APPROACH TO SEPSIS DIAGNOSIS

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PROJECT LEAD - ANTIMICROBIAL STEWARDSHIP AT AMERICARES INDIA FOUNDATION

SEPSIS

- Invasion of pathogens into the blood stream together with the host response to this invasion
- Consists of the systemic inflammatory response syndrome (SIRS)
- Complicated by remote organ dysfunction (severe sepsis) or arterial hypotension (septic shock)
- Among the most common causes of death in hospitalized patients
- Hospital mortality of patients with sepsis ranges from 28.3 to 41.1% in North America and Europe
- World Health Organization (WHO) has stated that the worldwide annual mortality due to sepsis is around 6 million, with most of these deaths being preventable
- Especially common in the elderly and is likely to increase substantially as the population ages

SEPSIS- INDIAN SCENARIO

Study by Chatterjee et al (5 year experience) showed:

1. Most frequent site of infection among severe sepsis patients was respiratory tract (53.3%), followed by abdomen (14.9%)
2. Severe sepsis was the reason for admission in 84% of severe sepsis cohort
3. Majority of infections were caused by Gram-negative organisms (73%); 6.2% of severe sepsis patients had fungal infections (candidemia, aspergillosis)
4. Commonly isolated microbes were *Acinetobacter baumannii* (21.2%), *Pseudomonas Aeruginosa* (17%) and equal prevalence of *Klebsiella* and *Escherichia coli* (15.4%)
5. The in-hospital mortality was 63.6%.

Chatterjee S et al. **Epidemiology of Adult-population Sepsis in India: A Single Center 5 Year Experience.** Indian J Crit Care Med. 2017 Sep; 21(9): 573–577.

THE CAUSE

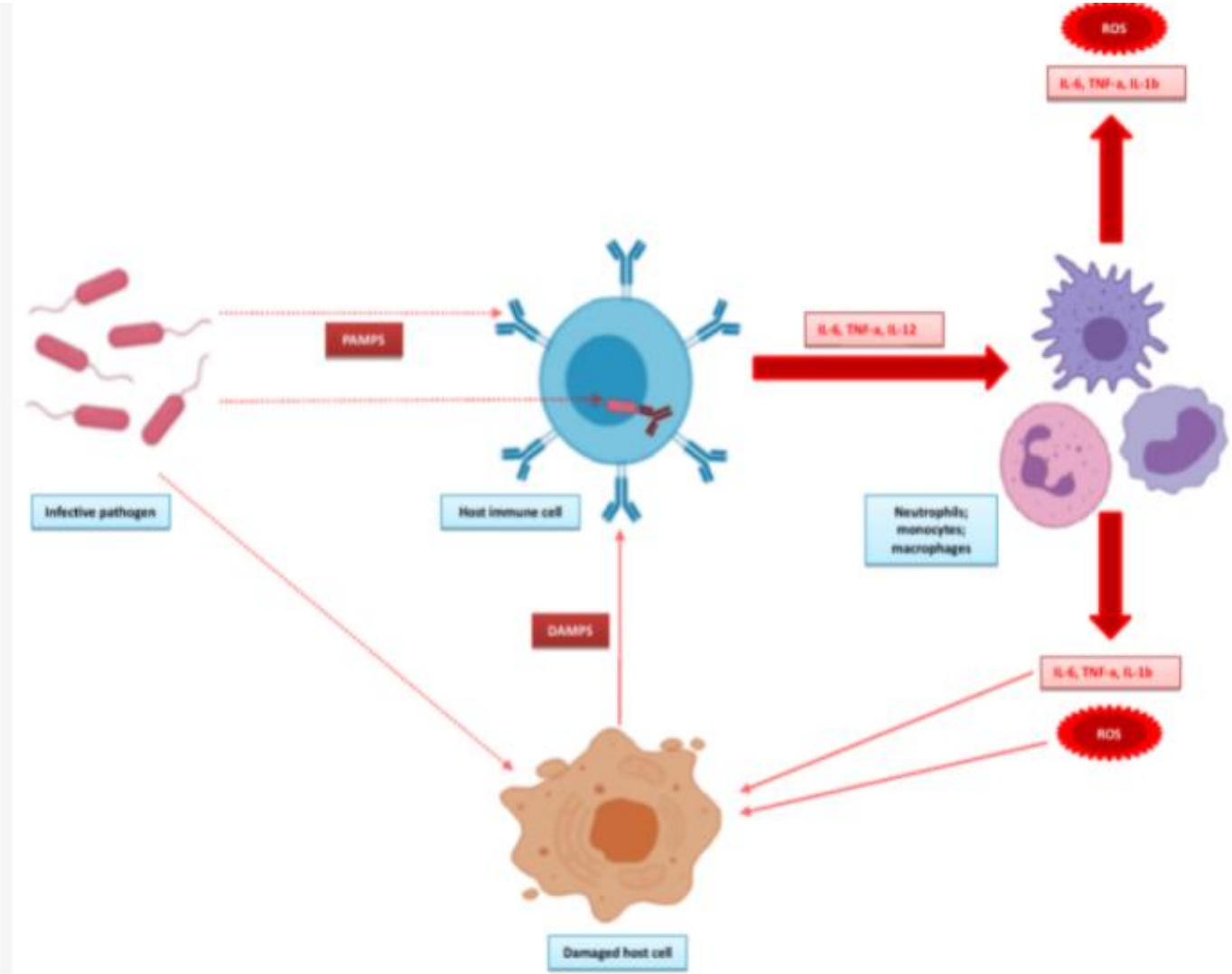
- Bacteria are recognized as the main infective agents that cause sepsis, viral and fungal infections are also responsible for a relatively small proportion of cases
- Most frequent infective bacteria were *escherichia coli* (7.35%), *streptococcus* (4%), methicillin-resistant *staphylococcus* (2.86%) and *staphylococcus* (1.9%)
- Viral sepsis should be considered in cases of sepsis with no clear bacterial infection or no other obvious causative agent
- Fungal infections that cause sepsis are almost always contracted after a patient has been admitted to hospital with similar presentation as that of bacterial sepsis
- Sepsis attributed to an infection acquired during hospital admission has been associated with a higher risk of mortality regardless of the infective pathogen

