Citometria e sepsi: un valore aggiunto

Arianna Gatti
ASST Ovest Milanese
Ospedale di Legnano (Milano).
arianna.gatti@asst-ovestmi.it

Background

- Sepsis is considered a major cause of health loss, but data for the global burden of sepsis are limited.

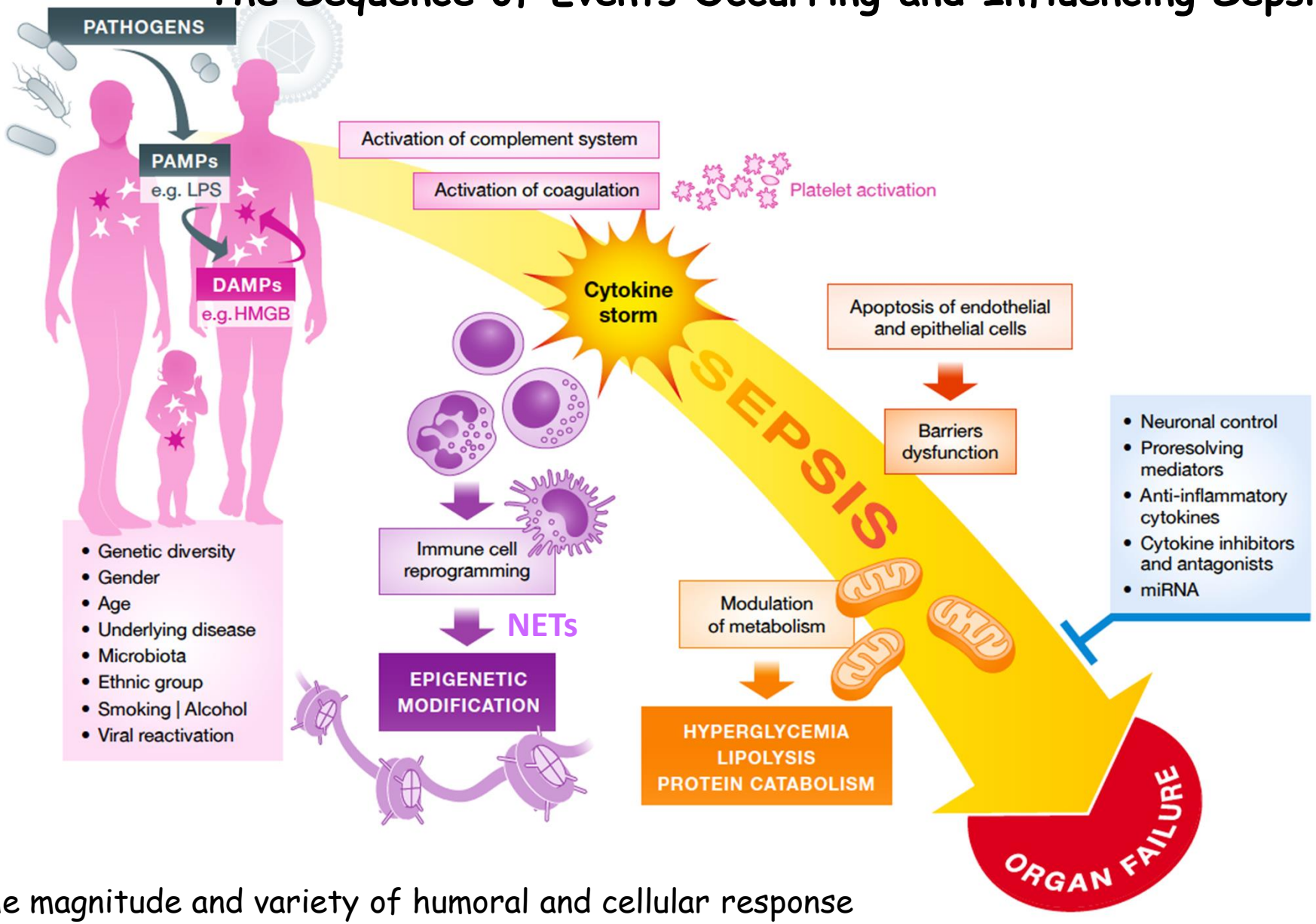
- In 2017, an estimated 49 million incident cases of sepsis were recorded worldwide.

- 11 million sepsis-related deaths were reported, representing 19.7% of all global deaths. The mortality decreased by 53% from 1990 to 2017.

- Sepsis incidence and mortality varies substantially across regions, with the highest burden in sub-Saharan Africa, Oceania, South Asia, East Asia, and Southeast Asia.

K. E Rudd et al. Lancet 2020; 395: 200–211

The Sequence of Events Occurring and Influencing Sepsis



The magnitude and variety of humoral and cellular response may lead to organ dysfunctions, which are a key denominator of sepsis in comparison with other forms of infection.

Cavaillon JM. EMBO Mol Med 2020; 12: e10128

JAMA. 2016 February 23; 315(8): 801–810. doi:10.1001/jama.2016.0287.

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

Mervyn Singer, MD, FRCP, Clifford S. Deutschman, MD, MS, Christopher Warren Seymour,

- **SEPSIS:** A life-threatening organ dysfunction caused by a dysregulated host response to infection.
- Organ dysfunction is identified by an acute increase of SOFA/qSOFA score ≥ 2 points with infection.
- qSOFA bedside score: Mental alteration (any Glasgow coma scale score); Mean blood pressure ≤ 65 mmHg or systolic bp ≤ 100 mmHg; Respiratory rate ≥ 22/min.

JAMA. 2016 February 23; 315(8): 801–810. doi:10.1001/jama.2016.0287.

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

Mervyn Singer, MD, FRCP, Clifford S. Deutschman, MD, MS, Christopher Warren Seymour,

- **SEPTIC SHOCK:** when the underlying circulatory and metabolic abnormalities are deep enough to increase substantially the mortality risk.
- Sepsis criteria plus need of vasopressors to ensure mean blood pressure ≥ 65 mmHg despite adequate volume replacement and plasma Lactate > 2mMol/L.

- **Lack of diagnostic golden standards. Role of Sepsis Biomarkers remains undefined.**

- Both pathogen source and organ dysfunction may be occult.

- Sepsis is shaped by pathogen and host factors, with characteristics that may evolve with time.

Blood and Body Fluid Microbiological Cultures

The "Golden Standard" to Diagnose Sepsis ?

Blood cultures require multiple samplings (≥ 8mL blood each), > 48 hours for initial results, and the avoidance of previous antibiotic treatment.

....even when microbiological tests are completed, culture-positive "sepsis" is observed in only **30% to 40% of cases**

(***Lack of sensitivity***)

In many cases **falsely positive** blood cultures due to skin bacterial contamination produce misleading findings

(***Low specificity***)

Singer M. JAMA 2016; 315(8): 801-810

GUIDELINES

Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021

Check for updates

Antibiotic Timing

	Shock is present
Sepsis is definite or probable	✔ Administer antimicrobials ***immediately***, ideally within 1 hour of recognition.
Sepsis is possible	✔ Administer antimicrobials ***immediately***, ideally within 1 hour of recognition.

Shock is absent
✔ Administer antimicrobials ***immediately***, ideally within 1 hour of recognition.
✔ Rapid assessment* of infectious vs. noninfectious causes of acute illness.
✔ Administer antimicrobials ***within 3 hours*** if concern for infection persists.

Evans L. Intensive Care Med. 2021 Nov; 47(11): 1181-1247

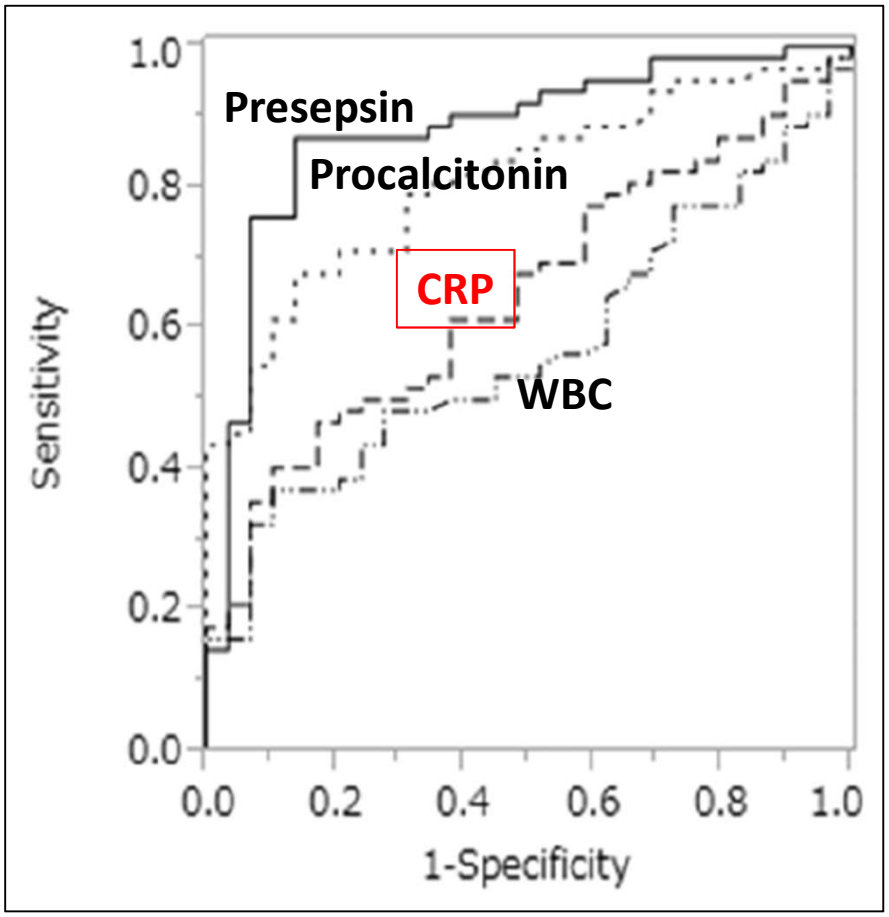
Old and New SEPSIS Biomarkers

- *Mostly Clinical, plus Soluble Analytes*
  - WBC Count
  - Body Temperature
  - Respiratory Rate
  - Blood Pressure
  - Blood pH
  - Use of Vasopressors
  - Lactate
  - CRP
  - Procalcitonin
  - Presepsin
  - Progranulin
  - DAMPs
  - PAMPs
  - IL-1β, IL-6, IL-12, IFNs
  - TNF-α
  - Complement
  - Coagulation Factors

