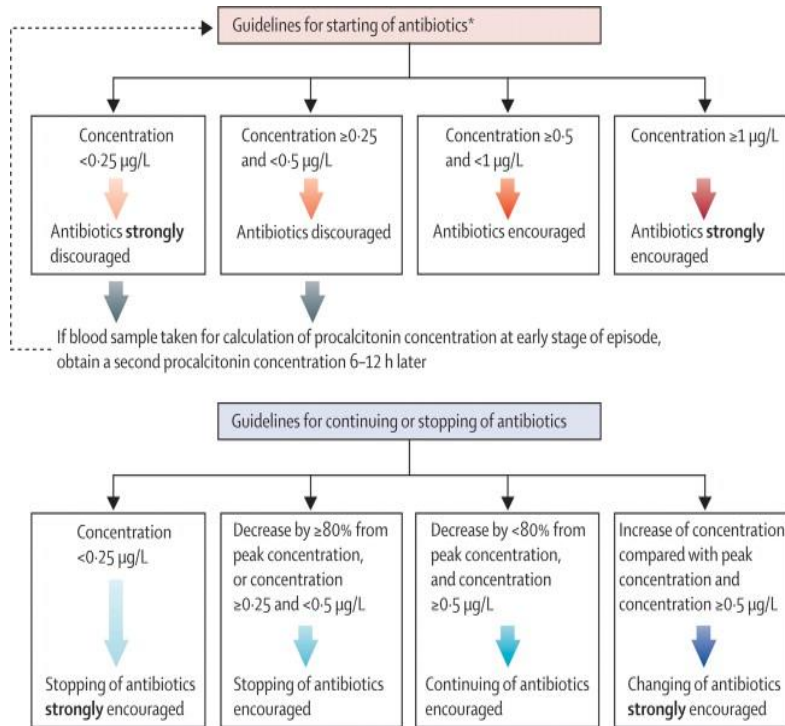


Figure 2: Antibiotic prescriptions in different subgroups of lower respiratory tract infection comparing standard group and procalcitonin group

CAP=community-acquired pneumonia. AECOPD=acute exacerbations of COPD.

- Factors inducing PCT production
 - exotoxins, *TNF*- α , and other cytokines

The Prorata trial



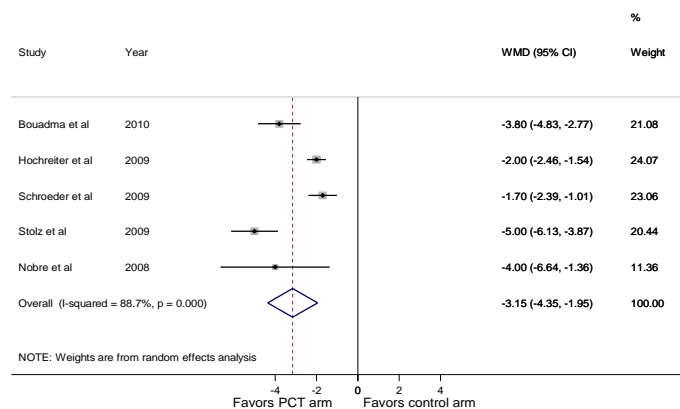
Patients receiving antibiotics for days 1–28

Significantly fewer patients assigned to the procalcitonin group received antibiotics than did those assigned to the control group ($p < 0.0001$, generalised linear model test for repeated measures).

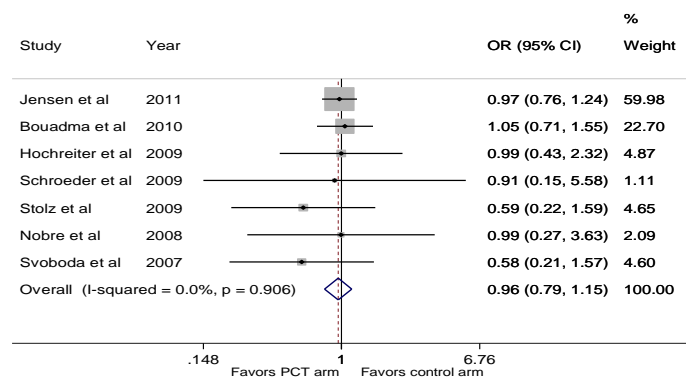
An ESICM systematic review and meta-analysis of procalcitonin-guided antibiotic therapy algorithms in adult critically-ill patients

- 7 RCTs
- ↓ in the duration of first episode of antibiotic treatment
- No difference in 28-day mortality
- ↑ in antibiotic free days within the first 28 days of hospitalization
- No difference between regarding the remaining outcomes
- Sensitivity analyses yielded similar results

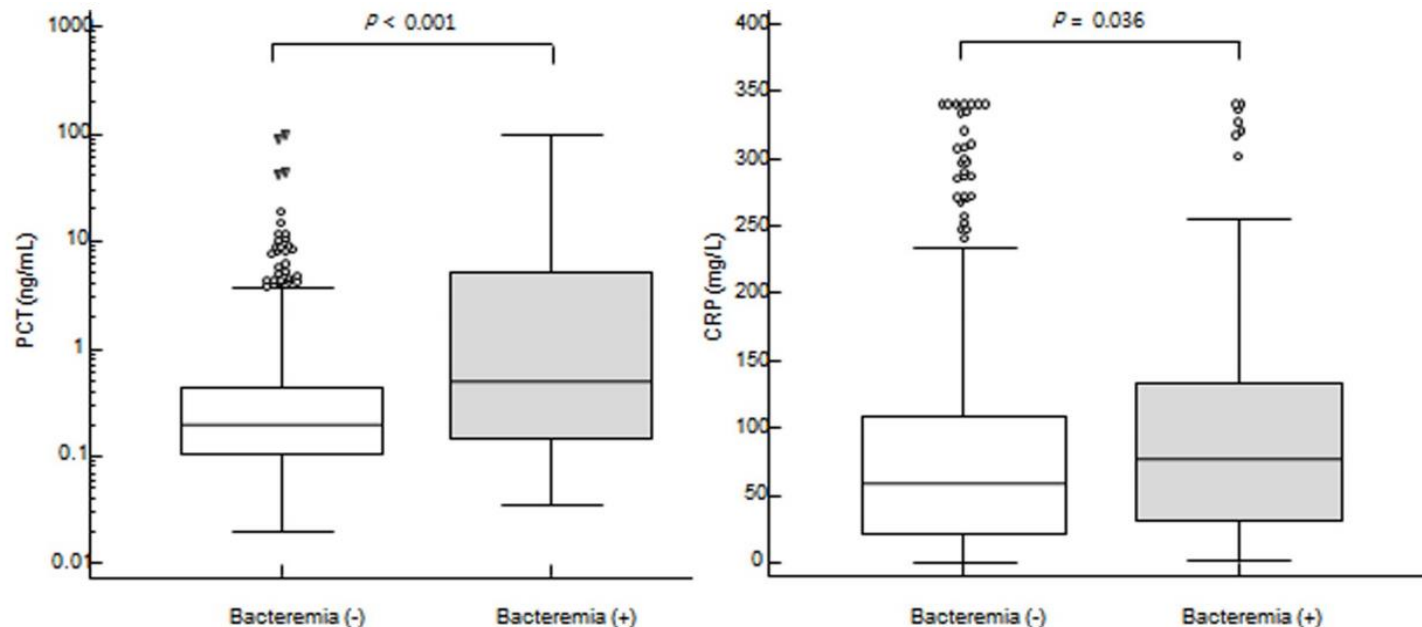
Weighted mean difference of duration of first episode of antibiotic treatment.



