Preanalytical issues in CSF analysis

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Disclosures

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- Corresponding member, EFLM’s WG-PRE
- Member of the Scientific Committee of this Conference
The nine steps in the performance of any laboratory test. The brain-to-brain turnaround time loop.

Lundberg, 1981
Summary

1. What kind of test is ordered?
2. Collection
3. Identification, Transportation, Preparation
4. “Closing the loop”
5. Conclusions
1. What kind of test is ordered?

2. Collection

3. Identification, Transportation, Preparation

4. “Closing the loop”

5. Conclusions
“procedures, especially when they are divorced from serious cognitive work, can easily be mastered by individuals without advanced education”

Ronald Dworkin

in The Cultural Revolution in Health Care

The Public Interest, Number 139, 2000
1. What kind of test is ordered?

Total CSF measurements_2018

- Total: 5,836,996
- CSF: 11,392

CSF < 0.002%
1. What kind of test is ordered?
Some particular characteristics of Cerebrospinal fluid (CSF):

- it is closely related to the brain as it bathes its surfaces;
Some particular characteristics of Cerebrospinal fluid (CSF):

- it is closely related to the brain as it bathes its surfaces;

- it reflects many brain and central nervous system (CNS) specific processes;
Some particular characteristics of Cerebrospinal fluid (CSF):

- it is closely related to the brain as it bathes its surfaces;

- it reflects many brain and central nervous system (CNS) specific processes;

- it is separated from the blood by the blood-brain barrier;
Indications for lumbar puncture can be:

1. meningeal infections,
Indications for lumbar puncture can be:

1. meningeal infections,
2. subarachnoid hemorrhage,
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1. meningeal infections,
2. subarachnoid hemorrhage,
3. primary or metastatic malignancy,
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1. meningeal infections,
2. subarachnoid hemorrhage,
3. primary or metastatic malignancy,
4. neurodegenerative diseases,
Indications for lumbar puncture can be:

1. meningeal infections,
2. subarachnoid hemorrhage,
3. primary or metastatic malignancy,
4. neurodegenerative diseases,
5. traumatic conditions,
Indications for lumbar puncture can be:

1. meningeal infections,
2. subarachnoid hemorrhage,
3. primary or metastatic malignancy,
4. neurodegenerative diseases,
5. traumatic conditions,
6. reduce pressure,
Indications for lumbar puncture can be:

1. meningeal infections,
2. subarachnoid hemorrhage,
3. primary or metastatic malignancy,
4. neurodegenerative diseases,
5. traumatic conditions,
6. reduce pressure,
7. inject anesthetics, chemotherapy drugs or other medications,
Indications for lumbar puncture can be:

1. meningeal infections,
2. subarachnoid hemorrhage,
3. primary or metastatic malignancy,
4. neurodegenerative diseases,
5. traumatic conditions,
6. reduce pressure,
7. inject anesthetics, chemotherapy drugs or other medications,
8. inject dye or radioactive substances for diagnostic images
Indications for lumbar puncture can be:

1. meningeal infections,
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3. primary or metastatic malignancy,
4. neurodegenerative diseases,
5. traumatic conditions,
6. reduce pressure,
7. inject anesthetics, chemotherapy drugs or other medications,
8. inject dye or radioactive substances for diagnostic images
9. biobanking purposes.
1. What kind of test is ordered?

**TABLE 29-1**

Diseases Detected by Laboratory Examination of CSF

- High sensitivity, high specificity*
  - Bacterial, tuberculous, and fungal meningitis

- High sensitivity, moderate specificity
  - Viral meningitis
  - Subarachnoid hemorrhage
  - Multiple sclerosis
  - Central nervous system syphilis
  - Infectious polyneuritis
  - Paraspinal abscess

- Moderate sensitivity, high specificity
  - Meningeal malignancy

- Moderate sensitivity, moderate specificity
  - Intracranial hemorrhage
  - Viral encephalitis
  - Subdural hematoma
1. What kind of test is ordered?

