SEPSIS AND THE HEMATOLOGY LABORATORY

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PRESENTATION OUTLINE

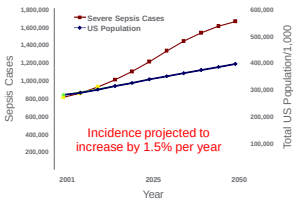
- Introduction to sepsis epidemiology and formal definitions
- Biological considerations and how they impact the role of the laboratory
- Differential diagnosis to be considered by the hematology laboratory
- Recently proposed markers for sepsis management and their limitations

SEPSIS AND ITS IMPACT TO SOCIETY

Today

>750,000 cases of severe sepsis/year in the U.S.*

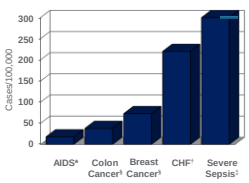
Future



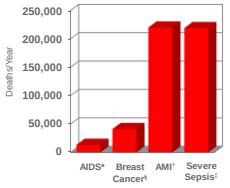
Angus DC. Crit Care Med. 2001;29(7):1303-1310.
*Slide source–Surviving Sepsis campaign

COMPARISON WITH OTHER MAJOR DISEASES

Incidence of Severe Sepsis



Mortality of Severe Sepsis



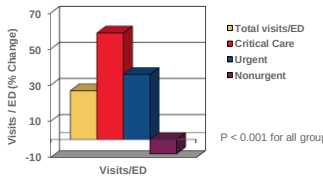
†National Center for Health Statistics, 2001. §American Cancer Society, 2001. *American Heart Association. 2000. ‡Angus DC et al. Crit Care Med. 2001;29(7):1303-1310.
Slide source–Surviving Sepsis campaign

COMPARABLE GLOBAL EPIDEMIOLOGY

- 95 cases per 100,000
  - 2 week surveillance
  - 206 French ICUs
- 95 cases per 100,000
  - 3 month survey
  - 23 Australian/New Zealand ICUs
- 51 cases per 100,000
  - England, Wales and Northern Ireland.

EMERGENCY DEPARTMENT CRITICAL CARE VOLUME INCREASES

- 102 million national ED visits in 1999
- 17% (17.5 million) "immediately life threatening"[1]
- 57 California emergency departments (1990-1999)[2]
- 50% (387,616) severe sepsis cases initially go to ED



P < 0.001 for all groups

1. National Center for Health Statistics; 2001
2. Ann Emerg Med 2002;39:389-96
3. Curr Opin Crit Care Dec 2002
Slide source–Surviving Sepsis campaign

EPIDEMIOLOGY OF NEONATAL SEPSIS

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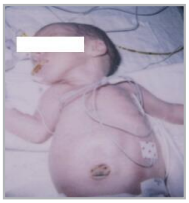
LOW INCIDENCE BUT SIGNIFICANT CONSEQUENCES

- 0.5-1% live births
- 50% NICU admissions
- 20-40% mortality rate



Haque KN. Definitions of bloodstream infection in the newborn. Pediatr Crit Care Med. 2005 May;6 (3 Suppl):S45-9.

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CURRENT IMPACT IN THE UNITED STATES

- Up to 50,000 neonates suffering from sepsis
- 10-20,000 neonatal deaths
- Incidence and mortality declining in developed nations but not altered in developing world

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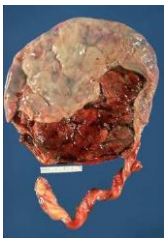
PATHOGENESIS–EARLY ONSET SEPSIS

- Vertical transmission
  - Vaginal delivery, bacteria colonizing the birth canal
  - Ascending chorioamnionitis prior to delivery
- Chorioamnionitis: 4-fold increase in the risk of neonatal sepsis (1 to 4%)
- Most important pathogen: group B streptococci (GBS)



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CLINICAL FINDINGS IN NEONATAL SEPSIS

- Subtle and non-specific, thus requiring a very high level of suspicion for prompt diagnosis and treatment
- Diagnosis should take into account possible risk factors
- Any deviation from an infant's usual pattern of activity or feeding should raise the suspicion of sepsis

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CLINICAL FINDINGS

Finding	Frequency*
Hyperthermia	+++
Respiratory distress	++
Anorexia	++
Vomiting	++
Jaundice	++
Hepatomegaly	++
Lethargy	++
Cyanosis	+
Hypothermia	+
Irritability	+
Apnea	+
Abdominal distension	+
Diarrhea	+

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RISK FACTORS

- Intrapartum maternal temperature ≥38ºC (100.4ºF)
- Delivery at <37 weeks gestation
- Chorioamnionitis
- Five-minute Apgar score ≤6
- Evidence of fetal distress
- Maternal GBS colonization*
- Membrane rupture ≥18 hours

*Maternal intrapartum antibiotic prophylaxis (IAP) reduces the risk of GBS infection.