- Types of organisms in culture-positive infected patients

	Frequency (%)	OR (95% CI)
Gram-positive	46.8	
Staphylococcus aureus	20.5	0.8 (0.6–1.1)
MRSA	10.2	1.3 (0.9–1.8)
Enterococcus	10.9	1.6 (1.1–2.3)
S. epidermidis	10.8	0.9 (0.7–1.1)
S. pneumoniae	4.1	0.8 (0.5–1.4)
Other	6.4	0.9 (0.7–1.2)
Gram-negative	62.2	
Pseudomonas species	19.9	1.4 (1.2–1.6)
Escherichia coli	16.0	0.9 (0.7–1.1)
Klebsiella species	12.7	1.0 (0.8–1.2)
Acinetobacter species	8.8	1.5 (1.2–2.0)
Enterobacter	7.0	1.2 (0.9–1.6)
Other	17.0	0.9 (0.7–1.3)
Anaerobes	4.5	0.9 (0.7–1.3)
Other bacteria	1.5	1.1 (0.6–2.0)
Fungi		
Candida	17.0	1.1 (0.9–1.3)
Aspergillus	1.4	1.7 (1.0–3.1)
Other	1.0	1.9 (1.0–3.8)
Parasites	0.7	1.3 (0.5–3.3)

OR, odds ratio; CI, confidence interval; MRSA, methicillin-resistant *S. aureus*

PATHOBIOLOGY OF SEPSIS



DIAGNOSIS

When to suspect?

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, i.e.,

Alteration in mental status,	**SYSTOLIC BLOOD PRESSURE 100 MM HG, OR**	**Respiratory rate 22/min.**

Can be used to identify patients at risk of sepsis in out of- hospital, emergency department and general hospital ward settings

Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent JL, Angus DC. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016 Feb 23;315(8):801-10
Treatment Guidelines for Antimicrobial Use in Common Syndromes. 2nd edition. Indian Council of Medical Research 2019.