Odds ratios of 28-day mortality

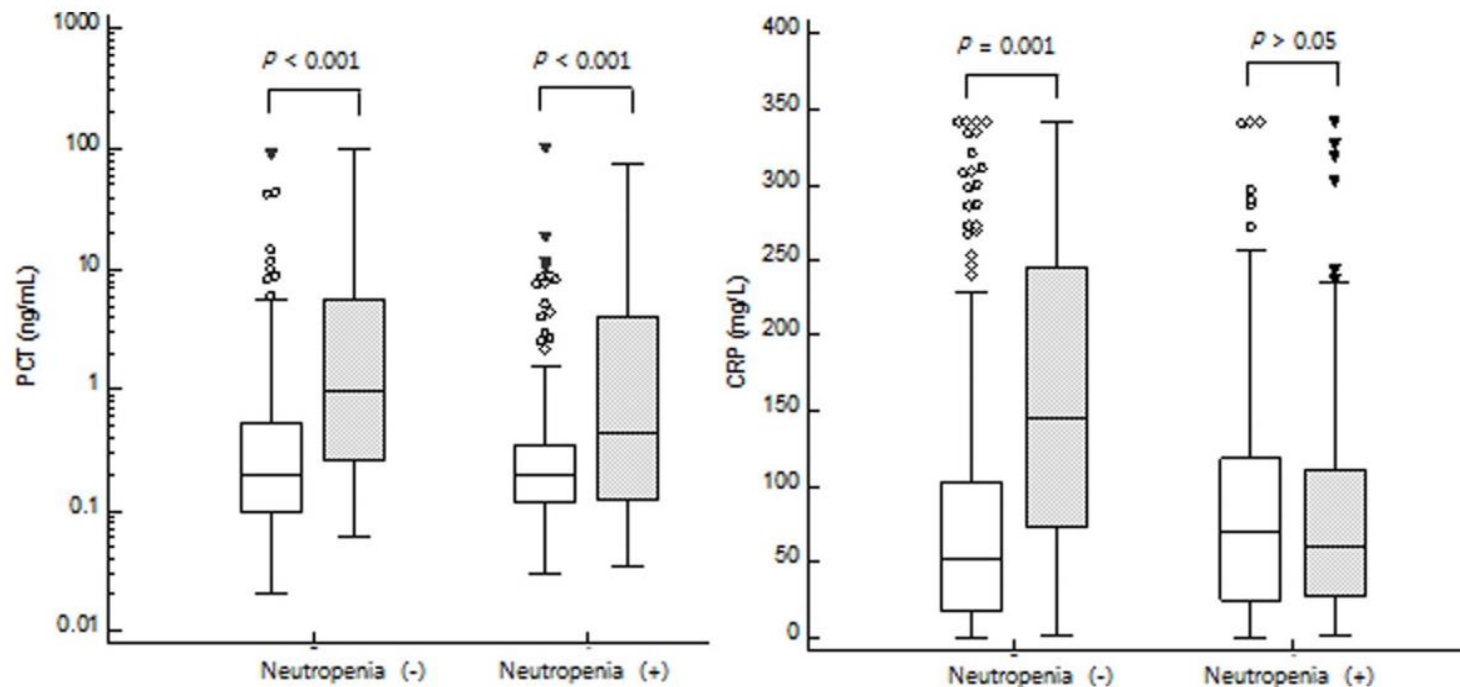


Serum PCT in hematologic malignancies



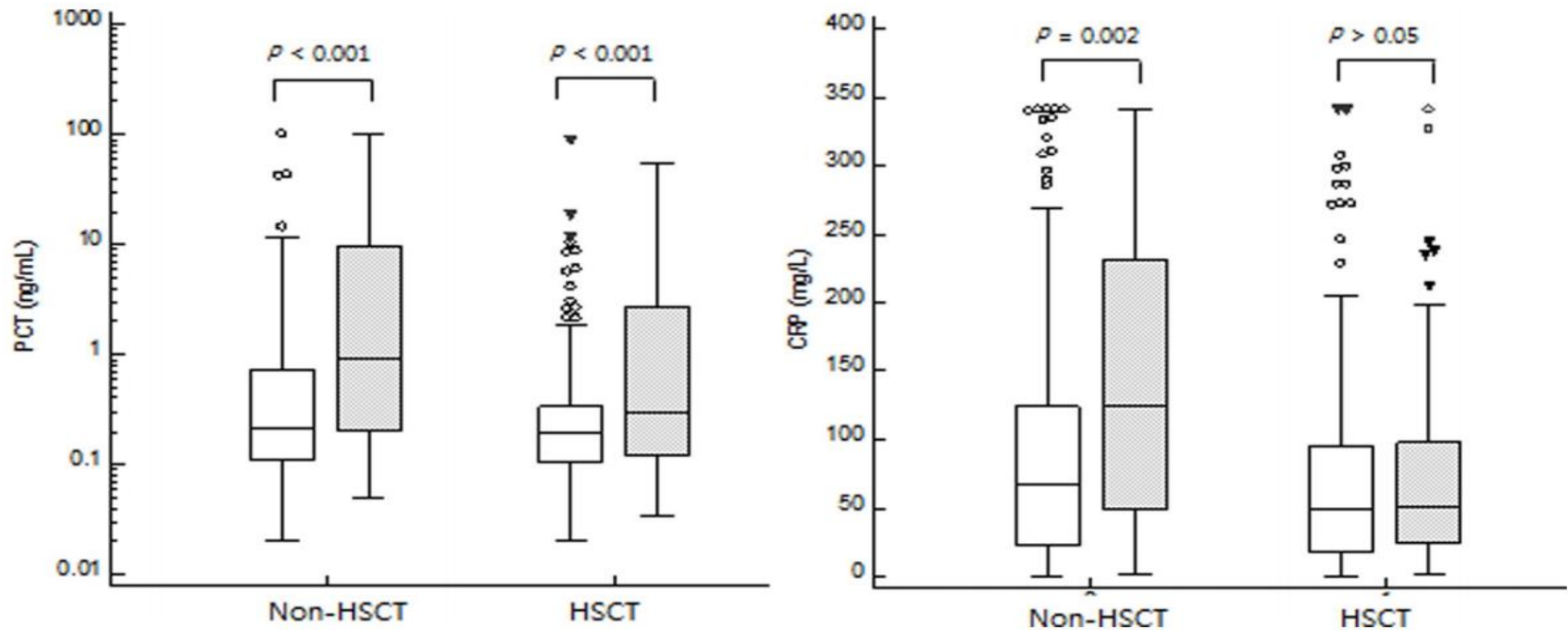
Ninety-nine systemic bacterial infection episodes (colored box) showed higher PCT and CRP levels than nonbacterial events [median (95% CI) (PCT: 0.49 (0.26–0.93) ng/mL vs. 0.20 (0.18–0.22) ng/mL, $P < 0.001$; CRP: 76.6 (50.5–92.8) mg/L vs. 58.0 (51.1–66.5) mg/L, $P = 0.036$)] by Mann-Whitney U test.

Serum PCT in hematologic malignancies



- PCT levels discriminated bacteremia (gray box) from non-bacteremia (white box) in neutropenia (-)
- CRP levels discriminated bacteremia (gray box) from non-bacteremia (white box) in neutropenia (-)
- CRP levels were not different between bacteremia and non-bacteremia in neutropenia (+) patients

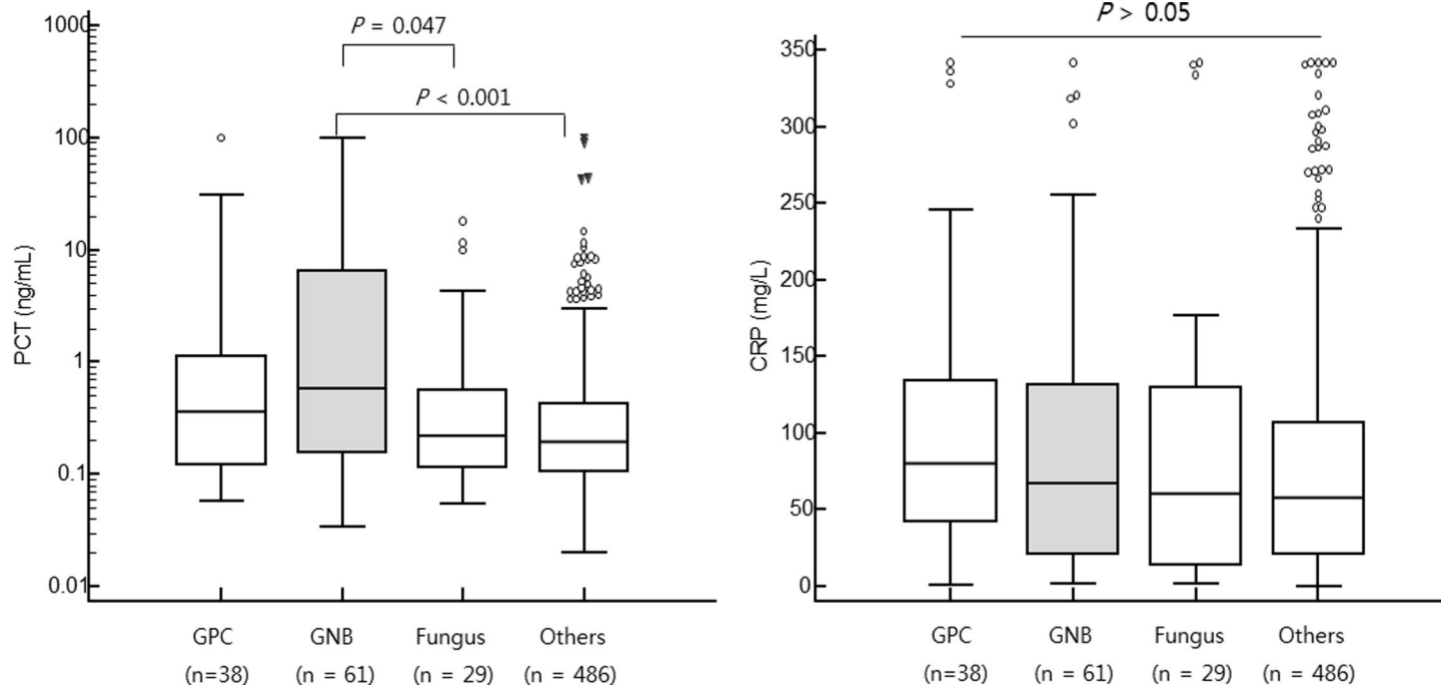
PCT and CRP levels in HSCT and non-HSCT



- PCT levels discriminated bacteremia from non-bacteremia in non-HSCT patient and HSCT patients
- CRP levels discriminated bacteremia from non-bacteremia in non-HSCT patient
- CRP levels were not different between bacteremia and non-bacteremia in HSCT patients

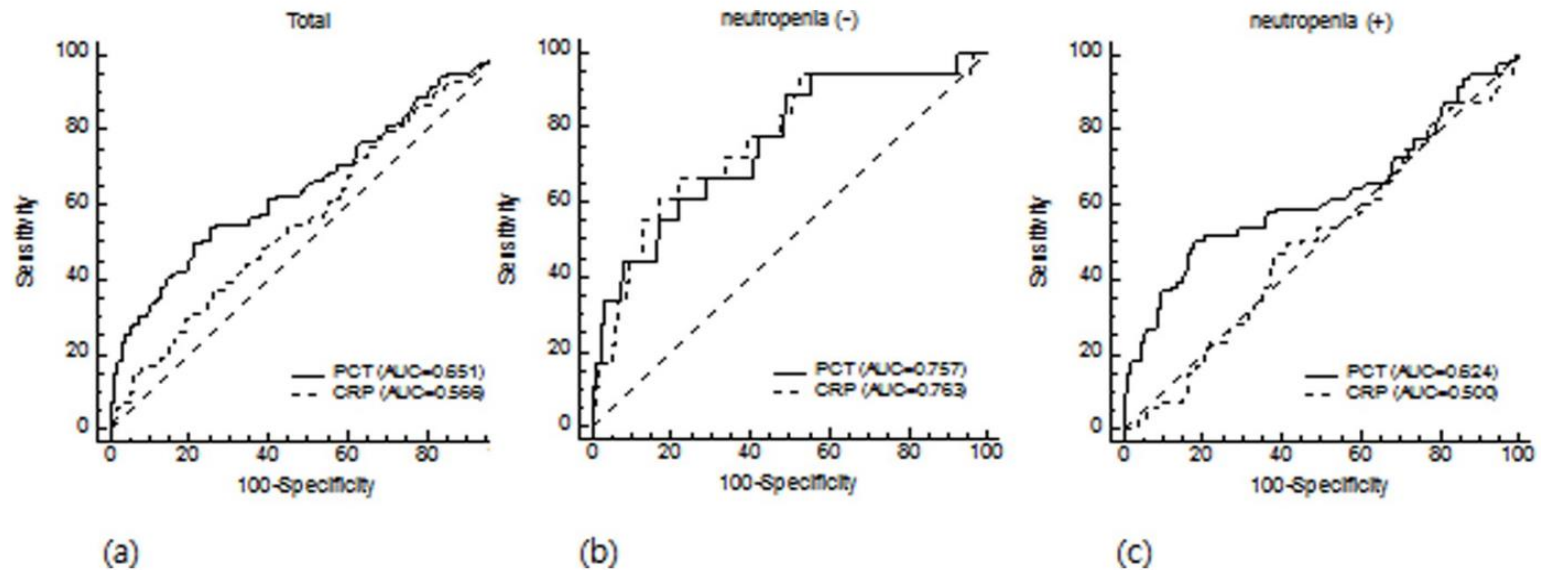
HSCT : hematopoietic stem cell transplantation

PCT and CRP : different etiologies of fever.



- PCT levels were significantly higher in GNB infectious episodes than those in febrile episodes caused by fungal infection or other etiology
- CRP levels were not significantly different

PCT and CRP : detection of bacteremia



- ROC curves of PCT and CRP for detecting bacteremia
- 614 febrile episodes : total
- 341 non-neutropenic and 273 febrile neutropenia episodes
- PCT discriminated bacteremia from non-bacterial infection
- CRP could not detect bacteremia

Procalcitonin key points

Procalcitonin key points

- PCT is useful in distinguishing bacterial from viral pneumonia.
- PCT greater than 0.5 ng/mL is considered to indicate high probability of bacterial infection, which requires antibiotic treatment.
- PCT increases along with increasing severity of CAP.
- PCT is a good predictor of mortality.
- PCT-guided therapy in patients with respiratory tract infections may reduce antibiotic exposure and cost of care without an increase in mortality and treatment failure.

PCT, procalcitonin; CAP, community-acquired pneumonia.

Adrenomedullin (ADM)

Proadrenomedullin (Pro-ADM)

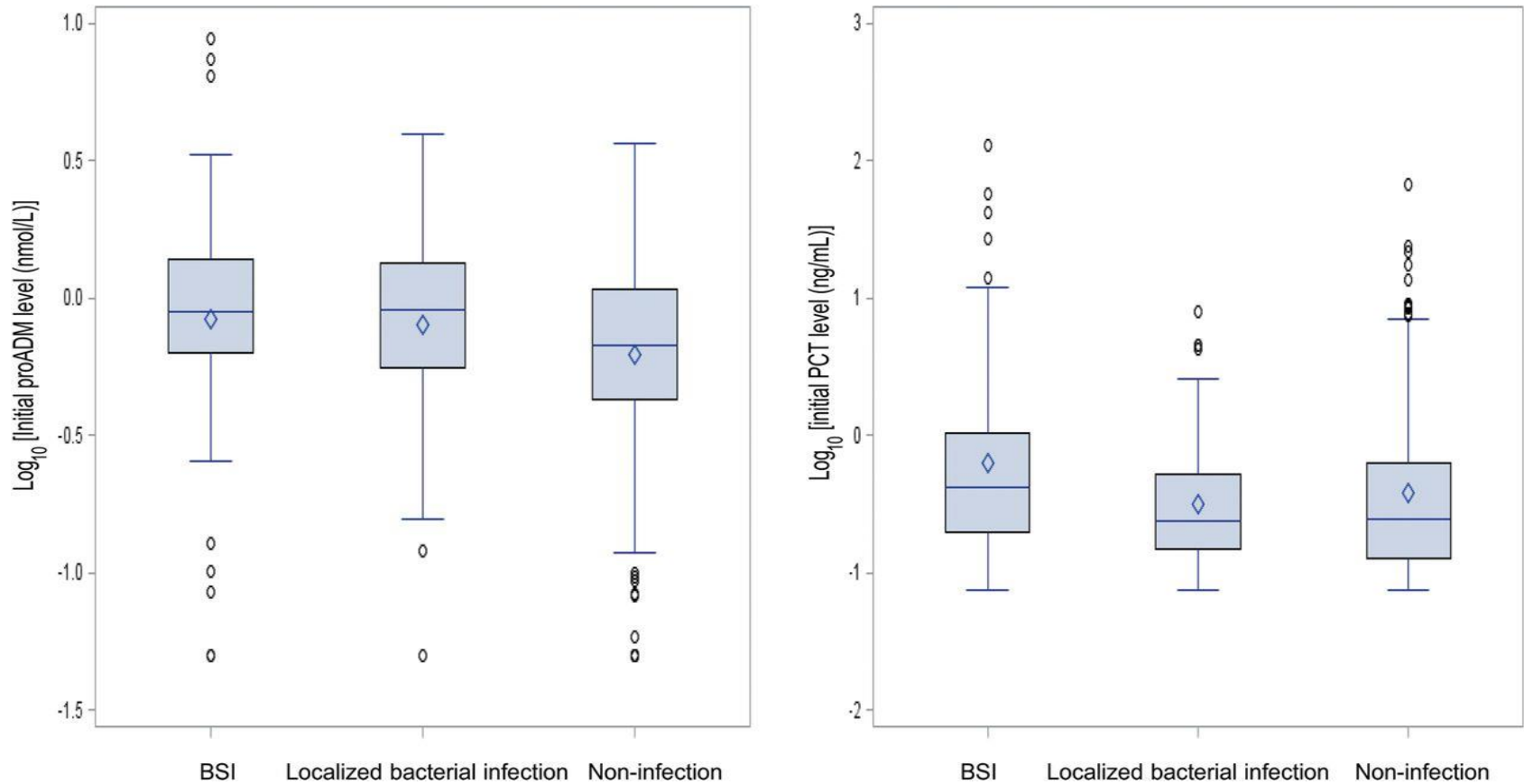
ADM

- ❑ Belongs to calcitonin peptide family
- ❑ Strong endogenous vasodilator
- ❑ Numerous effects on various organs
- ❑ A role in cell growth /apoptosis and in sepsis
- ❑ Bactericidal properties
- ❑ Highly upregulated during the hyperdynamic phase of sepsis and down-regulates the inflammatory response