2. Collection

3. Identification, Transportation, Preparation

4. “Closing the loop”

5. Conclusions
Cerebrospinal fluid (CSF) formation and reabsorption

1. **Arachnoid villus**
   - Tight junctions between arachnoid cells
   - Endothelium of venous sinus
   - CSF in subarachnoid space
   - Dura mater
   - Arachnoid membrane
   - Pia mater
   - Cerebral cortex

2. **Choroid plexus**
   - CSF secreted into ventricle
   - Capillary
   - Ventricular ependymal cell
   - Tight junctions

3. **CSF reabsorbed into blood supply**

**Labels**
- Lateral ventricle
- Superior sagittal sinus
- Third ventricle
- Cerebral aqueduct
- Fourth ventricle

*UpToDate®*
Some CSF characteristics:

- is produced in the choroid plexus and from interstitial fluid drainage;

- it has a very different cellular and molecular composition from other body fluids, especially the blood;

- there is a concentration gradient for total protein along the neuraxis (*lumbar puncture vs shunt drainage*);

- it may change its characteristics acutely or accommodate slowly occurring changes.
Lumbar puncture

- it is considered as relatively invasive;
- it is mainly performed for clinical indications and rarely for research purposes (rarely done in healthy controls- ethical considerations);
- CSF can be collected non-fasting.

Usually it is performed by medical doctors.
Aseptic technique is used!

- the overlying skin should be cleaned;

- the antiseptic should be allowed to dry;

- caution with the products chosen because of a concern that it can cause arachnoiditis;
Aseptic technique is used!

- a sterile drape with an opening over the lumbar spine is placed on the patient;

- face masks should be used;

- local anesthesia is infiltrated into the previously identified lumbar intervertebral.
Lumbar puncture

The most common risks or complications:

1- headache,
2- pain in the lower back,
3- bleeding at the puncture site.
Lumbar puncture

**Location**- Intervertebral space L3–L5 (S1)

The spinal needle may be advanced slowly, angling slightly toward the head, as if aiming towards the umbilicus.
2. Collection

Cerebrospinal fluid, collected from the thecal sac that surrounds the spinal cord.
Lumbar puncture

CSF pressure
should be measured with a manometer in a patient lying flat in the lateral decubitus position with the legs extended. It may change with:

- patient’s position,
- relaxation of the patient,
- skill of the performer,
- obesity.
Lumbar puncture

CSF aspect- it is clear and colorless (as few as 200 WBCs/μL or 400 RBCs/μL will cause CSF to appear turbid)

Preferred volume- 8-15 mL [partitioned into three to four sterile tubes. Larger volumes (10 – 15 ml) are necessary for certain pathogens like Mycobacterium tuberculosis, fungi, or parasites]

Type of needle- atraumatic
In normal adults, the CSF volume is in the range of:

a. 175 to 225 mL
b. 150 to 200 mL
c. 125 to 175 mL
d. 100 to 150 mL
e. 75 to 125 mL
In normal adults, the daily production of CSF is close to:

a. 600 mL
b. 500 mL
c. 400 mL
d. 300 mL
e. 200 mL
Diagnostic:

1. meningeal infections,
2. subarachnoid hemorrhage,
3. primary or metastatic malignancy,
4. neurodegenerative diseases,
5. traumatic conditions,
6. reduce pressure,
7. inject anesthetics, chemotherapy drugs or other medications,
8. inject dye or radioactive substances for diagnostic images
9. biobanking purposes.
Therapeutic or imaging:

1. meningeal infections,
2. subarachnoid hemorrhage,
3. primary or metastatic malignancy,
4. neurodegenerative diseases,
5. traumatic conditions,
6. reduce pressure,
7. inject anesthetics, chemotherapy drugs or other medications,
8. inject dye or radioactive substances for diagnostic images
9. biobanking purposes.
Research:

1. meningeal infections,
2. subarachnoid hemorrhage,
3. primary or metastatic malignancy,
4. neurodegenerative diseases,
5. traumatic conditions,
6. reduce pressure,
7. inject anesthetics, chemotherapy drugs or other medications,
8. inject dye or radioactive substances for diagnostic images
9. biobanking purposes.
The order of tubes for CSF collection should be?