- *Mostly Cellular Markers*
  - Monocyte Position Parameters
  - Monocyte Subset Changes
  - CD64 on Neutrophils and Monocytes
  - HLA-DR Down-Regulation on Mono
  - CD169 on Mono
  - CCR2 on Inflammatory Mono
  - CX3CR1 on Patrolling Mono
  - Integrin α3β1 VLA-3 on Neutrophils
  - TLR family (*DAMPs & PAMPs Receptors*)
  - NLR family (*DAMPs & PAMPs Receptors*)
  - P2X & P2Y Receptors
  - MR-pro-ADrenoMedullin (ADM)
  - HMGB-1
  - Pancreatic Stone Protein (PSP)
  - Soluble CD25
  - Genomewide WBC Transcriptome

- Trzeciak A. Immune Netw 2020 Jun; 20(3): e23
- Povoa P. Semin Respir Crit Care Med 2021; 42: 662-671

SEPSIS: Clinical Biomarkers – Diagnostic Performance of C-Reactive Protein (CRP)

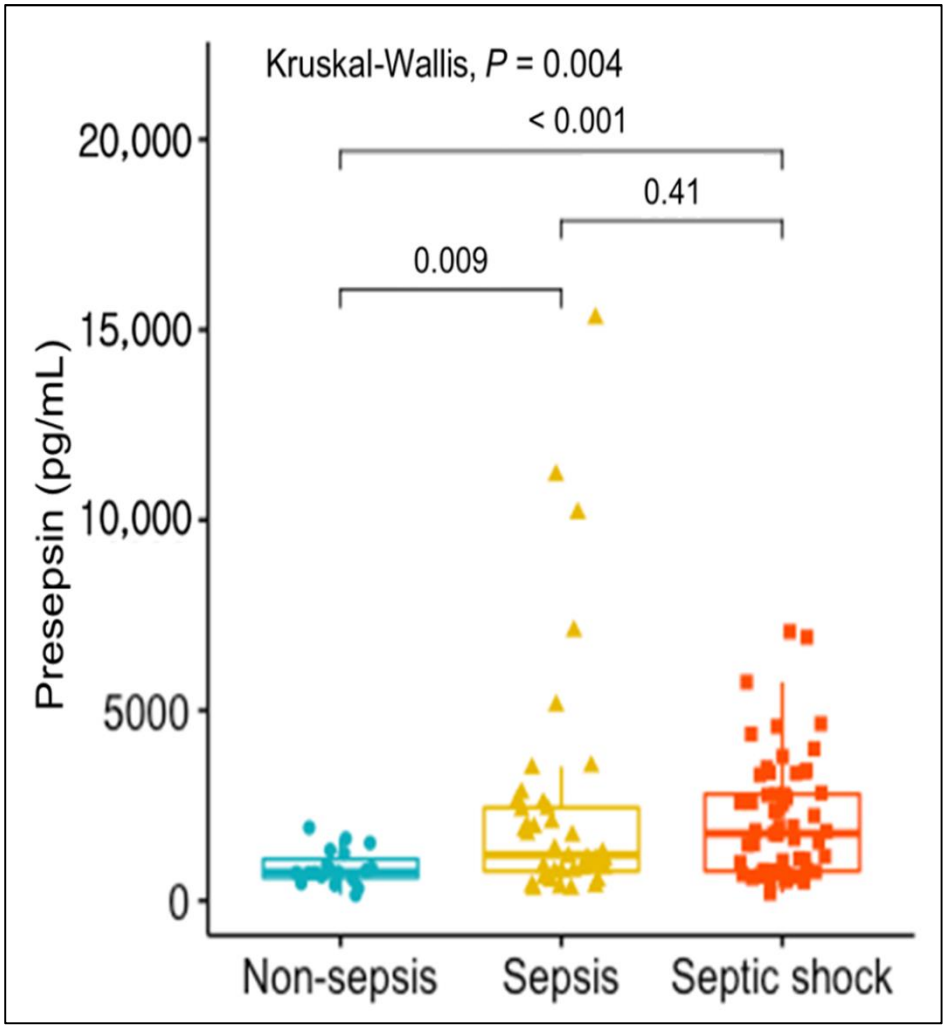


- CRP's role in multiple inflammation and coagulation pathways.
- Rapidly consumed during Sepsis.
- Decreased transcription in patients with Sepsis.
- Very low CRP levels in severe liver dysfunction despite infection.

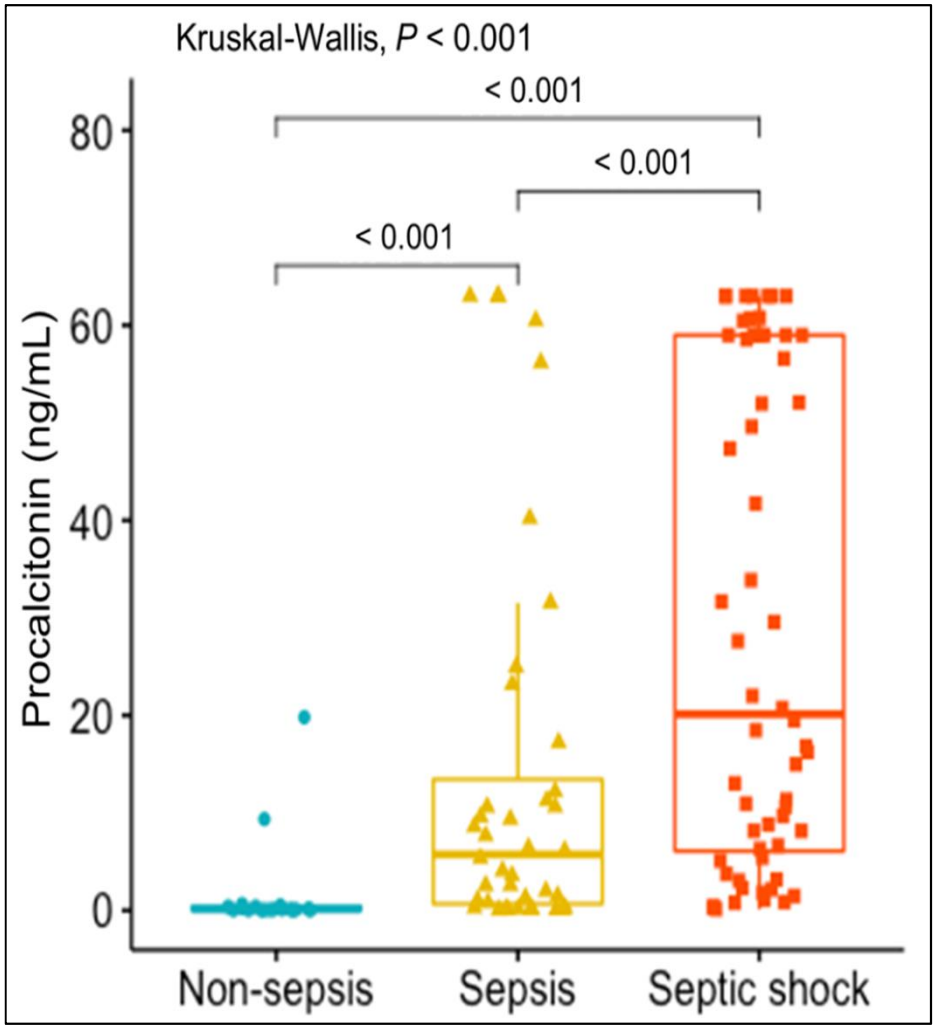
- Yamamoto T. Am J Emerg Med 2019 Oct; 37(10): 1936-1941.

SEPSIS: Clinical Biomarkers – Diagnostic Performance of Presepsin and PCT

Presepsin



PCT

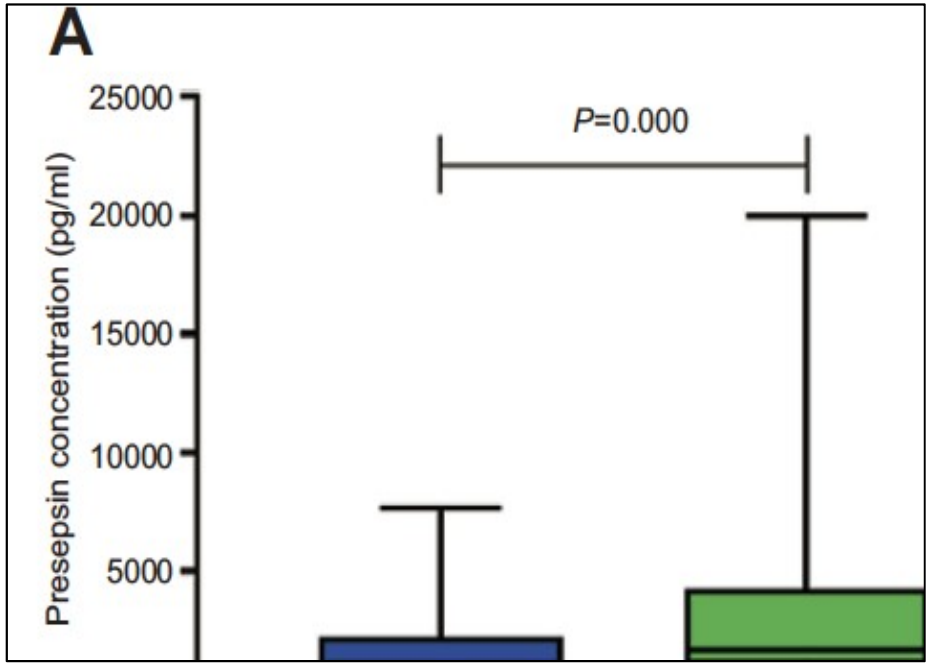


Lee J. Diagnostics 2021; 11: 60.

