Autopsy symposium

Diagnosis of infection, sepsis and SIRS at autopsy

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Case: S-15-28

**Clinical**

- Male 64, obese, hypertensive, diabetic
- D0: morning - felt cold and shaky
- GP diagnosis = gastroenteritis
- D0: evening, admitted after collapse
- Hospital diagnosis = septic shock
- All tests negative
  - Cultures, PCR for meningococcus
- ITU care: antibiotics, life support
- D1: dead within 24 hours of admission

**Autopsy**

- Lungs congested
- Large heart – 780gm, LVH
- Fatty liver – 3450gm
- Spleen 300gm, not soft
- Large vague red ?bruise inside left arm
Lung

Lung - DIC

Lung – CD54+ endothelial cells
Bone marrow – haemophagocytosis (HPC)
Steatosis, and HPC of Kupffer cells
Spleen: congested, and white pulp atrophy
Skin – subcutis necrosis and cocci++. A form of ‘necrotising fasciitis’
Case conclusion

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Pathogenesis</th>
</tr>
</thead>
</table>
| • Toxic shock syndrome  
  • The most severe form of ‘septic shock’ | • Minor skin injury |
| • Group A Streptococcus pyogenes (GAS) infection  
  • gram+ve cocci in chains | • GAS transfer from nose to skin |
| | • ? Host susceptibility factors  
  • Defence capacity - *genetic*  
  • Obesity  
  • Steatosis |
Diagnostic gross appearance... and microscopic?
Diagnostic gross appearance.... and microscopic

Gram+ve diplococci = pneumococcus
Schema of presentation

• Illustrative case material

• SEPSIS – a bit of theory and epidemiology

• The main autopsy organ pathologies, gross and microscopic

• Differential diagnosis of sepsis

• Sampling and testing autopsy material

• What is this all for?

• Is not all sepsis sorted out?
  • a) by clinical ID, ITU and microbiology
  • b) in life
What is sepsis?

Systemic Inflammatory Response Syndrome (SIRS)

• Temperature >38 deg C or <36 deg C
• Heart rate >90/min
• Respiratory rate >20/min or PaCO$_2$ <32mmHg
• Blood white cell count >12x10$^9$/L or <4x10$^9$/L, or >10% immature band forms.
### 3 Grades of sepsis – definitions modified from 1991 to ?

<table>
<thead>
<tr>
<th><strong>SEPSIS:</strong></th>
<th><strong>SEVERE SEPSIS:</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- SIRS + infection</td>
<td>- Sepsis with associated organ dysfunction, hypoperfusion, or hypotension including lactic acidosis, oliguria, or acute alteration in mental state.</td>
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</tbody>
</table>
3 grades of sepsis – defined *physiologically*

- **SEPTIC SHOCK**
  - Sepsis-induced hypotension despite adequate fluid resuscitation, along with presence of severe sepsis.

- **Problem:**
  - Nearly everyone in an ITU could be diagnosed on the physiological criteria as *SEPSIS*.
  - ~40% cases of ‘sepsis’ are culture negative
  - So how can we better treat and do clinical trials?
Pathogenesis of sepsis

- Main concept:
  - A focus of infection
    - Organ
    - Cavity
    - Blood only
  - With distant organ damaging effects
  - Multi-organ failure
  - Due to cytokine secretion and amplification

- Is *acute septic shock* a special case?
- Experimental and clinical evidence:
  - *Macrophage activation and release of TNFα & other mediators*
  - *Necessary and sufficient*
- Sepsis & severe sepsis are failures of homeostasis
Non-septic sepsis

- Pancreatitis
- Bypass surgery
- Major trauma
- Burns
- Heat stroke
- Thyroid storm

- ...and some others......

