SEPSIS: Did you know?

The Clinical and Lab Approach

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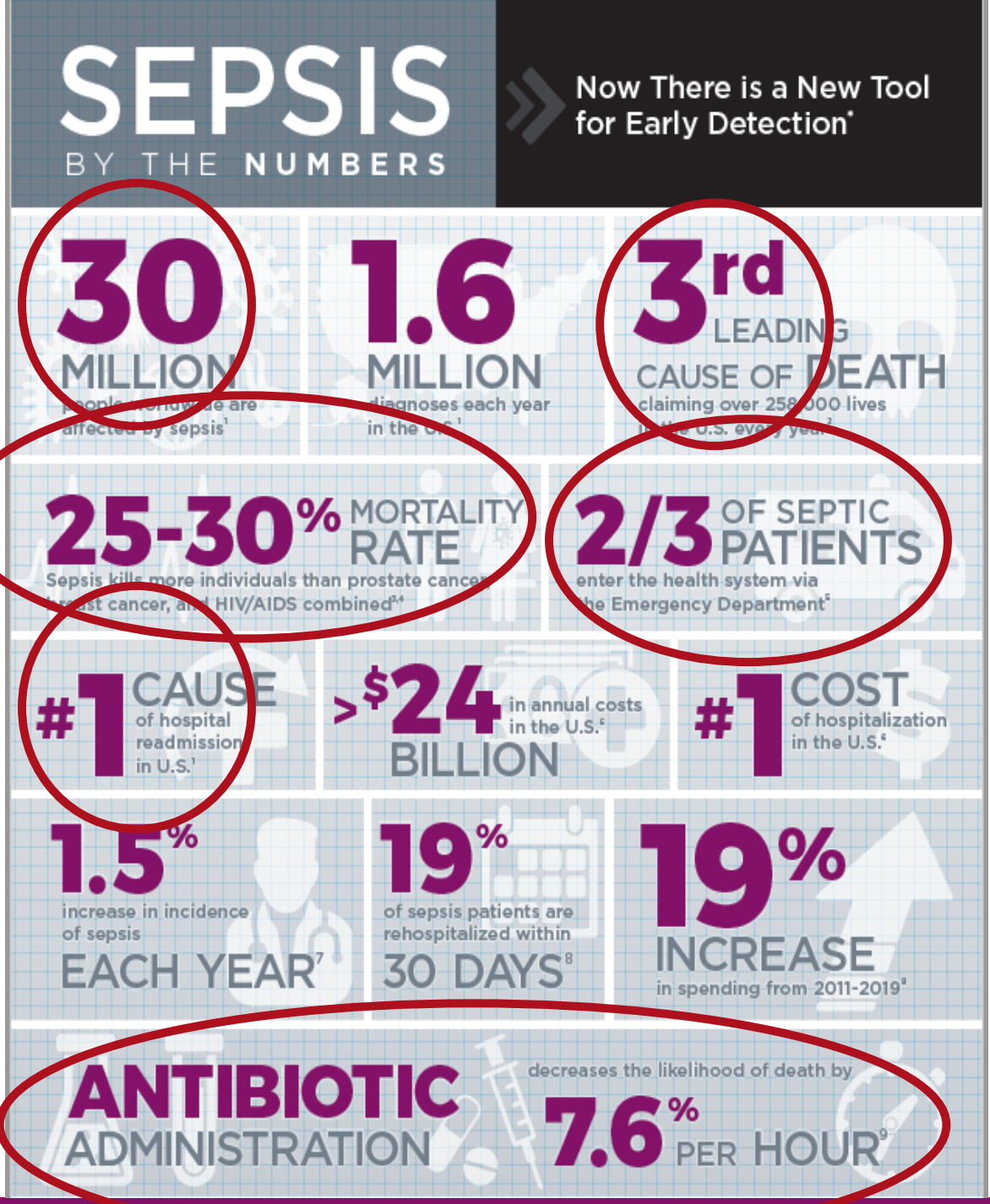
Objectives

To understand:

- The costly healthcare burden of sepsis
- Basic physiology of sepsis
- The challenges to rapidly diagnose sepsis
- The role of the laboratory in the diagnosis and management of sepsis

What is Sepsis?

A life-threatening **organ dysfunction** caused by a dysregulated host response to infection



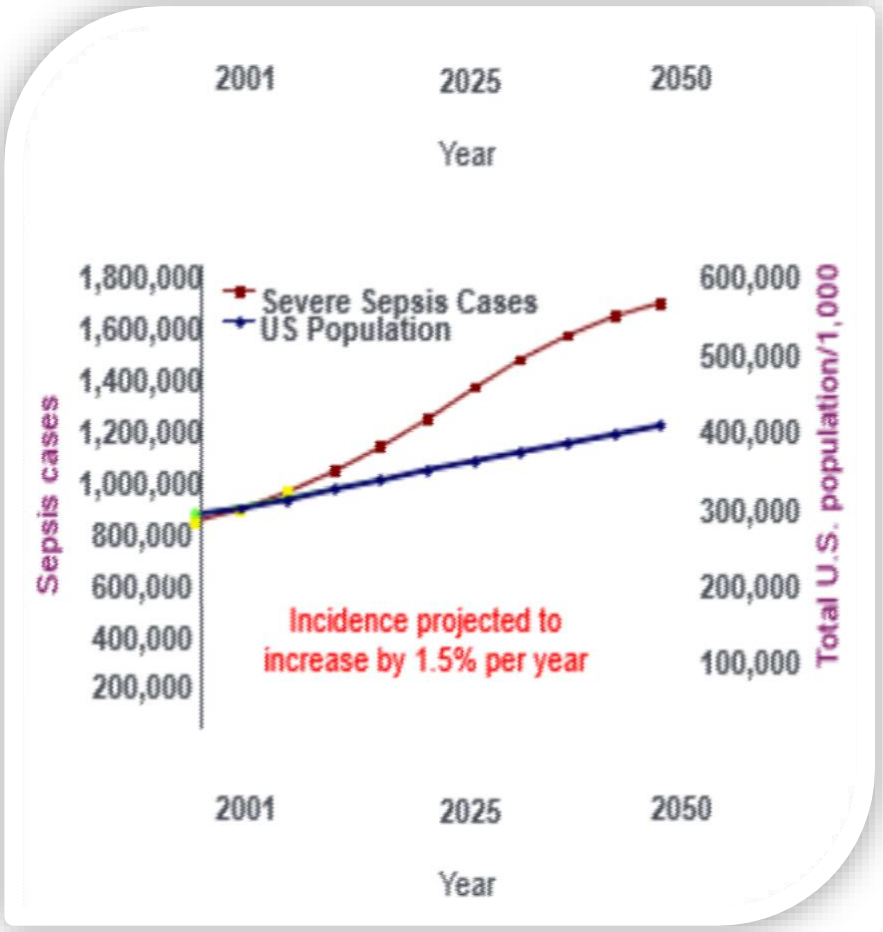
Singer M et al. "The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)." *JAMA*. 2016;315 (8):801-810).

*A, Roberts D, Wood KE et al. "Duration of Hypotension Before Initiation of Effective Antimicrobial Therapy is the Critical Determinant of Survival in Human Septic Shock."Crit Care Med, vol. 34. 2006, pp. 1589–96.

Sepsis: Its Impact on Healthcare

Increasing incidence of sepsis is due to:

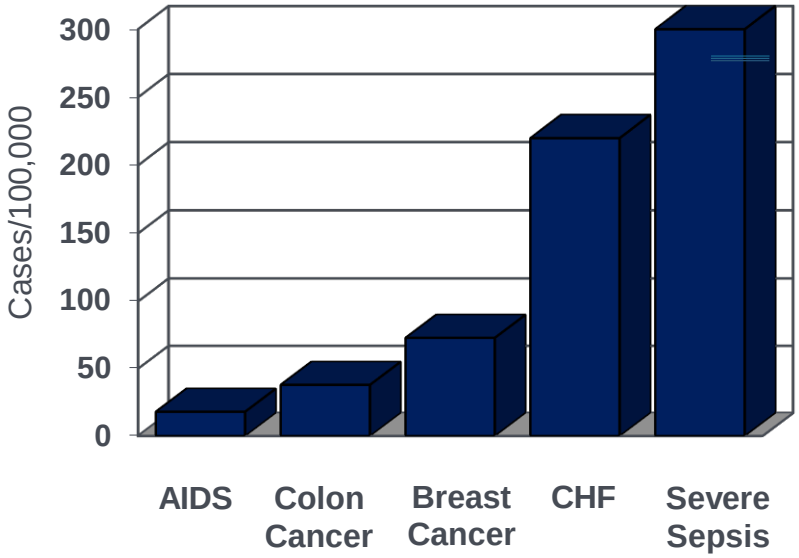
- Increased awareness and tracking
- Aging population
- Longevity of people with chronic Diseases (co-morbitdies)
- Antibiotic-resistant organisms
- Increase in invasive procedures
- Broader use of immunosuppressive and chemotherapeutic agents



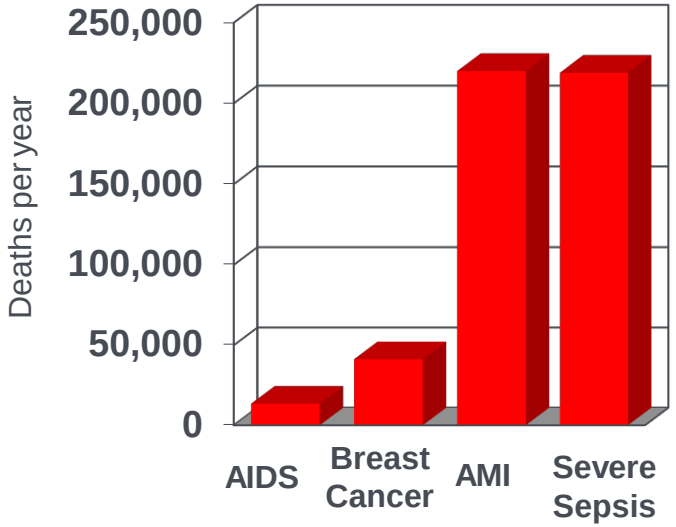
Source: Sepsis Review, Expert Rev Anti Infect Ther 2012; June 10-6 701-706.
CDC - Surviving Sepsis campaign
www.nigms.nih.gov/education/pages/factsheet_sepsis.aspx

Comparison With Other Major Diseases

Incidence of Severe Sepsis



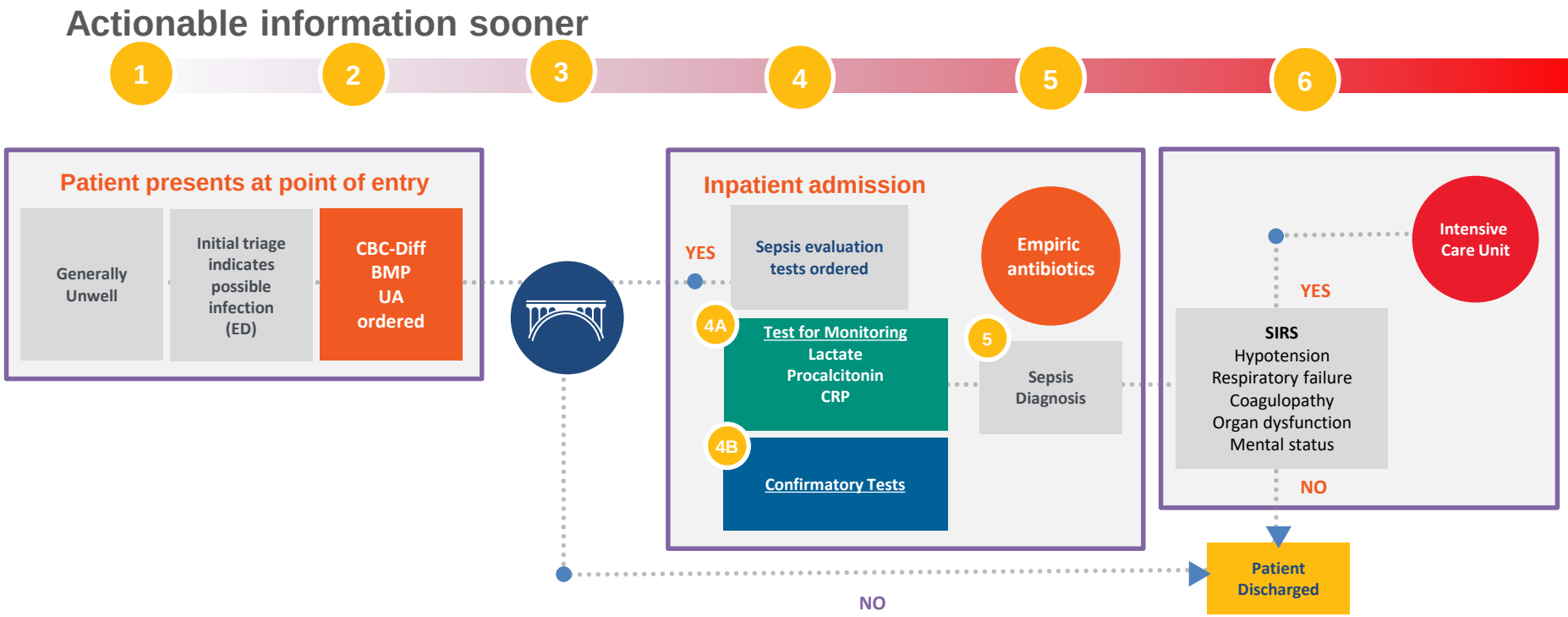
Mortality of Severe Sepsis



- 30%-50% of severe sepsis patients die
- More than deaths from prostate cancer, colon cancer, breast cancer and AIDS ***combined***