a. 1\textsuperscript{st} microbiology, 2\textsuperscript{nd} cytology, 3\textsuperscript{rd} chemistry

b. 1\textsuperscript{st} cytology, 2\textsuperscript{nd} microbiology, 3\textsuperscript{rd} chemistry

c. 1\textsuperscript{st} chemistry, 2\textsuperscript{nd} microbiology, 3\textsuperscript{rd} cytology

d. 1\textsuperscript{st} chemistry, 2\textsuperscript{nd} cytology, 3\textsuperscript{rd} microbiology

e. 1\textsuperscript{st} cytology, 2\textsuperscript{nd} chemistry, 3\textsuperscript{rd} microbiology
The CSF specimen is usually divided into serially collected sterile tubes:

- Tube 1 for chemistry and immunology studies,
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- Tube 1 for chemistry and immunology studies,

- Tube 2 for microbiological examination,
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- Tube 1 for chemistry and immunology studies,

- Tube 2 for microbiological examination,

- Tube 3 for cell count and differential,
The CSF specimen is usually divided into serially collected sterile tubes:

- Tube 1 for chemistry and immunology studies,
- Tube 2 for microbiological examination,
- Tube 3 for cell count and differential,
- Tube \( n \) (last one) for biobanking.
Some variations are critical:

- if tube 1 is hemorrhagic because of a traumatic puncture, it should not be used when protein studies are the most important aspect of the analysis (if necessary discard the initial 1-2 mL);

- tube 1 should never be used for microbiology;

- tube 3 should be examined for the major purpose of CSF collection;
Lumbar puncture:

Type of collection tube

- CSF biomarkers for the diagnosis of Alzheimer disease should be collected in polypropylene tubes;

- never use heparin tubes for the measurement of proteins.
Cerebrospinal Fluid Collection Tubes: A Critical Issue for Alzheimer Disease Diagnosis

Table 1. Impact of polypropylene collection tubes on Alzheimer disease biomarkers and analysis of surface polymer composition with differential scanning calorimetry and Fourier transform infrared spectroscopy.

<table>
<thead>
<tr>
<th>Tube ID</th>
<th>Provider</th>
<th>Catalog numbers</th>
<th>Volume, mL</th>
<th>Peak maximum, °C</th>
<th>Peaks superposition</th>
<th>Composition</th>
<th>Aβ1–42 Median percentage</th>
<th>hTau Median percentage</th>
<th>p-Tau181P Median percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Greiner</td>
<td>18 82 80</td>
<td>15</td>
<td>151.61</td>
<td>2</td>
<td>PP-PE® copolymer</td>
<td>92%</td>
<td>1</td>
<td>108%</td>
</tr>
<tr>
<td>B</td>
<td>Greiner</td>
<td>18 82 81</td>
<td>15</td>
<td>150.75</td>
<td>2</td>
<td>PP-PE copolymer</td>
<td>88%</td>
<td>0.429</td>
<td>99%</td>
</tr>
<tr>
<td>C</td>
<td>Deltalab</td>
<td>401402</td>
<td>12</td>
<td>149.99</td>
<td>2</td>
<td>PP-PE copolymer</td>
<td>112%</td>
<td>&lt;0.0001</td>
<td>108%</td>
</tr>
<tr>
<td>D</td>
<td>Evergreen</td>
<td>222-3529-G8D</td>
<td>30</td>
<td>150.32</td>
<td>2</td>
<td>PP-PE copolymer</td>
<td>52%</td>
<td>&lt;0.001</td>
<td>99%</td>
</tr>
<tr>
<td>E</td>
<td>CML</td>
<td>TC15PP</td>
<td>15</td>
<td>150.39</td>
<td>2</td>
<td>PP-PE copolymer</td>
<td>86%</td>
<td>0.159</td>
<td>105%</td>
</tr>
<tr>
<td>F</td>
<td>Sørstedt</td>
<td>629 924 284</td>
<td>10</td>
<td>149.83</td>
<td>3</td>
<td>PP-PE + ?</td>
<td>92%</td>
<td>0.448</td>
<td>92%</td>
</tr>
<tr>
<td>G</td>
<td>Sørstedt</td>
<td>62 610.201</td>
<td>10</td>
<td>150.16</td>
<td>3</td>
<td>PP-PE + ?</td>
<td>131%</td>
<td>&lt;0.0001</td>
<td>101%</td>
</tr>
<tr>
<td>H</td>
<td>Falcon</td>
<td>BD 35 2006</td>
<td>14</td>
<td>150.54</td>
<td>2</td>
<td>PP-PE copolymer</td>
<td>79%</td>
<td>0.029</td>
<td>100%</td>
</tr>
<tr>
<td>I</td>
<td>Nalgene</td>
<td>34 28 05</td>
<td>2</td>
<td>151.63</td>
<td>3</td>
<td>PP-PE + ?</td>
<td>111%</td>
<td>&lt;0.0001</td>
<td>101%</td>
</tr>
<tr>
<td>J</td>
<td>Falcon</td>
<td>BD 35 2096</td>
<td>15</td>
<td>150.75</td>
<td>2</td>
<td>PP-PE copolymer</td>
<td>98%</td>
<td>0.003</td>
<td>98%</td>
</tr>
<tr>
<td>K</td>
<td>Gosselin</td>
<td>TK75-085</td>
<td>5</td>
<td>168.25</td>
<td>1</td>
<td>PP</td>
<td>101%</td>
<td>0.001</td>
<td>96%</td>
</tr>
</tbody>
</table>

