Theme 3: Pathogen Detection & Discovery

BRAIN INFECTION AETIOLOGY

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NIHR Global Health Research Group on Brain Infections
Club Mak, Malawi - 16th Jan 2020
Incidence and healthcare costs of viral meningitis in adults – a multicentre prospective observational study in England.

- Total number of patients with meningitis by etiology (630):
  - Total patients screened: 2,119
  - Patients with meningitis: 42%
  - Unknown cause in 31%
  - Average 16 hrs to LP
  - Average stay: 4 days
  - Meningitis estimated NHS cost: £30 million year
Testing suspected meningitis or encephalitis

Children (n=355)
Focus CSF pleocytosis cases

Culture - 11% (excluding contaminants)
PCR - 21%
Serology - 10%
Unknown - 58%

Testing children with febrile encephalopathy
(n=106 [interim analysis])

Among Non-malaria
Culture - 7%
PCR - 32% (CSF) 7% (blood)
Unknown - 54%

Lack of diagnosis is a global problem

Indonesia (Yogyakarta)
Testing children with febrile encephalopathy (n=106 [interim analysis])
Among Non-malaria
Culture - 7%
PCR - 32% (CSF) 7% (blood)
Unknown - 54%

Malawi (Blantyre)
Culture - 7%
PCR - 32% (CSF) 7% (blood)
Unknown - 54%

Brazil (Rio / Recife)
Flaviviruses have short vireamia (e.g. ZIKA, JEV)
PCR not accurate after 7 days
Serology not always accurate
False positives/negatives
Wrong diagnosis critical implications to local families
Why lack of diagnosis?

1. Samples not being collected/stored appropriately
2. **Appropriate tests not being requested /undertaken**
   - 71.4% culture negative suspected bacterial meningitis not had bacterial PCR (UK data)
   - 56.3% of suspected viral meningitis not having full viral panel (UK data)
3. Inefficient use of limited samples
4. Tests often not 100% sensitive / specific
5. Difficulty detecting novel pathogens
Why lack of appropriate tests requested?

• Lack of large prospective studies of brain infection aetiology in LMICs
• Surveillance data limited by repertoire of available tests
• Makes knowing which pathogens to focus on difficult:
  • Intervention
    • Diagnostics
    • Management algorithms
  • Systematic pathogen detection
  • Gaps for pathogen discovery
Theme 3 objectives

• 3.1 Determine the range of pathogens causing acute brain infections in Brazil, India and Malawi.

• 3.2 Determine feasibility for and pilot introduction of a staged approach to brain infection diagnostics, involving near-patient, secondary care/district laboratory and advanced and novel molecular tests.
What causes suspected brain infections in LMIC settings?

How can novel methods help pathogen and syndromic diagnosis?

Aetiological assessment using staged diagnostic approach

Use of novel tools: TRIM and next-generation sequencing
Figure 2: Staged approach to diagnostics

- **Unconfirmed diagnosis**
  - Ongoing suspicion of brain infection

- **Core tests**
  - Examples: CSF cell count, protein, glucose
  - Blood counts, biochemistry
  - CSF dipstick

- **Syndromic tests**
  - Examples: Rapid blood/CSF serology
  - Viral and bacterial CSF PCR

- **Advanced tests**
  - Examples: Multiplex pathogen-specific PCR
  - High-cost complex serology
Theme 3: Pathogen Detection & Discovery

1. Finalise criteria for achievement of syndromic and microbiological diagnosis
2. Agree panels of pathogens for detection in retrospective testing, and prioritizing as new tests within the intervention
3. Plan a quality assurance system for ensuring equivalence of tests used in each centre
4. Decide an approach for employing next-generation sequencing tools on stored samples

Breakout sessions today: late morning & afternoon
1. Finalise criteria for achievement of syndromic and microbiological diagnosis

• Primary outcome of the study
  • Should have received and reviewed documents from Bhagteshwar – to confirm today.
2. Agree panels of pathogens for detection in retrospective testing, and prioritizing as new tests within the intervention