Source: CDC - Surviving Sepsis campaign: www.nigms.nih.gov/education/pages/factsheet_sepsis.aspx

Bridging the Care Gap



Challenges to Identifying Sepsis

SIRS is not specific to sepsis, making “well looking” patients difficult to identify

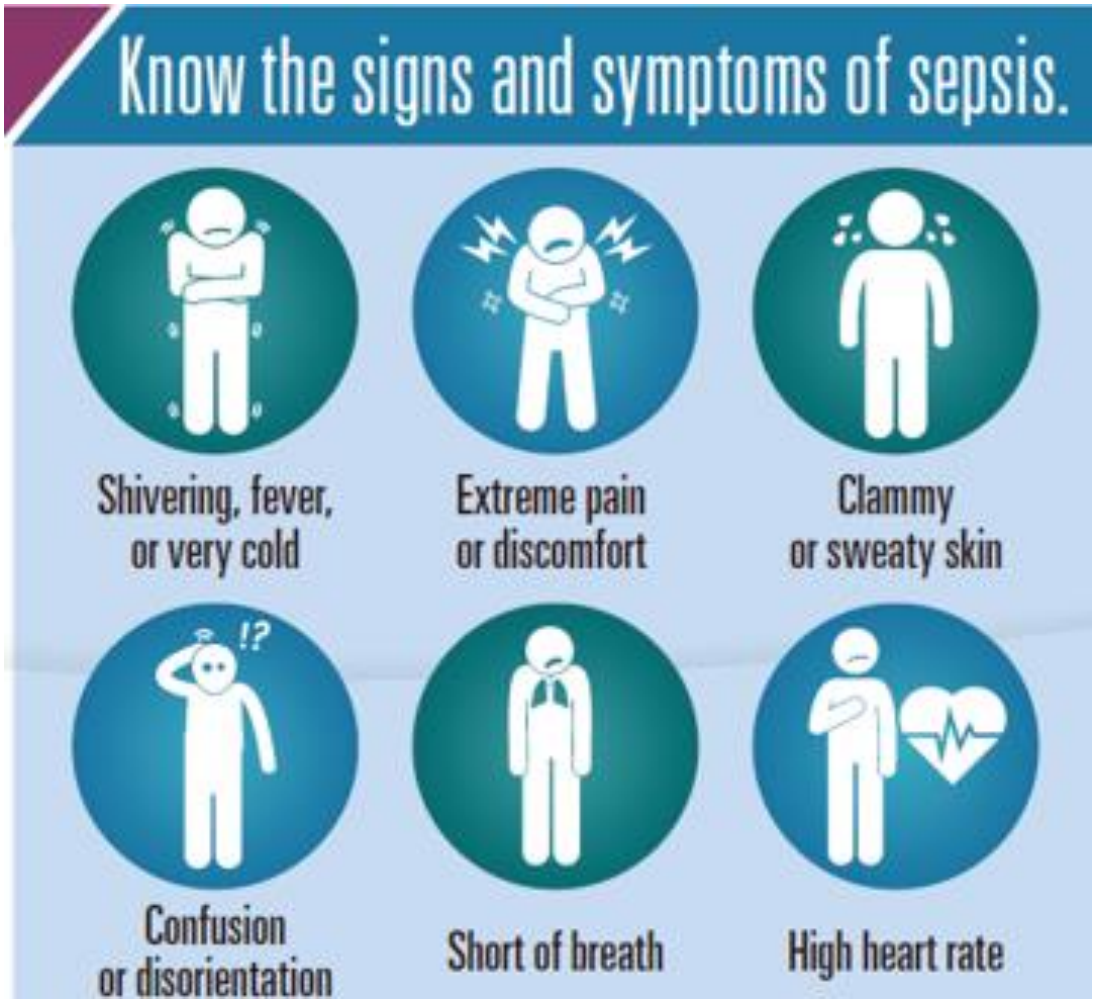
Temperature >38.3ºC, or <36ºC

Heart rate >90 bmp

Respiratory rate >20 bpm

White cell count <4 or >12 x 10^3/mL

New **altered mental state**



SIRS = systemic inflammatory response syndrome

Source: www.cdc.gov/vitalsigns/sepsis

Alycia K.

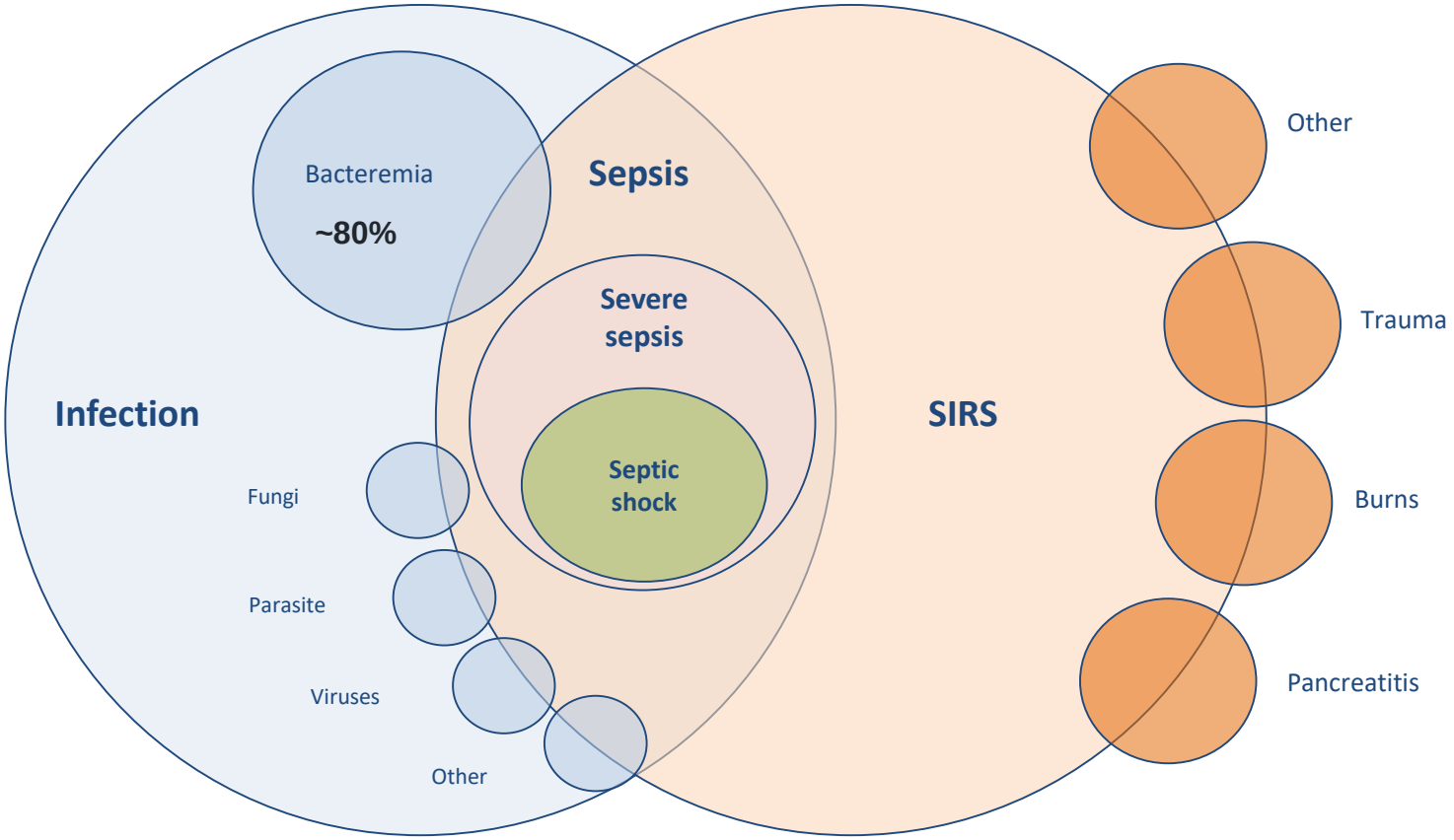
- **Multiple physician visits**
  - Primary Doctor x2
  - Urgent Care
  - Telemedicine nurse consult
  - Emergency Room
- **Multiple diagnoses/test**
  - Sinus Cold
  - Flu test, chest X-ray
  - Mononucleosis
  - Pneumonia
  - **Sepsis**
    - Mixed Viral and Bacterial
- **15 Months plus, recovery**

"It has been made brutally clear that there is not enough knowledge or awareness out there about sepsis and that needs to change, and I hope to be a part of that change…"

SEPSIS ALLIANCE
Suspect Sepsis. Save Lives.

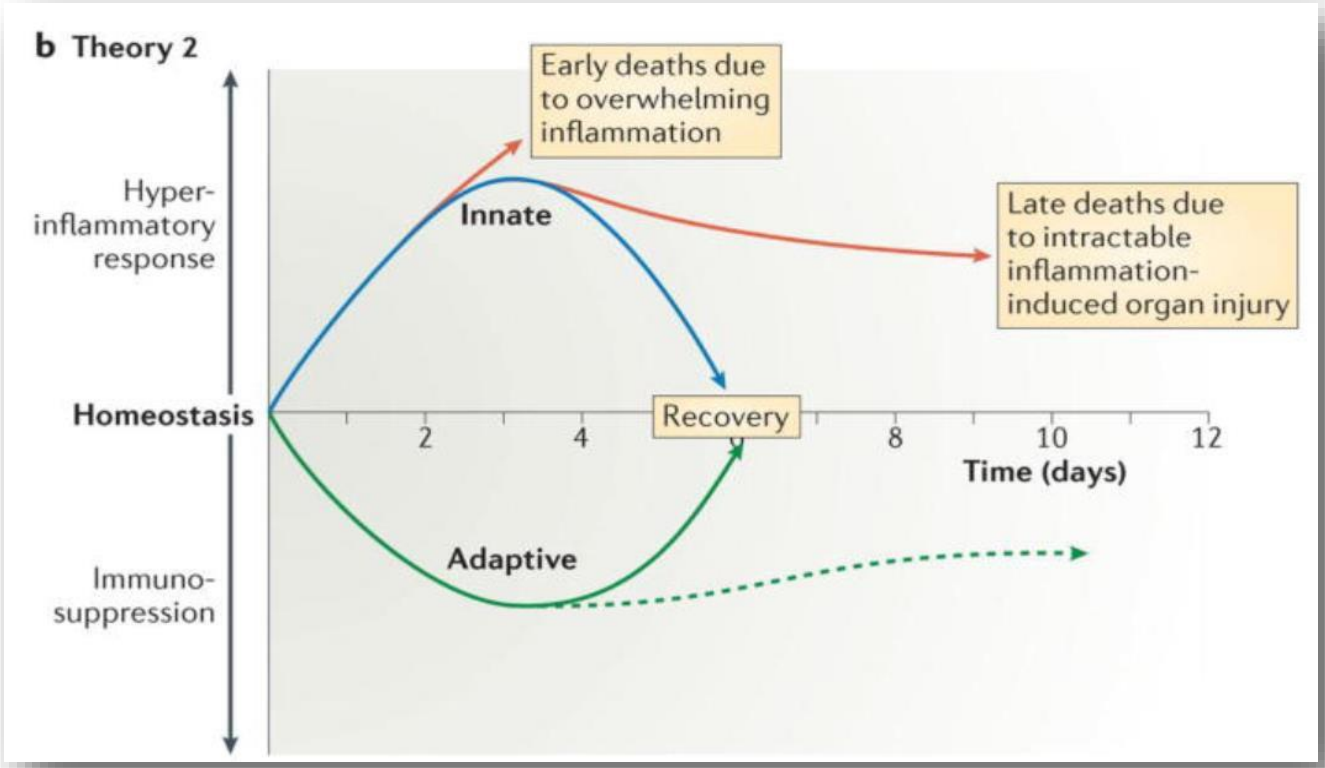
https://www.sepsis.org/faces/alycia-k/

Sepsis: The Intersection of Infection and SIRS



Source: http://emedicine.medscape.com/article/168943-overview
https://www.researchgate.net/figure/Criteria-for-Systemic-Inflammatory-Response-Syndrome-SIRS-Adapted-from-McClelland-H_fig2_306927533

Hypothesis Posits That Immunosuppression Occurs Concurrently to the Cytokine Storm



Richard S. Hotchkiss, *Nat Rev Immunol*. 2013 December; 13(12): 862–874

…"we hypothesize that protracted sepsis is predominantly characterized by systemic immunosuppression leading to a failure to eradicate primary infections and acquisition of lethal secondary infections."