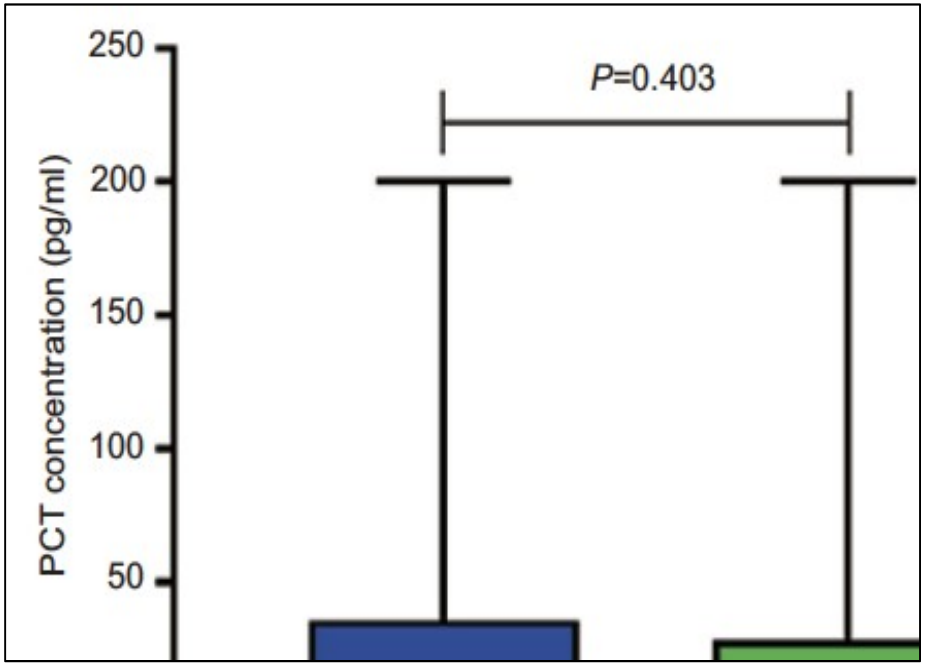
SEPSIS: Clinical Biomarkers – Diagnostic Performance of Presepsin and PCT (2)

Presepsin level in predicting patients' in
mortality from sepsis under sepsis-3 cr

Presepsin



PCT



Miao-Yun Wen. Therapeutics and Clinical Risk Management 2019:15 733–739

GUIDELINES

Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021

Check for updates

Biomarkers to start antibiotics

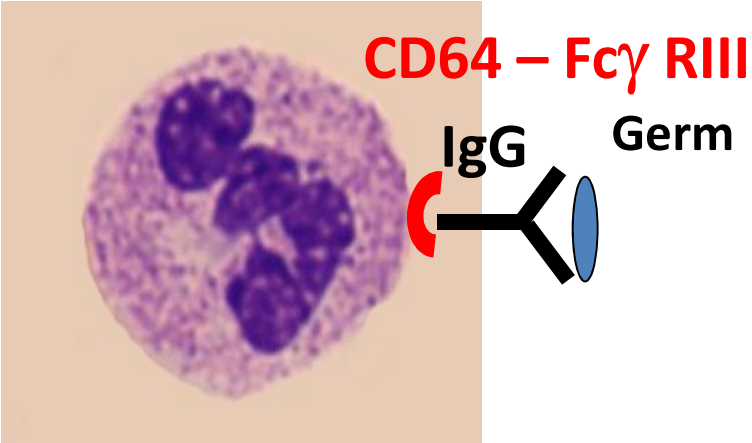
Recommendation
16. For adults with suspected sepsis or septic shock, we **suggest against** using procalcitonin plus clinical evaluation to decide when to start antimicrobials, as compared to clinical evaluation alone *Weak recommendation, very low quality of evidence*

Recommendation
2. We **recommend against** using qSOFA compared to SIRS, NEWS, or MEWS as a single screening tool for sepsis or septic shock *Strong recommendation, moderate-quality evidence*

Evans L. Intensive Care Med. 2021 Nov; 47(11): 1181-1247

SEPSIS: Cellular Biomarkers

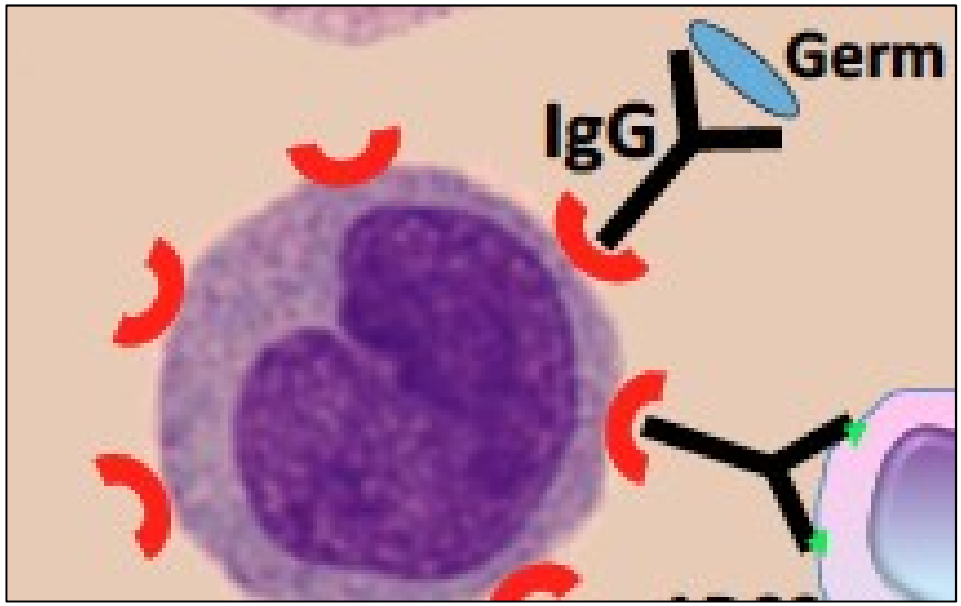
- **Neutrophil CD64 – Fcγ RIII**



- Constitutively expressed at **very low** density on **Neutrophils (<800 ABC)**, but promptly up-regulated under stimulation (*bacterial products, endotoxins, LPS etc.*).

SEPSIS: Cellular Biomarkers

- **Monocyte CD64 – Fcγ RIII**



- Mediates opsonic phagocytosis and Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC).
- Constitutively expressed at **high** density on **Monocytes (20-25,000 ABC),** further up-regulated under stimulation.
- CD64 modulation is **relatively independent from the overall immune system function**.
- The ups and downs of CD64 density correlate with the clinical course of sepsis.