*Indicated are peak maximum (°C) and peaks superposition results produced by differential scanning calorimetry and Fourier transform infrared spectroscopy analyses, which allowed determination of the polymer composition of the tubes. Six CSF supernatants from 6 freshly collected samples were placed in 6 tubes for each of the 11 tube types for 15 min. Then Aβ1−42, hTau, and p-Tau181P were measured with commercially available ELISAs. For each sample, the measured concentration in each of the 6 tubes was converted to a percentage of the mean of the values obtained for the 11 tube types. The median percentage for the 6 tubes is reported in the table for each tube type. The P value reported for each biomarker column is the result of the nonparametric Kruskal–Wallis test result for the comparison of tube A with the results for the 10 other tubes. P values <0.05 are in boldface.

b PE, polyethylene.
Other body may be collected simultaneously:

- In the setting of suspected bacterial meningitis, it is important to obtain **blood cultures**;

- The **glucose** concentration in CSF should be related to the **blood** concentration. Therefore CSF glucose/serum ratio is preferable.
1. What kind of test is ordered?

2. Collection

3. Identification, Transportation, Preparation

4. “Closing the loop”

5. Conclusions
- type and volume of the aliquoting tubes are determinant, especially for biobanking;

- **transport conditions** (they can be sent through PTS);

- temperature between collection and analysis or storage;

- **effects of freeze/thaw cycles** (increasing the number of cycles decreases CSF concentrations of certain proteins);
- **centrifugation conditions** (results remain inconsistent for some biomarkers)

- **storage duration and temperature**;

- **sample treatment** (additives, detergents, etc);

- **coding** - unique codes, freezing-proof labels (ideally barcodes);
Problems with CSF samples:

- haemolysis;

- xanthochromia;
3. Identification, Transportation, Preparation

Legend

- **Yellow**: Weak evidence Ab42, weak evidence tTau, limited evidence pTau
- **Light Grey**: Limited evidence Ab42, pTau, tTau
- **Orange**: Strong evidence Ab42, weak evidence tTau, limited or no evidence pTau
- **Red**: Strong evidence Ab42, limited evidence tTau pTau
- **Red with diagonal stripes**: Strong evidence Ab42, pTau and tTau
- **Green**: Strong evidence of no effect on Ab42, pTau or tTau

