Stop Sepsis: Challenges and Approaches to the Laboratory Diagnosis of Sepsis

Esther Babady, PhD, D (ABMM)
Memorial Sloan-Kettering Cancer Center
New York, New York
Learning Objectives

After this presentation you should be able to:

- Define the sepsis continuum
- List standard and novel laboratory methods used for diagnosis of sepsis
- Describe advantages and challenges of laboratory methods used for diagnosis of sepsis
Case

- 32 yo man
- Patient presented to ED with a 2-day history of weakness, general malaise, and fever associated to retro-orbital headache
- Admits having some vomiting and loose stools but no abdominal pain or nausea
- Investment banker, lives in NYC, no recent travel history
Case

- In ER
  - Temperature: 38.0°C
  - Blood pressure: 120/78 mm Hg
  - Heart rate: 92 beats/min

- Given IV fluids and Tylenol
- Discharged home
Case

- No improvement overnight and returns to ER following day
  - Temperature: 39.1°C
  - Blood pressure: 110/70 mm Hg
  - Heart rate: 94 beats/min

- On further questioning, recalls scraping his knee during a basketball game a week prior to presentation
Case

- ER physician orders lactate and CBC

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>6</td>
<td>[0.3-1.3 mM/L]</td>
</tr>
</tbody>
</table>

- Patient admitted and Sepsis Protocol initiated
- Additional testing requested:
  - Blood cultures
  - Wound cultures
Background: Sepsis continuum

- Two components
  - Infections (probable or documented)
  - Host response to infection: Systemic Inflammatory Response syndrome (SIRS)

Dellinger et al., CCM 2013, v 41, #2, 580-670
Background: Sepsis continuum

<table>
<thead>
<tr>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepsis</td>
</tr>
<tr>
<td>Infection and SIRS*</td>
</tr>
<tr>
<td>Severe sepsis</td>
</tr>
<tr>
<td>Sepsis and organ dysfunction or tissue hypoperfusion</td>
</tr>
<tr>
<td>Septic shock</td>
</tr>
<tr>
<td>Sepsis-induced hypotension (despite adequate fluid resuscitation)</td>
</tr>
</tbody>
</table>

*Temperature, Heart rate, Respiratory rate and WBC

Dellinger et al., CCM 2013, v 41, #2, 580-670
Sepsis: Epidemiology

Figure 1. Hospitalizations for and with septicemia or sepsis

NOTE: Significant linear trend from 2000 through 2008 for both categories.
Sepsis: Epidemiology

- Sixth most common cause of hospitalization (~2.1%)
- Most costly cause of hospitalization (~$20 billions in 2011)
- High in-hospital mortality (~16%)

Sepsis: Epidemiology

- Kaiser Permanente Northern California
  - 2010-2012
  - 1 in every 2 to 3 deaths
  - Most patients had sepsis at admission
  - Patients with initially less severe sepsis made up the majority of sepsis deaths

Liu, V. JAMA July 2, 2014 Volume 312, pp 91-92
Surviving Sepsis Campaign

- An international effort:
  - 2004
    - Initial resuscitation (≤ 6 hrs): ↑ serum lactate (hypotension/lactic acidosis).
  - Diagnosis:
    - Blood cultures and other appropriate cultures before start of antimicrobial therapy.

Dellinger et al., CCM 2004, v 32, 858-873
Surviving Sepsis Campaign

An international effort:

- 2008
  - Initial resuscitation (≤ 6 hours): serum lactate ≥ 4 mmol/L.
  - Diagnosis:
    - Blood cultures (≥ 2) and other appropriate cultures before start of antimicrobial therapy. Gram stains of samples,
    - Procalcitonin: ?
    - Rapid diagnostics?

Dellinger et al., CCM 2008, v 36, 296-327
Surviving Sepsis Campaign

- An international effort:
  - 2013
    - Initial resuscitation (≤ 6 hrs): serum lactate ≥ 4 mmol/L.
    - Routine screening of seriously ill patients for sepsis.
  - Diagnosis:
    - Blood cultures (≥ 2) and other appropriate cultures before start of antimicrobial therapy. Gram stains of samples,
    - Fungal markers: BD glucans, mannan and anti-mannan abs.
    - Rapid influenza tests (during Flu season)
    - Procalcitonin and CRP: No recommendations provided
    - Non-culture methods: No data

Dellinger et al., CCM 2013, v 41 , 580-670
Sepsis: States Regulations

The New York Times

N.Y. / REGION

 Cuomo Plans New Rules in Fight Against Sepsis

By JIM DWYER  JAN. 7, 2013

Gov. Andrew M. Cuomo will announce in his State of the State Message this week that every hospital in New York must adopt aggressive procedures for identifying sepsis in patients, including the use of a countdown clock to begin treatment within an hour of spotting it, a state official said.