Table 1

The Sequential Organ Failure Assessment (SOFA) score[a]

Organ system	SOFA score				
	0	1	2	3	4
Respiratory, PO_2/FiO_2, mmHg (kPa)	≥400 (53.3)	<400 (53.3)	<300 (40)	<200 (26.7) with respiratory support	<100 (13.3) with respiratory
Coagulation, Platelets, $\times 10^3/mm^3$	≥150	<150	<100	<50	<20
Liver, Bilirubin, mg/dL	<1.2	1.2–1.9	2.0–5.9	6.0–11.9	>12.0
Cardiovascular	MAP ≥70 mmHg	MAP <70 mmHg	Dopamine <5 or dobutamine (any dose)[b]	Dopamine 5.1–15 or epinephrine ≤0.1 or norepinephrine ≤0.1[b]	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1[b]
Central nervous system, Glasgow Coma Scale	15	13–14	10–12	6–9	<6
Renal, Creatinine, mg/dL. Urine output, mL/d	<1.2	1.2–1.9	2.0–3.4	3.5–4.9 <500	>5.0 <200

[a], adapted from Vincent *et al*. (7); [b], Catecholamine doses are given as μg/kg/min for at least 1 hour. FiO_2, fraction of inspired oxygen; MAP, mean arterial pressure; PO_2, partial pressure of oxygen.

Diagnosing sepsis

APPROPRIATE ROUTINE MICROBIOLOGIC CULTURES SHOULD BE OBTAINED BEFORE INITIATION OF ANTIMICROBIAL THERAPY FROM ALL POTENTIAL SITES OF INFECTION

These may include blood, respiratory secretions, urine, cerebrospinal fluid, wounds, and other body fluids

If it is not logistically possible to obtain cultures promptly (45 minutes), the appropriate antimicrobials should be administered

In sepsis or septic shock patients, the diagnosis includes identification of a specific anatomic site of infection requiring emergent source control

Treatment Guidelines for Antimicrobial Use in Common Syndromes. 2nd edition. Indian Council of Medical Research 2019.

Hour-1 Surviving Sepsis Campaign Bundle of Care: The Golden Hour

Treatment Guidelines for Antimicrobial Use in Common Syndromes. 2nd edition. Indian Council of Medical Research 2019.

1. Measure lactate level. Re-measure if initial lactate level > 2 mmol/L.*

2. OBTAIN BLOOD CULTURES BEFORE ADMINISTERING ANTIBIOTICS.**

3. Administer broad-spectrum antibiotics.

4. Begin rapid IV administration of 30mL/kg crystalloid for hypotension or lactate level ≥ 4 mmol/L.***

5. Apply vasopressors if patient is hypotensive during or after fluid resuscitation to maintain MAP ≥ 65 mm Hg. ****

*"Time zero" or "time of presentation" is defined as the time of triage in the emergency department or, if presenting from another healthcare facility, from the earliest time-point in the treatment chart wherein, the patient had all the features consistent with sepsis.
**Two or more sets of blood cultures are recommended before initiation of any new antimicrobial in all patients with suspected sepsis.
***Hydroxyethyl starches (HESs) are not recommended for intravascular volume replacement in patients with sepsis or septic shock.
**** Norepinephrine is the first-choice vasopressor.

Diagnostic criteria	Threshold
Fever	>38.3°C
Tachycardia	>120/minute
Systolic blood pressure	<90 mmHg
Procalcitonin	>0.5 ng/ml
Bandemia	>5%
Lymphocytopenia	$<0.5 \times 10^3$ ul
or neurophil/lymphocyte ratio	>10
Thrombocytopenia	$<150 \times 10^3$ ul
Lactate	>2.0 meq/l

DIAGNOSTIC FEATURES SUGGESTIVE OF SEPSIS

THE CHALLENGES

The initial signs and symptoms of sepsis are frequently non-specific, leading to a delay in diagnosis

Elevated white blood count, neutrophilia or eosinopenia are frequently used to diagnose bacterial sepsis; however, these variables have low diagnostic value

Blood cultures are only positive in 20 to 30% of patients with sepsis; moreover, it takes 2 to 3 days before the results become available

The clinical diagnosis of sepsis can be challenging, and microbiological tests are unhelpful, several biomarkers have been developed to assist in the early diagnosis of sepsis, including procalcitonin (PCT), c-reactive protein (CRP) and, more recently, circulating cell-free DNA (cfdna)