Timing of CSF sampling

Location of sampling and volume of CSF

Type of puncture needle*

Collection method

Blood contamination

Tube material

Aliquot tube volume†

Additives

Shaking

Heat

Centrifugation

Temperature

Freeze/thaw cycles

Storage time -80°C
### 3. Identification, Transportation, Preparation

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overview of studies addressing cerebrospinal fluid stability using immunoassays or the mass spectrometry measurements of Aβ40, Aβ42, tau and phosphorylated tau. h, hours; AD, Alzheimer's disease; MCI, mild cognitive impairment; RT, room temperature; Hb, haemoglobin; TTR, transthyretin; CSF, cerebrospinal fluid.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ref</th>
<th>Subjects</th>
<th>Conditions analysed using immunoassays</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southwick et al. (1996)</td>
<td>AD = 39, non-AD = 20, other = 49</td>
<td>Freeze/thaw</td>
<td>Decrease of Aβ during freeze/thaw cycles</td>
</tr>
<tr>
<td>Jensen et al. (2000)</td>
<td>–</td>
<td>Delayed storage, Freeze/thaw, 4°C within 5 days vs. storage at −80°C for 6 months</td>
<td>Decrease of Aβ40 and Aβ42 after 24 h at 20°C or 15 freeze/thaws</td>
</tr>
<tr>
<td>Vanderstichele et al. (2000)</td>
<td>N = 97</td>
<td>Freeze/thaw</td>
<td>Decrease of Aβ42 after 2 freeze/thaws</td>
</tr>
<tr>
<td>Sjogren et al. (2001)</td>
<td>N = 231</td>
<td>Freeze/thaw</td>
<td>No difference in tau and Aβ42 levels between freeze/thaw cycles</td>
</tr>
<tr>
<td>Bibl et al. (2004)</td>
<td>7 = healthy, 8 = AD</td>
<td>Delayed storage, varying temperatures, freeze/thaw, delayed centrifugation</td>
<td>Absolute levels of Aβ peptides stable, discrimination between groups disappeared after 6 months of storage</td>
</tr>
<tr>
<td>Schoonenboom et al. (2005)</td>
<td>N = 4–10</td>
<td>Delayed storage, heated/cooled</td>
<td>Decrease of Aβ42 after 2 days or 3 freeze/thaws. Decrease of tau after 12 days 37°C. Tau stable for 6 freeze/thaws</td>
</tr>
<tr>
<td>Kaiser et al. (2007)</td>
<td>N = 12–20</td>
<td>Delayed storage, RT</td>
<td>Increased levels of Aβ42 after 24 h but stable levels of tau and P-tau</td>
</tr>
<tr>
<td>Bjerke et al. (2010)</td>
<td>N = 15–20</td>
<td>Delayed storage, varying temperatures.</td>
<td>Aβ42 no significant differences between conditions.</td>
</tr>
<tr>
<td>Sanchesario et al. (2010)</td>
<td>AD/MCI = 27, other = 24, CTRL = 23</td>
<td>Delayed storage, heated/cooled</td>
<td>Heat of sample increase Aβ42 in AD/MCI group; maintaining samples at 37°C prevents a decrease in Aβ42 levels.</td>
</tr>
<tr>
<td>Fronek et al. (2011)</td>
<td>N = 11</td>
<td>Sample volume, Shaking of samples</td>
<td>Lower tau levels in shaken samples. Volume has a small effect.</td>
</tr>
<tr>
<td>Schipke et al. (2011)</td>
<td>N = 56</td>
<td>Long-term storage</td>
<td>Oldest samples denatured when thawing. No degradation of tau or Aβ42 after several years of storage, but Aβ40 was vulnerable to degradation. CSF proteins were relatively stable at RT for 4 days. Three freeze/thaw unsystematic variation</td>
</tr>
<tr>
<td>Zimmermann et al. (2011)</td>
<td>N = 6–10</td>
<td>Temperature, freeze/thaw</td>
<td>Overall peptide decrease after 24 h. No effect of freeze/thaw. Cystatin C and truncated cystatin C and complement C3 peptide difference −20°C from −80°C</td>
</tr>
<tr>
<td>Jimenez et al. (2007)</td>
<td>N = 3–5</td>
<td>Delayed storage, RT; −20°C for 2–3 months. Freeze/thaw</td>
<td>Overall peptide decrease after 24 h. No effect of freeze/thaw. Cystatin C and truncated cystatin C showed a difference in stability between −20°C and −80°C. OK at RT for 5 h. No effect by the protease inhibitor Temperature variations had no effect; freeze/thaw altered mass spectra.</td>
</tr>
<tr>
<td>Berven et al. (2007)</td>
<td>N = 2–3</td>
<td>Delayed storage, Protease inhibitor.</td>
<td>Cystatin C and truncated cystatin C showed a difference in stability between −20°C and −80°C. OK at RT for 5 h. No effect by the protease inhibitor Temperature variations had no effect; freeze/thaw altered mass spectra.</td>
</tr>
<tr>
<td>Bruegel et al. (2009)</td>
<td>N = 4</td>
<td>Long-term storage, freeze/thaw.</td>
<td>Proteomic profile changed within 120 min after lumbar puncture. Freeze/thaw altered TTR levels. 4°C: time-dependent decrease of peak intensities. No significant decrease for albumin and cystatin C. Two peptides show a significant decrease</td>
</tr>
<tr>
<td>Zimmermann et al. (2011)</td>
<td>Porcine CSF</td>
<td>Delayed storage, freeze/thaw, left at 4°C for 1 month</td>
<td>Proteomic profile changed within 120 min after lumbar puncture. Freeze/thaw altered TTR levels. 4°C: time-dependent decrease of peak intensities. No significant decrease for albumin and cystatin C. Two peptides show a significant decrease</td>
</tr>
<tr>
<td>Rosenling et al. (2011)</td>
<td>N = 5–6</td>
<td>Temperature, freeze/thaw before analysis</td>
<td>Proteomic profile changed within 120 min after lumbar puncture. Freeze/thaw altered TTR levels. 4°C: time-dependent decrease of peak intensities. No significant decrease for albumin and cystatin C. Two peptides show a significant decrease</td>
</tr>
</tbody>
</table>
1. What kind of test is ordered?

