Case Summary

- HPI: 39 y/o previously healthy Hispanic man presented to an Emergency Department at an outside hospital in rural South Texas with shortness of breath, massive hemoptysis and vomiting. He was welding at home that morning and reportedly felt fine. He had sudden onset of SOB and cough. Initially he had a dry cough but then quickly developed hemoptysis with bright red blood. He went to the outside ED within 2 hours of onset of his first symptoms.
Issues to be Discussed
- Adequacy criteria for various respiratory specimens
- Sensitivity, specificity of respiratory cytology
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- Critical Values in Anatomic pathology
- Banbury Project and role of Pathology/Pathologists

Respiratory, Adequacy
- Adequate if it explains the clinical condition
- Sputum - Alveolar macrophages, no numerical cut point.
- Bronchial wash & brush - Cells or agents diagnostic of a pathologic process are present.
- Well preserved, optimally stained ciliated columnar cells, goblets and macrophages.

- BAL - Adequate if demonstrates specific pathologic process
- Numerous alveolar macrophages(93+/−5%) and few lymphocytes(7+/−1%). Occasional neutrophils and rare ciliated or squamous cells
Respiratory Adequacy

BAL (Chamberlain et al.) Inadequate if:
- Paucity of alveolar macrophages (<10/10 hpf or <25/hpf with*)
- *excess epithelial cells, degenerative changes or > # of macrophages
- *Mucopurulent exudate of PMNs
- RBCs, deg changes, artifacts

Acta Cytol 1987;31:599

Unsatisfactory BAL

- Paucity of alveolar macrophages (<10/hpf)
- Too many ciliated or squamous cells
- Mucopurulent exudate (polys)
- Degenerated cells and lab artifacts

Respiratory FNAs

- Cells that explain the radiographic and clinical presentation
- Alveolar macrophages, mesothelial cells, resp epithelial cells
- Blood, low cellularity, tracheobronchial secretions air drying etc contribute to unsats

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GMS Stain

- Gomori/Grocott Methamine Silver stain
- Stains Fungi and PCP
- But notorious for precipitating on other things
- Non specific precipitate if poorly performed

Comparison of Error rates in Respiratory Cytology (PCP)

From CAP Interlaboratory Comparison Program in Non Gyn Cytology

Comparison of Error rates in Respiratory Cytology for Infectious Organisms

From CAP Interlaboratory Comparison Program in Non Gyn Cytology 2010
Bronchial Washings: Reporting Errors in General Dx (Lab)

General Diagnosis Reporting Rates

Adenocarcinoma
Squamous Ca
Non Small Ca
Fungus

Microbiology

BAL on presentation
Blood culture

Cultures of tracheal aspirate, BAL fluid and 2 different blood samples all grew a pure culture of gram positive rods, identified provisionally as Bacillus species.

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Clinical Presentations of B anthracis Vs B cereus

**B anthracis**

- Cutaneous Anthrax (90-95% worldwide, untreated mortality 10-15%)
- Pulmonary or Inhalation Anthrax (80-90% mortality if untreated)
- Oropharyngeal or G1 anthrax (high mortality)

**B cereus**

- Food borne from eating contaminated food (acute self limited gastroenteritis, not reportable)
- Immune compromised hosts potentially fatal
- Infections from direct contamination
- Mostly nuisance in cultures and considered contaminants
Virulence of B. anthracis Vs. B. cereus

**B. anthracis**
- Produces toxins composed of 3 distinct proteins: protective antigen (PA), edema factor (EF), and lethal factor (LF).
- Structural genes for these reside on a plasmid called pXO1.

**B. cereus**
- Toxins related to GI illness

Hospital Course

Only 10 hours after he presented to the initial outlying hospital he remained hypotensive and hypoxic with multisystem organ failure. He was placed on veno-venous extracorporeal membrane oxygenation and given high dose methylprednisolone in addition to the antibiotics. He continued to deteriorate with renal failure, rhabdomyolysis, metabolic acidosis and abdominal compartment syndrome requiring decompressive laparotomy. He expired on hospital day 4 less than 72 hours after initial presentation to the OSH.

Antimicrobials

He received multiple antimicrobials including vancomycin, piperacillin-tazobactam and ciprofloxacin.