BLOOD CULTURE

- The only diagnostic testing that currently has a confirmed role in sepsis diagnosis is pathogen identification by blood cultures
- Obtaining blood cultures is recommended by all the previously described guidelines (it is important that this be done before the administration of antibiotics, as these can quickly sterilise blood cultures once administered
- Blood culture considered to be the gold standard test for diagnosing bloodborne infections

KEY STEPS

- Best available site for culture
- Aseptic technique
- Adequate volume of blood
- Sufficient number of blood culture sets
- Timing of blood culture

BEST AVAILABLE SITE

- Easily accessible readily available & minimal discomfort to the patient
- Low contamination rate
- Antecubital veins- preferred
- Arterial blood = venous blood
- Avoid blood drawing through lines

ASEPTIC TECHNIQUE

- To avoid contamination
- Method
  - Cleansed with 70% isopropyl or ethyl alcohol and allowed to air dry.
  - A second cleansing should be performed using 1% to 2% tincture of iodine or 10% povidone-iodine solution applied concentrically;(this should be allowed to air dry before the vein is punctured)
- Contamination of Blood cultures could be
  - Initial Contaminants Prevent contamination during collection of Blood for culture – from skin flora – skin flora –CoNS, Diptheroids etc
  - Latent Contamination- usually bacterial and is often observed long after cultures are initiated

BLOOD VOLUME

- Direct relationship between the volume and detection of bacteremia or fungemia
- Blood volume of each culture (Culture set) is the single most important variable in recovering microorganisms
- When volume of blood increased from 2ml to 20ml, the yield increased 30% to 50%
- Cocerill et al showed that for adults optimum volume is 20ml for a culture

NUMBER OF BLOOD CULTURE SETS

- Two or three blood cultures are adequate for detecting episodes - caused by common microbial pathogens.
- Using conventional, non-automated blood culture found that total of 20ml per set gives proper result
- Useful to interpreting clinical significance of positive blood culture
- One positive test is sufficient for primary pathogens
- Opportunistic pathogens and Regional flora need more than one culture result for interpretation
- It is not appropriate to collect only a single blood specimen for culture. A single blood culture will not have sufficient volume for optimal detection of bacteremias and fungemias

TIME FOR BLOOD CULTURE

- Chills(and rigors) – occur 1 hour of lag period
- Fever follows chills and rigors
- Therefore, some recommend collection of blood culture soon before chills/rigors or fever spike
- The optimal time for collection - just before the onset of a shaking chil
- Not possible to anticipate the precise timing. - Common practice to draw blood cultures when fever is detected
- It is reasonable to obtain two blood culture sets simultaneously, especially if antibiotic therapy is going to be initiated
- In less urgent situations - blood cultures may be spaced at intervals

MOLECULAR STRATEGIES TO IMPROVE PATHOGEN DETECTION

- A number of molecular approaches to improve conventional culture-based identification, including PCR, have been suggested such as matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry
  - Decrease the time to result to 4 hours once the BC has become positive
- Alternative strategy is the extraction and amplification of microbial nucleic acids from a positive BC and subsequent hybridization on a microarray platform to detect the *gyrb*, *pare*, and *meca* genes of 50 bacterial species
  - The assay was 18 hours faster than conventional BC
  - Shortcomings include an incomplete coverage of pathogens, the inability of the test to be applied directly to a biological sample, and restricted information regarding antimicrobial susceptibility

BIOMARKERS

- Origins of sepsis biomarkers in relation to the pro-inflammatory cascade activated during sepsis
- CRP: c-reactive protein; CD64: cluster of differentiation 64; IL-6: interleukin-6; PCT: procalcitonin; strem-1: soluble triggering receptor expressed by myeloid cells-1.