Sepsis Can Affect Major Organs

Sources of sepsis	
Respiratory	38%
Urinary tract	21%
Intra-abdominal	16.5 %
Others	11.3%
CRBSI	2.3%
Device	1.3%
CNS	0.8%

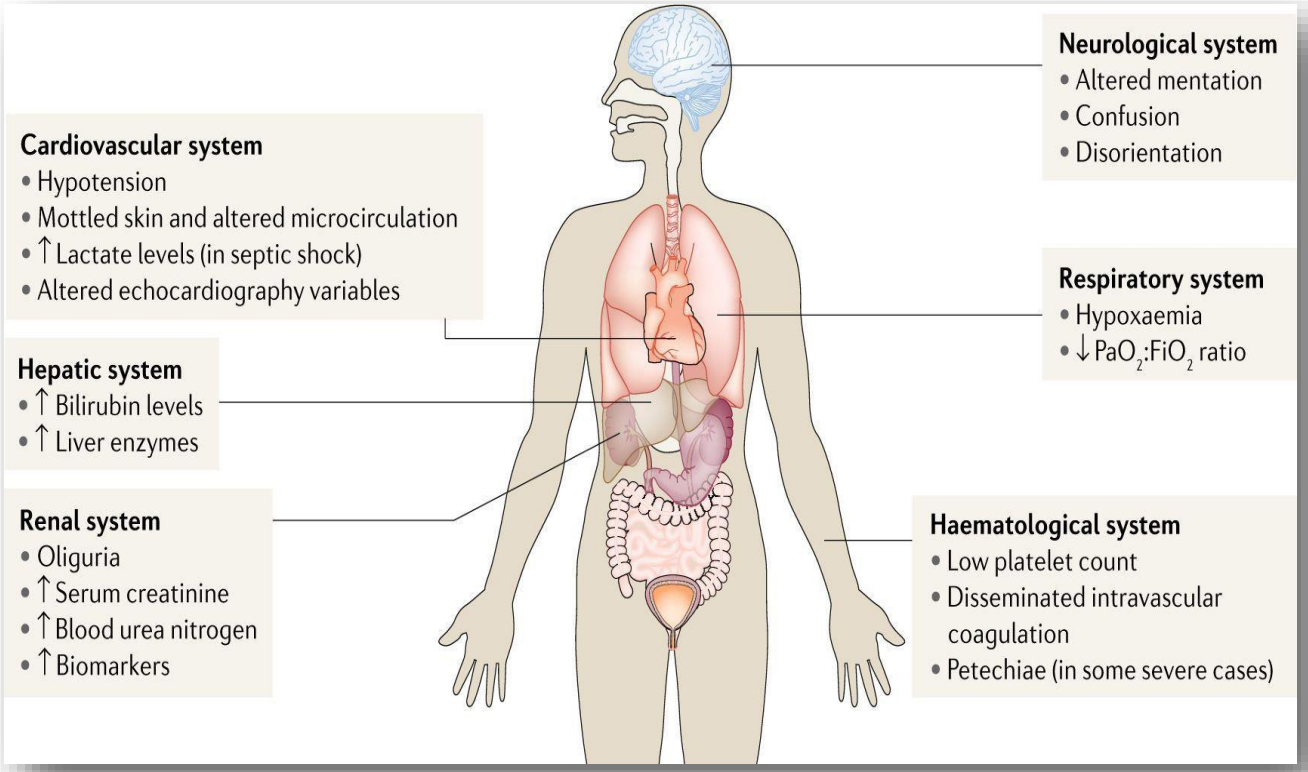


Image from: https://www.nature.com/articles/s41581-018-0005-7

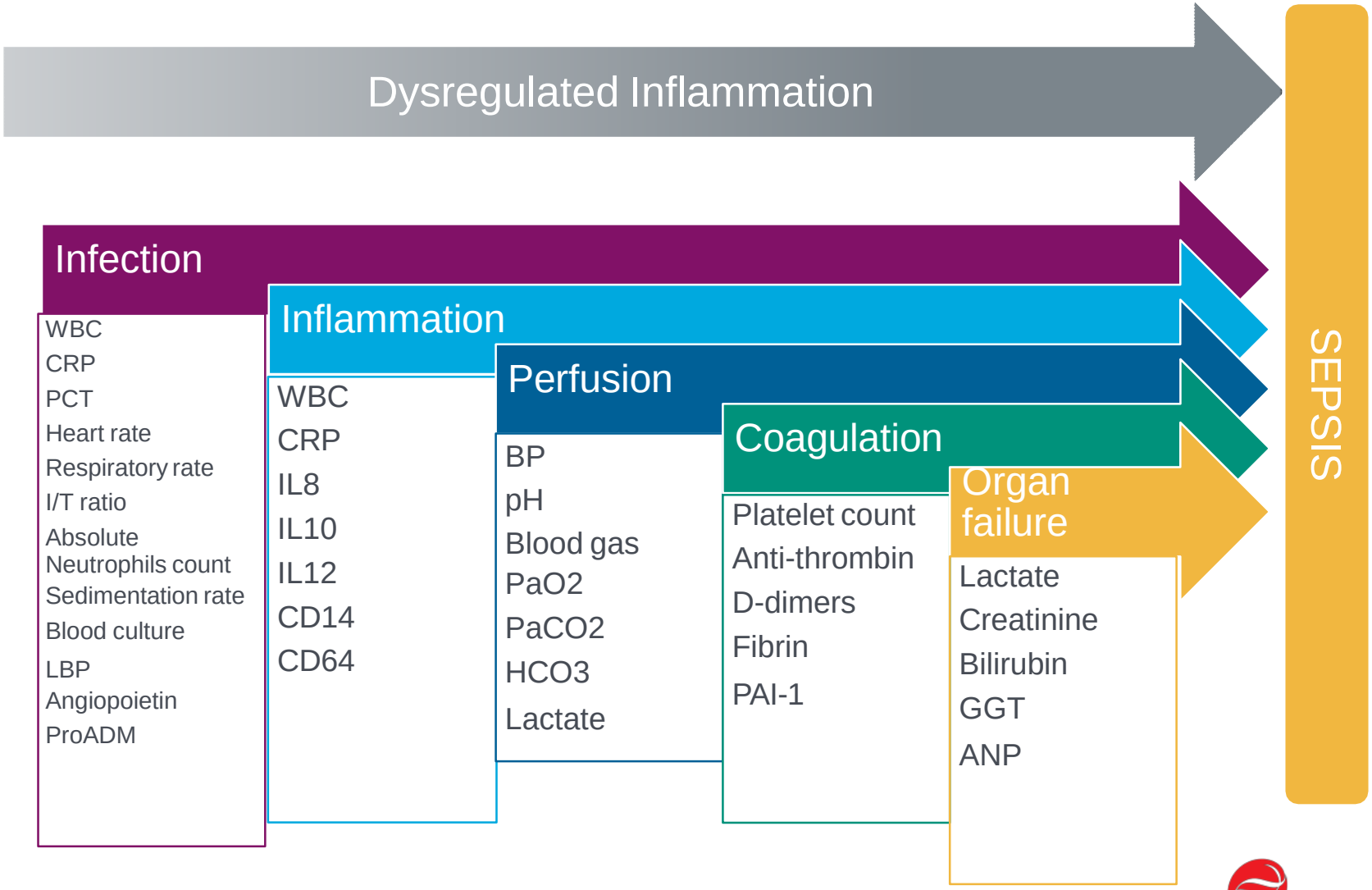
The more organs affected, the higher the risk of death

The Clinical Laboratory in the Management of Sepsis

- Key challenges
  - Early diagnosis with better sensitivity than current tests
  - Discrimination between infection and other causes of SIRS
  - Monitoring antibiotic therapy
  - Discriminate bacterial from viral infection

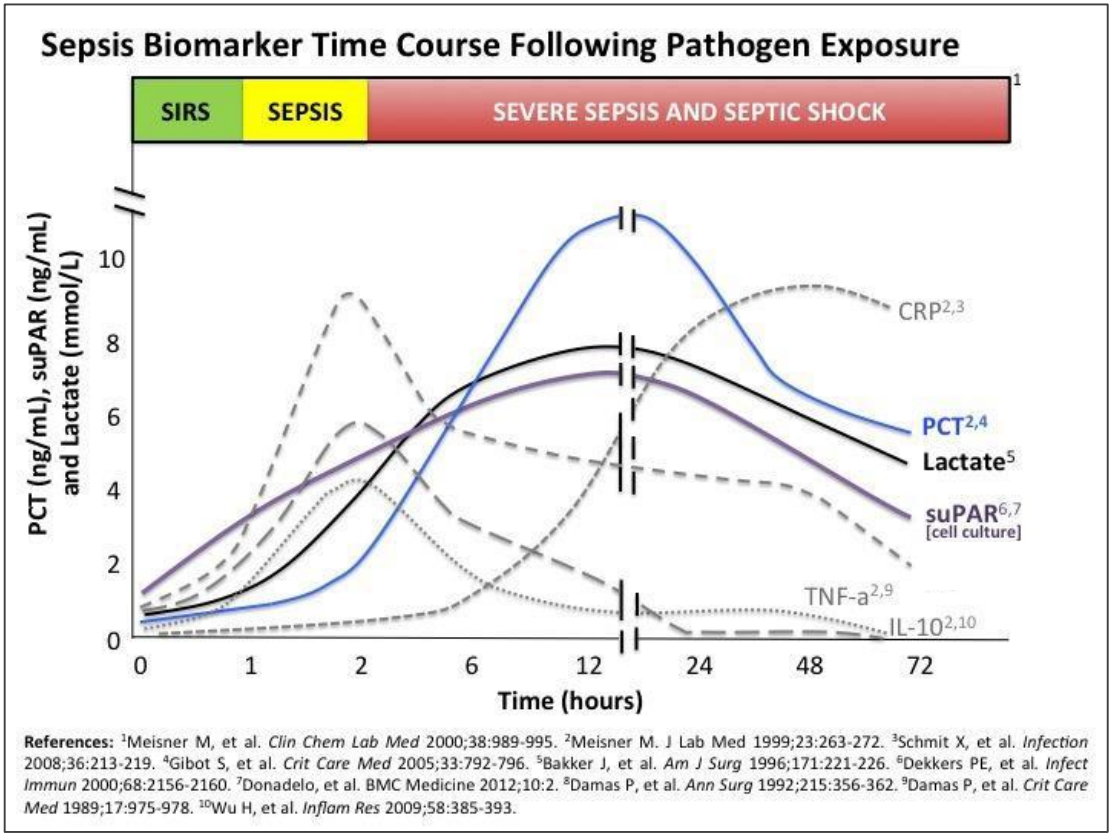
- Lab tests for these uses are less likely to impact the current standard of care
  - Confirming diagnosis – treatment starts regardless, gold standard for confirmation is the culture
  - Prognostication – therapy will be aggressive regardless

Biomarkers of Sepsis



Other Current Key Biomarkers for Sepsis

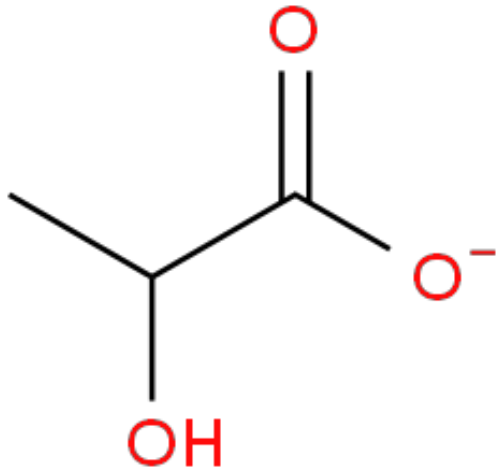
- Lactate
- Procalcitonin
- C-reactive Protein
- WBCs
  - elevated count
  - immature forms
  - leukocyte morphology
- Other: IL-10, TNF-α



References: [1]Meisner M, et al. *Clin Chem Lab Med* 2000;38:989-995. [2]Meisner M. J Lab Med 1999;23:263-272. [3]Schmit X, et al. *Infection* 2008;36:213-219. [4]Gibot S, et al. *Crit Care Med* 2005;33:792-796. [5]Bakker J, et al. *Am J Surg* 1996;171:221-226. [6]Dekkers PE, et al. *Infect Immun* 2000;68:2156-2160. [7]Donadelo, et al. BMC Medicine 2012;10:2. [8]Damas P, et al. *Ann Surg* 1992;215:356-362. [9]Damas P, et al. *Crit Care Med* 1989;17:975-978. [10]Wu H, et al. *Inflam Res* 2009;58:385-393.