Characteristics of *Bacillus cereus* Isolates Associated with Fatal Pneumonia

- Strain: Not closely related to *Bacillus anthracis* strain
- *B. anthracis* Virulence Genes
  - Structural genes for these reside on a plasmid called pXO1

Hospital Course

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Antimicrobials

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**B. cereus group**

The Pathogenic Bacillus Troika

- *Bacillus anthracis*
- *Bacillus cereus*
- *Bacillus thuringiensis*

All three “species” very closely related – plasmids are crucial for virulence

The next few slides are courtesy Drs R. Olsen and J. Musser

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**Possibilities:**

- Unusual presentation of *Bacillus cereus*?
- Illegitimate release of *Bacillus anthracis*?
- Genetically altered form of *Bacillus cereus* group member
- Unusual, naturally occurring strain of *Bacillus cereus* group member

We had the strains, sequencer, motivation, expertise, and responsibility to perform whole genome sequencing.

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**Summary of genome sequence data**

<table>
<thead>
<tr>
<th>Genetic Feature</th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. cereus</em> group chromosome</td>
<td>✔️</td>
<td></td>
</tr>
<tr>
<td>Anthrax toxin genes (pXO1-like plasmid)</td>
<td>✔️</td>
<td></td>
</tr>
<tr>
<td>Mutant plcR allele</td>
<td>✔️</td>
<td>✗</td>
</tr>
<tr>
<td>Four prophage</td>
<td>✔️</td>
<td>✗</td>
</tr>
<tr>
<td><em>B. anthracis</em> pXO2-like plasmid</td>
<td>✔️</td>
<td>✗</td>
</tr>
<tr>
<td><em>B. thuringiensis</em> insecticidal toxin genes</td>
<td>✔️</td>
<td>✗</td>
</tr>
<tr>
<td>Foreign (non-<em>B. cereus</em> group) DNA</td>
<td>✔️</td>
<td>✗</td>
</tr>
</tbody>
</table>

Overall, these genomic features are most consistent with an unusual *B. cereus* strain that has acquired genes encoding the tripartite anthrax toxin.

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**Rapid, whole genome analysis**

- Identified etiologic agent of fatal Anthrax-like infection
  - *B. cereus* with anthrax toxin genes, not *B. anthracis*
- Ruled out the likelihood of bioterrorism
  - No foreign DNA or evidence of other genetic manipulation
- Guided TMH infection control response
  - Offered prophylaxis to exposed health care personnel
- Gained new insight to molecular pathogenesis

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**Estimates of genetic relationships**

Strain Elc2 (blood) is more closely related to *B. cereus* & *B. thuringiensis* than *B. anthracis*.

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**Infection Prevention and Control Perspective**

- 52 employees exposed
- 43 (83%) received prophylaxis
- 9 (17%) declined prophylaxis
- 0 secondary cases
Rapidly Progressive, Fatal, Inhalation Anthrax-like Infection in a Human

Case Report, Pathogen Genome Sequencing, Pathology, and Coordinated Response

November 2011

CID 2007:44 (1 February) Brief report

The New Generation of Molecular Herbs

The New Generation of Microbial Fungi

Care providers and laboratory personnel should consider Bacillus species as pathogens in critically ill patients with respiratory illness. If such illnesses are not on a state’s list of notifiable conditions, they should still be reported to the state health department, because they qualify as unusual, severe diseases that can pose a public health threat. Timely reporting facilitates the determination of these isolates’ prevalence and their public health significance. Knowledge gained facilitates the development of rapid diagnostic tests to differentiate these virulent isolates from the majority of B. cereus isolates and the determination of whether these isolates express and elaborate B. anthracis toxins in infections.
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Blue Dot Projects (Seven Proposed)

- Mandatory curriculum in genomics and personalized medicine (ACGME programs)
- Inventory of current testing in labs and see what can be replaced by NGS or other high-throughput technologies
- Establish a clinical grade variant database

Blue Dot Projects—continued

- Identify and validate operational models for whole genome analysis in human diagnostics and preventative medicine
- Formulate regulatory guidelines
- Define concept of primary care pathologist in genome-era medicine
- Address reimbursement issues