First state to require all hospitals to have a sepsis protocol
Sepsis: Regulations

https://www.health.ny.gov/regulations/public_health_law/section/405/
Sepsis

- *Early recognition of sepsis is key*

- Survival from septic shock is directly related to the duration of hypotension before initiation of effective antimicrobial therapy

Laboratory Testing:

- Abnormal vital signs
- Blood cultures
- Fungal markers
- Lactate
- Rapid influenza tests
- Non-culture methods
- Procalcitonin
- C-reactive protein
Laboratory Tests: Lactate

- First laboratory measurement in support of sepsis
- Usually ordered STAT. Performance may be time sensitive.
- Marker of hypotension:
  - Lactate $\geq 4\text{mmol/L}$
  - Serial monitoring for normalization

Dellinger et al., CCM 2013, v 41, 580-670
Laboratory tests: Blood Culture

- Gold standard for diagnosis of bacteremia
- Positive in ~30-50% of sepsis cases
- Yield affected by:

  PRE-ANALYTICAL VARIABLES
Laboratory tests: Blood Culture

- Timing of collection re: antibiotic administration
  - Ideally prior to antibiotic administration
  - Should not substantially delay start of therapy
- Volume collected
  - 20-30 mL/set
- Number of sets
  - @ least two sets
- Time from collection to incubation
  - As soon as possible

Blood Culture: Traditional Methods

Preliminary Report: Gram positive cocci
Traditional Diagnostic tools
Blood Culture: Traditional Methods

Preliminary Report: Gram positive cocci

Final Report: Sensitive S. aureus

T=0  T=hours/days  Minutes  Minutes  Days
# New technologies

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Test name</th>
<th>Resistance marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>AdvanDx, Inc.</td>
<td>Staphylococcus QuickFISH BC</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>S. aureus</em> and/or other Staph species PNA FISH</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>S. aureus</em> PNA FISH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mecA Xpress FISH</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td>E. faecalis/OE PNA FISH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. faecalis PNA FISH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GNR Traffic light PNA FISH</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td>E. coli/P. aeruginosa PNA FISH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EK/P aeruginosa PNA FISH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. coli PNA FISH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. albicans PNA FISH</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td>C. albicans/C. glabrata PNA FISH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yeast Traffic Light PNA FISH</td>
<td></td>
</tr>
</tbody>
</table>

As of 04/2015
New technologies

- **DNA Probe**: A,T,C,G; negatively charged phosphate backbone
- **PNA Probe**: A,T,C,G: non-charged polyamide or “peptide” backbone
- Peptide nucleic acid Fluorescent in-situ hybridization (PNA-FISH)

*Enterococcus faecalis/OE*  
*Staphylococcus aureus/CoNS*
## New technologies

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Test name</th>
<th>Method</th>
<th>Resistance marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cepheid</td>
<td>Xpert MRSA/SA BC</td>
<td>PCR</td>
<td>✓</td>
</tr>
<tr>
<td>Nanosphere, Inc.</td>
<td>Verigene Gram-Positive Blood Culture test</td>
<td>Multiplex Gold nanoparticle Probes</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Verigene Gram-Negative Blood Culture Test</td>
<td>Multiplex Gold nanoparticle Probes</td>
<td>✓</td>
</tr>
<tr>
<td>bioMérieux</td>
<td>BioFire FilmArray Blood Culture Identification Panel</td>
<td>PCR</td>
<td>✓</td>
</tr>
<tr>
<td>BD Diagnostics</td>
<td>BD GeneOhm StaphSR Assay</td>
<td>PCR</td>
<td>✓</td>
</tr>
</tbody>
</table>

As of 04/2015
New technologies

- Verigene, Nanosphere
- FilmArray, BioFire
- MALDI-TOF MS
- GeneXpert, Cepheid
Laboratory testing: Performance

- 146 positive monomicrobial BC

<table>
<thead>
<tr>
<th></th>
<th>Accuracy (%)</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuickFISH</td>
<td>98</td>
<td>60-100</td>
</tr>
<tr>
<td>MALDI-TOF MS</td>
<td>88</td>
<td>50-100</td>
</tr>
<tr>
<td>Verigene BC-GP</td>
<td>98</td>
<td>67-100</td>
</tr>
</tbody>
</table>

