Anaerobic infections

PART 2: Infection with Gram-positive obligate anaerobes (toxigenic Clostridium spp.)

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Sources of Anaerobic Infections

• Usually endogenous
  – Intestinal anaerobes
  – Oral anaerobes

• Usually exogenous
  – *Clostridium tetani* (tetanus)
  – *Clostridium botulinum* (botulism)
  – *Clostridium difficile* (antibiotic-associated colitis)

• Either endogenous or exogenous
  – Other Clostridial infections (e.g., gas gangrene)
What are these lectures about?

- **Part 1: Invasive Clostridium spp.**
  - gas gangrene/myonecrosis: *C. perfringens, C. septicum, C. histolyticum, C. novyi, etc.*
  - wound infection/abscess: *C. perfringens*
  - food poisoning

- **Part 2: Toxigenic Clostridium spp.**
  - tetanus: *C. tetani*
  - botulism: *C. botulinum*
  - antibiotic-associated colitis: *C. difficile*

- **Part 3: Gram-negative anaerobes**
  - abscesses: *B. fragilis, Bacteroides spp, Prevotella, Porphyromonas, Fusobacterium, anaerobic cocci*
  - other
Case: back spasms in a newborn

• A 10 day old newborn male develops spastic rigidity of the face, neck and back. Minimal movement of the infant’s cradle causes repetitive whole body spasms.

• On examination, the infant has a heart rate of 140/min but is afebrile. The umbilical stump appears moist and cyanotic.
Rigidity (tetany)
Clinical features of tetanus

- No fever or sepsis
- Early localized spastic paralysis
- Generalized spastic paralysis
  - Toxin blocks central motor inhibitory impulses
  - Reflex spasms
- Trismus, risus sardonicus, opisthotonos are key signs
Organisms grow in the anaerobic wound

Tetanus toxin enters a peripheral nerve and migrates centrally

Release of GABA and glycine is inhibited

Toxin enters pre-synaptic, inhibitory neurons of the spinal cord and brain stem

Stimulatory motor impulses are uninhibited, and tetany occurs
Tetanus toxin mechanism

- 150kDa protein exotoxin
  - A-B two-chain toxin, connected by a -S-S- bridge
  - A is a zinc endopeptidase, B is a binding protein
- Toxin enters α-motor neurons at the wound site, is discharged across synapses, and is taken up by presynaptic neurons (B subunit binds to specific receptors)
- A subunit is released into cytoplasm
- Degrades synaptobrevin, preventing release of vesicle contents
- Note: There is no significant toxemia
Risus sardonicus
Opisthotonos in an adult
Tetanus-who is at risk?

- Unvaccinated persons with puncture wounds
- Neonates with unsanitary umbilical care
- IV drug users
Treatment & Prevention

• Antiserum to toxins to neutralize any free toxin
• Antibiotics (e.g., metronidazole) to kill live organisms
• Physical and respiratory support
• Primary tetanus vaccination (toxoid); priority for unvaccinated pregnant woman
  – N.B. tetanus is a non-immunizing event
How does toxoid vaccine work?

**INFECTION**

- wound

**VACCINATION**

- culture

**Peripheral Nerve**

No uptake by antigen-presenting cells

- toxoid

Anti-toxoid antibodies produced

**Y Y Y**
How does toxoid vaccine work?

Anti-toxoid antibodies bind to and inactivate toxin
Case: descending paralysis

- 18 hours after eating home-canned string beans, a 38 year old man develops blurred vision, slurred speech, and dry mouth. Within hours, he notes weakness of the neck and arms and is having labored breathing.
- On physical examination, his vital signs are normal. He is drooling.
- His 34 year old wife also ate some of the beans and is now beginning to have some difficulty swallowing.
Botulism

Improper sterilization; *C. botulinum* spores inoculated

Toxin inhibits acetylcholine release at myoneural junction

Muscle cells

Motor paralysis and respiratory failure
How toxic is it?

- 400mg of pure botulinum toxin is enough to kill everyone on Earth!!
Mechanism of botulinum toxin

Normal neurotransmission at the neuromuscular junction:
- Vesicle containing acetylcholine
- Synaptobrevin
- SNAP-25
- Syntaxin
- Acetylcholine receptor
- Muscle fiber

Inhibitory effect of botulinum toxin:
- Toxin A subunit enters cytoplasm and proteolytically cleaves components of the SNARE complex
- Membranes cannot fuse; acetylcholine not released

Source undetermined
Other forms of botulism

• Wound botulism
  – (analogous to tetanus)

• Infant botulism
  – flaccidity at 3-20 weeks
  – ingestion of large numbers of organisms that proliferate and sporulate in the intestine
  – Honey implicated in a large outbreak
  – (+/-analogous to clostridial food poisoning)
Treatment and prevention

• Prompt antitoxin can be life-saving
  – (mortality 100% → 25%)
• Airway protection and respiratory support
• There is no vaccine
• Prevention relies on regulated food manufacturing
Case: diarrhea

• An 81-year-old male invalid with dementia has a fever of 38.5°C for 5 days. He was previously well, except for a UTI 4 weeks ago. At that time, he was hospitalized and given ampicillin.

• On P.E., he was comfortable, but confused. Temp = 39°; other vital signs - normal. There were no localized physical findings; abdominal examination-normal.