LABORATORY EVALUATION

- Blood culture
- CBC with differential
- Lumbar puncture
- Culture of urine and other fluids
- Other inflammatory markers
  - C-reactive protein
  - Cytokines (IL 2, 4, 6, 10, TNF, interferon gamma)
  - Procalcitonin

THE LIMITATIONS OF EACH TEST

- Blood Culture: gold standard, but sensitivity can vary depending on volume of blood and number of bottles
  - Cytokines (IL 2, 4, 6, 10, TNF, interferon gamma)
  - Procalcitonin
- CBC with Differential
  - Poor sensitivity
  - Band counts are very cumbersome for the lab
- Other cultures and lumbar punction
  - Less than ideal sensitivity

NEW INFLAMMATORY MARKERS FOR SEPSIS

- C-Reactive Protein
  - Poor specificity
- Cytokines (IL 2, 4, 6, 10, TNF, interferon gamma) procalcitonin
  - Both options very expensive and have sub-optimal sensitivity and specificity
- All tests above require additional sample and additional costs

SOME FORMAL DEFINITIONS

SEPSIS DEFINITIONS

- **Sepsis**: a clinical syndrome that complicates severe infection…characterized by the cardinal signs of inflammation (vasodilation, leukocyte accumulation and increased microvascular permeability) occurring in tissues that are remote from the infection
- **Systemic inflammatory response syndrome (SIRS):** an identical clinical syndrome that complicates a noninfectious insult (e.g., acute pancreatitis, pulmonary contusion)
- Both: dysregulation of the inflammatory response with massive and uncontrolled release of proinflammatory mediators initiating a chain of events that lead to widespread tissue injury

SEPSIS DEFINITIONS

- Dysregulation of the inflammatory response with **massive and uncontrolled release of pro-inflammatory mediators** initiating a chain of events that lead to widespread tissue injury
- Measuring these inflammatory mediators does not provide much help in the discrimination between the two possible causes of this process (infectious sepsis, versus non-infectious SIRS)

USING THE RIGHT DEFINITIONS

- **Infection**: the invasion of normally sterile tissue by organisms
- **Bacteremia**: the presence of viable bacteria in the blood
- **Systemic inflammatory response syndrome (SIRS):** the clinical syndrome that results from a dysregulated inflammatory response to a noninfectious insult, such as an autoimmune disorder, pancreatitis, vasculitis, thromboembolism, burns or surgery

USING THE RIGHT DEFINITIONS

- **Sepsis**: the clinical syndrome that results from a dysregulated inflammatory response to an infection. Sepsis is defined as the presence (probable or documented) of infection together with systemic manifestations of infection
- **Severe sepsis:** sepsis-induced tissue hypoperfusion or organ dysfunction due to the infection
- **Septic shock:** sepsis-induced hypotension persisting despite adequate fluid resuscitation, which may be defined as infusion of 30 mL/kg of crystalloids (a portion of this may be albumin equivalent)

CRITERIA FOR ORGAN DYSFUNCTION NEEDED TO DEFINE SEVERE SEPSIS

- Sepsis-induced hypotension
- Lactate above upper limits of laboratory normal
- Urine output <0.5 mL/kg/hr for more than two hours despite adequate fluid resuscitation
- Acute lung injury with PaO2/FIO2 <250 in the absence of pneumonia as infection source
- Acute lung injury with PaO2/FIO2 <200 in the presence of pneumonia as infection source
- Creatinine >2 mg/dL (176.8 micromol/L)
- Bilirubin >2 mg/dL (34.2 micromol/L)
- Platelet count <100,000 microL–1
- Coagulopathy (INR >1.5)

CRITERIA FOR SYSTEMIC INFLAMMATION (EITHER SEPSIS OR SIRS)