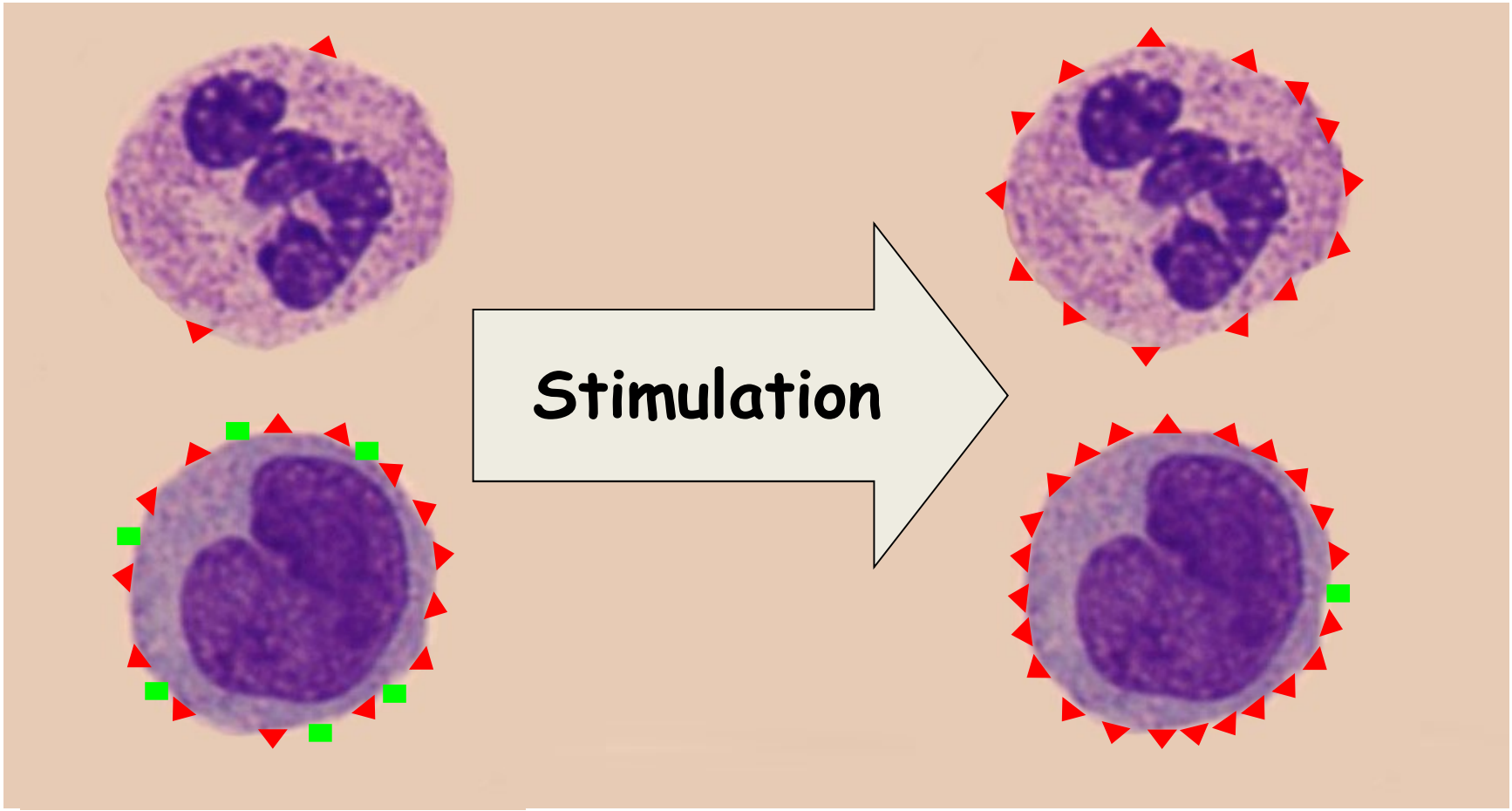
- Qureshi SS. Clin Exp Immunol 2001; 125: 258 - 265.
- Hoffmannn JJ. Clin Chem Lab Med 2009; 47: 903 - 916.

Inactive Neutrophil

neCD64 <1000 ABC

Active Neutrophil

neCD64 > 2.500 ABC



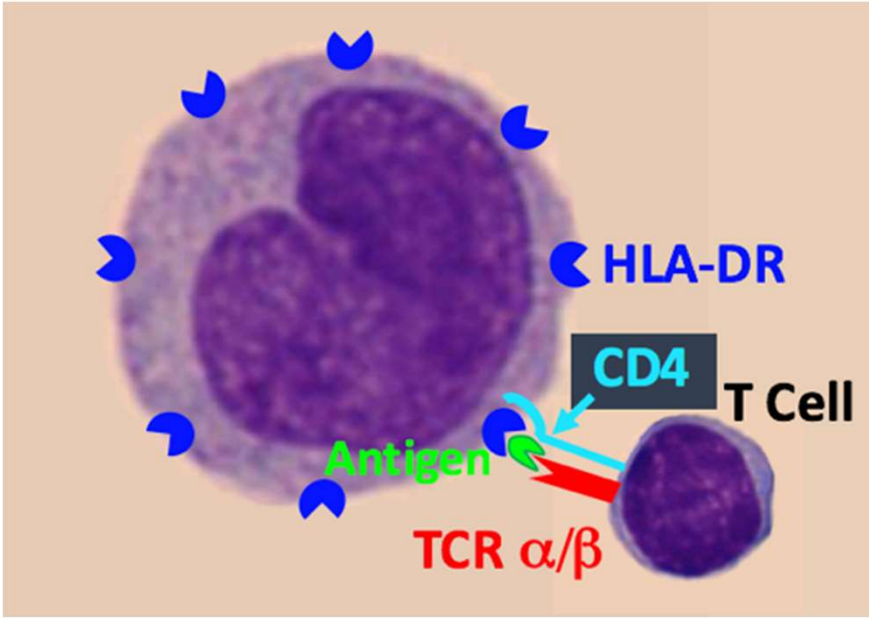
Inactive Monocyte

Active Monocyte

▲ CD64 ■ HLA-DR

SEPSIS: Cellular Biomarkers

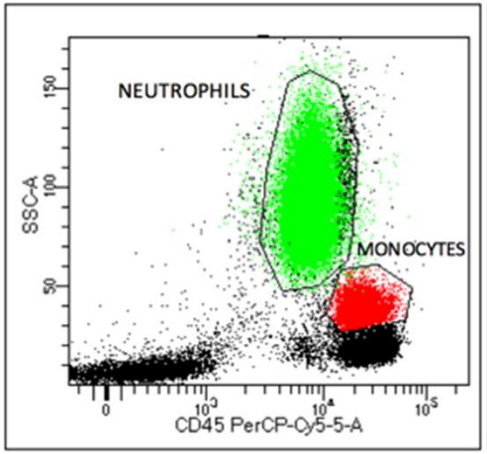
Monocyte HLA-DR Expression in Sepsis

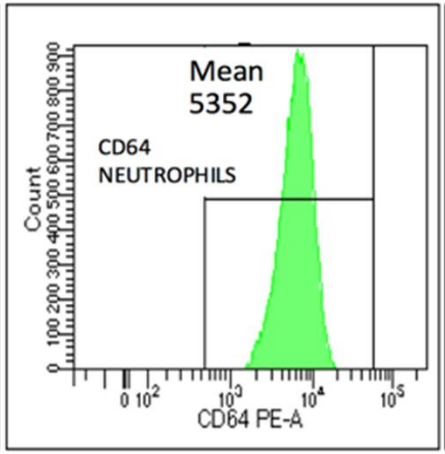


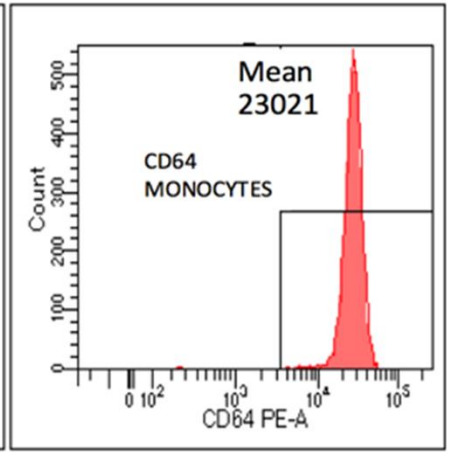
- Monocyte HLA-DR is an indicator of the global status of immune response.
- Inflammatory cytokines reduce the **number** of HLA-DR+ Monocytes and the HLA-DR surface **density**.
- Reduced Antigen-Presenting Cell function → **Immunosuppression**.
- Reduced responsiveness to secondary antigen challenge.
- The persistence of a **down-regulated Monocyte HLA-DR** over the clinical course is associated with **poor prognosis and increased mortality**.

- Landelle C. Intensive Care Med 2010; 36: 1859 – 1866.
- Monneret G. Cytometry Part A 2019; 95A: 431 - 441

CD64 QUANTIBRITE:

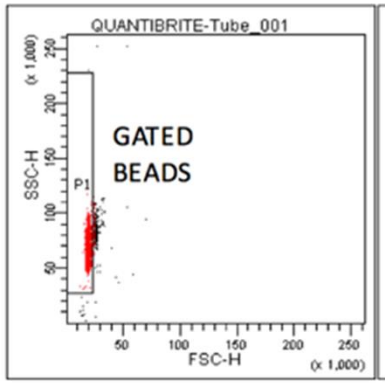


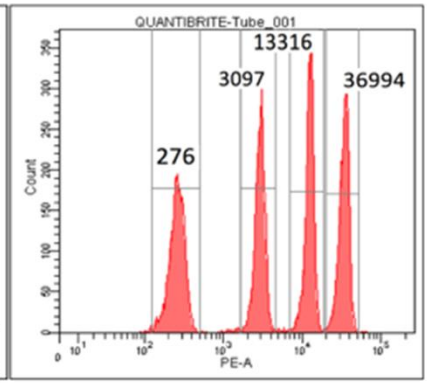


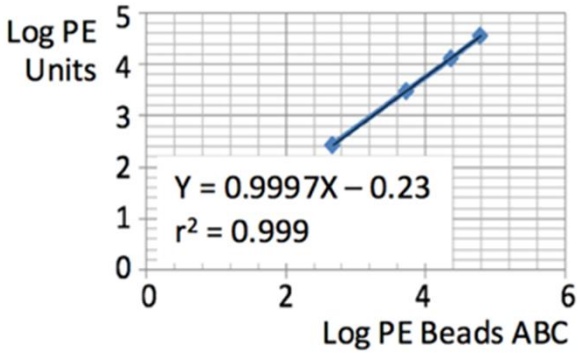


Translating FCM intensity values into ABC Units (Antibody Binding Capacity)

QUANTIBRITE
PE Calibration Beads







CALIBRATION LINE - PE

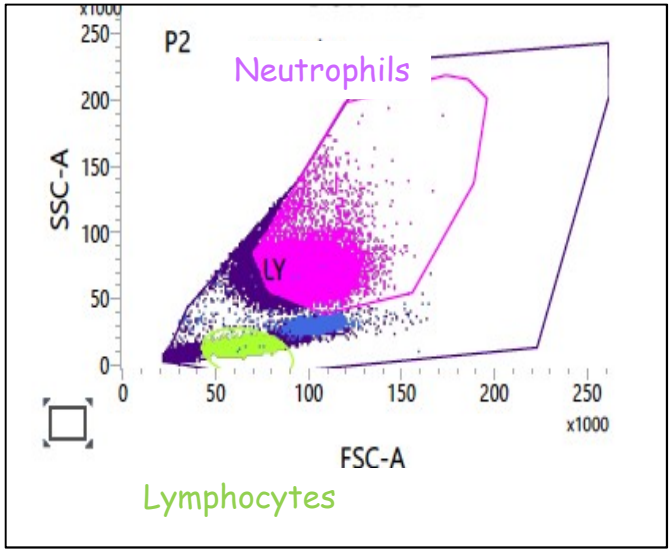
PE Beads ABC	X Log PE Beads	Y Log PE Units
474	2.676	2.441
5359	3.729	3.491
23843	4.377	4.124
62336	4.795	4.568