• A WBC count was 25,000/mm³
Case (continued)

• The next morning, the patient passed two loose bowel movements during the night and another in the morning.
• A stool specimen was positive for occult blood.
• Assay of stool for *Clostridium difficile* toxin was positive.
• Treatment was begun with oral metronidazole.
• The patient became afebrile within 36 hours, and he returned to his home without further laboratory investigations within 72 hours.
Questions to consider

• Where do the causative organisms come from?
• Is the history of previous treatment with ampicillin pertinent to *C. difficile* infection?
• What is the role of the spores of *C. difficile* in the disease process?
• What caused the patient’s symptoms?
• Could this illness have been fatal?
Background

- Cause of “clindamycin-associated colitis” established in 1978
- Cytotoxin assay on stool filtrate
  - *Most reliable diagnostic test*

Clinical features of CDI

- Diarrhea, abdominal cramps, fever, fecal WBCs → pseudomembranous colitis (advanced stage)
- Protein-losing enteropathy → hypoalbuminemia and anasarca
- Leukocytosis → leukemoid reaction
- Ileus → megacolon (previously rare)
Endoscopic view of PMC
CDI: predisposing factors

• Antibiotic use:
  - Clindamycin, Ampicillin, Amox (1970s)
  - Cephalosporins (1980s)
  - Fluoroquinolones (1990s onward)

• Hospitalization:
  - Colonization 10x higher in hospitalized adults

• Advanced age:
  - Attack rate 20-fold higher in patients >65 vs. <20yrs

• GI surgery/procedures
C. difficile pathogenesis

• CDI is a disease of the colon (generally it does not affect other parts of the GI tract)
• Establishes itself in the colon only when normal flora is disrupted
• The bacteria are non-invasive
• The disease is caused by bacterial toxins
  – Toxin A = enterotoxin (in most, but not all strains)
  – Toxin B = cytotoxin
• Some asymptomatic patients are culture-positive, but toxin-negative
A Change Noted in Canada

Pepin et al. CMAJ 2004;171(5):466-72
### Mortality attributable to CDI, Quebec

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Group; no. (%) of patients</th>
<th>Case subjects</th>
<th>Control subjects</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group; no. (%) of patients</td>
<td>n = 161</td>
<td>n = 656</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within 30 d</td>
<td></td>
<td>37 (23.0)</td>
<td>46 (7.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Within 90 d</td>
<td></td>
<td>48 (29.8)</td>
<td>75 (11.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Within 6 mo</td>
<td></td>
<td>58 (36.0)</td>
<td>96 (14.6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Within 1 yr</td>
<td></td>
<td>60 (37.3)</td>
<td>135 (20.6)</td>
<td>&lt; 0.001</td>
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<tr>
<td>Total duration in hospital, mean, d</td>
<td></td>
<td>33.7</td>
<td>23.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Admission to ICU</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All causes</td>
<td></td>
<td>51 (31.7)</td>
<td>158 (24.1)</td>
<td>0.06</td>
</tr>
<tr>
<td>CDAD-related</td>
<td></td>
<td>16 (9.9)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>CDAD-related colectomy</td>
<td></td>
<td>4 (2.5)</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

{~16%}
Pathogenicity loci in *C. difficile*

Positive regulator of toxin production

Toxin B gene

Toxin A gene

Toxin excretion pore

Negative regulator of toxin production
Characteristics of the epidemic strain

- Single clone
- Resistant to fluoroquinolones
- Deletion in $tcdC$
- Encodes a novel binary toxin

@PD-INEL McDonald et al. NEJM 2005; 353:2433-41)
**BI/NAP1 and severity of disease**

<table>
<thead>
<tr>
<th>Presence of ΔtcdC and binary toxin</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Non-Severe</td>
<td>110</td>
<td>25</td>
</tr>
</tbody>
</table>

P=0.03

(from Loo et al. NEJM 2005; 353: 2442-9)
Diagnosis of CDI

• Cytotoxin B assay ("gold standard")
• Toxin ELISA test
  – Only ~70-80% sensitive, hence must be repeated to have adequate sensitivity
• Culture alone is not useful
• Culture plus cytotoxin assay
• Endoscopy
• Response to metronidazole or vancomycin
Treatment of CDI

- Luminal antibiotics
  - Oral metronidazole,
  - Oral vancomycin (not absorbed)
- ? Probiotics (none proven effective)
- No antimitotility agents (contraindicated)
CDI recurrence

- Common among the elderly with severe underlying disease or continued antibiotics
- Persistence of spores in the GI tract
- Treated with long, tapering courses of vancomycin
Where are the spores?
Questions to consider

• Where do the causative organisms come from?
• Is the history of previous treatment with ampicillin pertinent to *C. difficile* infection?
• What is the role of the spores of *C. difficile* in the disease process?
• What causes the patient’s symptoms?
• Could this illness have been fatal?
Environmental methods to control the spread of CDI

- Hand hygiene: washing with antiseptic soap; not alcohol-based hand gels!
- Environmental surfaces can be cleaned with 1:10 sodium hypochlorite mixed fresh daily
- Isolate and/or cohort patients with CDI in the hospital
- Control 2nd and 3rd generation cephalosporin and fluoroquinolone use
- Treatment of asymptomatic carriers is not helpful
Vaccine?

• There is evidence that luminal antitoxin prevent disease; however,
• There is no effective vaccine currently
Generalizations about clostridia

- Sporulation is important for survival in the environment and for transmission between hosts.
- Disease is mediated by exotoxin-release from vegetative cells.
- Simple antibiotics are effective; resistance is not a problem.
- Active and passive immunization targets exotoxins.
Additional Source Information
for more information see: http://open.umich.edu/wiki/CitationPolicy

Slide 7: CDC, Neonatal tetanus, Public Health Image Library, #6374


Slide 11: CDC, Risus sardonicus, Public Health Image Library, #2857

Slide 12: CDC, Opisthotonus, Public Health Image Library, #6373


Slide 20: Source undetermined, Source undermined


Slide 40 (left to right):
- Chris McKenna, Bathroom Sink, Wikimedia Commons, http://commons.wikimedia.org/wiki/File:Bathroom_sink.JPG