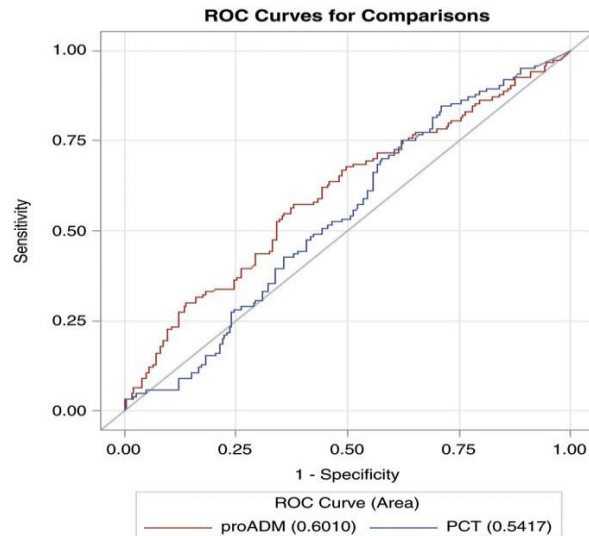
Pro-ADM

- ❑ A peptide generated from ADM precursor along with ADM and other molecules
 - ❑ More stable than ADM, metabolically inactive and is produced indirectly correlated amounts with ADM.
 - ❑ Thus, it is preferred to be measured in septic patients instead of ADM
 - ❑ Rapid clearance from the circulation
 - ❑ Mean plasma concentration in healthy individuals varies from 0.33 to 0.46 nmol/L
-

Initial Pro-ADM and PCT in definite sepsis, with and without infections



ROC curve and AUC comparison: All bacterial infections (bloodstream + localized) vs noninfection.



ROC Association Statistics							
ROC Model	Mann-Whitney				Somers' D (Gini)	Gamma	Tau-a
	Area	Standard Error	95% Wald Confidence Limits				
proADM	0.6010	0.0332	0.5359	0.6660	0.2019	0.2020	0.0971
PCT	0.5417	0.0328	0.4774	0.6060	0.0834	0.0837	0.0401

ROC Contrast Estimation and Testing Results by Row						
Contrast	Estimate	Standard Error	95% Wald Confidence Limits		Chi-Square	Pr > ChiSq
proADM - PCT	0.0592	0.0388	-0.0168	0.1352	2.3327	0.1267

Currently, there are no studies focusing on optimizing treatment duration with pro-ADM-based algorithms.

Its importance lies in using pro-ADM as a diagnostic and prognostic tool

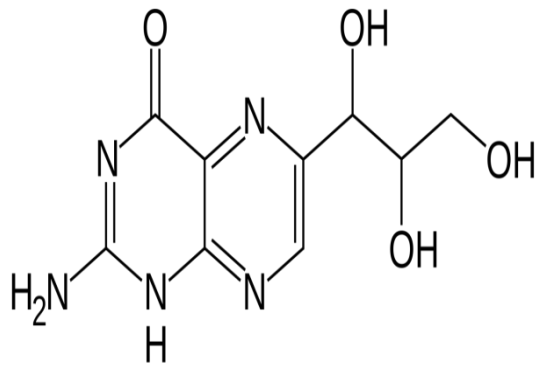
Proadrenomedullin key points

Proadrenomedullin key points

- Pro-ADM levels increase with increasing severity of CAP.
- Pro-ADM cannot be used for the discrimination of CAP etiology.
- Pro-ADM is inhibited by steroid pretreatment in a dose-dependent manner.
- Pro-ADM is a better predictor of CAP and COPD severity than of mortality.

Pro-ADM, proadrenomedullin; CAP, community-acquired pneumonia.

Neopterin



A pyrazino-(2,3-d)-pyrimidine molecule

- chemical group of pteridines (aromatic pteridines)
- is synthesized almost exclusively in monocytes and macrophages although may be detectable in microglial cells of CNS

- Neopterin

- indirect marker of macrophage activation
- is regulated by INF- γ stimulation
- can be measured by ELISA, radioimmunoassay (RIA) and HPLC
- it may be detected in various body fluids
 - serum, urine, pleuritic fluid, cerebrospinal fluid, ascetic fluid, pancreatic juice, BAL and synovial fluid.

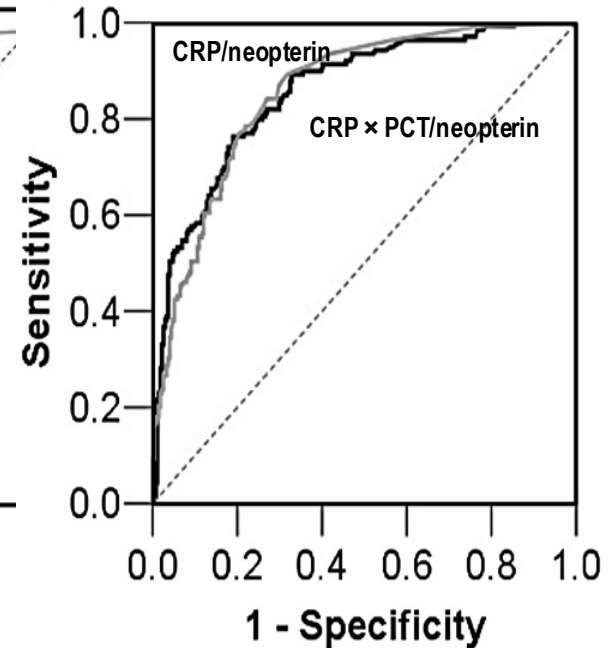
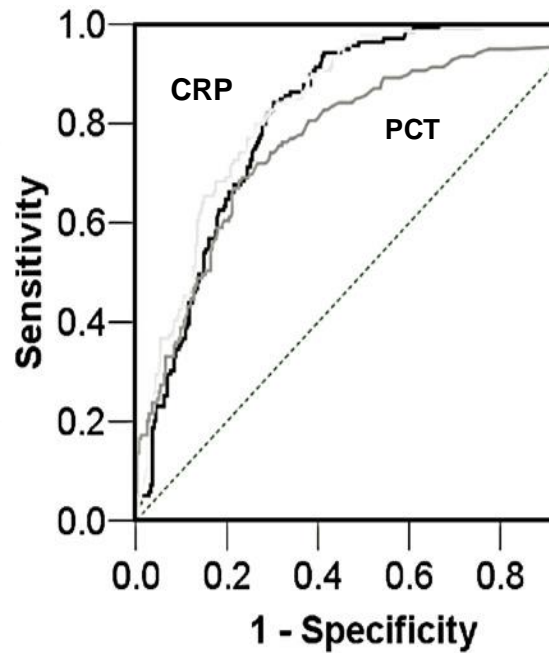
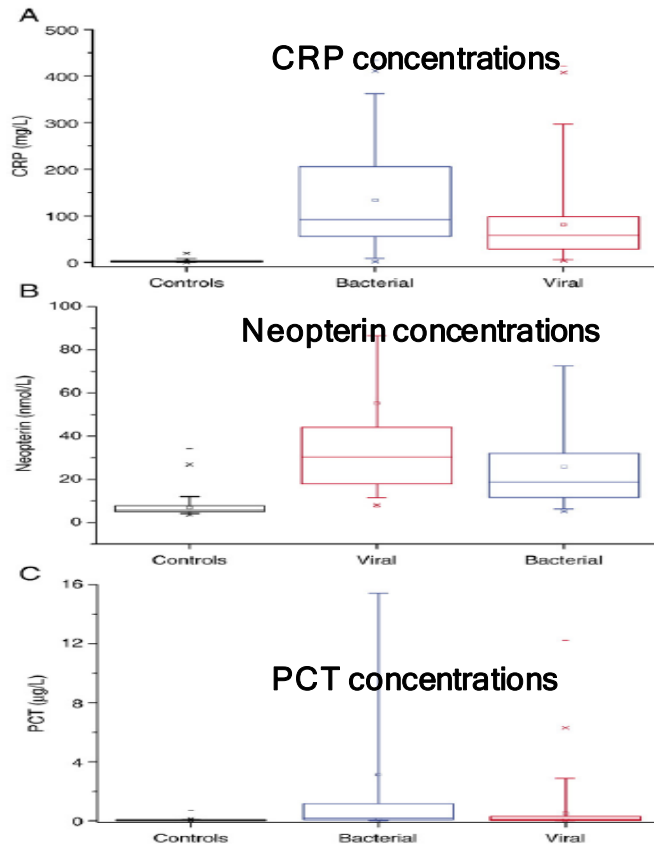
Neopterin

Neopterin levels in blood

- mean value : 6 nmol/L until adulthood
- lowers down to 5 nmol/L till the 8th decade of life and
- then rises sharply to a mean of 8.5 nmol/L

These variations are associated with renal function.

CRP, neopterin, and PCT in healthy controls, bacterial and viral LRTI.



Distinguishing bacterial from viral infections and healthy subjects

Monitoring the efficacy of treatment and compliance of patients with tuberculosis

Studies reporting neopterin concentrations in various body fluids of patients with tuberculosis

Study (Ref)	Serum Mean±SD (nmol/L)	Urine Mean±SD (μmol/mol creatinine)	Pleural Mean±SD (nmol/L)	BAL fluid Mean±SD (nmol/L)	Assay
Turgut ⁷⁶	69.5±29.4	NR	NR	NR	ELISA
Tozkoparan ⁷⁷	38.3±14.2	759.2±622.7	39±14.2	NR	HPLC
Immanuel ⁷⁹	39.9 (range 32.1–47.7)	NR	NR	NR	HPLC
Mohamed ⁸⁰	61.3±29.4	NR	NR	88.6±27.4	RIA
Yuksekol ⁷⁸	20.6±12.1	718.5±594.4	NR	33.3±18.6	HPLC
Baganha ⁸¹	41.3±25	NR	42±23	NR	RIA

SD, standard deviation; BAL, bronchoalveolar lavage; NR, not reported; HPLC, high-performance liquid chromatography; RIA, radioimmunoassay.

Neopterin

Neopterin key points

- Neopterin is of clinical value in conditions associated with cell-mediated immunity.
- Neopterin increases in infections caused by intracellular bacteria and viruses.
- Neopterin is useful in distinguishing between bacterial and viral etiology of LRTIs
- Neopterin is useful for monitoring the efficacy of treatment and the compliance of patients with tuberculosis.

LRTIs, lower respiratory tract infections.

