Meningoencephalitis: Next-Generation Approaches

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Talk Overview

- Encephalitis epidemiology
- Encephalitis of unknown etiology
- Next-generation sequencing (NGS)
  - Case examples

Disclosures

None

Acute Encephalitis Burden: U.S.

- 20,000 hospitalizations annually
- $2 billion annual inpatient costs
- 6% mortality overall
- 10% mortality with HIV co-infection
- 17% mortality with organ transplant

Encephalitis of Unknown Etiology

- > 50% unknown cases in 26 of 41 studies
  - Not explained by number of diagnostic tests
- California Encephalitis Project
  - 7 yrs (1,570 cases): 63% of cases unknown

Why Is Diagnosis So Difficult?

- > 100 pathogens cause encephalitis
- Is it even infectious?
  - Autoantibody-mediated
  - Vasculitis
  - Collagen vascular disorders

Traditional Diagnostic Techniques

- Virus-specific polymerase chain reaction
- Acute and convalescent sera
- Virus culture
- Brain biopsy
  - Histological staining
  - Microorganism stains
  - Electron microscopy

Tissue Sample → RNA/DNA → Fragment and PCR Amplify → Illumina Sequence

- Granerod J. et al. Neurology 2010;75:924-932
- Glaser CA, et al. CID 2006;43:1868-1877
- Tunkel AR, et al. CID 2008;47:303-327
**NGS: Three Niches to Fill**

1) Identify novel, highly divergent pathogens

2) Identify pathogens not previously associated with a clinical phenotype

3) Identify rare pathogens not frequently on the differential

- Stenglein MD, et al. Mbio 2012;14:e00180-12
- Chiu CY, et al.. CID 2006;43:71-76

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**Case #1**

- 14 year-old boy with severe combined immunodeficiency
- Adenosine deaminase deficiency
- Partially corrected with 2 haploidentical bone marrow transplants
  - Required monthly IV immunoglobulins


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- 14 year-old boy with severe combined immunodeficiency
- Adenosine deaminase deficiency
- Partially corrected with 2 haploidentical bone marrow transplants
  - Required monthly IV immunoglobulins
- Developed subacute meningoencephalitis

• Family trip to Puerto Rico
  – Swam in fresh water and the ocean

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• Fever and bilateral conjunctivitis
• Thrombocytopenia
• Conditions resolved over 2 months
  – Prednisolone (ophthalmic)
  – Ciprofloxacin (ophthalmic)
  – Rituximab for presumed ITP

• Family vacation to Naples, Florida

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  • Admitted for 6 days of headache and fever to 103°F
  • Discharged 1 day later with resolution of fever and headache
  • Negative infectious work-up
    – Epstein-Barr virus
    – Cytomegalovirus
    – Respiratory virus panel
    – Enterovirus
• Second admission for fevers and headache
• New photophobia
• Occasional neck stiffness
• Intermittent abdominal pain
• 5 pound unintentional weight loss over 1 month

• Cerebrospinal fluid
  – 125 leukocytes/mm³ (18% neutrophils, 52% lymphocytes)
  – 0 red blood cells/mm³
  – Protein 97 mg/dL
  – Glucose 24 mg/dL
  – Gram stain and culture negative
• MRI brain normal
• Additional negative infectious work-up
• Discharged after observation

• Third admission for fever, headache, diffuse weakness, myalgias, nausea and vomiting
• 2 doses of rituximab given

• CSF
  – 109 WBC (16% polymononuclear cells, 38% lymphocytes)
  – 0 RBC
  – Protein 162 mg/dL
  – Glucose 17 mg/dL
  – Gram stain and culture negative
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![MRI Images](image1.png)

![Histopathology Image](image2.png)
- Trialed on IV methylprednisolone for possible neurosarcoid
- Extraventricular drain for worsening communicating hydrocephalus
- Status epilepticus required placement in a medically-induced coma


- Emergency enrollment in UCSF research protocol for pathogen discovery by next-generation sequencing
- 48 hours after sample receipt...

- Methylprednisolone discontinued
- IV penicillin initiated
- Dramatic response
- Discharged to rehab 2 weeks later
- Returned home now back to baseline
Case #2

- 74 year old woman
- Rheumatoid arthritis on plaquenil
- Vision loss in the left eye due to endophthalmitis
- Treated empirically with intravitreal antibiotics and anti-toxoplasmosis therapy

Case #2

- Referred to City Tuberculosis clinic for left lower lobe cavitary lung lesion
- Glasgow coma scale of 6 at TB clinic
- Sent to Emergency Department
Case #2

- **CSF**
  - 347 WBCs (57% PMN, 31% lymphs, 11% monos, 1% eosinophils)
  - 13 RBCs
  - Glucose 30 mg/dL
  - Protein 71 mg/dL

Case #2

- RNA extracted from 500 uL of CSF
- 20,000,000 total reads
- 15,000 non-human reads
- 119 reads 99% similar to *Balamuthia mandrillaris*
- Only 3 of the likely 15,000+ Balamuthia genes are sequenced
Necrotizing Vasculitis

Immunohistochemistry

• First identified in a baboon in 1986
• > 200 meningoencephalitis cases worldwide
• Likely contracted from soil exposure
• 4 patients have survived in the U.S.

Balamuthia mandrillaris

Cyst Form

• Schuster FL, et al. CID 2009;48:879-887
Trophozoite

UCSF Meningitis and Encephalitis Center

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Epilogue

- Ethical and regulatory issues raised by case
- CDC confirmatory PCR