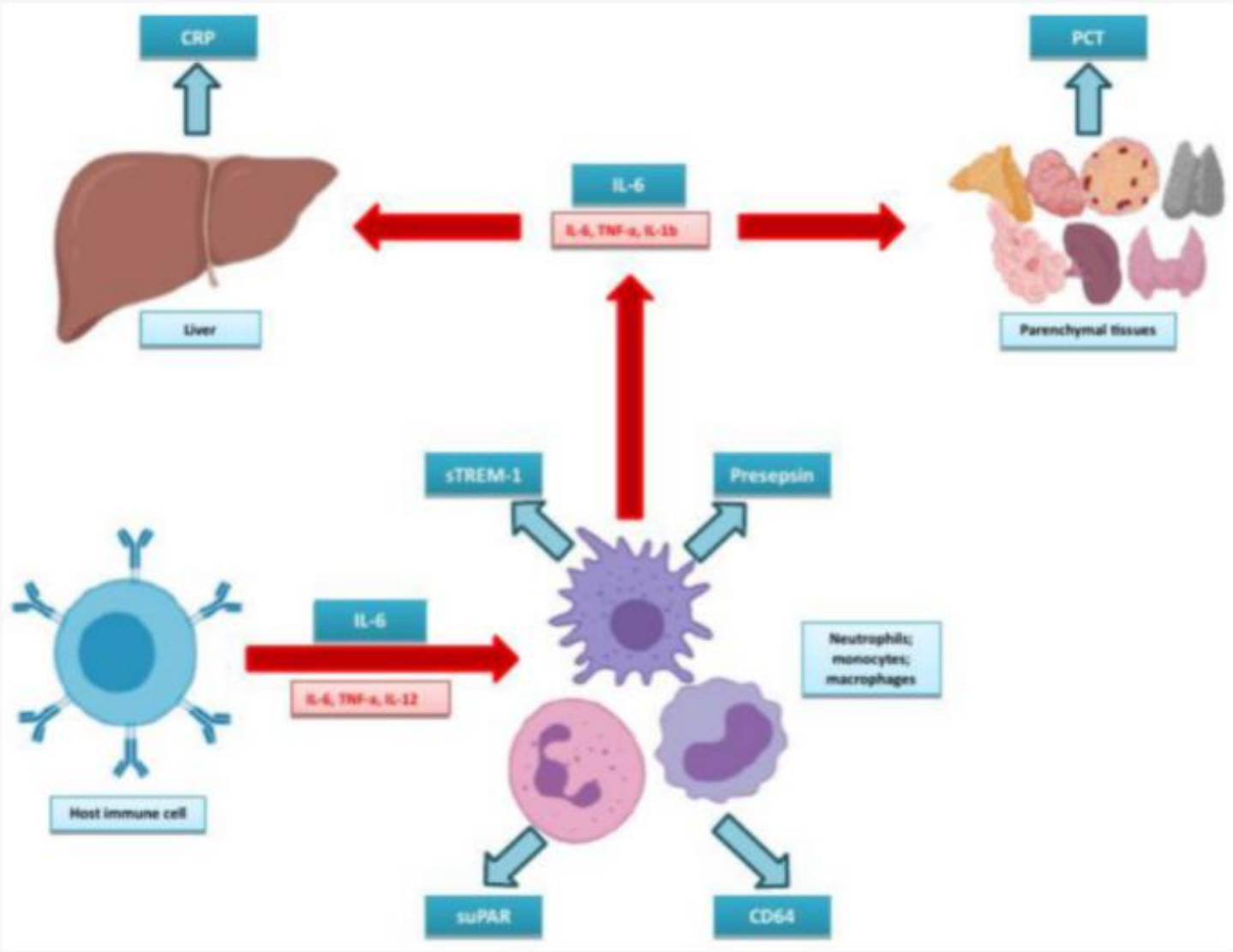


Table 1 of 1

Table 1. Diagnostic value and limitations of biomarkers to separate infectious from non-infectious causes of inflammation

Biomarker	Source	Sens.	Spec.	AUC	LR^+	LR^-	Limitations
C-reactive protein[21]	Metaanalysis (*n* = 1386)	0.75	0.67	-	2.43	0.42	Slow kinetic, independent of infection severity, increased in many inflammatory diseases
Procalcitonin[35]	Metaanalysis (*n* = 3244)	0.77	0.79	0.89	4.0	0.29	Increased in various non-infectious causes of SIRS (i.e., cardiac arrest, severe trauma)
Interleukin-6[57]	Cohort study (*n* = 327)	0.82	0.75	0.86	-	-	Limited data, conflicting results
sTREM-1[78]	Metaanalysis (*n* = 1795)	0.79	0.80	0.87	4.0	0.26	Present in inflammatory disease without infection
LBP[57]	Cohort study (*n* = 327)	0.57	0.85	0.73	-	-	Non-specific marker of inflammation
suPAR[98]	Cohort study (*n* = 273)	-	-	0.62	-	-	Limited data; low diagnostic value for sepsis