- Temperature >38.3 or <36ºC
- Heart rate >90 beats/min or more than two standard deviations above the normal value for age
- Tachypnea, respiratory rate >20 breaths/min
- Altered mental statusSignificant edema or positive fluid balance (>20 mL/kg over 24 hours)
- Hyperglycemia (plasma glucose >140 mg/dL or 7.7 mmol/L) in the absence of diabetes
- Arterial hypotension (systolic blood pressure SBP <90 mmHg, MAP <70 mmHg, or an SBP decrease >40 mmHg in adults or less than two standard deviations below normal for age)

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ALL CLINICAL CRITERIA, NO LAB WORK NEEDED!
The real challenge is not to diagnose the inflammatory process–it is to know if this process is caused by an infection

THE CLINICAL LABORATORY IN THE MANAGEMENT OF SEPSIS

- Clinical aspects of sepsis care where lab tests may be used
  - Screening
  - Confirming diagnosis
  - Ruling out diagnosis
  - Monitoring therapy
  - Determining prognosis
- New lab tests that address any of these needs will have to be assessed for patient care and for health economics

THE CLINICAL LABORATORY IN THE MANAGEMENT OF SEPSIS

- Key challenges
  - Early diagnosis with better sensitivity then current tests
  - Discrimination between infection and other causes of SIRS
  - Rule out ("EKG for sepsis"), but need ~100% NPV
  - Monitoring antibiotic therapy (procalcitonin)
  - Discriminate bacterial from viral infection
- Lab tests for these uses 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

THE REAL CHALLENGE

DOES THE PATIENT HAVE AN INFECTION?

WHY IS IT SO DIFFICULT TO KNOW?

INFECTION–THE PRESENCE OF VIABLE BACTERIA IN A STERILE BODY SITE

What happens?

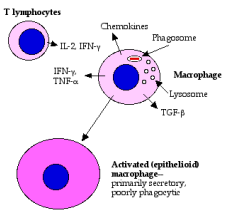
- Step 1: Macrophages phagocyte bacteria
- Step 2: Macrophages RELEASE CYTOKINES
- Step 3: Cytokines activate circulating WBC (neutrophils)
- Step 4: Cytokines stimulate bone marrow
- Step 5: Released granulocytes must stay in the circulation and reach the site of infection
- Step 6: Bone marrow releases more granulocytes in blood, some of which are immature (left shift)

STEPS 1 AND 2–MACROPHAGE PHAGOCYTOSIS AND CYTOKINE RELEASE

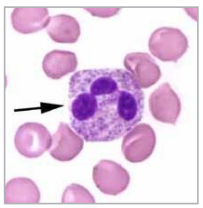
- STEP 1 TESTS: blood culture and gram stain
- STEP 2 TESTS: CRP, ESR, IL-6

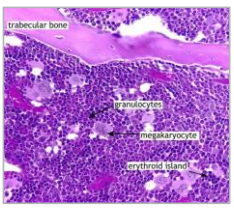
Neutrophil stimulus: toxic granules contain toxic enzymes that kill bacteria opsonized by Ab

Bone marrow stimulus: more granulocytes released in circulation



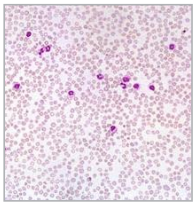
STEPS 3 AND 4–MORPHOLOGIC CHANGES DETECTABLE AT THIS STAGE–MICROSCOPIC REVIEW





STEPS 5 AND 6–LEUCOCYTOSIS AND LEFT SHIFT NEUTROPHILIA

Leucocytosis



Left shift neutrophilia
(Increased bands)

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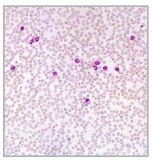
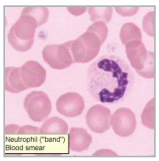
STEPS 5 AND 6–LEUCOCYTOSIS AND LEFT SHIFT NEUTROPHILIA

Increased Bands

- This is what we use today to diagnose infection in the CBC-DIFF
- The **last steps** in the entire process!





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THERE IS A NEED FOR NEW SEPSIS MARKERS

- Current tests routinely ordered in practice, such as the CBC-diff, have very limited specificity and sensitivity
- The changes expected to be seen in sepsis may be absent in a large proportion of cases
- The same changes may be present in a series of other conditions
- The left shift, neutrophilia and leucocytosis expected to be seen in bacterial infection including sepsis, are in fact markers of inflammation
- Other inflammatory conditions, ranging from SIRS to a simple surgery, also lead to these changes

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OVER THE LAST DECADE, SEVERAL SEPSIS BIOMARKERS HAVE BEEN PROPOSED

- Review of 3,370 articles discussing 178 biomarkers
- Different clinical applications, including diagnosis, discrimination from SIRS, and prognostication