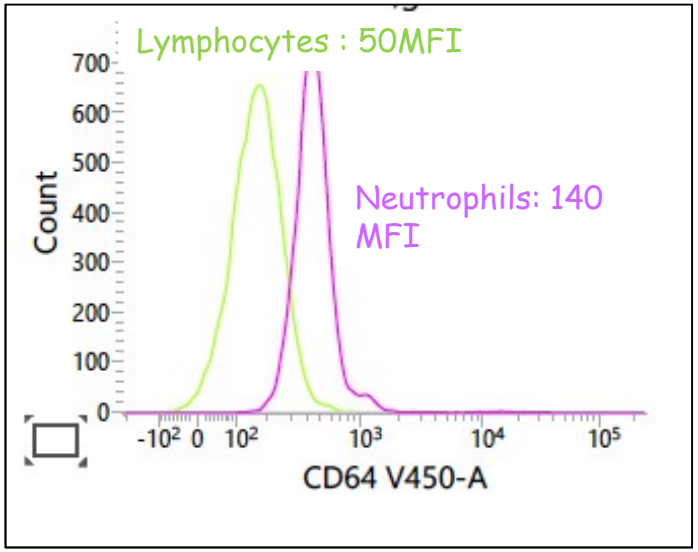
CONVERSION PE Units → ABC

	Mean PE Units	**ABC Units**
Neutrophils	5352	**9238**
Monocytes	23021	**39737**

neCD64 ratio and moHLA-DR

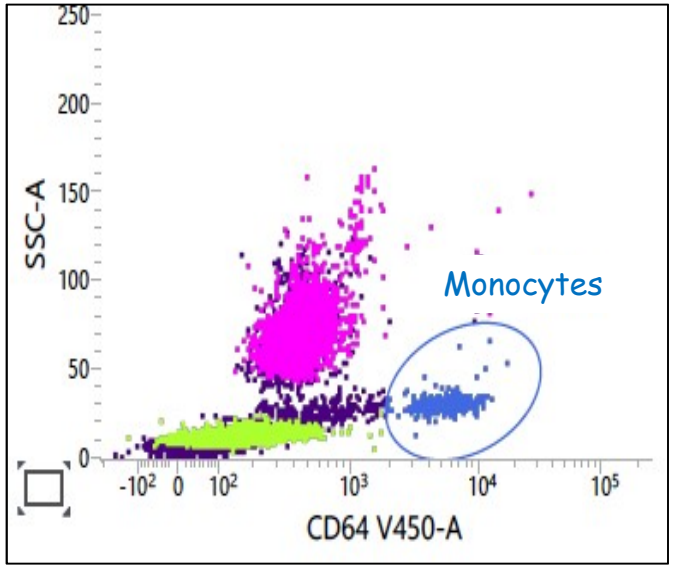
NORMAL SUBJECT

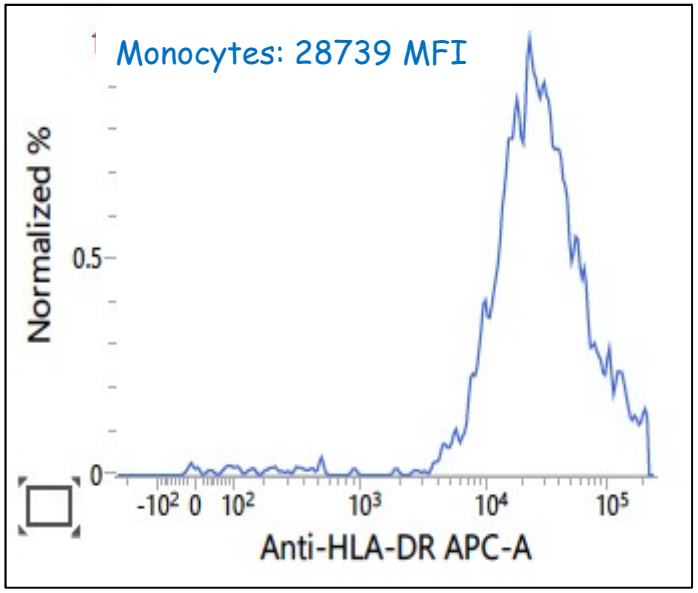




neCD64 ratio:
140/50= 2.8
(normal value <3)

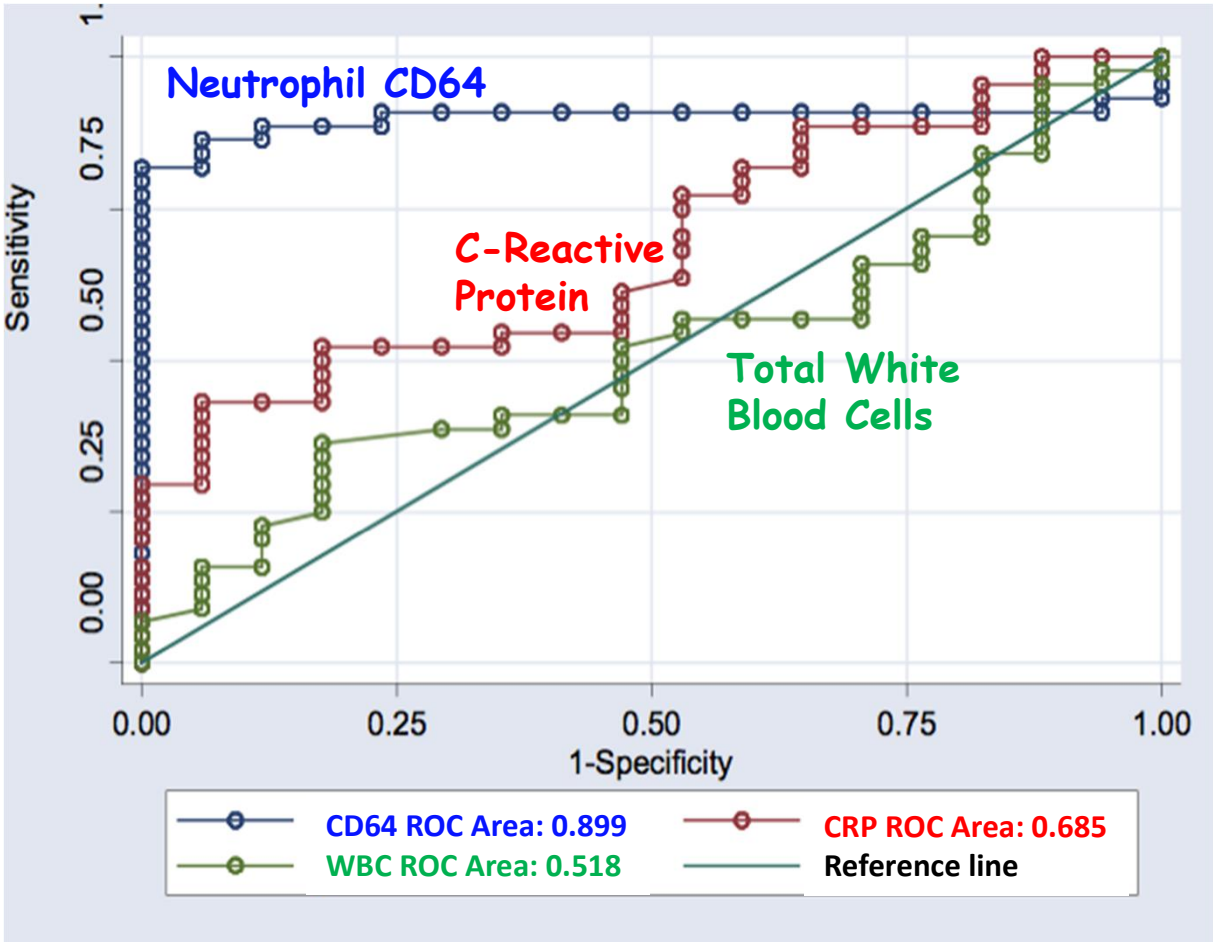
Lymphocytes used as Negative Internal Control