Clinical diagnosis: necrotising fasciitis

Autopsy diagnosis: pancreatitis
The autopsy pathology of sepsis: gross + histopathology – focus on generalised features

<table>
<thead>
<tr>
<th>Left Side</th>
<th>Right Side</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Spleen</td>
<td>• Gut</td>
</tr>
<tr>
<td>• Lymph nodes and gut lymphoid tissue</td>
<td>• Lung</td>
</tr>
<tr>
<td>• Bone marrow</td>
<td>• Kidney</td>
</tr>
<tr>
<td>• Liver</td>
<td>• Heart</td>
</tr>
<tr>
<td></td>
<td>• Adrenal</td>
</tr>
<tr>
<td></td>
<td>• [Brain</td>
</tr>
<tr>
<td></td>
<td>• Selective neuronal damage</td>
</tr>
<tr>
<td></td>
<td>• Research only]</td>
</tr>
<tr>
<td></td>
<td>• Skin</td>
</tr>
<tr>
<td></td>
<td>• Genital tract in pregnancy</td>
</tr>
</tbody>
</table>
GAS Toxic Shock Syndrome
Spleen

• White pulp atrophy

• Diffluent??
• Soggy red and runny
• ....not specific!!

• Haemophagocytosis by red pulp macrophages

• Focal fibrinoid necrosis

Problems: autolysis
HPC in bone marrow – please remember to sample marrow!
Leptospirosis liver - HPC

CD68+ Kupffer cells
Gut case – rapid death at home
Case – ileum submucosa
Case — fixed, paraffin-embedded ileum

Both amplicons sequenced:

*Shigella flexneri* 98% homology

*Shigella boydii* 98% homology

Diagnosis: shigellosis made worse by liver cirrhosis
Lungs
Acute lung injury (ALI)

- Shock lung
- Hyaline membrane disease
- Adult respiratory distress syndrome
- Acute respiratory distress syndrome (ARDS)

- NB – all histopathological definitions
- Not diagnosable by naked eye alone

- Also
- DIC
- And upregulated endothelial cell ICAM-1 expression
ALL progression
Lung CD54 (ICAM-1) - upregulated by TNFa & HMGBP-1

‘Endothelial cell up-regulation = septic shock”?

Dr M. Tsokos (Germany)
Kidney: disseminated intravascular coagulation (DIC)

- Most frequently seen in
  - Meningococcal infection
  - Strep pyogenes (group A)

- Fibrin & PLTs
  - Platelets alone = TTP

Also: acute tubular injury/necrosis & myoglobin casts
Heart: gross - myocarditis?
Gross – can be misleading........

B19 viral myocarditis

CPR haemorrhage

Toxic shock syndrome – firm muscle

TTP-related ischaemia
Toxic myocarditis

Direct effect

- Drugs
- Catecholamines
  - Resuscitation
  - Phaeochromocytoma
- Cocaine
- Amphetamines

Indirect - SIRS

- Secondary transient pathology eg in sepsis
- Cytokine storm
- T-cell activation

Histopathology

- Pleomorphic inflammation
- +/- small foci of necrosis
- +/- contraction bands
- Interstitial oedema
SIRS heart:
TSS from GAS infection

CD54
Adrenals: Waterhouse-Friedrichson syndrome
Skin septic rashes

- GAS rashes are more blurred and less haemorrhagic
- ‘scarlet fever rash’

- Bacteria are more scanty unless it is ‘necrotising fasciitis-like’
  - Bland necrosis
  - No cellular reaction
  - Vast numbers of GPC

Meningococcaemia
Case: Meningococcal skin
Case: Meningococcal skin

Brown-Hopps gram
Meningococcal meningitis IHC+
Schema of presentation

• Illustrative case material

• SEPSIS – a bit of theory and epidemiology
  • In UK, ~150,000 cases a year [300/100,000 population]

• The main autopsy organ pathologies, gross and microscopic

• Sampling and testing autopsy material

• Differential diagnosis of sepsis

• What is this all for?