Pierrakos and Vincent Critical Care 2010, 14:R15
http://ccforum.com/content/14/1/R15

CRITICAL CARE

RESEARCH Open Access

Sepsis biomarkers: a review

Charalampos Pierrakos, Jean-Louis Vincent*

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Table 1: Cytokine/chemokine biomarkers identified in the literature search (with some selected references)

Sepsis marker	Evaluated in experimental studies	Evaluated in clinical studies	Evaluated as a prognostic factor	Comment
GRO-alpha [49,50]	✓	C (m)	✓	Higher in septic shock than in sepsis
High mobility group-box 1 protein (HMGB-1) [51,52]	✓	C	✓	No difference between survivors and non-survivors at 28 days
IL-1 receptor antagonist [53,55]	✓	A	✓	Correlation with SOFA score
IL-1β [56,57]	✓	A		Increased in septic compared with non-septic individuals
IL-2 [58]		B	✓	Increased in parallel with disease severity
IL-4 [59]		C (s)	✓	Increased levels associated with development of sepsis
IL-6 [48,60]	✓	B	✓*	Distinguished between survivors and non-survivors at 28 days
IL-8 [61,62]		B	✓***	Prediction of MOF, DIC
IL-10 [63-65]	✓	B	✓**	Higher in septic shock than sepsis, distinguished between survivors and non-survivors at 28 days
IL-12 [66,67]	✓	C	✓	Predictive of lethal outcomes from postoperative sepsis
IL-13 [68,69]	✓	B	✓	Higher in septic shock than sepsis
IL-18 [37,70]	✓	B (s)	✓	Distinguished between survivors and non-survivors at 28 days
Macrophage inflammatory protein (MIP)-1 and -2 [71,72]	✓	A	✓	Increased sepsis compared with healthy controls
Macrophage migration inhibitory factor (MIF) [42,73]	✓	A	✓**	Distinguished between survivors and non-survivors at 28 days
Monocyte chemotactic protein (MCP-1 and 2 [42,74]	✓	B	✓*	Distinguished between survivors and non-survivors at 28 days
Osteopontin [75]		B		Increased in sepsis compared with healthy controls
RANTES [76,77]	✓	C	✓	Distinguished between survivors and non-survivors at 28 days in patients with septic shock
TNF [78,79]	✓	C	✓	Distinguished between survivors and non-survivors at 28 days in patients with septic shock

*sensitivity and specificity of less than 80%, **sensitivity of more than 90% but specificity of less than 60%, ***sensitivityand specificity more than 90%. A: Clinical study with less than 20 patients; B, Clinical study with 20 to 50 patients; C, Clinical study with more than 50 patients; (s), surgical patients only; (m), medical patients only.

DIC: disseminated intravascular coagulopathy; MOF: multiple organ failure; SOFA: sequential organ failure assessment.

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Table 2: Cell marker biomarkers identified in the literature search (with some selected references)

Sepsis marker	Evaluated in experimental studies	Evaluated in clinical studies	Evaluated as a prognostic factor	Comment
CD10 [80,81]	✓	A		Decreased in septic shock compared with healthy controls
CD11b [82,88]	✓	B (s)	✓	Correlation with SOFA score
CD11c [84]		A		Decreased in septic shock compared with healthy controls
CD14 (cellular and soluble) [85]		C	✓	Distinguished between survivors and non-survivors at 28 days
CD18 [86]	✓			
CD25 (cellular and soluble) [87]		A		Distinguished between survivors and non-survivors at 28 days
CD28 (soluble) [88]		B	✓	Distinguished between survivors and non-survivors at 28 days
CD40 (cellular and soluble) [89]		B	✓	Distinguished between survivors and non-survivors at 28 days
CD48 [90]		B		Increased in sepsis compared with healthy controls
CD64 [91]		B	✓	Correlation with APACHE II and SOFA scores
CD 69 [92]		A		Increased in sepsis compared with healthy controls
CD80 [88]		B	✓	Distinguished between survivors and non-survivors at 28 days
CD163 (soluble) [93]		C	✓	Distinguished between survivors and non-survivors at 28 days
mHLA-DR (soluble) [94]		C	✓*	Distinguished between survivors and non-survivors at 28 days with septic shock

*sensitivity and specificity of less than 80%, **sensitivity of more than 90% but specificity of less than 60%, ***sensitivityand specificity more than 90%; A Clinical study with less than 20 patients; B, Clinical study with 20 to 50 patients; C, Clinical study with more than 50 patients; (s), surgical patients only; (m), medical patients only.