- Specific to country and adult vs child (and <6mo? – depends on average age of children seen in each centre)
- Aetiology review should help
- Surveillance data may be even more helpful – but struggling to get
- Focus on:
  - Common
  - Treatable
  - Achievable (intervention)
  - Validated test available
<table>
<thead>
<tr>
<th>Step</th>
<th>Pathogen</th>
<th>Sample</th>
<th>Technique</th>
<th>Common?</th>
<th>Treatable?</th>
<th>Validated test available?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pneumococcus</td>
<td>CSF</td>
<td>PCR</td>
<td>Yes - most common: 30% [14-48] of all participants in meta-analysis (6 studies)</td>
<td>Yes</td>
<td>Yes</td>
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<td></td>
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<td></td>
<td>Yes: 12% [2-28] of those tested for Cryptococcus in meta-analysis (2 studies)</td>
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<td>Cryptococcus</td>
<td>CSF/serum</td>
<td>Lateral flow</td>
<td>Yes: pooled 6% [3-10] of participants in meta-analysis (2 adult studies)</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Yes: pooled 5% [4-8] of adults tested for bacteria in meta-analysis (2 studies)</td>
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<tr>
<td></td>
<td>M. tuberculosis</td>
<td>CSF</td>
<td>PCR</td>
<td>Yes: pooled 5% [4-8] of adults tested for bacteria in meta-analysis (2 studies)</td>
<td>Yes</td>
<td>Yes</td>
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<td></td>
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<td></td>
<td>Yes: pooled 6% [3-10] of all participants in meta-analysis (6 studies)</td>
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<td>Meningococcus</td>
<td>CSF/serum</td>
<td>PCR/Ag test?</td>
<td>Yes: 7% [3-12] of 1793 participants in 2 studies; but most febrile patients have routine malaria testing</td>
<td>Yes</td>
<td>Yes but difficult to interpret</td>
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<td></td>
<td>Plasmodium falciparum</td>
<td>Blood/serum</td>
<td>PCR</td>
<td>Yes: 7% [3-12] of 1793 participants in 2 studies; but most febrile patients have routine malaria testing</td>
<td>Yes</td>
<td>Yes but difficult to interpret</td>
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<td>2</td>
<td>HSV</td>
<td>CSF</td>
<td>PCR</td>
<td>Probably moderate - 2/53 tested in Benjamin 2013</td>
<td>Yes</td>
<td>Yes</td>
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<td>Enteroviruses</td>
<td>CSF</td>
<td>PCR</td>
<td>Probably - common in HIC; 2% [1-3] in children (Mallewa 2013)</td>
<td>No</td>
<td>Yes</td>
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<td>E. coli</td>
<td>CSF</td>
<td>PCR</td>
<td>Not rare: 1% [0-2] in 2622 participants (4 studies: 3 adult)</td>
<td>No</td>
<td>Yes</td>
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<td>Syphilis</td>
<td>CSF/serum</td>
<td>Serology</td>
<td>Marks 2017 syst rev</td>
<td>Yes</td>
<td>Yes</td>
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<td>Salmonellae</td>
<td>CSF</td>
<td>PCR</td>
<td>Not rare: 2% [1-4] of 4088 participants (5 studies: 2 adult)</td>
<td>Yes</td>
<td>Yes</td>
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<td>0/53 PCR-positive in Benjamin 2013, but expected to be prominent with high HIV prevalence</td>
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<td>3</td>
<td>VZV</td>
<td>CSF</td>
<td>PCR/PCRserology</td>
<td>Probably not rare, but not reported in Malawi studies; 25% in HIV-infected adults in Ghana (Opintan 2018)</td>
<td>Yes</td>
<td>Yes</td>
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<td>Toxoplasma</td>
<td>CSF/serum</td>
<td>Serology</td>
<td>No Malawi-based studies available, but as seen in India &amp; Brazil, could well be present</td>
<td>Yes</td>
<td>Yes but difficult to interpret</td>
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<td>Dengue</td>
<td>CSF/serum</td>
<td>Serology</td>
<td>No Malawi-based studies available, but as seen in India &amp; Brazil, could well be present</td>
<td>No</td>
<td>Yes but difficult to interpret</td>
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<td>Chikungunya</td>
<td>CSF/serum</td>
<td>Serology</td>
<td>No Malawi-based studies available, but as seen in India &amp; Brazil, could well be present</td>
<td>No</td>
<td>Yes but difficult to interpret</td>
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<td>Adenovirus</td>
<td>CSF</td>
<td>PCR</td>
<td>42/513 children tested in Mallewa 2013; adult commonality plausible</td>
<td>No</td>
<td>Yes</td>
</tr>
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<td></td>
<td>CMV</td>
<td>CSF</td>
<td>PCR</td>
<td>Yes in HIV</td>
<td>Yes</td>
<td>Yes</td>
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</table>
Also be challenges in selecting right tests

Accuracy in your population
Serology - cross reaction
PCR – maintain sensitivity/specificity
Lateral Flow Assay
Availability and Sustainability
3. Plan a quality assurance system for ensuring equivalence of tests used in each centre

• Use what you already have in place - IQA
• Random selection of cross checking of samples – EQA
• Use of stock samples - EQA
4. Decide an approach for employing next-generation sequencing tools on stored samples

- Brief overview of some of the options (Chitra, Fiona and Bhagteshwar)
  - Minion
  - Illumina
  - VirCapSeq
  - BactCapSeq
  - Others....
The Team

**Malawi:**
- Sam Moody
- Jen Cornick
- Stephen Ray
- Greta Wood

**India:**
- Chitra Pattabiraman
- Divya Mathew
- Sharon Jose
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- V Ravi

**Brazil:**
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**Liverpool:**
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- Fiona McGill
- Lance Turtle
- Mike Griffiths
- Tom Solomon
Questions & Discussion...