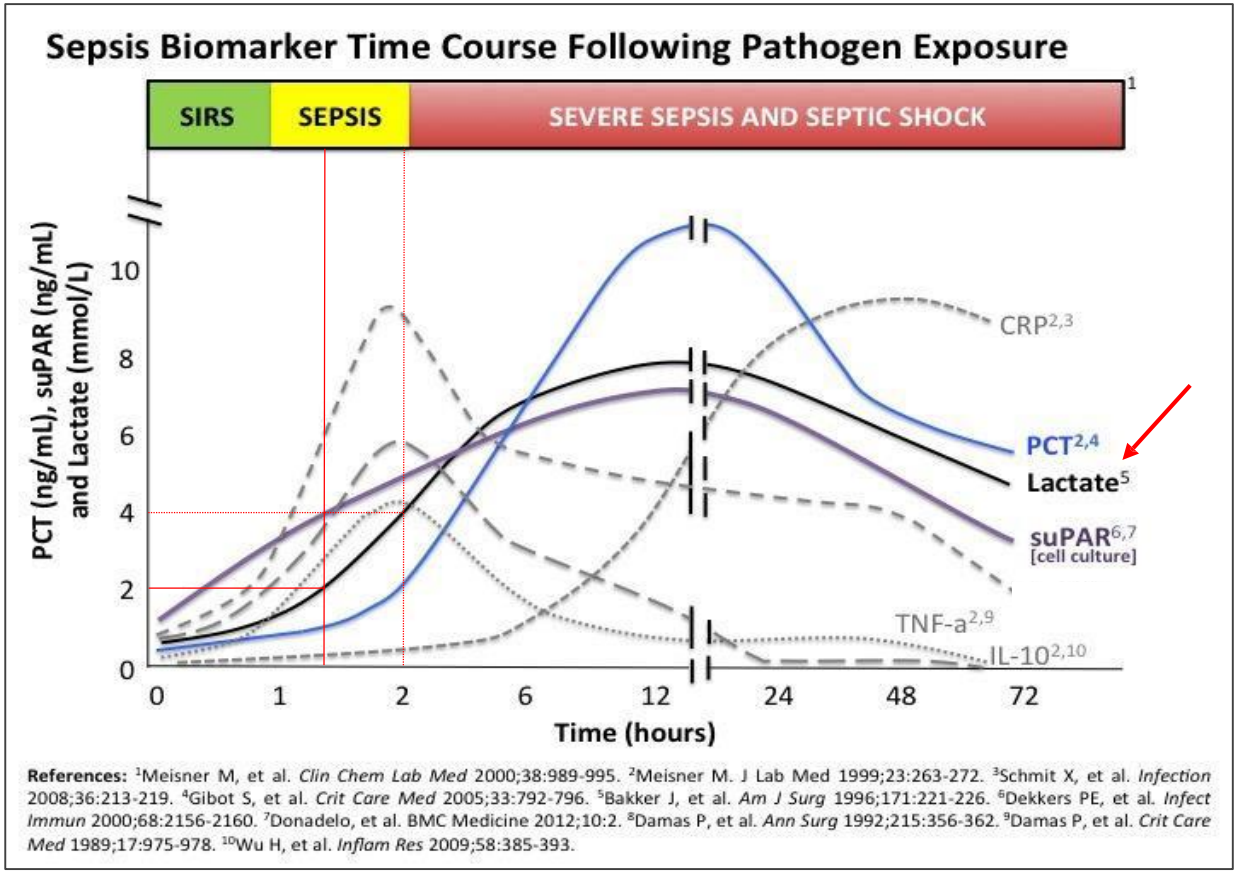
Image from: https://blog.ucdmc.ucdavis.edu/labbestpractice/index.php/laboratory-best-practice-blog-author-guidelines/

Lactate



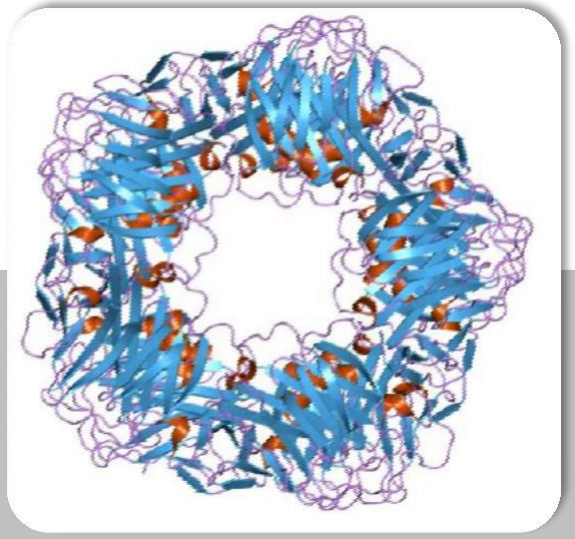
- Lactate is a byproduct of anaerobic metabolism from pyruvate
- With insufficient oxygenation, cells and tissues move from aerobic metabolism to anaerobic metabolism
- Used as a measure of tissue perfusion (oxygenation of tissues) irrespective of blood pressure

Lactate

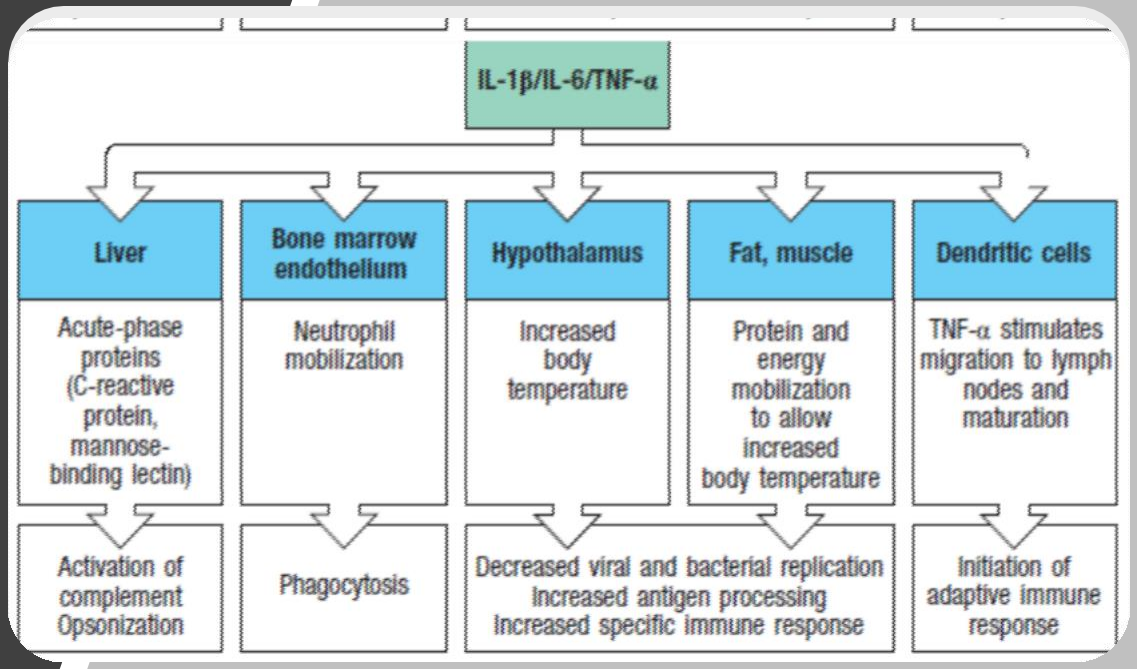


- Cutoff was reduced to **>2 mmol/L** from >4 mmol/L
- Mortality is positively correlated in septic patients with lactate >2 mmol/L

C-reactive Protein



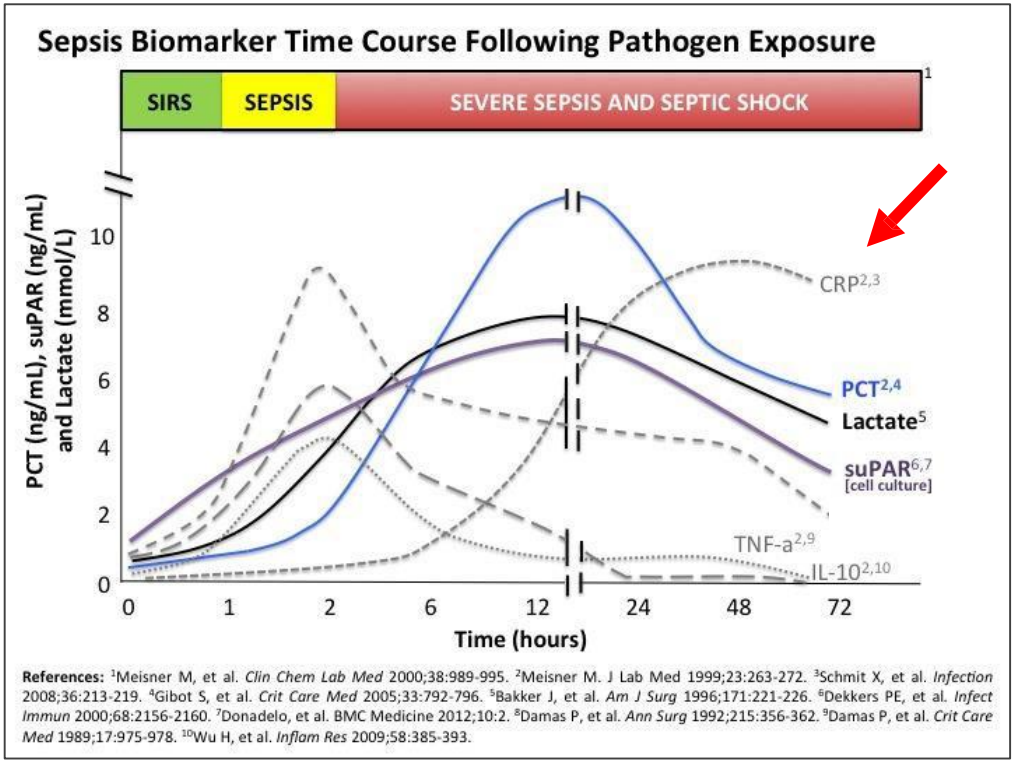
- Macrophages secrete ILs which stimulate the liver to initiate the acute-phase response and produce CRP
- Binds to phosphocholine, for uptake by phagocytes
- Bacteria binding to CRP can activate the complement cascade



Janeway's Immunology, 9th edition

C-reactive Protein

- Maximum production at 24-38 hours after the onset of inflammation
- The concentration of CRP in healthy subjects is **<5mg/l**
- Used to distinguish **viral** and bacterial infections.
- CRP is not a specific parameter for the presence of infectious inflammation



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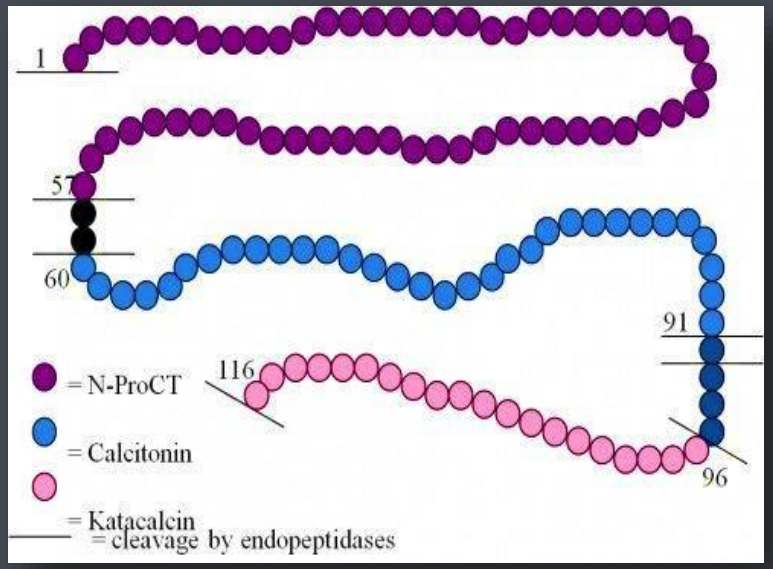
Procalcitonin



A prohormone of calcitonin

In physiological conditions, calcitonin is secreted by parafollicular cells of the thyroid.