Presepsin

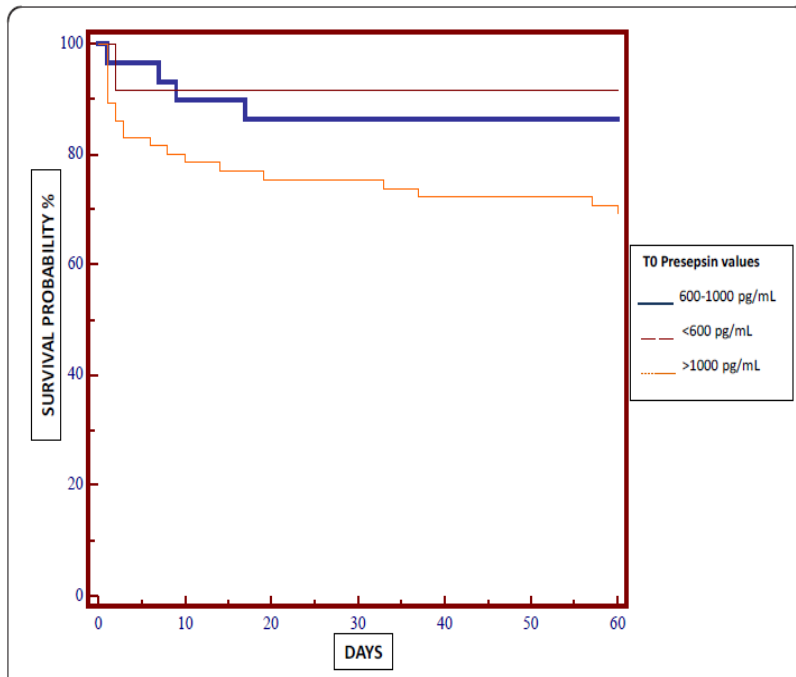
Presepsin (sCD14-ST)

Cluster of Differentiation 14 (CD14) is a gene encoding a protein taking part in the innate immune response

This protein is mostly expressed in macrophages and monocytes

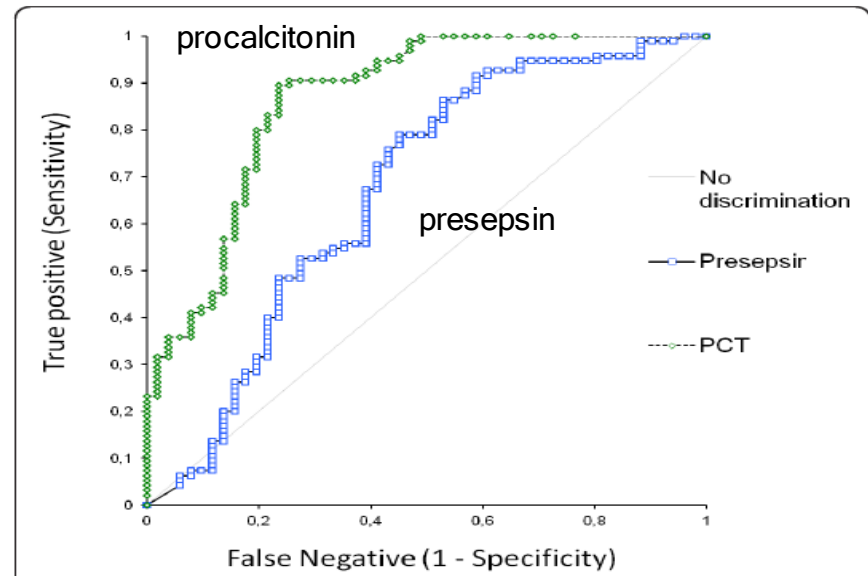
- mediate bacterial phagocytosis by inducing cytokine secretion
 - cytokine secretion takes place after the ligation of CD14 indirectly to LPS (with the help of LPS binding protein -LBP LPS-LBP binds to CD14 and to TLR-4 (stimulate the immune response))
- CD14 is integrated into the cellular membrane
- Also found in a soluble form
- CD14 in its soluble form is called presepsin
- in adults 3 ng/mL and 7 ng/mL in children

Presepsin : Diagnostic and prognostic value



Correlation between initial values (time 0) of presepsin and survival in patients with sepsis, severe sepsis and septic shock.

- 60th day in-hospital mortality
- higher in patients with initial values of presepsin >1,000 pg/ml than in groups with lower values (P = 0.04).



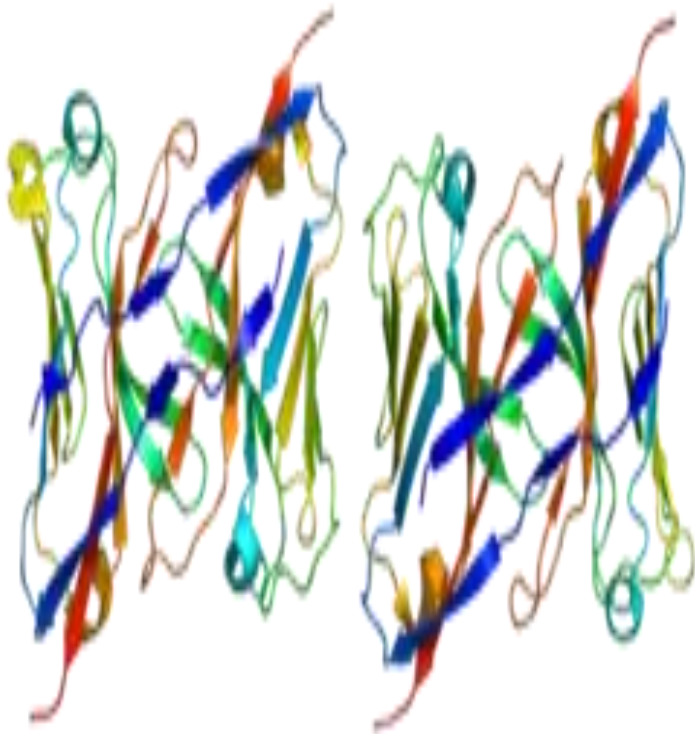
Patients with definitive diagnosis of sepsis, severe sepsis or septic shock.

Presepsin

Presepsin key points

- Presepsin is useful as a biomarker in the diagnosis of sepsis.
- Presepsin levels may have a prognostic value for sepsis.
- Presepsin levels in patients with Gram (-) sepsis are significantly higher at the day of diagnosis compared to other groups of patients.
- Presepsin may also increase in noninfectious conditions.

soluble Triggering Receptor expressed on Myeloid cells-1 (sTREM-1)



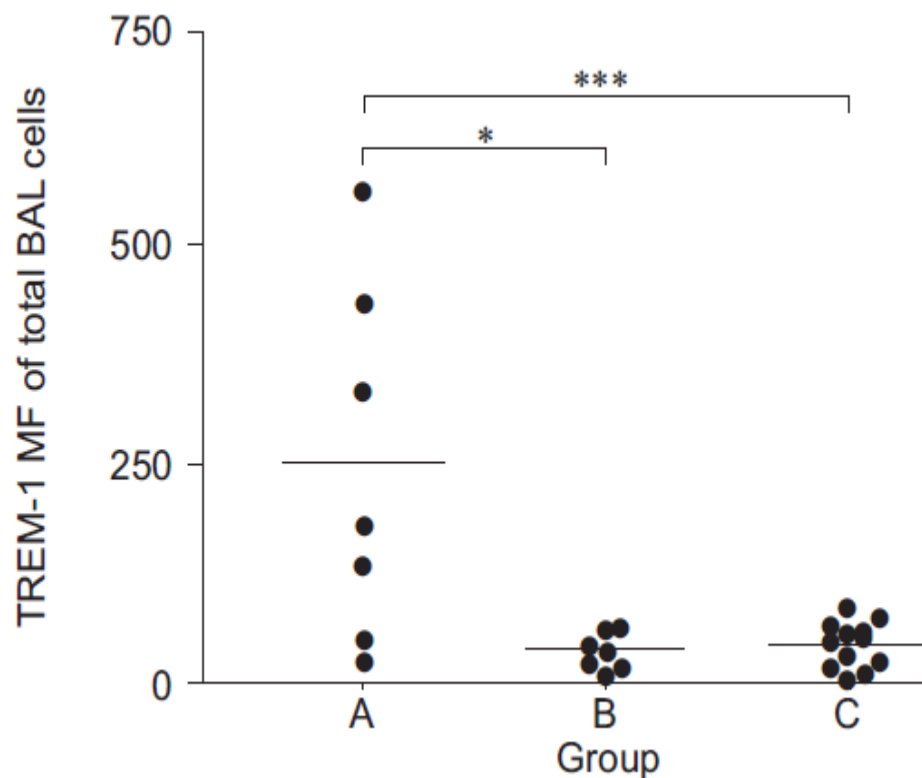
TREM-1 belongs to the immunoglobulin superfamily

Is expressed on the surface of immune cells, like neutrophils, monocytes and macrophages after exposure to infectious agents.

sTREM-1 is released from activated phagocytes and can be found in plasma, urine, cerebrospinal fluid, alveolar lining fluid and pleural fluid

It may serve as a more direct marker of infection than CRP and PCT.

sTREM-1 and respiratory infections

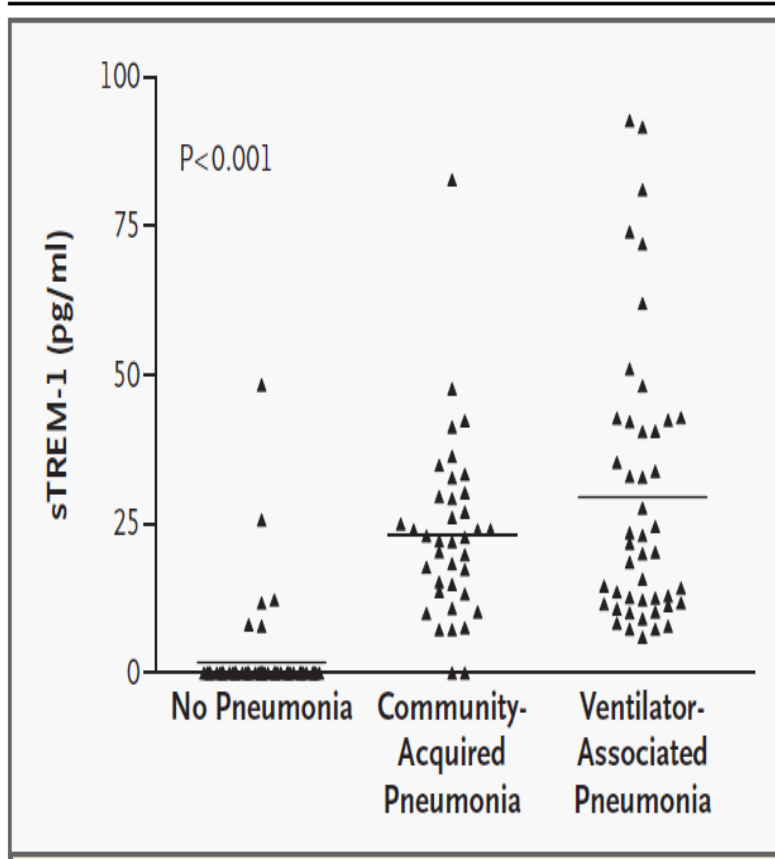


TREM-1 expression on total BAL cells is significantly higher in patients

- with CAP likely to be caused by extracellular bacteria (group A) than
- in patients with pulmonary tuberculosis (group B) or
- patients with noninfectious interstitial lung diseases (group C)

Data are presented as TREM-1 mean fluorescence (MF) of total BAL cells, subtracted from isotype control.

sTREM-1 in the diagnosis of pneumonia



- Levels of sTREM-1 in BAL from
- 64 pts without pneumonia
- 38 pts with CAP and
- 46 pts with VAP

sTREM-1

sTREM-1 key points

- sTREM-1 in combination with other markers may help in diagnosing sepsis.
- sTREM-1 kinetics in sepsis may have predictive value.
- Elevated baseline sTREM-1 levels may be a protective factor, while a progressive decline of plasma sTREM-1 concentration correlate with a favorable outcome in patients with sepsis.

sTREM-1, soluble form of TREM-1; TREM-1, triggering receptor expressed on myeloid cells-1.