• Is not all sepsis sorted out?
  • a) by clinical ID, ITU and microbiology
  • b) in life
Risk factors for severe sepsis

**Specific**
- Hyposplenism
  - Spleenectomy
  - Sickle cell disease
  - Congenital
- Cirrhosis
- HIV disease
- Transplantation
- Immunosuppressive therapies

**General**
- Malignancy
- Diabetes
- Alcohol
- Autoimmune disorders
- Extremes of age
### Infections in sepsis (USA 2000)

- 37% gram-negative infections
- 52% gram-positive infections
- 5% polymicrobial infections
- 1% anaerobes
- 5% fungi

**However:** other studies..........

2-4% viral and parasitic infections (but probably underestimated)

### In real life:

- ~30-40% negative cultures (community-acquired sepsis treated with antibiotics before admission)

![Candida in kidney](image)
Blood cultures – how to sample

**Blood Circulation**
Principal Veins and Arteries

- Neck veins
- Heart
- Femoral veins

**NOT intra-abdominal veins** – OK for toxicology samples, NOT for cultures.
Other sampling processes

- CSF
- Solid organs – care against contamination
- Culture
  - Bacteria, mycobacteria, fungi
- Parasites and viruses
- Histology
  - Special stains
  - ISH
  - PCR
- Cytology
- Serology

Spleen dab: giemsa & HIVp24
## Two similar cases – clinically “MOF/sepsis”

<table>
<thead>
<tr>
<th><strong>#1: F 24yrs</strong></th>
<th><strong>#2: F 30yrs</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Phillipino</td>
<td>African</td>
</tr>
<tr>
<td>No medical history</td>
<td>Recurrent joint pains – hands and knees</td>
</tr>
<tr>
<td>Unwell, abdo pain</td>
<td>Skin rash</td>
</tr>
<tr>
<td>Progress to MOF</td>
<td>Seronegative for RhF etc</td>
</tr>
<tr>
<td></td>
<td>• Similar history to other case</td>
</tr>
<tr>
<td></td>
<td>• Died on D14 admission</td>
</tr>
<tr>
<td></td>
<td>• AUTOPSY- GROSSLY NEGATIVE</td>
</tr>
<tr>
<td>• Liver</td>
<td></td>
</tr>
<tr>
<td>• Lung</td>
<td></td>
</tr>
<tr>
<td>• Kidney</td>
<td></td>
</tr>
<tr>
<td>• All imaging negative</td>
<td></td>
</tr>
<tr>
<td>• All microbiology negative</td>
<td></td>
</tr>
<tr>
<td>• Died on D8 of admission</td>
<td></td>
</tr>
<tr>
<td>• AUTOPSY – ENLARGED NODES IN MEDIASTINUM only</td>
<td></td>
</tr>
</tbody>
</table>
Bone marrow – similar in both
#1: Hilar node
#1: Hilar node
Diagnosis – #1

• MOF
• Cytokine storm
• Haemophagocytosis
• Not sepsis (pre & post mortem studies)

• T-cell lymphoma – occult mediastinal nodes, not mass lesion
• ?diagnosable in life?
Diagnosis – #2

- Haemophagocytosis
- No lymphoma
- Not sepsis (pre & post mortem studies)
- ?

- Adult-onset Still’s disease [seronegative]
- Can trigger fatal cytokine storm
- Mimicking septic shock
• Infection (1108)
  • a) Viruses (762)
  • Epstein-Barr virus (330)
  • HIV (173)
  • Herpes viruses (74)
  • Cytomegalovirus (69)
  • Viral hepatitis (20)
  • Influenza (14)
  • Human parvovirus B19 (14)
  • Other viruses or not specified (68)
  • b) Bacteria (206)
  • Mycobacterium tuberculosis (78)
  • Rickettsia spp (17)
  • Staphylococcus spp (15)
  • Escherichia coli (11)
  • Other bacteria or not specified (85)
  • c) Parasites (53)
  • Leishmania spp (17)
  • Plasmodium spp (14)
  • Toxoplasma spp (10)
  • Other parasites (12)
  • d) Fungi (37)
  • Histoplasma spp (18)
  • Other fungi (19)
  • e) Infection not specified (50)

• Neoplasms (1047)
  • a) Haematological (981)
    • T-cell or natural-killer lymphoma (369)
    • B-cell lymphoma (333)
    • Leukaemia (67)
    • Hodgkin’s lymphoma (61)
    • Not specified lymphoma (35)
    • Castleman’s disease (22)
    • Other haematological neoplasms or not specified (94)
  • b) Solid (32)
  • c) Not specified neoplasm (34)