In sepsis, the main producers of PCT are macrophages and monocytic cells of different organs, especially liver.

Normal value PCT <0.05 ng/mL

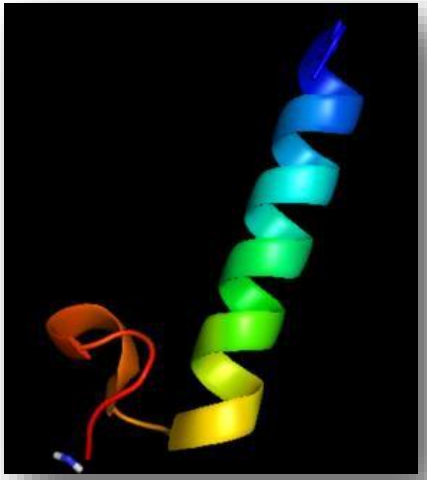
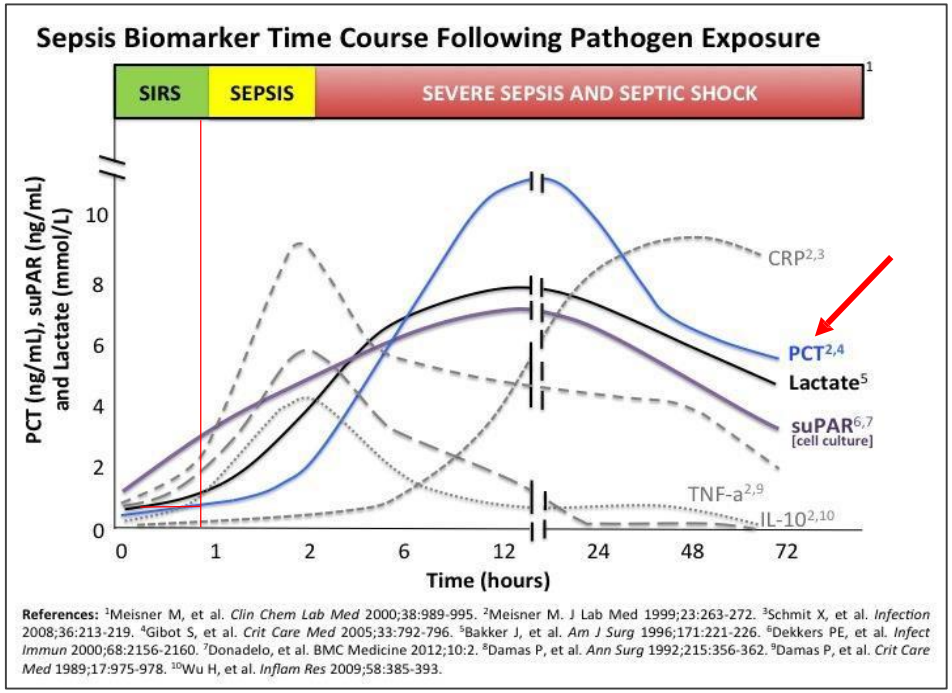


Image from: https://en.wikipedia.org/wiki/Procalcitonin

Procalcitonin

- Minimum elevation of PCT concentration in viral infections
- As stated in the previous slide normal value is < 0.05 ng/mL
- More specific and more sensitive than CRP, although
  - PCT is not specific for sepsis
  - PCT is not sensitive for patients with abscesses or fungal infections



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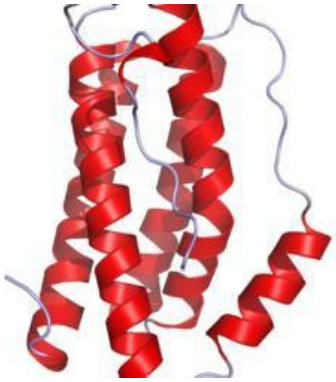
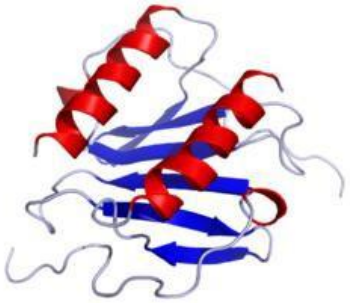
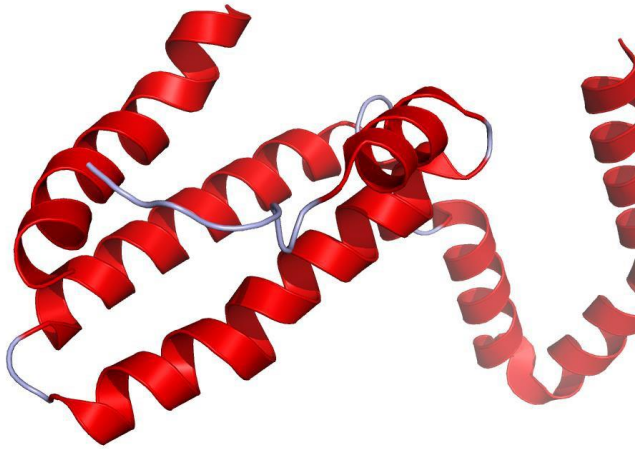
Cytokines

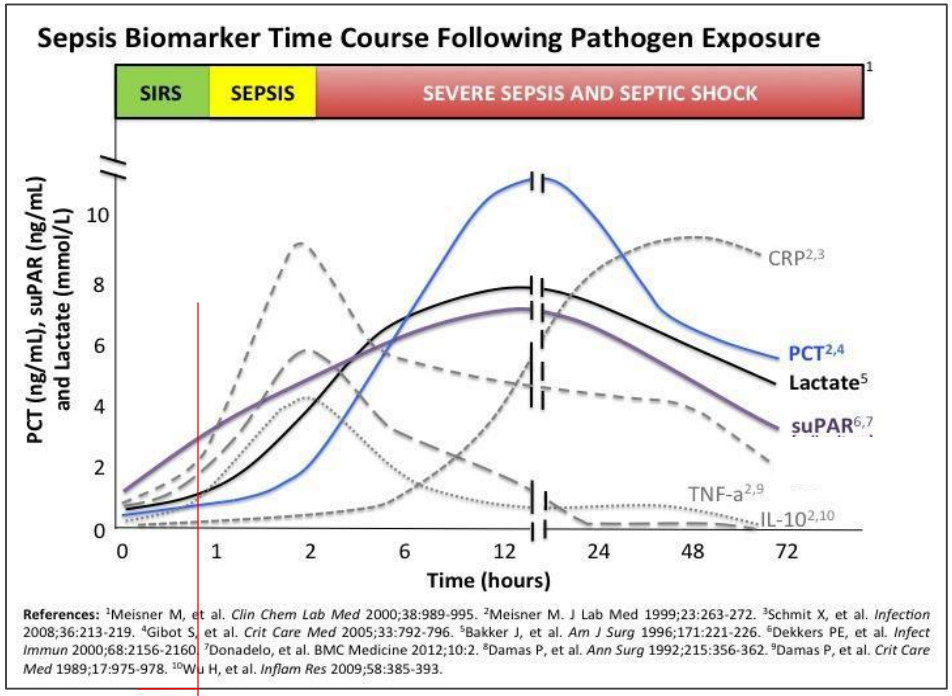
- IL-6 is produced by monocytes, fibroblasts, endothelial cells, keratinocytes, T-cells, and tumor cells.
  - Released into the bloodstream for 4–6 h, decreasing over the next 24–48 h.
- IL-8 produced by macrophages and endothelial cells.
- IL-10 is an anti-inflammatory cytokine produced by monocytes, macrophages, T and B cells, neutrophils and mesangial cells







Cytokines



- Measurements of CRP or PCT are more sensitive.
  - Elevated cytokine levels are **also seen in SIRS of noninfectious origin.**
- No studies which would prove that the treatment of sepsis based on these markers influences the treatment strategy or improves the clinical result.

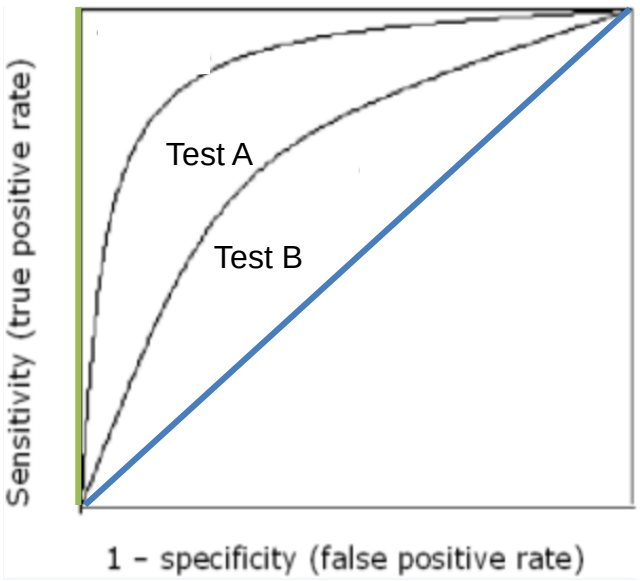
Current Biomarkers not Sufficient for Less Severe Sepsis

AREA UNDER THE CURVE FOR CURRENT BIOMARKERS

None of the current biomarkers reach AUC of 0.8 for sepsis of all severities

Monocyte Distribution Width (MDW) + WBC show increased value over WBC alone

Representation of a ROC Curve



AUC	Category
0.9-1.0	very good
0.8-0.9	good
0.7-0.8	fair
0.6-0.7	poor
0.5-0.6	fail

IG: not an adequate biomarker

Table 1
Odds ratio (OR), area under the receiver operating characteristic curve (AUC), optimal cut-off by receiver operating characteristic analysis, and sensitivity and specificity at optimal cut-off for prediction of sepsis. p value < 0.05 indicates that the odds ratio for prediction of sepsis was statistically different from 1.0. 95% CI indicate 95th percentile confidence intervals for selected values.

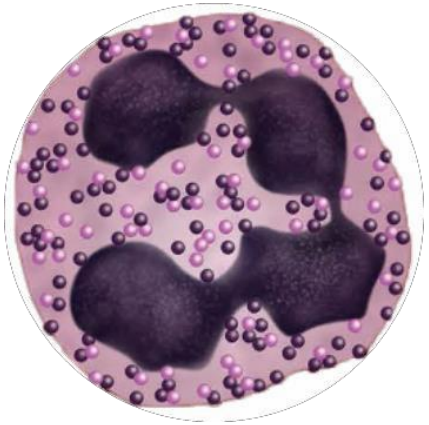
Biomarker	OR (95% CI)	p-Value	AUC (95% CI)	Optimal cut-off	Sensitivity (95% CI)	Specificity (95% CI)
Lactate	1.44 (1.20, 1.73)	< 0.0001	0.63 (0.58, 0.68)	1.3 mmol/L	55.1% (48.7, 61.4)	62.7% (56.8, 68.4)
Neutrophil #	1.10 (1.06, 1.14)	< 0.0001	0.63 (0.58, 0.68)	7.5 × 10^9/L	60.7% (54.3, 66.7)	60.7% (54.7, 66.3)
Neutrophil %	1.05 (1.03, 1.06)	< 0.0001	0.69 (0.64, 0.74)	79%	63.2% (56.9, 69.2)	63.3% (57.4, 68.9)
IG #	1.45 (0.64, 3.29)	0.37	0.61 (0.56, 0.66)	0.02 × 10^9/L	49.6% (43.2, 55.9)	67.3% (61.4, 72.7)
IG %	1.06 (0.96, 1.19)	0.26	0.60 (0.55, 0.64)	0.2%	45.7% (39.5, 52.1)	67.0% (61.2, 72.4)
WBC	1.04 (1.01, 1.07)	0.004	0.59 (0.54, 0.64)	9.7 × 10^9/L	57.7% (51.3, 63.8)	57.7% (51.7, 63.5)
Procalcitonin	1.01 (0.99, 1.04)	0.28	0.68 (0.63, 0.72)	0.20 ng/mL	63.0% (56.4, 69.1)	63.0% (56.8, 68.8)

method for detecting immature granulocytes (promyelocytes, myelocytes, metamyelocytes) compared to peripheral smear review [9]. In this study we evaluated the diagnostic utility of conventional sepsis biomarkers: lactate, PCT, WBC, neutrophil count, and IG; for the prediction of sepsis in ED patients.