Neutrophil CD64 as Early Indicator of Sepsis in ICU Patients

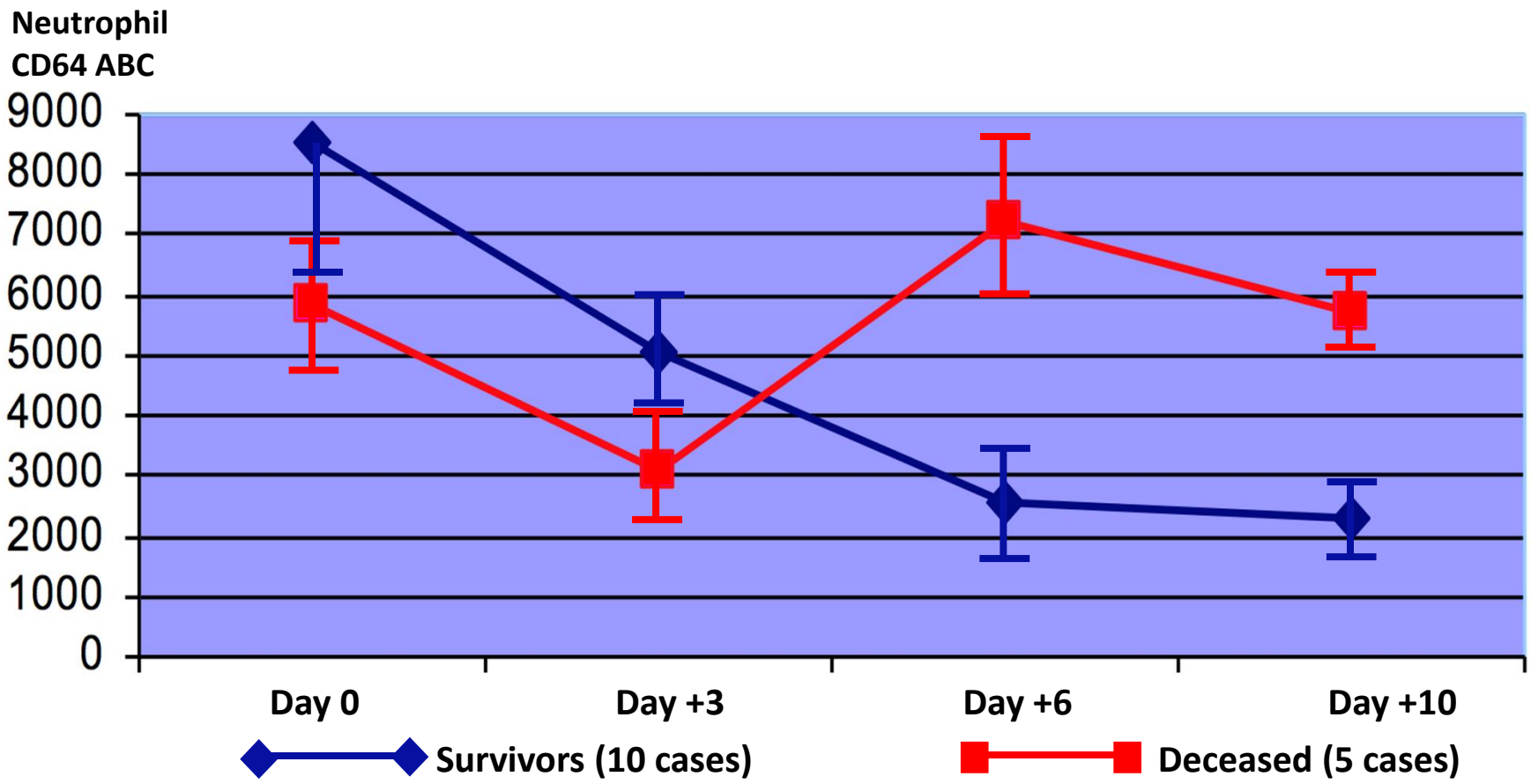
61 ICU patients with sepsis, mostly from lower airway infections



Intensive Care Unit - Legnano General Hospital 2009-2011

Kinetics of Flow Cytometric Parameters as Prognostic Indicators (3)

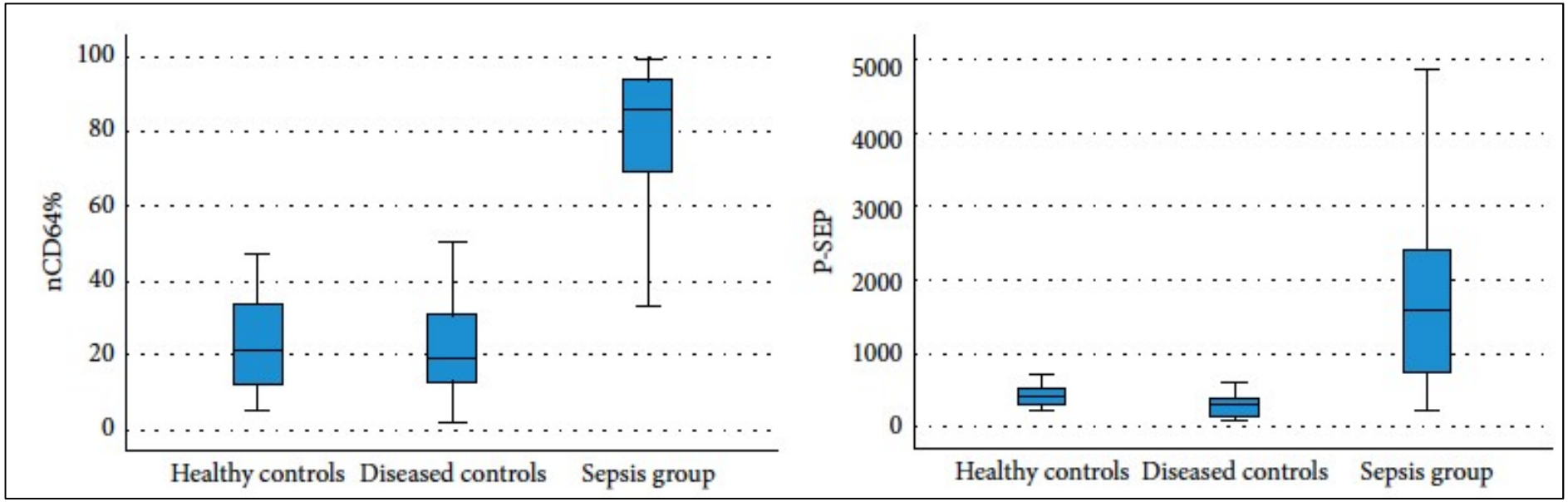
Early changes of Neutrophil CD64 Expression may Predict Middle-Term Mortality



Intensive Care Unit - Legnano General Hospital 2009-2011

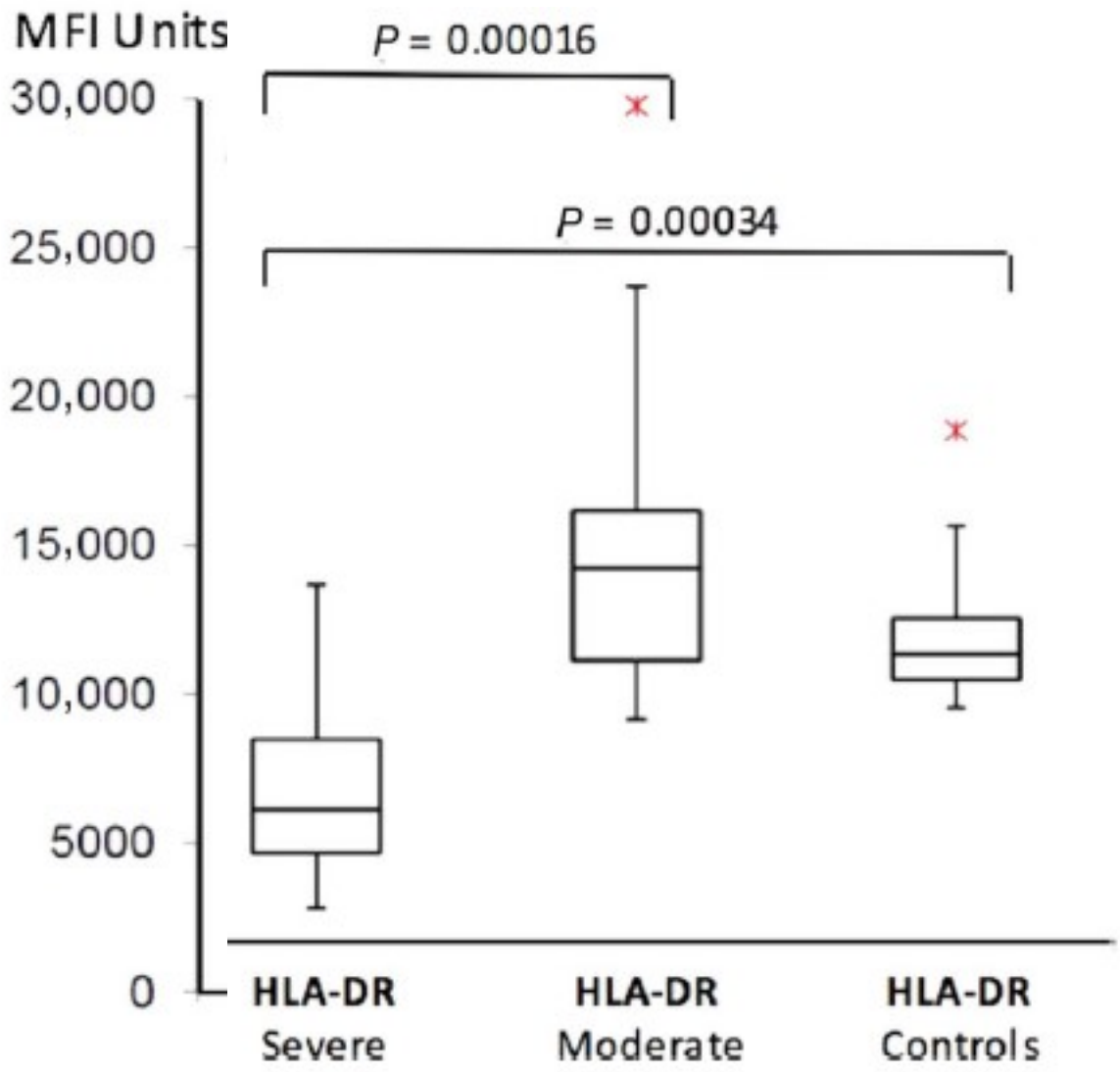
Research Article

The Utility of Neutrophil CD64 and Presepsin as Diagnostic, Prognostic, and Monitoring Biomarkers in Neonatal Sepsis