- Challenge with polymicrobial BC

Martinez, R.M. et al., JCM 2014, 52: 2521-2529
Laboratory testing: Performance

- Conventional methods vs. Verigene BC GP/GN and FilmArray BC

New technologies

- FDA cleared 2014
- T2 magnetic resonance
- *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*
- Directly from whole blood
- Sensitivity: 88-94%
- Specificity: 99%
- TAT: ~4 hours

Laboratory Tests: Biomarkers

Most common biomarkers:

- Procalcitonin
- C-reactive protein
- Interleukins (6 and 8)
- ADM
- LBP
- PTX3
- EAA

Laboratory Tests: Biomarkers

- Procalcitonin
  - 116-amino acid precursor to calcitonin
  - Marker of inflammation:
    - Increases in response to bacterial toxins, malaria, fungi
    - Increases in noninfectious syndromes (i.e. burn, trauma)

Laboratory Tests: Biomarkers

- Procalcitonin utility
  - Diagnostics
  - Prognostics

# Laboratory Tests: Procalcitonin

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Test name</th>
<th>Method</th>
<th>Functional sensitivity (ng/mL)</th>
<th>Cut off</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAHMS</td>
<td>BRAHMS PCT LIA</td>
<td>Chemiluminescence</td>
<td>0.3</td>
<td>&gt;2  high risk</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.5 low risk</td>
</tr>
<tr>
<td>BRAHMS</td>
<td>BRAHMS PCT sensitive Kryptor® test</td>
<td>Immunofluorescent</td>
<td>0.06</td>
<td>&gt;2  high risk</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.5 low risk</td>
</tr>
<tr>
<td>bioMérieux</td>
<td>VIDAS BRAHMS PCT</td>
<td>Enzyme-Linked Fluorescent Assay immunoassay</td>
<td>0.05</td>
<td>&gt;2  high risk</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.5 low risk</td>
</tr>
</tbody>
</table>

As of 04/2015
Does it or Doesn’t it?

- Challenge with interpretation of procalcitonin data
  - Different study populations
  - Different measurement methods
  - Different comparator methods
  - Different cut-off values
Laboratory testing: Performance

- 295 adult patients, 15 months
- Emergency room, Baltimore, MD
- Blood culture (GS) and Procalcitonin, 24h

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT (0.1475 ng/mL)</td>
<td>75</td>
<td>78.9</td>
<td>17</td>
<td>98.2</td>
</tr>
</tbody>
</table>

Screening assay: Rule-out sepsis

Reidel, S. et al., AJCP 2011, 135: 182-189
Laboratory testing: Performance

- Blood culture as reference
- 387 publications, 17 included: 2,008 patients
- Pooled sensitivity/specificity:
  - 76% and 70% (cut-off 0.5 or 0.4 ng/mL)
- Moderate marker
Procalcitonin and AST

- 6 published randomized controlled trials
  - Procalcitonin guided vs standard care
  - Significant reduction in antibiotic duration
  - No deleterious effect on relapse and mortality
Procalcitonin and AST

- Syndromes
  - Bacterial pneumonia
  - Sepsis

- Predictive
  - Low PCT (2 with 4-6 hrs): delay or no initiation of therapy
  - Sequential values: falling trend to discontinue therapy (i.e. 0.1 ng/mL)

Gilbert, D.N. J Clin Micro 2010, 48: 2325–2329
Dellinger et al., CCM 2013, v 41 , 580-670
Conclusions

- Sepsis remains an important syndrome associated with high costs and high mortality.
- Prompt identification of sepsis remains a challenge.
- Rapid and sensitive commercial assays for identification of microorganisms are now available.
- Several serum/plasma biomarkers including PCT have been evaluated for diagnosis of sepsis.
- Implementation of these rapid methods may positively impact the outcome of sepsis.
Self-Assessment Questions

1. Which of the following test is recommended for severe sepsis evaluation in the 2012 SSC guidelines?
   A. Blood cultures  
   B. Procalcitonin  
   C. CRP  
   D. C. difficile PCR

2. Procalcitonin levels in serum may increase for all of the following conditions except?
   A. Surgery  
   B. Bacterial pneumonia  
   C. Sepsis  
   D. Viral pneumonia
Self-Assessment Questions

3. Broadly multiplexed molecular assays are currently available for detection of pathogens from positive blood culture?
   A. True
   B. False

4. Which of the following statement about sepsis is correct?
   A. Caused by infection only
   B. Caused by infection and inflammation
   C. Caused by inflammation only
   D. None of the above