were calculated for the AUC, along with 95% CI on the odds ratio [10]. Statistical significance of the odds ratio was defined as $p < 0.05$. Sensitivity and specificity confidence intervals were calculated using the method of Agresti [11]. AUC analysis was performed with SAS, version 9.4; and recursive partitioning utilized the rpart function and

Karon BS, et al., Clinical Biochemistry 50 (2017) 956–958

Innate Immune System Cells



Neutrophils

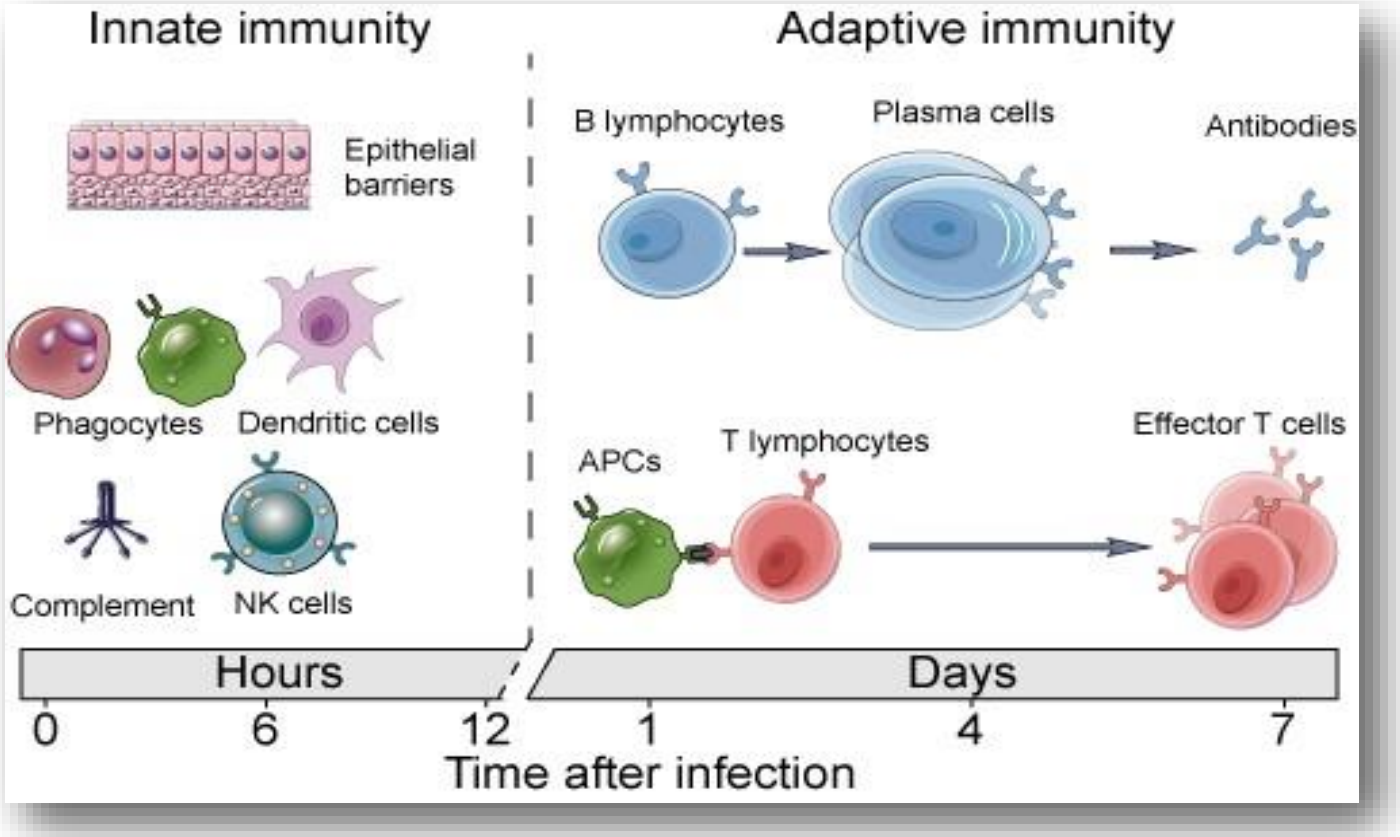
- Phagocytosis
- Neutrophils contain granules that release enzymes to help kill and digest bacteria

Key Functions In Infection

Monocytes

- Phagocytosis
- Ag presentation
- Cytokine production
- Activation of the acquired immune system

Innate Immune System – First Line of Defense



- **Mechanical and chemical barriers**
- **Protein in the blood**
- **Cells**

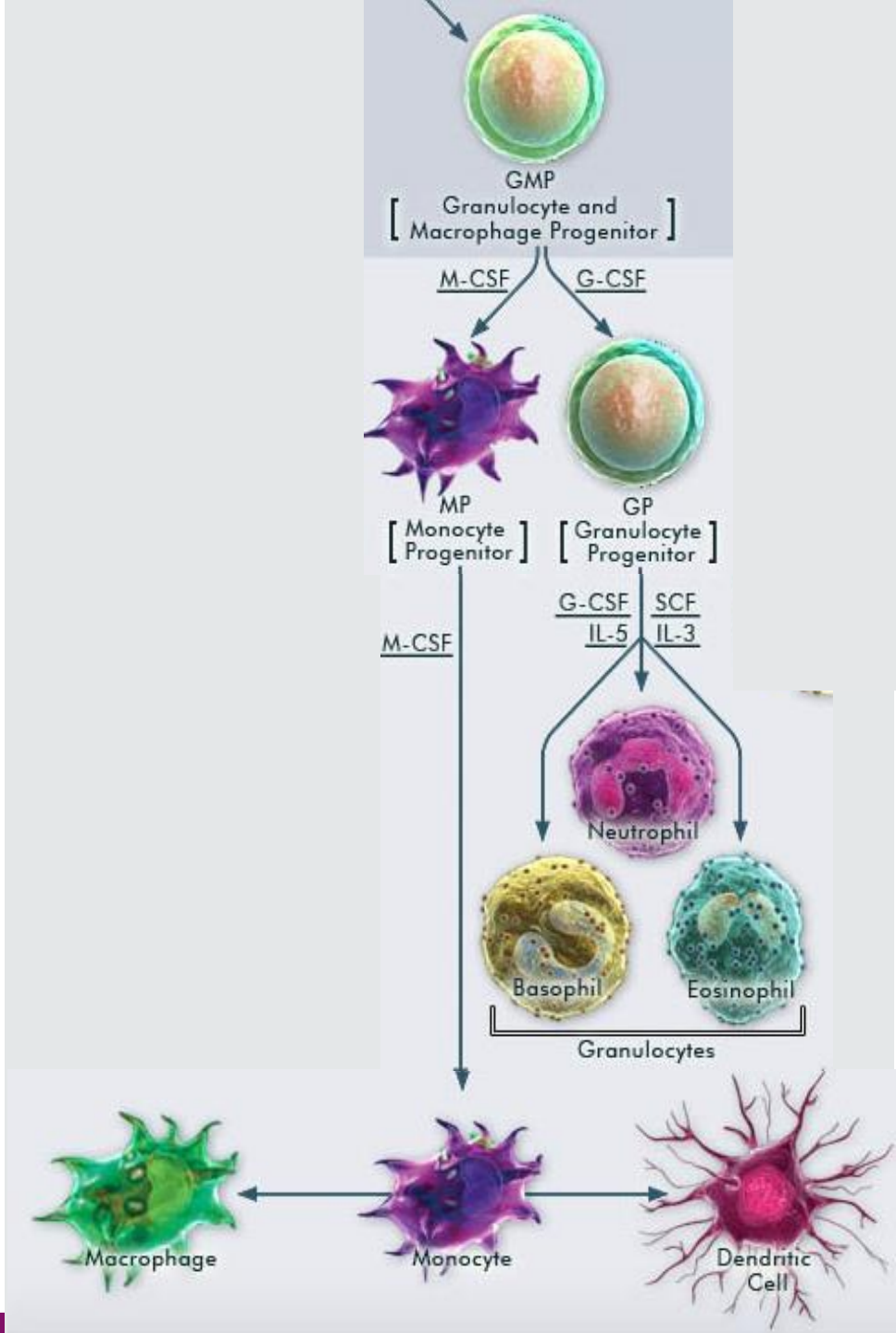
Image from: https://www.creative-diagnostics.com/innate-and-adaptive-immunity.htm

What Are Monocytes?

Monocytes can:

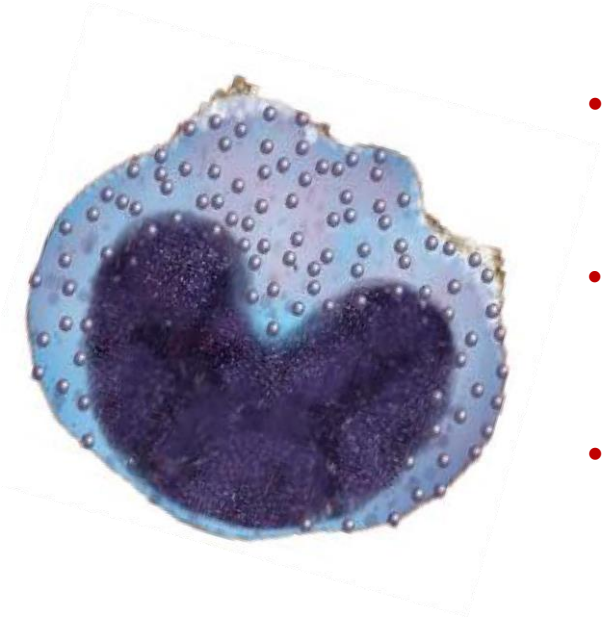
- Migrate to and from the blood, bone marrow and tissue
- Stored in bone marrow or spleen
- Further differentiate to macrophages or myeloid dendritic cells

Monocytes are myeloid cells of the innate immune system, protecting against **bacterial, viral and fungal infections**.



Adapted from: https://beyondthedish.files.wordpress.com/2016/01/hematopoiesis-from-multipotent-stem-cell.jpg

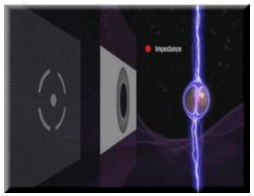
Stepwise Activation Of Innate Immune Response



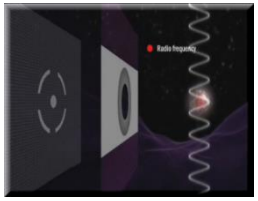
- **Step 1: Macrophages** phagocytose **bacteria**
- **Step 2:** Macrophages and monocytes RELEASE CYTOKINES (cytokine storm)
- **Step 3a:** Cytokines activate circulating WBC **(neutrophils)** –TOXIC GRANULATION
- **Step 3b:** Cytokines stimulate bone marrow – LEUKOCYTOSIS
- **Step 4:** Bone marrow releases more granulocytes in blood, some of which are immature (**BANDS-left shift**)

Janeway's Immunology, 9th edition

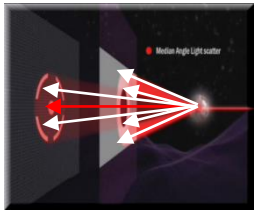
Flow Cytometric Digital Morphology



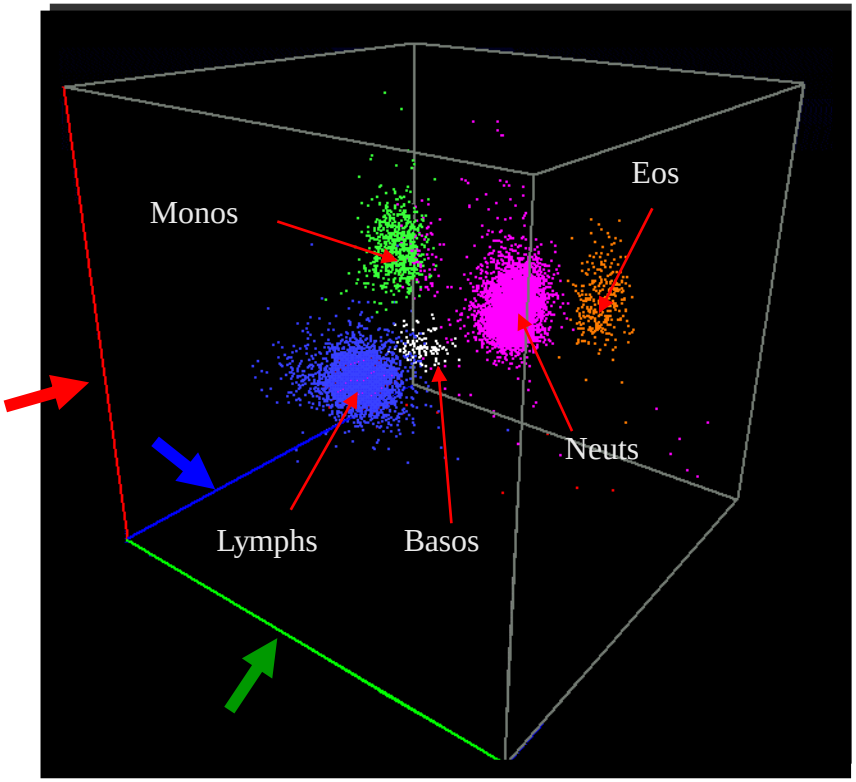
VOLUME (V)