What is uPAR ?

urokinase Plasminogen Activator Receptor

- ✓ a protein in blood (plasma)
 - 55 kD glycosylated protein
- ✓ measurable in all individuals
- ✓ stable *in vivo* and *in vitro*

expressed on



- vascular endothelial cells
- monocytes
- neutrophils and
- activated T-cells

involved in



- Migration
- Adhesion
- Angiogenesis
- Fibrinolysis
- Proliferation

and released

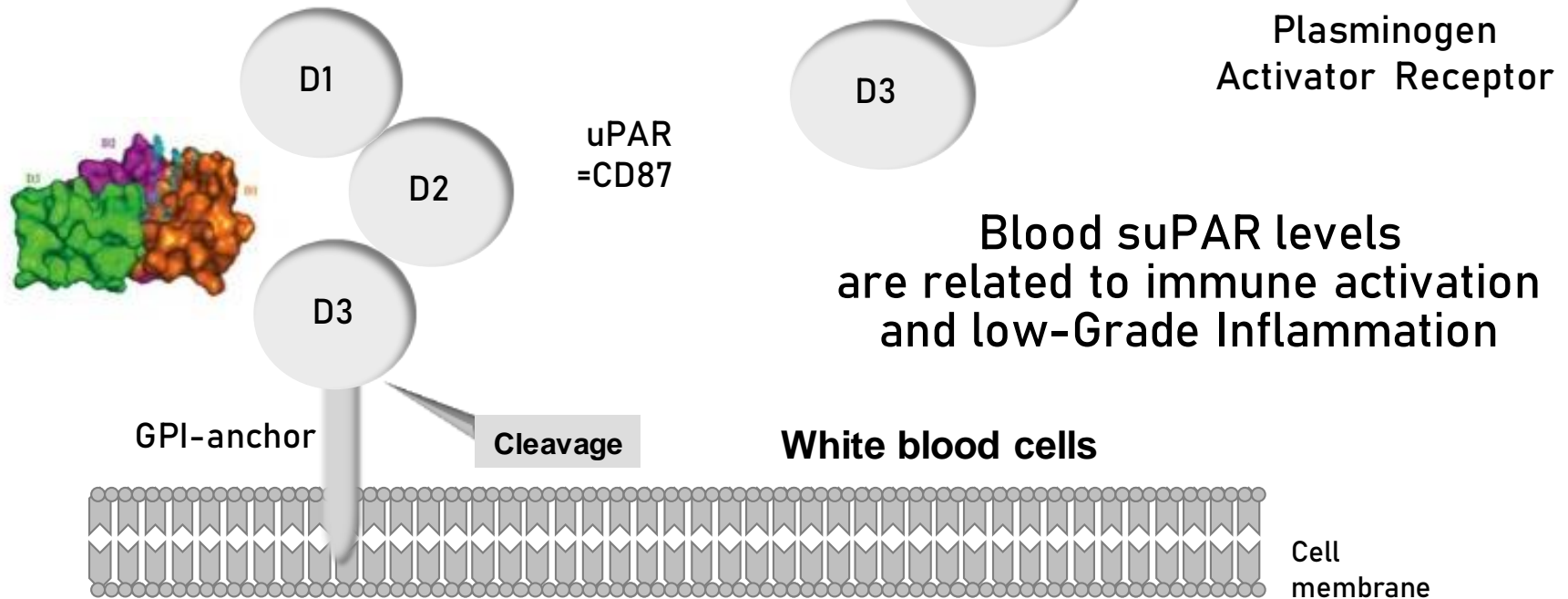


during inflammatory stimulation to generate soluble uPAR (suPAR) that is a highly flexible molecule with intrinsic chemotactic properties

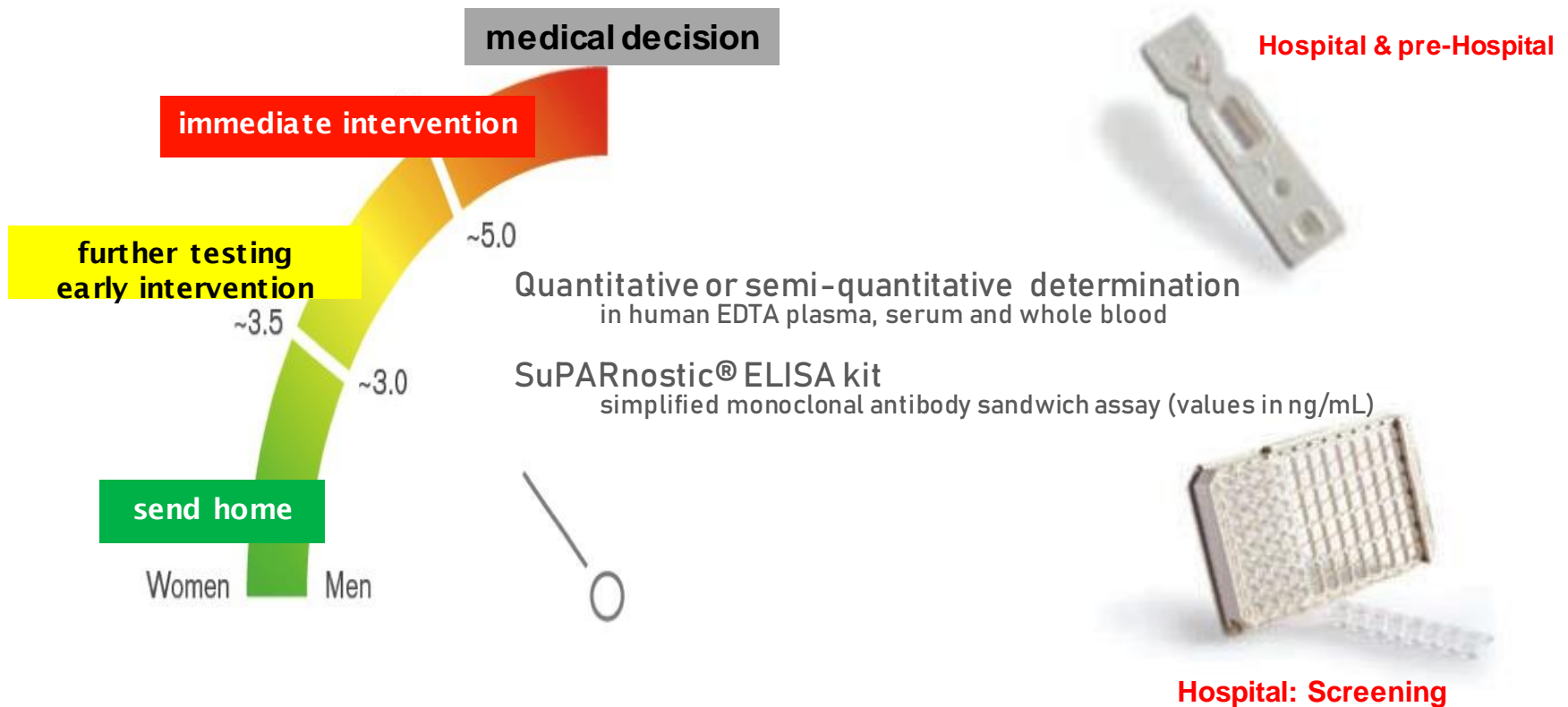
From uPAR to suPAR

The GPI-anchor links uPAR to the cell membrane making it available for uPA binding. When the receptor is cleaved between the GPI-anchor and DIII, it becomes soluble. suPAR is a stable protein that can be measured in various body fluids

GPI: glycosyl-phosphatidylinositol

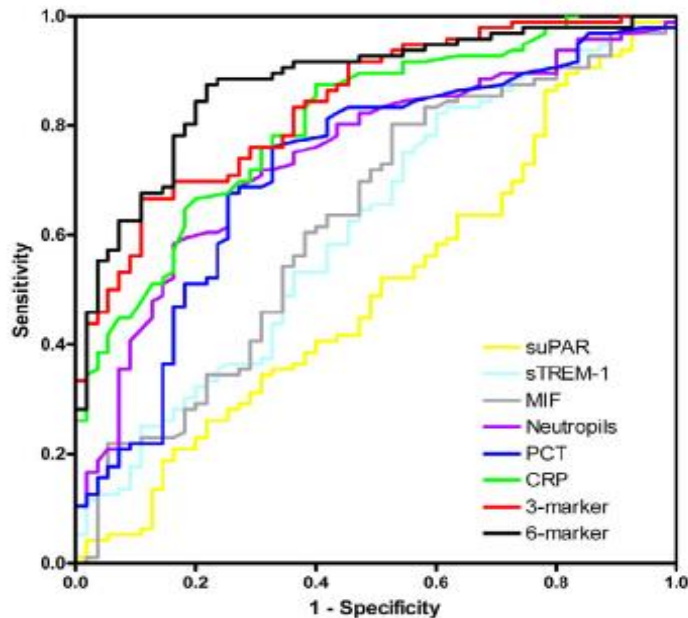


suPAR : the interpretation



suPAR

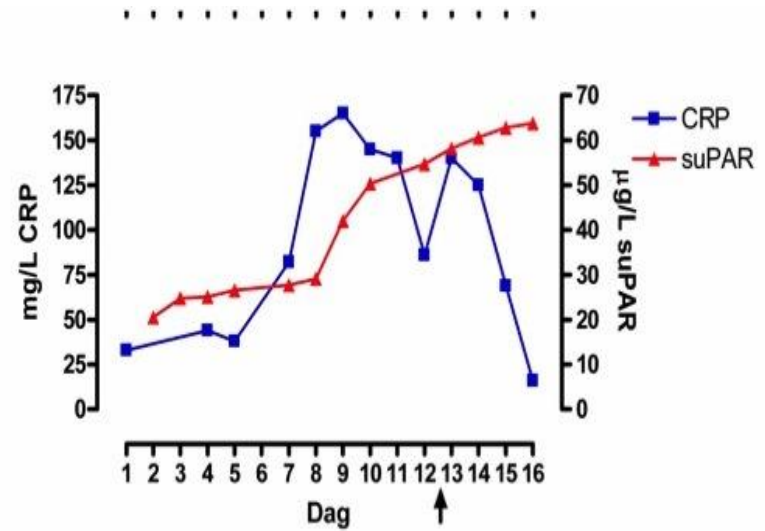
No value as diagnostic marker



SIRS cohort

- 151 patients
 - 96 bacterial infection,
 - 16 viral infection
 - 1 parasite

A Strong Prognostic Marker



28-year-old man with ulcerative colitis treated during 10 years with azathioprine (AZA)

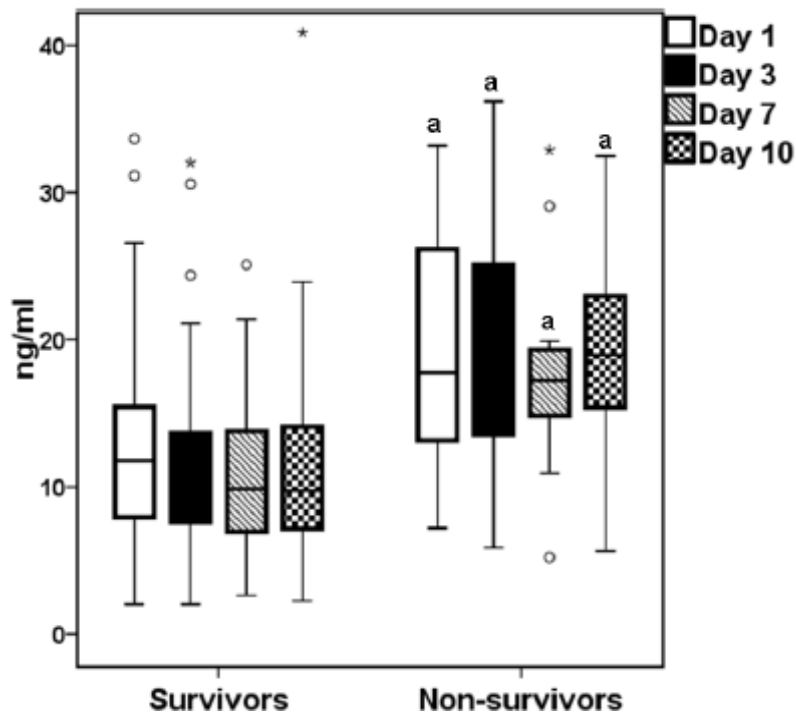
- fever, swollen lymph glands, hepato splenomegaly and pancytopenia
 - positive for acute Epstein-Barr virus (EBV) infection
 - before the final diagnosis of EBV associated Large B-cell lymphoma was confirmed
- He died from multiple organ failure.