APACHE: acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment.

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Table 3: Receptor biomarkers identified in the literature search (with some selected references)

Sepsis marker	Evaluated in experimental studies	Evaluated in clinical studies	Evaluated as a prognostic factor	Comment
CC chemokine receptor (CCR) 2 [95]	✓			
CCR 3 [96]		C	✓	Distinguished between survivors and non-survivors at 28 days
CSL2 [97]	✓	B	✓	Predicted development of MOF
CRTh2 [98]		C	✓	Distinguished between survivors and non-survivors at 28 days
Fas receptor (soluble) [99]		B (m)	✓	Predicted development of MOF
Fc-gamma RIII [100]		A	✓	Increased in sepsis compared with healthy controls, correlated with APACHE II score
FLT-1 (soluble) [101,102]	✓	C	✓	Correlated with APACHE II score
GP130 [103]		A		Increased in sepsis compared with healthy controls
IL-2 receptor (soluble) [104]		C	✓	Predicted development of septic shock
Group II phospholipase A2 (PLA2-11)	✓	B		Distinguished between survivors and non-survivors at 28 days
RAGE (soluble) [107]		B	✓*	Distinguished between survivors and non-survivors at 28 days
ST2 (soluble, IL-1 receptor) [108]		A (s)	✓	Increased sepsis compared with healthy controls
Toll-like receptor (TLR) 2 and 4 [109]	✓	B	✓	Increased in septic compared with non-septic critically ill patients
TREM-1 (soluble) [111, 112]	✓	C	✓	Distinguished between survivors and non-survivors at 28 days
TNF-receptor (soluble) [113]		B		Predicted development of MOF
Urokinase type plasminogen activator receptor (uPAR) (soluble) [114]		C (m)	✓	Distinguished between survivors and non-survivors at 28 days

*sensitivity and specificity of less than 60%; **sensitivity of more than 60% but specificity of less than 60%; ***sensitivity and specificity more than 60%; A Clinical study with less than 20 patients; B. Clinical study with 20 to 50 patients; C. Clinical study with more than 50 patients; (s) surgical patients only; (m) medical patients only

APACHE acute physiology and chronic health evaluation; MOF: multiple organ failure; TREM: triggering receptor expressed on myeloid cells; RAGE receptor for advanced glycation end-products

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ADDITIONAL TYPES OF SEPSIS BIOMARKERS

- Coagulation biomarkers
- Vascular endothelial damage biomarkers
- Vasodilation biomarkers
- Organ dysfunction biomarkers
- Acute phase protein biomarkers

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ADDITIONAL TYPES OF SEPSIS BIOMARKERS

- Coagulation biomarkers
- Vascular endothelial damage biomarkers
- Vasodilation biomarkers
- Organ dysfunction biomarkers
- Acute phase protein biomarkers

All these biological events are associated with a dysregulated inflammatory process

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PROPOSED SEPSIS BIOMARKERS– PRACTICAL CONSIDERATIONS

- Added cost to an already constricted health economics environment
- Not part of routine care; thus will only be ordered upon clinical suspicion–diagnosis already made at this point
- For neonates, additional blood required

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CRITICAL CLINICAL NEEDS A SEPSIS BIOMARKER SHOULD ADDRESS

- Earlier diagnosis of sepsis–information would have to be available as part of the routine workup of an ICU or ED patient
- Higher specificity for infection, lower sensitivity to the inflammatory process
- Indication of etiology (gram positive versus gram negative, versus viral or fungal)

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FINAL CONCLUSION FROM THE LARGEST LITERATURE REVIEW ON SEPSIS BIOMARKERS

Conclusions

Our literature review indicates that there are many bio-markers that can be used in sepsis, but none has sufficient specifically or sensitivity to be routinely employed in clinical practice. PCT and CRP have been most widely used, but even these have limited abilities to distinguish sepsis from other inflammatory conditions or to predict outcome. In view of the complexity of the sepsis response, it is unlikely that a single ideal biomarker will ever be found. A combination of several sepsis biomarkers may be more effective, but this requires further evaluation.

Key messages

- More than 170 different biomarkers have been assessed for potential use in sepsis, more for prognosis than for diagnosis
- None has sufficient specificity or sensitivity to be routinely employed in clinical practice
- Combinations of several biomarkers, but this requires further evaluation

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