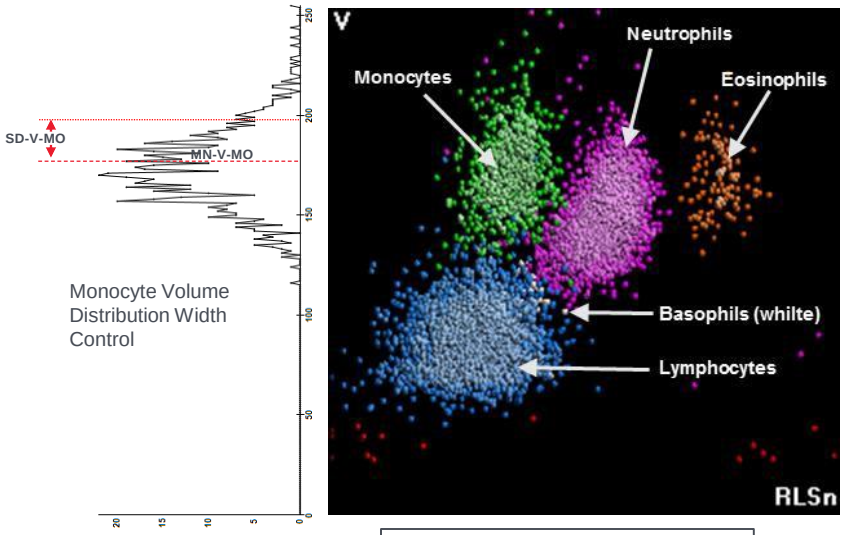
CONDUCTIVITY (C)



LIGHT SCATTER (S)

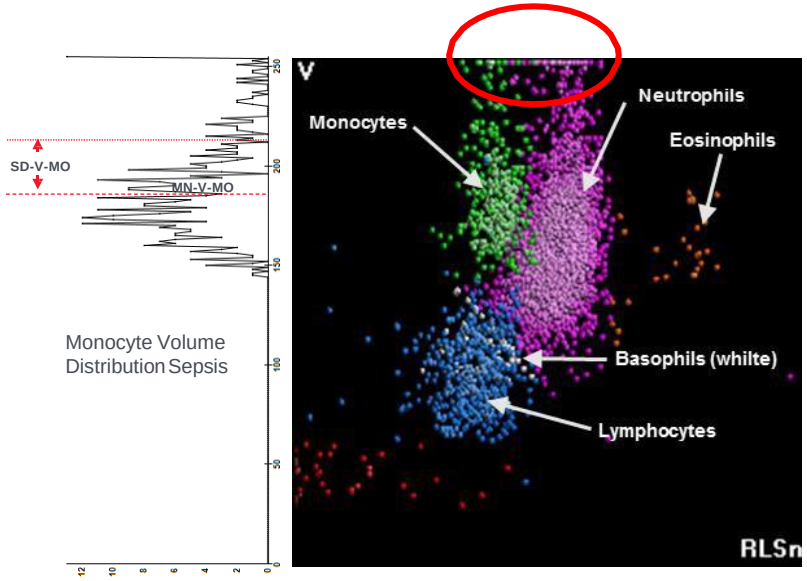


Sepsis Demonstrates Increased Variability In Monocyte Volume (MDW)



Non-septic

MDW = 19.1
WBC cells x10^3= 4.73



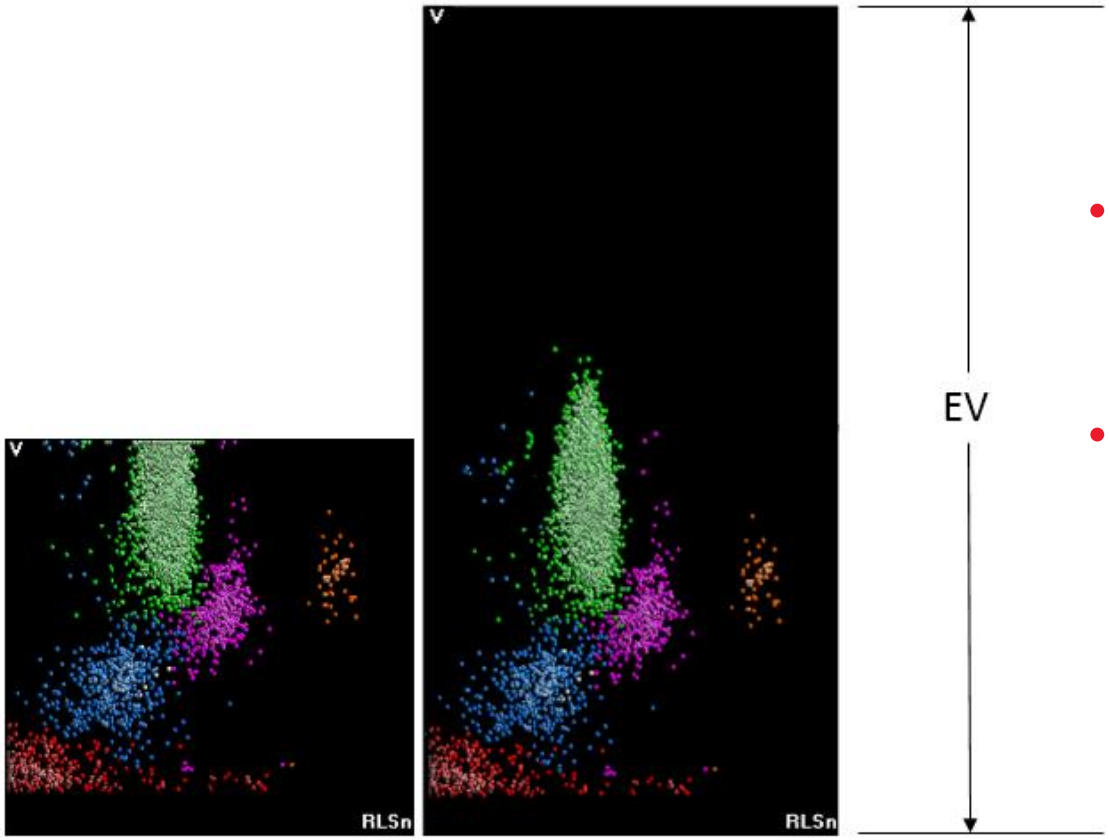
Septic

MDW = 24.3
WBC cells x10^3 = 10.27

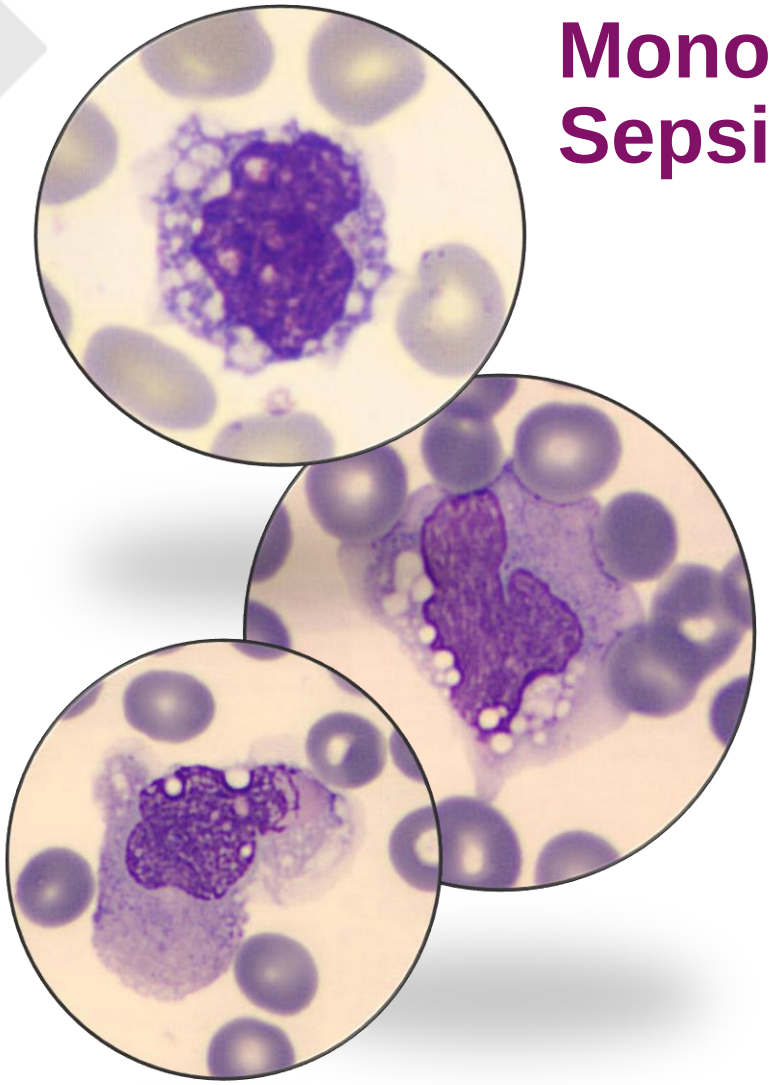
This is a representation of the MDW parameter that is 510(k) cleared by FDA.

Crouser ED et al. “Improved early detection of sepsis in the ED with a novel monocyte distribution width biomarker.” *Chest*, 2017 vol. 152, no. 3, pp. 518–526.

MDW is measured by Extended Volume range



- Monocyte volume values are accumulated on an **extended volume (EV)** range (available internally to the algorithm)
- The extended volume range accurately measures MDW above the standard 5-part differential range.



Monocytes in Infection and Sepsis: Changes in Morphology

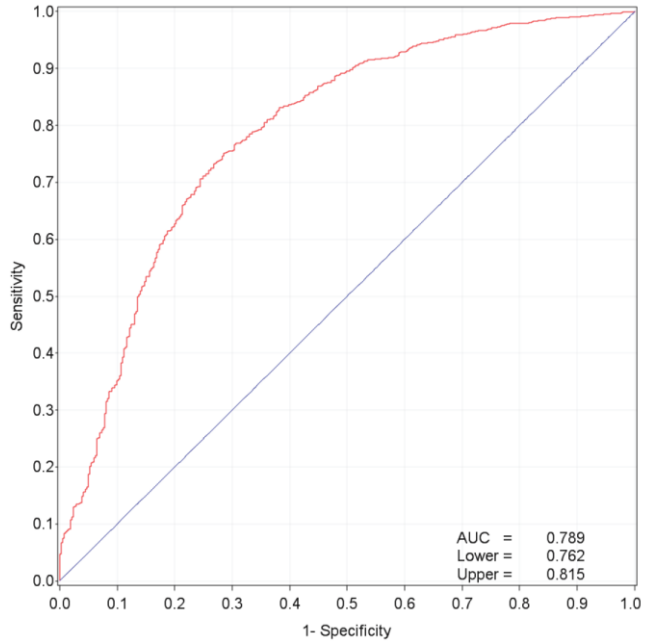
Functional changes of the monocytes, and, in parallel, changes in cellular morphology, were demonstrated for human THP-1 monocytic cell line, infected with viable *C. pneumonia* bacteria.

The differentiation of infected cells into macrophages was accompanied by a change to the amoeboid or diffused morphology.

Yamaguchi Y, Haranaga S, Widen R, Friedman H, Yamamoto Y. "Chlamydia pneumoniae infection induces differentiation of monocytes into macrophages." Infection and Immunity, 2002, vol. 70, pp. 2392–8.