• Autoimmune diseases (276)
  • a) Systemic (244)
    • Systemic lupus erythematosus (133)
    • Adult-onset Still’s disease (54)
    • Rheumatoid arthritis (18)
    • Vasculitis (11)
    • Other or not specified (28)
  • b) Organ-specific (32)
    • Inflammatory bowel disease (11)
    • Other diseases (21)

• Other circumstances or diseases (184)
  • a) Transplantation (95)
    • Kidney (53)
    • Haematological (29)
    • Other (13)
  • b) Other circumstances (76)

What is all this effort for?

• Diagnosis
  • Audit loop
  • Complications of ITU treatment

• Clinicians
• Coroners
• Family

• Research
• Clinical progress
• Clinical trials
  • New therapies for ‘sepsis’

• Case definitions of sepsis – *low specificity*

• Sepsis is like pornography, ie in the eye of the beholder? [RA Balk]

• Early diagnosis = early, more specific treatment = lower mortality

• The search for sepsis biomarkers
• >178 at last count
  • Eg procalcitonin
# Outcomes of sepsis in hospital care

<table>
<thead>
<tr>
<th>Direct</th>
<th>Indirect &amp; later</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Mortality</td>
<td>• Persistent critical illness (PCI)</td>
</tr>
<tr>
<td>• Sepsis: &gt;25-30%</td>
<td>• Cognitive dysfunction</td>
</tr>
<tr>
<td>• Septic shock 40-50%</td>
<td>• Functional disabilities</td>
</tr>
<tr>
<td></td>
<td>• Neuropathy</td>
</tr>
<tr>
<td></td>
<td>• Myopathy</td>
</tr>
<tr>
<td></td>
<td>• Immune dysfunction</td>
</tr>
<tr>
<td></td>
<td>• Post-severe sepsis survivors:</td>
</tr>
<tr>
<td></td>
<td>• Mean age 77yrs</td>
</tr>
<tr>
<td></td>
<td>• 5 yr mortality ~ 80%</td>
</tr>
</tbody>
</table>
What HIV does

INDIRECT EFFECTS

**Cell mediated immune dysfunction:**
- Opportunistic infections
- Opportunistic tumours
  - Virus-associated transformation

**Gut lymphoid depletion:**
- Microbial translocation
- Pro-inflammatory state
- “Immune activation syndromes”
  - Atherosclerosis
  - Premature ageing
  - Non-AIDS cancers
- COPD
- Liver fibrosis
- Osteopaenia
- Neurocognitive impairment?

DIRECT EFFECTS

**CD4+ T-cell destruction**

**Organ specific:**
- HV lymphadenitis
- HIV encephalopathies
- HIV glomerulopathies
- HIV enteropathy
- HIV pneumonitis
- HIV dermatitides
- Haemophagocytosis

- HIV cardiomyopathy
- HIV pulmonary hypertension
There is no specific treatment for sepsis

Failed trials

• Anti-TNF
• Tight glucose control
• Steroids
• APC
  
  • In progress
• Anti-C5a inhibitors
• Anti-HMGB1
• Pepducins
• etc

Case

• Male with congenital aplastic anaemia
• Elective bone marrow Tx failed
• Neutropaenic sepsis: gram-ve bacilli
  
  • Rx APC – activated protein C
  • Died within 2 days of lung failure
Case: lung haemorrhage only

- Rx aPC
- Known cause of intra cerebral haemorrhage

Trials:
- 8% survival benefit
- 2% risk of major bleed
Useful sepsis references

  - Google: ‘Lucas autopsy sepsis’
- RA Balk. *SIRS: where did it come from and is it still relevant today?* Virulence 2014, 5:120.
Conclusion

• The autopsy has a significant role in follow up of sepsis cases
  • Confirmation or refutation – other diagnosis
  • Medical education for all clinicians

• Assessment of complications of treatments

• Contribution to clinical trials – an aspiration at present

• A systematic approach to cadaveric examination
• Lots of histology – bone marrow etc
• Use of all diagnostic modalities