Biomarkers in diagnosis and prognosis

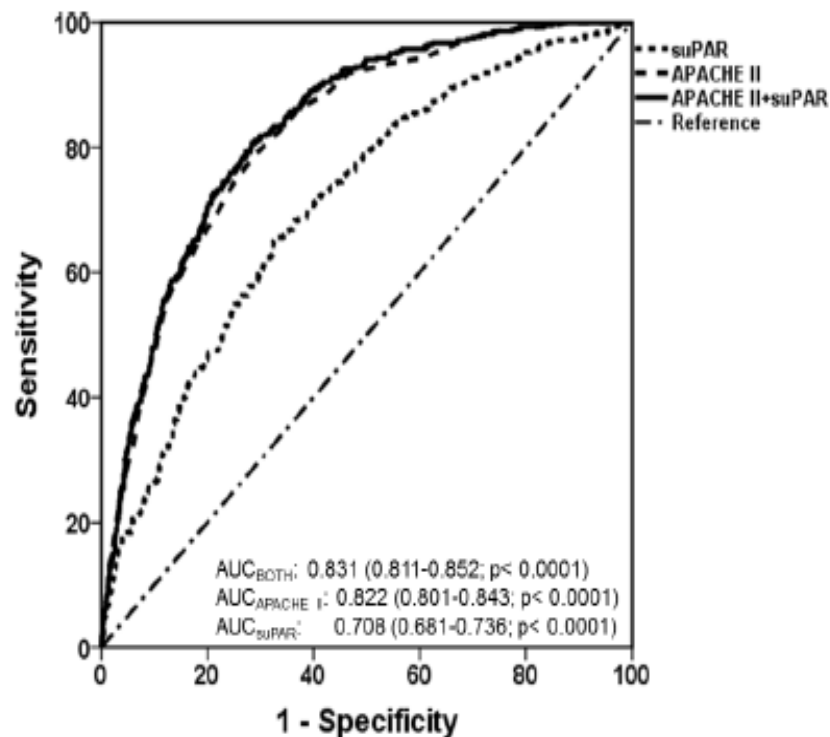
	SuPAR	PCT	Others
Diagnosis	No value	High value – bacterial infections	CRP – high value Neutrophils
Triaging	High value	Medium value – related to diagnostic power Restricted to proven bacterial infections	CRP – low value TREM1 – low value MIF – ? value
Prognosis	High value	PCT: Low value PCT kinetics: Some value within bacteraemia	CRP – no value TREM1 – low value MIF – low value

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

Evangelos J Giamarellos-Bourboulis^{1*}, Anna Norrby-Teglund², Vassiliki Mylona³, Athina Savva¹, Iraklis Tsangaris⁴, Ioanna Dimopoulou⁴, Maria Moutkaroudi¹, Maria Raftogiannis¹, Marianna Georgitsi¹, Anna Linnér², George Adamis⁵, Anastasia Antonopoulou^{1,4}, Eferpi Apostolidou⁶, Michael Chrisofos⁷, Christostomos Katsenos⁸, Ioannis Koutelidakis⁹, Katerina Kotzampassi¹⁰, George Koratzanis³, Marina Koupetori¹¹, Ioannis Kritselis¹², Korina Lymberopoulou³, Konstantinos Mandragos⁸, Androniki Marioli³, Jonas Sundén-Cullberg², Anna Mega¹³, Athanassios Prekates¹⁴, Christina Routsis¹⁵, Charalambos Gogos¹⁶, Carl-Johan Treutiger², Apostolos Armaganidis⁴ and George Dimopoulos⁴



Serum suPAR levels among 315 survivors and 52 non-survivors from sepsis over the course of 10 days.

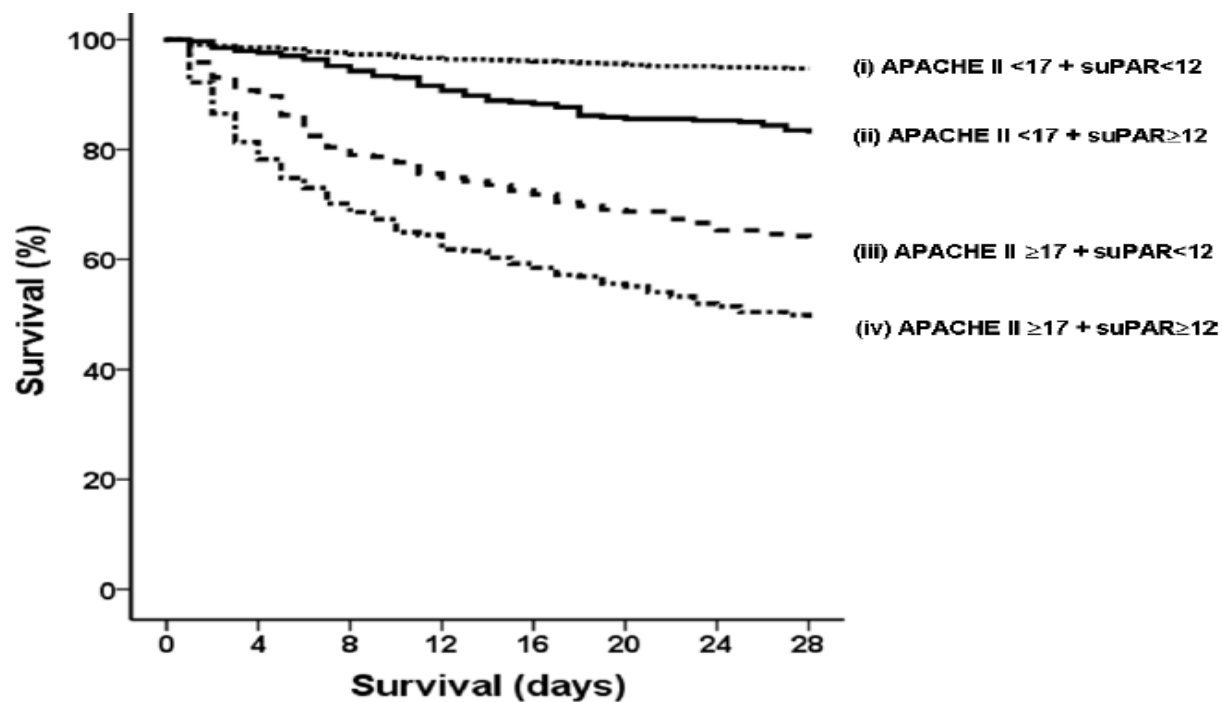


APACHE II score, serum suPAR, and their combination to define unfavorable outcome in a study cohort of 1,914 Greek patients

Table 2 Validation of the new stratification scheme

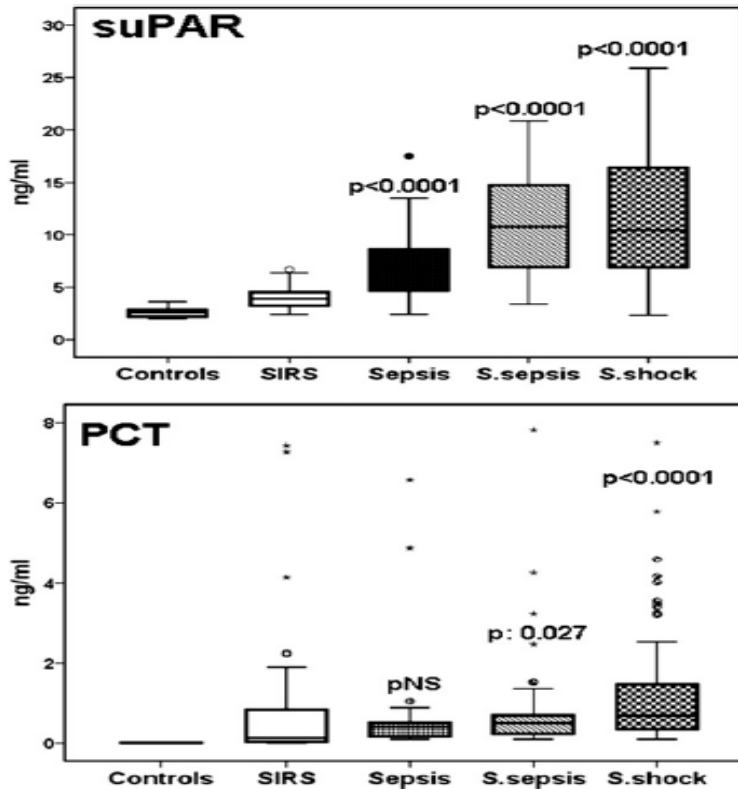
APACHE II score	suPAR, ng/mL	Survivors, number (percentage)	Non-survivors, number (percentage)	P value	OR	95% CI
<17	<12	844 (94.5)	49 (5.5)	<0.0001	3.62	2.42-5.42
	≥12	276 (82.6)	58 (17.4)			
≥17	<12	184 (62.8)	109 (37.2)			
	≥12	191 (48.5)	203 (51.5)			

APACHE II, Acute Physiology and Chronic Health Evaluation II; CI, confidence interval; OR, odds ratio; suPAR, soluble urokinase plasminogen activator receptor.

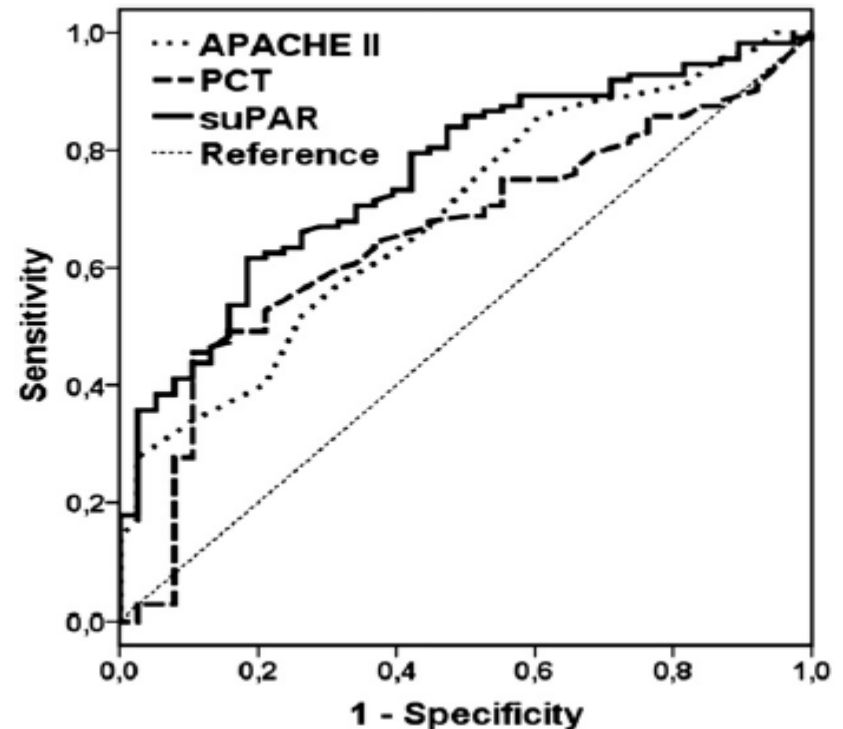


Soluble urokinase plasminogen activator receptor (suPAR) for assessment of disease severity in ventilator-associated pneumonia and sepsis