H.E. Hashem. International Journal of Microbiology 2020. https://doi.org/10.1155/2020/8814892

Flow cytometric analysis of moHLDR in SARS-Cov 2 infected patients and controls



A. Gatti. Cytometry Part A 2020; 97A: 887–890.

Legnano General Hospital 2020-2021

moHLADR MFI comparison between NC, alive SARS-CoV 2 patients with short hospital admission (<15 days), alive patients with long hospital admission (>15 days) and dead patients

- **Caso clinico con esito favorevole**

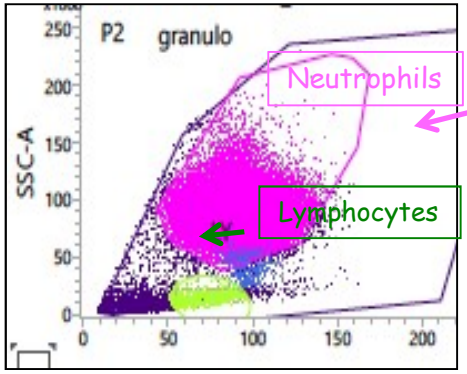
Uomo di 56 anni si presenta in PS con febbre. Tampone molecolare positivo per SARS- CoV2. Valori ematochimici evidenziano valori di PCR di 1,2 mg/dL; PCT di 0.41 ng/ mL e Globuli Bianchi pari a 3,9 * 10^3 /µL. Angio TAC con quadro di Ground Glass e TEP.

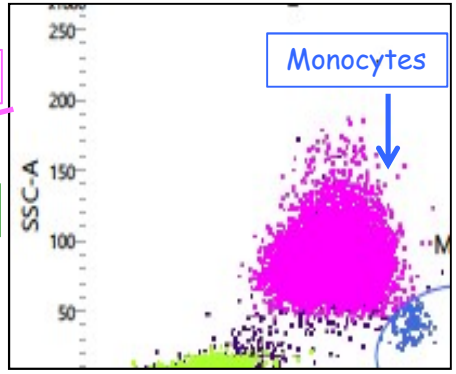
Eseguite emocolture, CD64 e HLADR in citofluorimetria a flusso
Valori di neCD64 ratio pari a 15,7 Valore di moHLADR pari a 12.122 MFI .

Somministrata terapia antibiotica ad ampio spettro.
A distanza di 48 ore emocolture positive per *Serratia marcenens*.

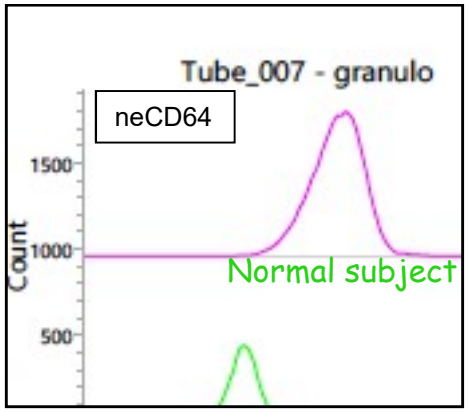
Dopo 5 giorni osservata una significativa riduzione di neCD64 ratio pari a 4,9 con un generale miglioramento del quadro clinico

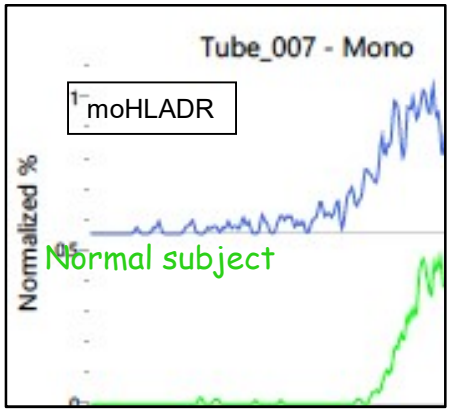
Caso clinico con esito favorevole





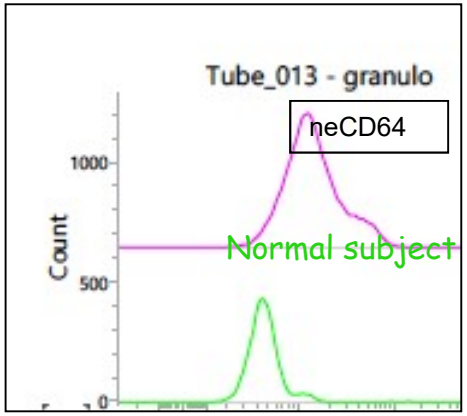
neCD64 ratio: 3095MFI/ 200 MFI: **15.5**
(valori normali <3)





Valore di moHLADR pari a **12.122 MFI**
(valori normali >25.000 MFI).

Dopo 5 giorni



neCD64 ratio: 1299MFI/ 265 MFI: **4.9**
(valori normali <3)

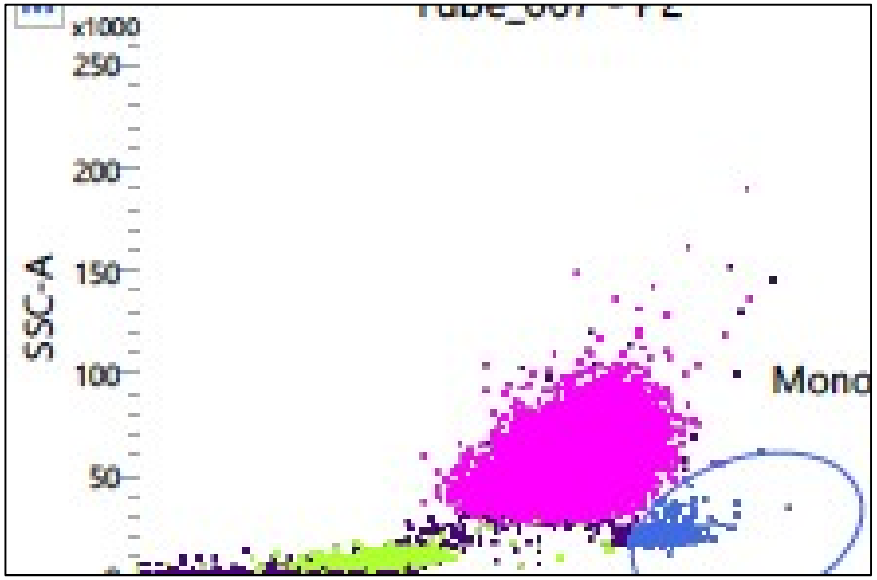
- **Caso clinico con esito sfavorevole**

Uomo di 67 anni cardiopatico e iperteso si presenta in PS con febbre. Tampone molecolare positivo per SARS- CoV 2.
Dopo 10 giorni dal ricovero in Terapia Intensiva per dispnea ingravescente si riscontrano valori ematochimici suggestivi per quadro settico con PCR di 34 mg/dL; PCT di 16 ng/ mL e Globuli Bianchi pari a 12 * 10^3 /µL.

Eseguite emocolture, CD64 e HLADR in citofluorimetria a flusso. Valori di neCD64 ratio pari a 11.5. Valore di moHLADR severamente ridotto (327 MFI).
Protocollo CVC positivo per *Staphilococcus capitis* con tempo di positività di 49-50 ore.

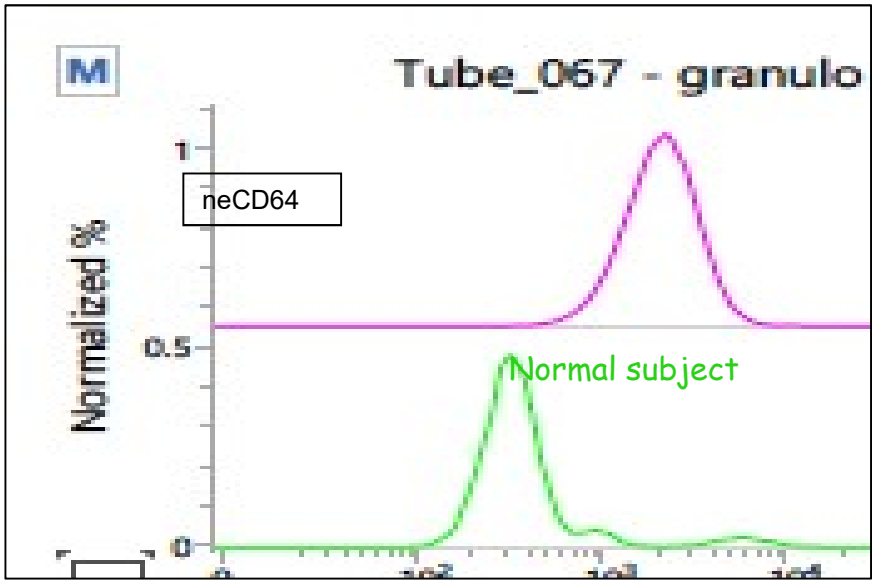
Dopo 24 ore morte per Multiorgan Failure

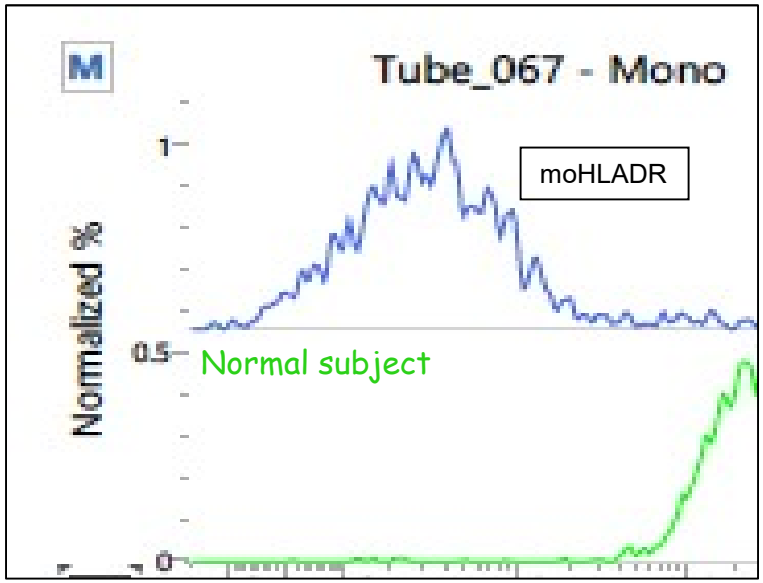
Caso clinico con esito sfavorevole



neCD64 ratio: 2172MFI/ 189 MFI: **11.5**
(valori normali <3)

Valore di moHLADR pari a **327 MFI**
(valori normali >25.000 MFI).