Multi-center Clinical Trial With Unselected Population

Blinded, prospective, observational cohort study was conducted across three sites in the U.S. with 2,158 consecutive adults entering emergency-departments



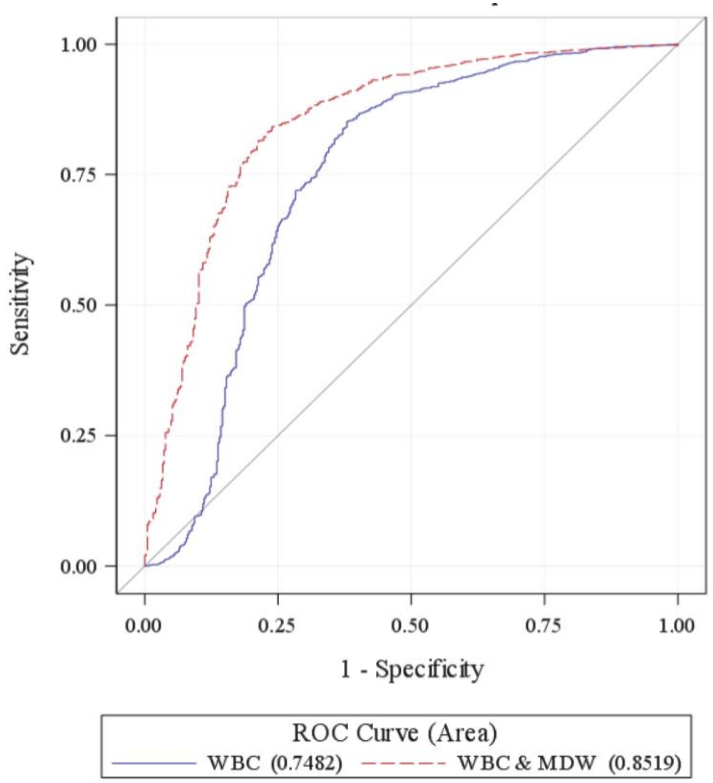
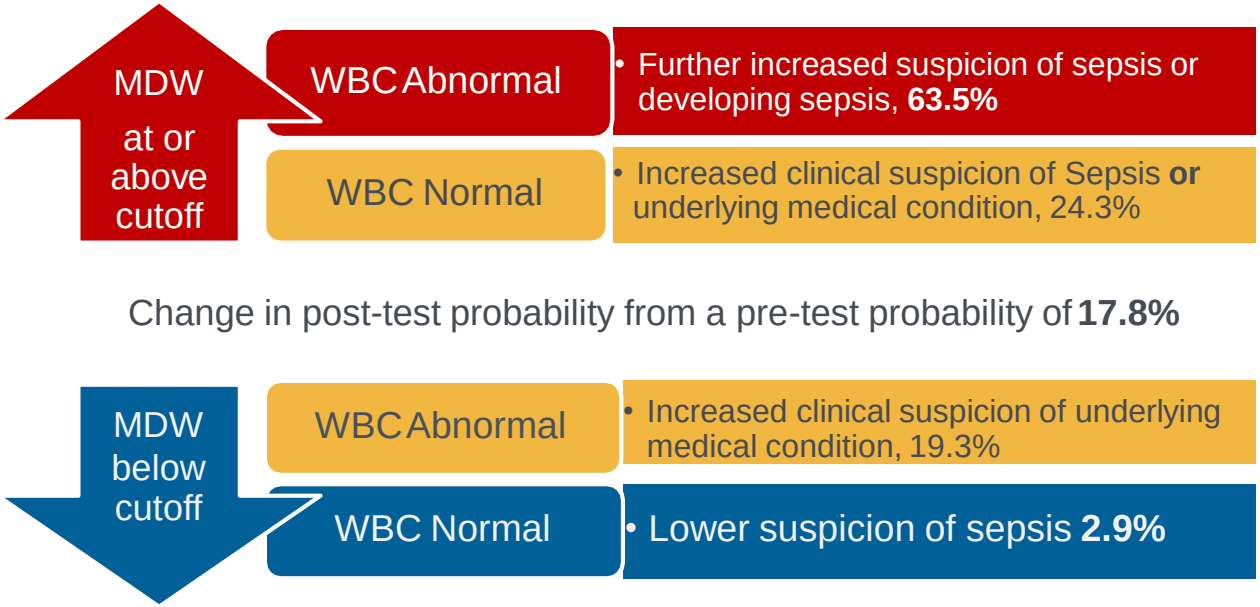


Table 1.5 Performance of MDW for Sepsis-2

C05728AB-IFU; pg. 1-7, 1-8

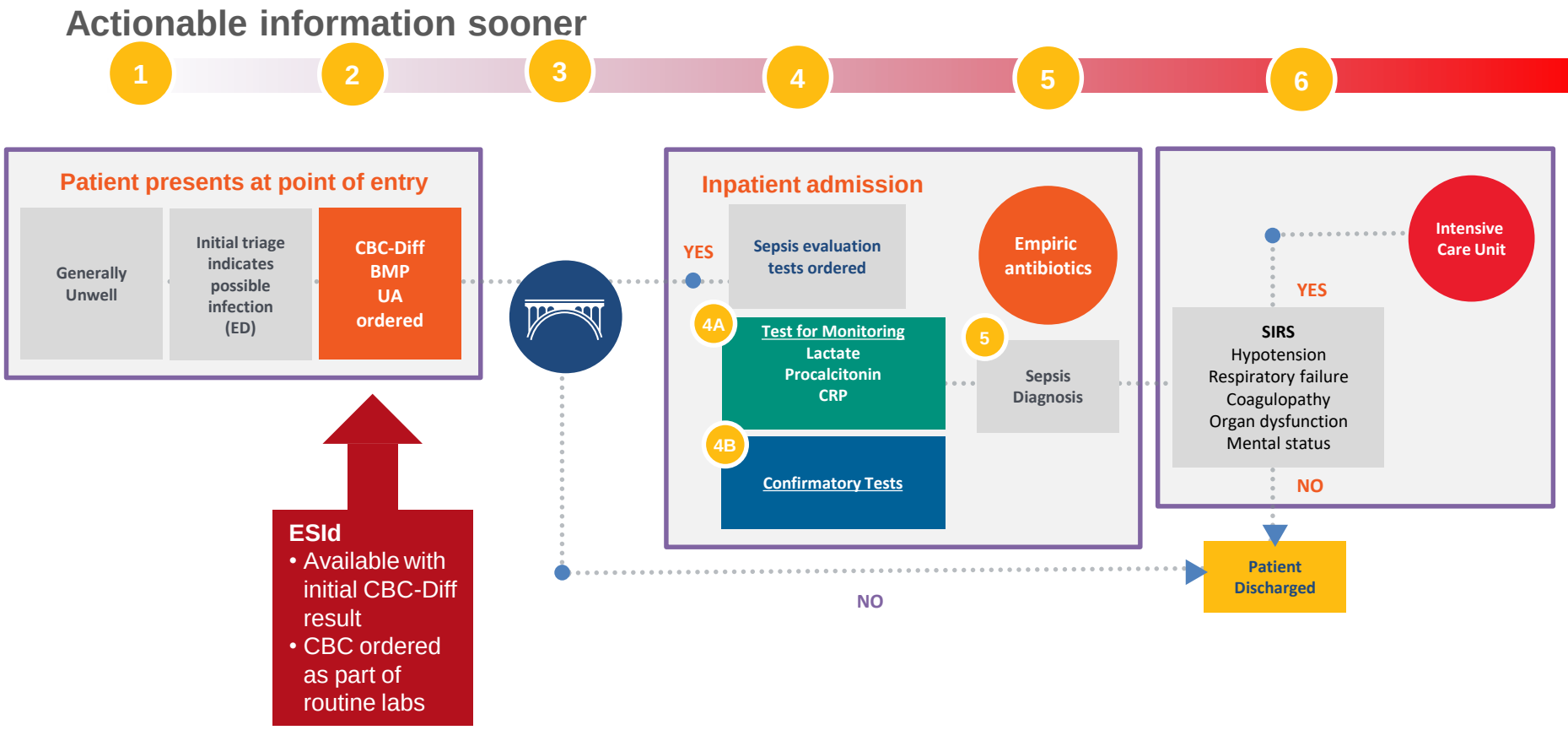
ESId is to Be Used With the Current Standard of Care

Early Sepsis Indicator adds value to the current standard of care. Combined with WBC count, Early Sepsis Indicator augments clinical suspicion of sepsis.[1,2]



1. UniCel DxH Series with System Manager Software. Early Sepsis Indicator Application Addendum. PN C05728AA. March 2018.
2. Sepsis Clinical Accuracy Performance on DxH 800 Test Summary Report. PN C07352. March 2018.

Bridging the Care Gap



Sepsis is a Global Healthcare Problem

- Strikes an estimated **30 million** people worldwide each year
- More common than **heart attacks, and claims more lives** than any cancer
- Healthcare cost (U.S.) is estimated to be $17 billion (**average of $50,000 per case**) per year
- **Primary cause of death from infection** despite advances in modern medicine, vaccines, antibiotics and advanced acute care

The Lab Plays a Major Role

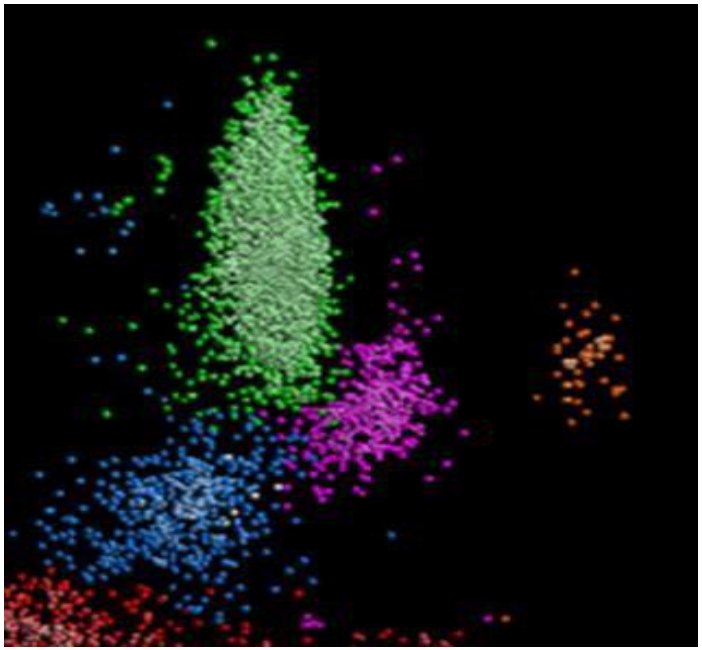
. Sepsis Alliance. “Critical Fact Sheet.” Sepsis.org. Accessed January 15, 2018.

Information to Help Diagnose Sooner

Early Sepsis Indicator

- Technology detects morphological changes in **monocytes**, cells that play a role in the dysregulated immune response to sepsis. Identifying these monocyte morphological changes provides insight into possible sepsis.

- Offered as part of a routine CBC with differential test, the **hematology-based** solution is intended to aid emergency department clinicians in the early detection of patients **with or developing sepsis**.



Lab Workflow Includes 6C Plus Controls and Early Sepsis Indicator Activation

Detection Tools

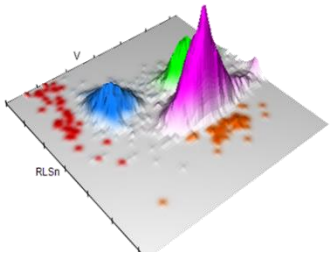
MDW Parameter included with Differential

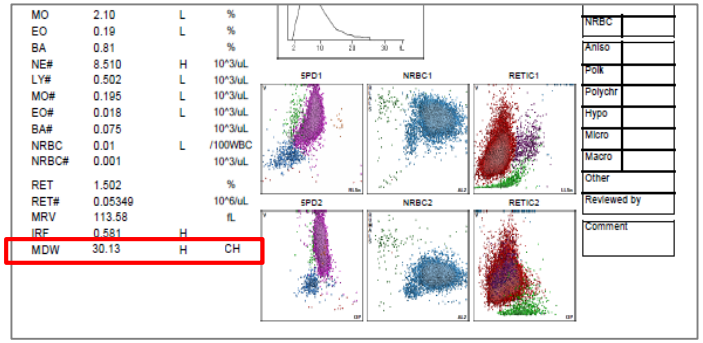
Alerts

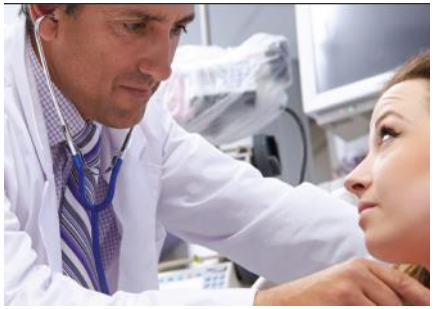
DxH Instrument

6C Plus QC

Early Sepsis Indicator







Decision rules to trigger alerts

Time = Life

- The most important element of sepsis care is **prompt recognition and treatment**

Thank you