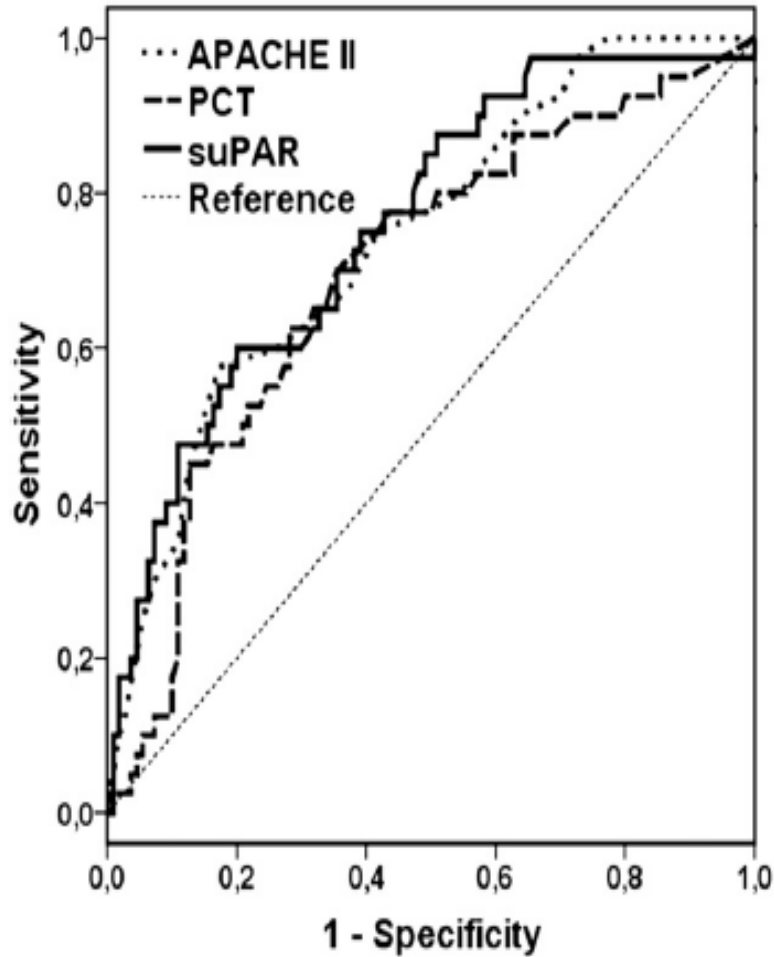
Athina Savva ^{a,*}, Maria Raftogiannis ^a, Fotini Baziaka ^a, Christina Routsis ^b, Anastasia Antonopoulou ^a, Pantelis Koutoukas ^a, Thomas Tsaganos ^a, Anastasia Kotanidou ^b, Efterpi Apostolidou ^c, Evangelos J. Giamarellos-Bourboulis ^a, George Dimopoulos ^d



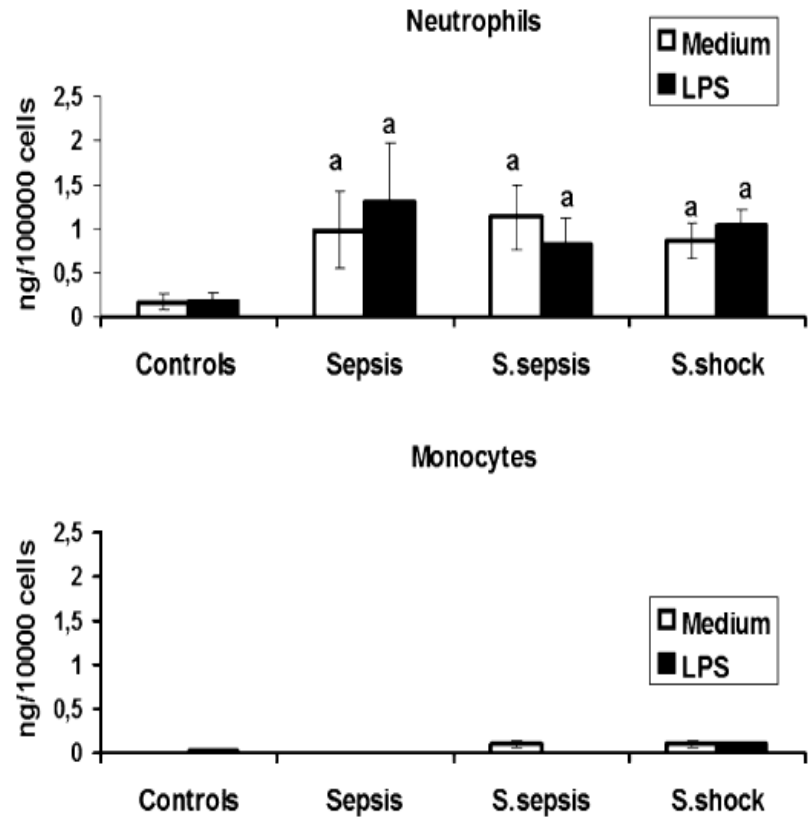
Serum concentrations of suPAR and of PCT after diagnosis of VAP in relation with the stage of sepsis. Respective values of 10 healthy volunteers and of 50 patients with SIRS



ROC analysis for serum concentrations of suPAR and of PCT and for APACHE II score to discriminate between sepsis and severe sepsis/shock.



ROC analysis for serum concentrations of soluble urokinase plasminogen activator receptor (suPAR) and of PCT and for APACHE II score to predict death.



Concentrations of suPAR in supernatants of monocytes and of neutrophils isolated on the first day in relation with the stage of sepsis. Respective concentrations of cells isolated from 10 healthy controls are shown. Cells were left untreated or treated with LPS of Escherichia coli

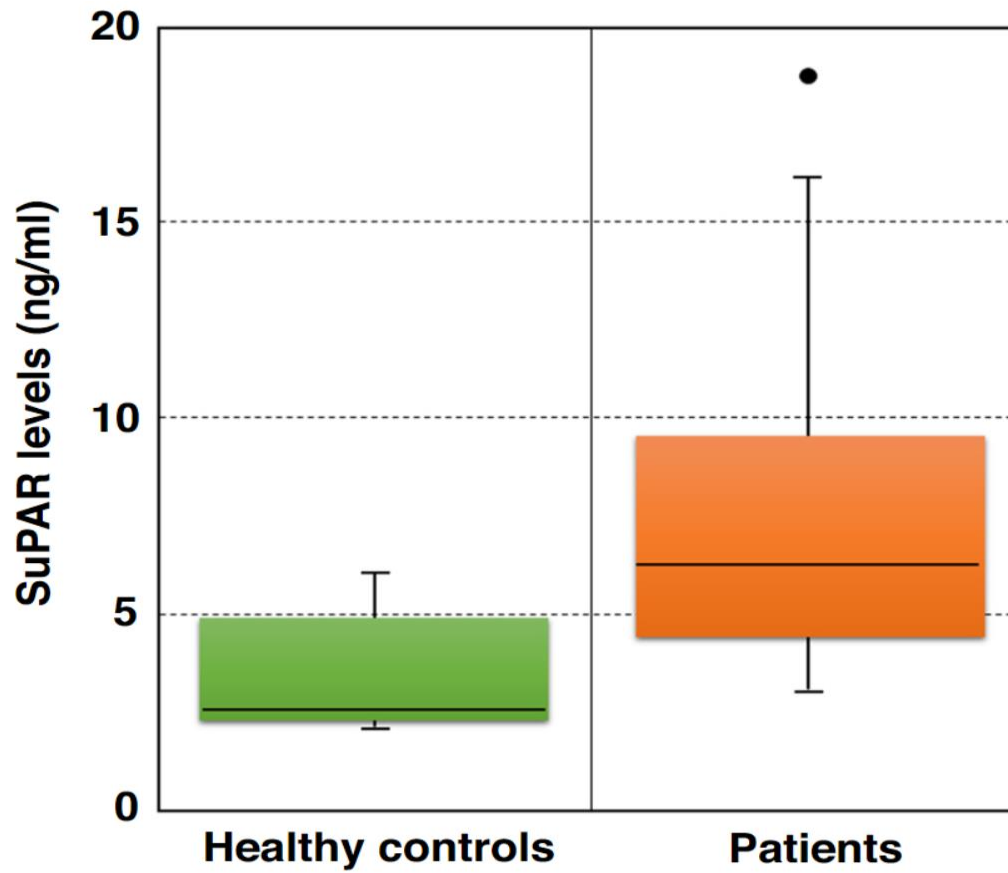
suPAR values in healthy and patients

TABLE 1

SuPAR levels (ng/mL) in serum, or otherwise specified, of healthy controls and patients^a

Pathology	suPAR levels (ng/ml)	
	Healthy controls	Patients
Diabetic nephropathy (DN)	2.3 ± 0.5	4.4 ± 1.6
Severe acute pancreatitis (SAP)	5.2 (2.0–8.0)	16.1 (12.6–24.2)
Moderate-severe acute pancreatitis (MSAP)	5.2 (2.0–8.0)	12.2 (9.6–17.0)
Moderate acute pancreatitis (MAP)	5.2 (2.0–8.0)	9.4 (6.9–12.0)
Asthma	2.5 (1.9–3.3)	5.6 (3.6–7.7) ^b
Systemic lupus erythematosus (SLE)	3.2 (2.9–3.0)	4.5 (3.8–5.2) ^c
Cirrhosis	2.6 (1.3–7.8)	7.2 (1–27.4) ^d ; 6.8 (1–29.4) ^e
Critical illness	2.1 (0.0–3.5)	5.9 (2.1–24.1) ^f ; 9.7 (0.4–38.0) ^g ; 8.3 (1.5–38.0) ^h ; 10.8 (0.4–38.0) ⁱ
Cardiovascular disease (CVD) ⁱ	3.9 (3.3–4.7)	4.6 (3.8–5.5) ^b
Ventilator-associated pneumonia (VAP) ^j	4.7 (3.6–6.3)	6.6 (5.7–7.7)
Community-acquired pneumonia ^j	2.7 ± 1.4	4.0 ± 2.3
Acute exacerbation chronic obstructive pulmonary disease (AECOPD)	2.4 ± 0.9	4.8 ± 1.9
Diabetes type 2	2.1 (1.9–2.4)	3.0 (2.5–3.5)
Diabetes type 1 ^j	2.3 (1.1–3.6)	3.0 (1.1–10.5) ^l ; 3.6 (1.6–15.1) ^m ; 4.9 (1.8–13.2) ⁿ
Cigarette smoke ^k	2.1 ± 0.1	3.3 ± 0.2
Sepsis ^k	6.0 (3.7–10.8)	18.8 (6.8–30.1)
Bacteremia in patients with systemic inflammatory response syndrome	5.6 (4.3–7.8)	8.1 (5.8–15.5) ^o ; 9.6 (6.5–11.7) ^p

suPAR values in healthy and patients



Soluble urokinase plasminogen activator receptor (suPAR) as an early predictor of severe respiratory failure in patients with COVID-19 pneumonia

Rovina et al. *Critical Care* (2020) 24:187
<https://doi.org/10.1186/s13054-020-02897-4>

Nikoletta Rovina¹, Karolina Akinosoglou², Jesper Eugen-Olsen³, Salim Hayek⁴, Jochen Reiser^{5*} and Evangelos J. Giamarellos-Bourboulis^{6,7*}

Independent variables at admission associated with the development of severe respiratory failure

	No need for MV or CPAP, n (%)	Need for MV or CPAP, n (%)	Univariate analysis		Forward Cox regression analysis	
			OR (95%CI)	p value	HR (95%CI)	p value
Male gender	15 (41.7)	19 (90.5)	0.07 (0.02–0.37)	< 0.0001	7.80 (1.75–34.76)	0.007
CCI > 2	17 (48.6)	17 (77.3)	7.00 (2.11–24.25)	0.002		ns
suPAR ≥ 6 ng/ml	3 (8.3)	18 (85.7)	66.00 (12.05–361.35)	< 0.0001	16.43 (4.56–59.19)	< 0.0001
Neutrophils ≥ 4200/mm ³	8 (22.2)	16 (72.2)	11.20 (3.13–40.08)	< 0.0001		ns
CRP ≥ 58 mg/l	7 (19.4)	13 (61.9)	6.73 (2.01–22.51)	0.002		ns

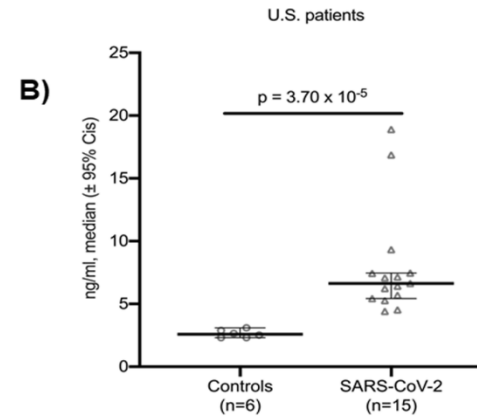
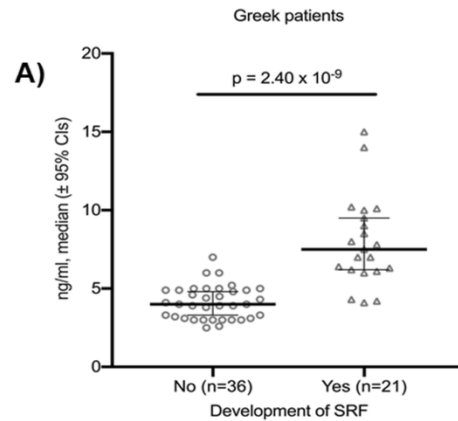
CCI Charlson's comorbidity index, CRP C-reactive protein, CI confidence interval, HR hazard ratio, OR odds ratio

Soluble urokinase plasminogen activator receptor (suPAR) as an early predictor of severe respiratory failure in patients with COVID-19 pneumonia

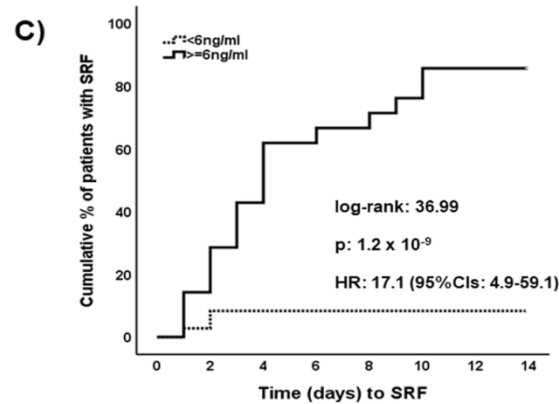
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Admission levels of suPAR among Greek patients who eventually developed or not SRF.