Caso clinico shock settico

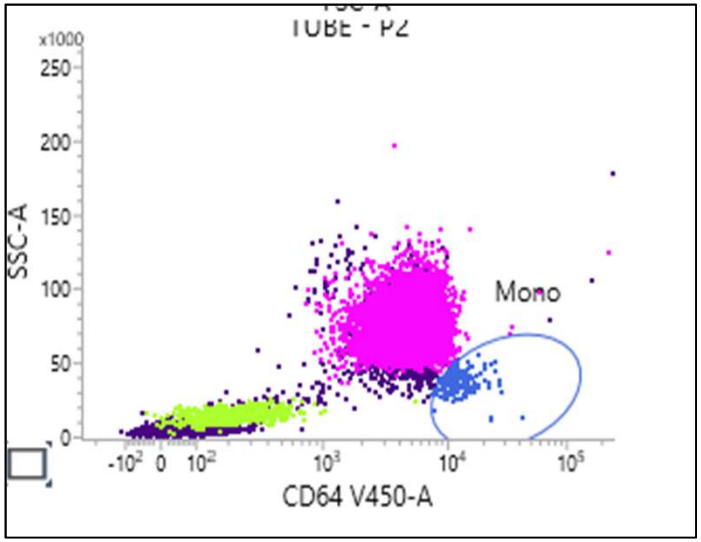
Uomo di 88 anni cardiopatico in shock settico.
Valori ematochimici : PCR di 15.7 mg/dL; PCT di 35.2 ng/ mL e Globuli Bianchi pari a 21.1 * 10^3 /µL.

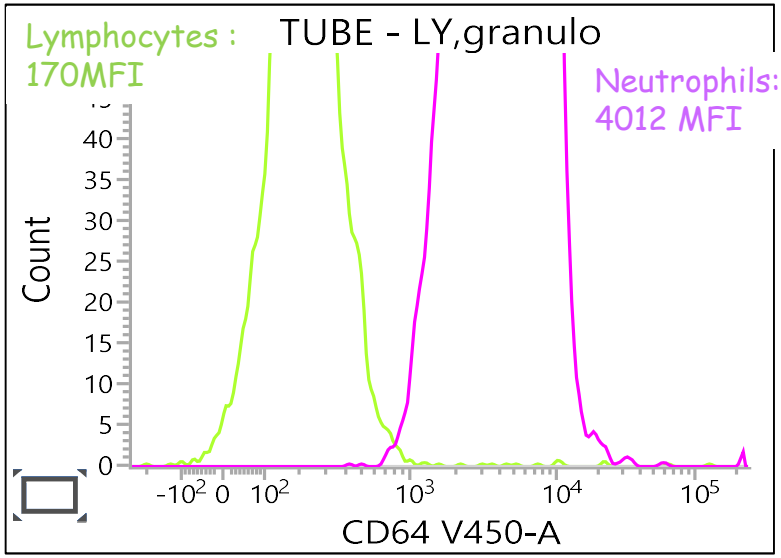
Eseguite emocolture, CD64 e HLADR in citofluorimetria a flusso.

Valori di neCD64 ratio pari a 23.6. Valore CD64 quantibrite:6612 ABC; Valore di moHLADR: 4639 MFI

Emocolture positive per *Streptococcus mitis* con tempo di positività di 8-9 ore.

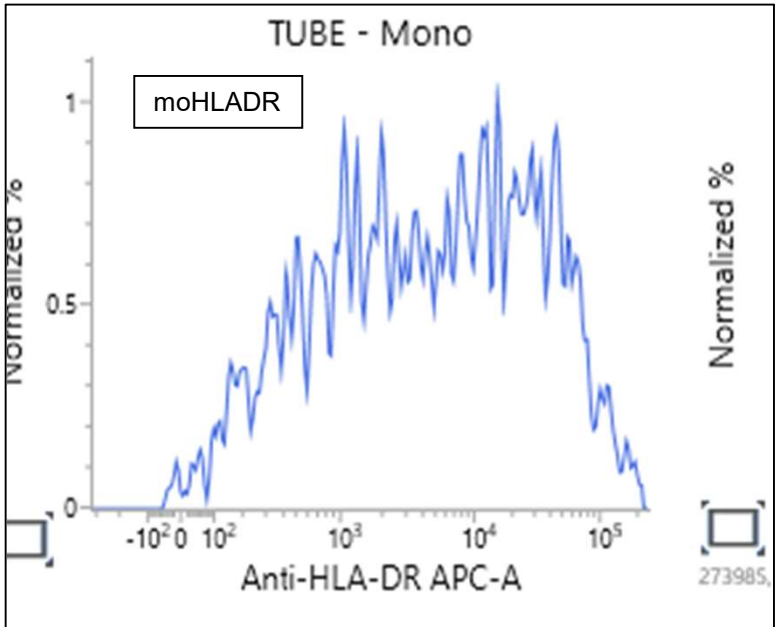
Caso clinico shock settico



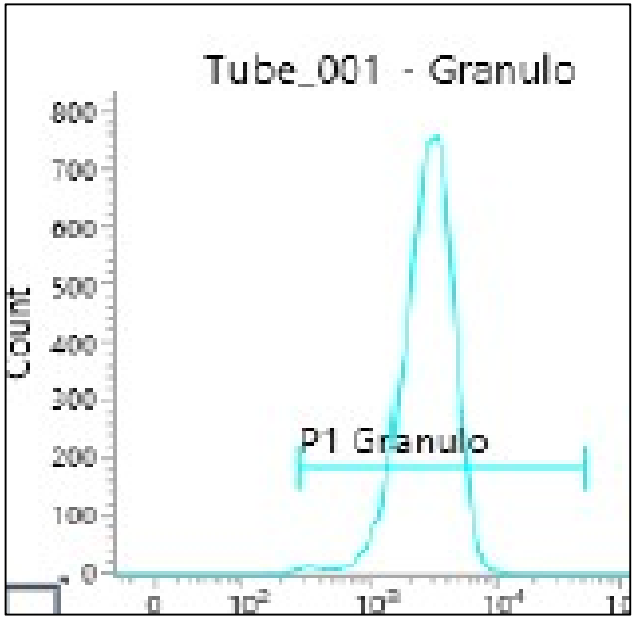


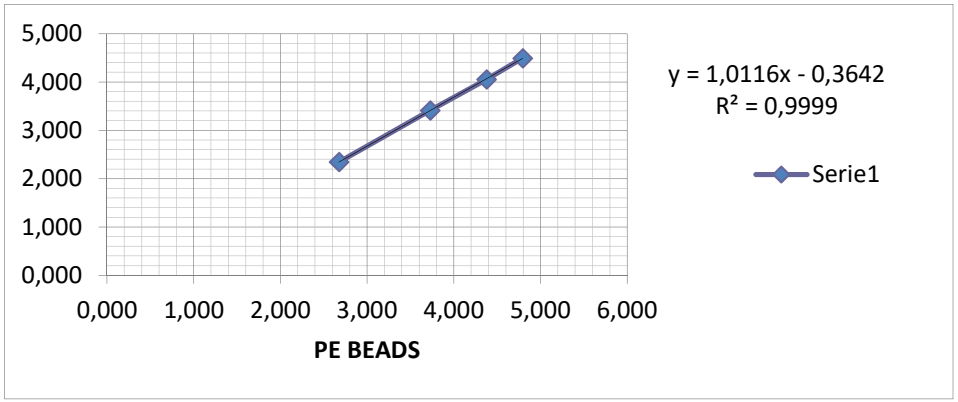
neCD64 ratio: 4012MFI/ 170 MFI: **23.6**
(valori normali <3)

Valore di moHLADR pari a **4639 MFI**
(valori normali >25.000 MFI).



Caso clinico shock settico





CD64 PE MFI neutrofilo: 2886 MFI
CD64 Quantibrite: 6612 ABC
(valori normali <1000 ABC)

Expression of Cellular Markers in the Immune Monitoring of Sepsis – A Practical Guide

Granulocyte CD64 (ABC Units)	neCD64 ratio	Monocyte HLA-DR	Diagnostic Clues
Slightly Increased (< 2500 ABC)	Slightly Increased (<10)	Normal	Infection, Immunocompetent Host
Increased (> 2500 ABC)	Increased (> 10)	Normal	Bacterial Sepsis Likely Immunocompetent Host
Increased (> 2500 ABC)	Increased (> 10)	Reduced	Bacterial Sepsis Likely Immunocompromised Host
Variable	Variable	Reduced/Absent	Anergic State, follow-up Immunocompromised Host

THANK YOU
for your
ATTENTION!