C-REACTIVE PROTEIN

- C-reactive (CRP) is an acute phase protein and is released from the liver after stimulation predominantly of IL-6 and other cytokines
- During infection, CRP has both pro-inflammatory and anti-inflammatory effects as it mediates elimination of pathogens but also inhibits interaction between endothelial cells and leukocytes
- Secretion is started 4 to 6 h after stimulation and peaks at 36 h. CRP is frequently used for the diagnosis of infection
- A metaanalysis showed only a sensitivity 0.75 and a specificity of 0.67 to differentiate bacterial from noninfectious causes of infection
- In critically ill patients with SIRS, elevated CRP-levels on ICU day 1 could differentiate between patients with and without sepsis
- CRP levels decrease over the first 48 h when successful antimicrobial therapy is initiated

PROCALCITONIN (PCT)

- Prohormone of calcitonin which is normally produced in the c-cells of the thyroid glands
- In healthy humans, all PCT is cleaved to calcitonin and only < 0.1 ng/ml is measured in the blood
- Massive release of PCT into the bloodstream which depends on sepsis severity
- PCT shows a more favorable kinetic profile than CRP and cytokines as its levels increase within 4 to 12 h after onset of infection
- A recent metaanalysis including 3244 patients from 30 studies calculated a sensitivity of 0.77 and a specificity of 0.79 to discriminate sepsis from non-infectious causes of sepsis
- For critically ill patients, cut-offs for sepsis diagnosis a median cut-off of 1.1 (interquartile range 0.5–2.0) ng/ml across the studies was reported
- Patients with septic shock have the highest PCT levels averaging between 4 and 45 ng/ml
- Circulating PCT levels decrease with a half-time of about 24 h when the infection is sufficiently treated
- Dropping PCT levels associated with improved survival rates while increasing or persistent elevated PCT levels are predictive for an unfavorable outcome
- Also increases in non infective situations such as severe trauma

CD64

- CD64 is an IgG-binding receptor expressed by neutrophils, monocytes and macrophages in response to cytokines released during bacterial infection

- It is bound to the cell, and the current method of choice for analysis is therefore flow cytometry.

- The available data indicate that neutrophil CD64 seems to have high sensitivity (86%) and specificity (87%)

PRESEPIN

- By activating a proinflammatory signaling cascade on contact with infectious agents, CD14 has a role as a recognition molecule in the innate immune response against microorganisms
- During inflammation, plasma protease activity generates soluble CD14 (sCD14) fragments
- One of them, called sCD14 subtype (sCD14-ST), or presepsin, is normally present in very low concentrations in the serum of healthy individuals and has been shown to be increased in response to bacterial infections
- In a multicenter prospective study (106 patients with suspected sepsis or septic shock were included and 83 SIRS patients without infection), elevated concentrations of presepsin were observed in septic patients compared to control patients
- The best diagnostic cutoff for presepsin was 600 pg/mL with sensitivity of 78.95% (95% CI, 69.4 to 86.6) and specificity of 61.90% (95% CI, 50.7 to 72.3)
- two recent studies have shown that presepsin is a useful biomarker for early diagnosis of sepsis and evaluation of prognosis in septic patients (sensitivity: 71-72%, specificity: 70–86%, and NPV: 52–71%)

STREM-1

Triggering receptor expressed on myeloid cells-1 (TREM-1) is a member of the immunoglobulin superfamily which is upregulated on phagocytes after exposure to bacteria and fungi

During sepsis, activated phagocytes release a soluble form of TREM-1 (strem-1) which among other body fluids can be found in the plasma

A recent metaanalysis included 11 studies with 1795 patients to calculate sensitivity and specificity for differentiating sepsis from noninfectious SIRS gave a result with sensitivity as 0.79 and specificity as 0.8

sTREM-1 was found to be present in other inflammatory disease without infection

LIPOPOLYSACCHARIDE-BINDING PROTEIN (LBP)