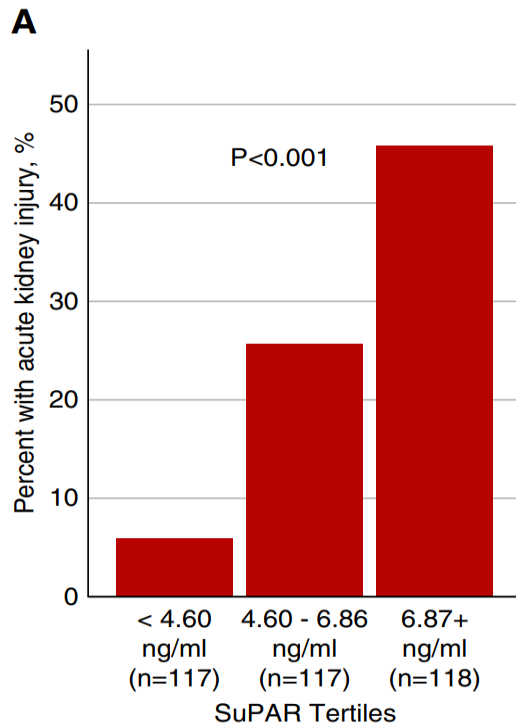


Levels of suPAR in patients with COVID-19 and controls.

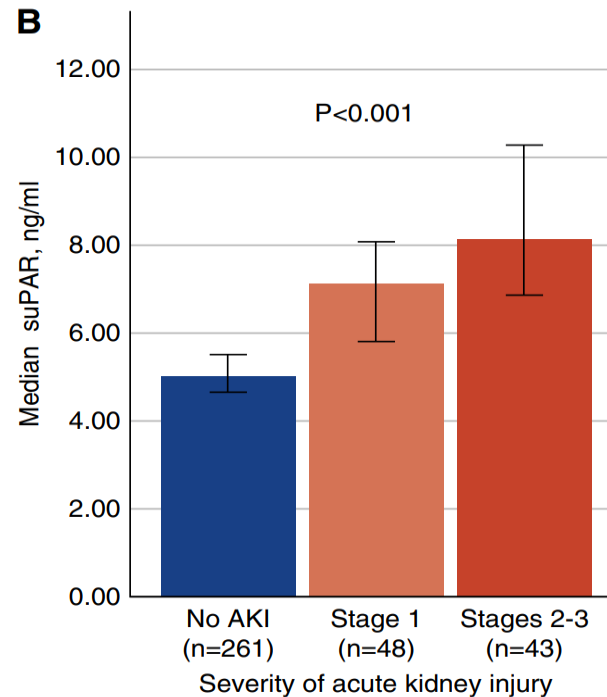


Time to SRF of Greek patients in relation to the admission levels of suPAR. CI, confidence interval; HR, hazard ratio

SuPAR in COVID-19-Related AKI



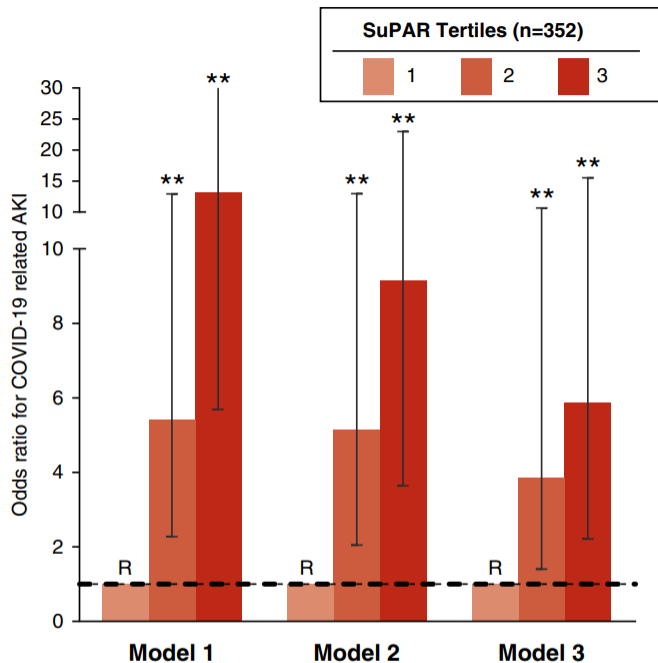
Patients with incident AKI stratified by suPAR tertiles,



Median suPAR levels across AKI stages.

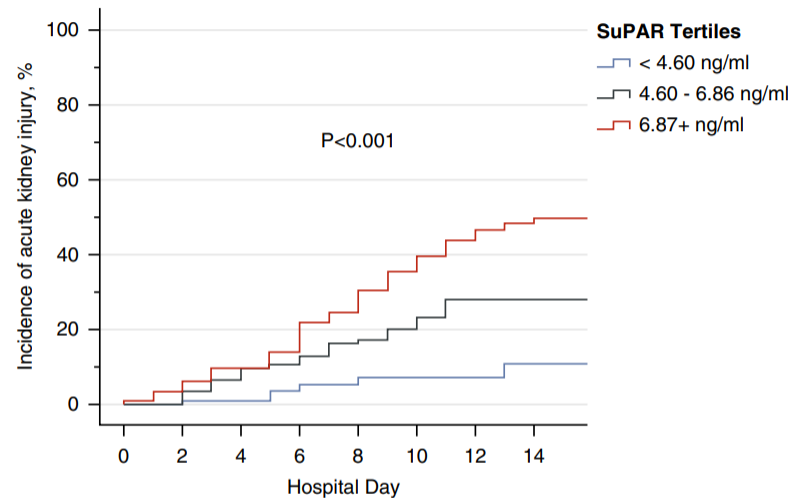
SuPAR in COVID-19-Related AKI

A



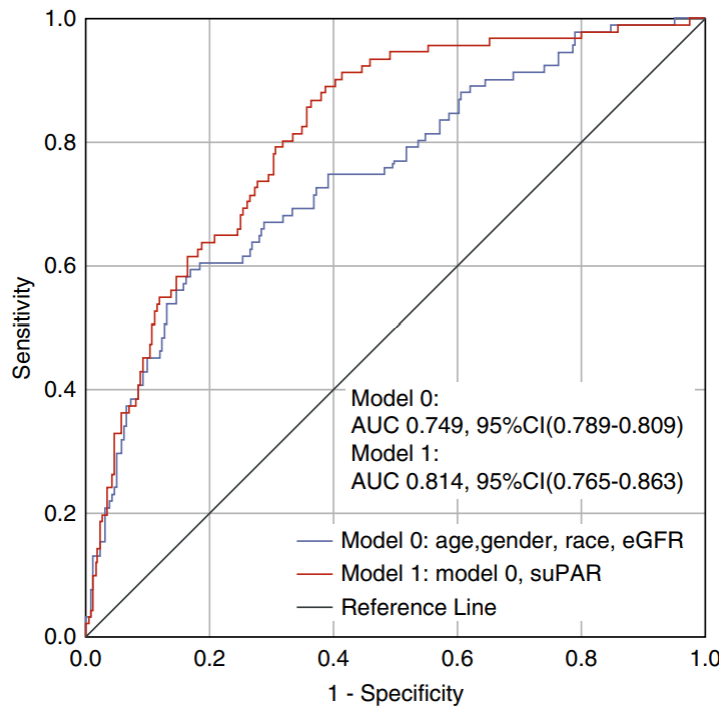
SuPAR, ng/ml	Odds ratio (95%CI)		
	Model 1	Model 2	Model 3
<4.60	Reference	Reference	Reference
4.60-6.86	5.42 (2.27-12.93)	5.16 (2.05-12.97)	3.87 (1.41-10.65)
>6.86	13.26 (5.69-30.88)	9.15 (3.64-22.93)	5.86 (2.21-15.52)
P trend	<0.001	<0.001	<0.001

B

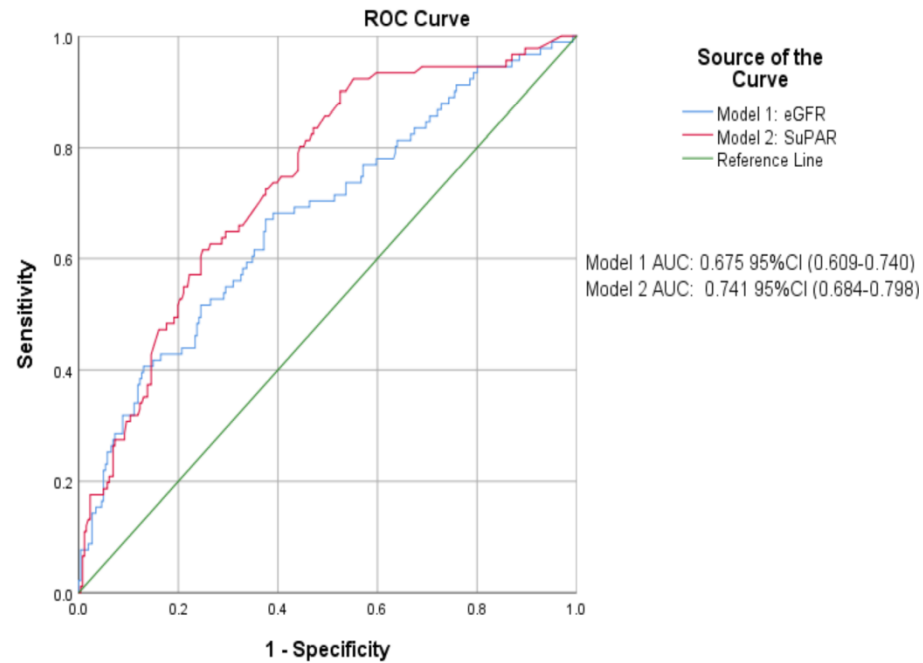


SuPAR, ng/ml	Hazard ratio (95%CI)		
	Model 1	Model 2	Model 3
<4.60	Reference	Reference	Reference
4.60-6.86	3.28 (1.44-7.47)	3.25 (1.40-7.54)	2.62 (1.03-6.70)
>6.86	5.76 (2.61-12.70)	4.36 (1.91-9.98)	3.74 (1.47-9.47)
P trend	<0.001	<0.001	0.005

SuPAR in COVID-19-Related AKI



Addition of suPAR improves the AUC for predicting AKI.



ROC Curves for SuPAR and Admission eGFR

suPAR Key points

suPAR is a marker of risk of death

- is not a diagnostic marker

Emergency Room

- General Screening
- Identifying risk patients (e.g. suspect SIRS/sepsis cases)

Intensive Care Unit

- Monitoring for risk of sepsis and other critical conditions
-

Βιοδείκτες ταξινόμησης στη Σήψη

More than 170 different biomarkers have been assessed

- for potential use in sepsis and infections
- more for prognosis than for diagnosis



How to use
them
CAREFULLY !!!