- An acute-phase reactant that forms a complex with LPS
- LPS-LBP complex binds to CD14 and to the toll-like receptor 4/md2-complex resulting in transcription of cytokines and other pro-inflammatory mediators
- In human serum, LBP is constitutively present at a concentration of 5 to 10 µg/ml. During sepsis, LBP levels increase to median peak levels of 30–40 µg/ml within 24 h
- Further studies did not confirm these findings showing that LBP is a rather non-specific marker of the inflammatory response
- LBP does not detect resolution of sepsis or is predictive of outcome

SUPAR

- Soluble form of urokinase-type plasminogen activator receptor (supar) is a new biological marker of immunologic activation
- Urokinase-type plasminogen activator receptor (upar) system participated in migration of inflammatory cells from the bloodstream into tissues against infection
- During inflammatory stimulation, upar is cleaved from the cell surface by proteases to create the soluble form of the receptor, supar, which can be detected in blood, urine, and cerebrospinal fluid
- Some studies have showed that supar levels were elevated in acutely ill patients but that their diagnostic value was not superior to other biomarkers such as CRP, PCT, or strem-1

PRO-ADM

Adrenomedullin (ADM) is a 52-amino-acid peptide with immune modulating, metabolic, and vasodilator activity

ADM has a bactericidal activity and could be helpful in the evaluation of sepsis diagnosis and prognosis and in monitoring such conditions

The midregional fragment of proadrenomedullin (MR-pro-ADM), included between amino acids 45–92, is the most stable part of the ADM, and it has been detected in plasma of patients with septic shock as a consequence of the ADM active peptide degradation

Table 1

Role of biomarkers of sepsis.

Biomarkers of sepsis	Prognostic value	Diagnostic value	Syndrome/disease
CRP	No	Yes	Sepsis
Procalcitonin	Yes	Yes	Sepsis/respiratory tract infections/pneumonia/
sTREM-1	Yes	Yes	Sepsis/pneumonia/ meningitis
Pro-ADM	Yes	No	Pneumonia
suPAR	Yes	No	Sepsis/tuberculosis
Presepsin	Yes	Yes	SIRS/sepsis

POINT-OF-CARE TESTING (POCT) FOR SEPSIS

- The biomarkers currently measured for the investigation of sepsis are generally measured in the central laboratory environment
- Usually performed on large, automated clinical chemistry analyzers that employ a combination of ion-selective electrodes and colourimetric, immunoturbidimetric and various immunoassay techniques
- There is an inherent delay in obtaining results due to sample transportation to the laboratory and pre-analytical steps
- Interest has therefore turned to point-of-care testing (POCT) devices that can be used to provide rapid results and reduce the time before appropriate treatment is started
- Lactate may be measured on portable POCT devices and on blood-gas analyzers equipped with a lactate-measuring electrode

ALARMING LEVELS OF ANTIMICROBIAL RESISTANCE !!!!!!

- According to the Centre for Disease Dynamics, Economics and Policy (CDDEP), 80% of the Indian *K. pneumoniae* isolates are resistant to cephalosporins and up to 60% resistant to carbapenems
- India has witnessed a rise in carbapenem resistance rates from 9% in 2008 to 44%
  in 2010
- Carbapenem resistant (CR) *K.pneumoniae* infections are associated with mortality rates as high as 30 to 44%

Veeraraghavan B, Shankar C, Karunasree S, Kumari S, Ravi R, Ralph R. Carbapenem resistant Klebsiella pneumoniae isolated from bloodstream infection: Indian experience. Pathog Glob Health. 2017 Jul;111(5):240-246.

INFECTION CONTROL PRACTICES

Careful choice of prophylaxis and antimicrobials to avoid selection of resistant strains

Shorter hospital stays before surgery will help to decrease risk of nosocomial infections

Early removal of in-dwelling urethral catheters reduces nosocomial urosepsis

Routine use of protective disposable gloves and frequent hand disinfection

Application of infectious disease control measures to prevent cross-infections

Bonkat G, Cai T, Veeratterapillay R, Bruyère F, Bartoletti R, Pilatz A, Köves B, Geerlings SE, Pradere B, Pickard R, Wagenlehner FM. Management of Urosepsis in 2018. European urology focus. 2018 Nov 15

THANK YOU..