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5. Conclusions
When collecting CSF you should not?

a. report xanthochromia using visual inspection

b. simultaneously measure serum glucose

c. remove up to 20 mL of CSF

d. puncture between L3 and L5

e. suspend it in the presence of severe thrombocytopenia
1. CSF should be analyzed immediately (i.e. < 1 h) after collection;

2. if storage is required for later investigation this can be done at 4 – 8 °C (short term) or at – 20 °C (long term).

3. only protein components and RNA (after appropriate preparation) can be analysed from stored CSF.
- **Cellular** morphology (cytological staining) should be evaluated whenever pleocytosis is found or leptomeningeal metastases or pathological bleeding is suspected.

- If cytology is inconclusive in case of query CSF bleeding, measurement of bilirubin is recommended up to 2 weeks after the clinical event.

- For standard **microbiological** examination sedimentation at 3000 × g for 10 min is recommended. Microscopy should be performed using Gram or methylene blue, Auramin O or Ziehl - Nielsen (*M. tuberculosis*), or Indian ink stain (*Cryptococcus*).
### Table 1.1 Typical constellation of CSF parameters in some neurological diseases.

<table>
<thead>
<tr>
<th></th>
<th>Total protein (g/l)</th>
<th>Glucose ratio</th>
<th>Lactate (mmol/l)</th>
<th>Cell count (per 3.2 μl)</th>
<th>Typical cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal values$^a$</td>
<td>&lt;0.45</td>
<td>&gt;0.4–0.5</td>
<td>&lt;1.0–2.9</td>
<td>&lt;15</td>
<td>MNC</td>
</tr>
<tr>
<td>Disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute bacterial</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>&gt;1000</td>
<td>PNC</td>
</tr>
<tr>
<td>meningitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral neuro-infections</td>
<td>=$/$↑</td>
<td>=$/$↓</td>
<td>=</td>
<td>10–1000</td>
<td>PNC/MNC</td>
</tr>
<tr>
<td>(meningoencephalitis)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autoimmune</td>
<td>↑</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>polyneuropathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MNC</td>
</tr>
<tr>
<td>Infectious</td>
<td>↑</td>
<td>=</td>
<td>=</td>
<td>↑</td>
<td>MNC</td>
</tr>
<tr>
<td>polyneuropathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subarachnoidal</td>
<td>↑</td>
<td>=</td>
<td>=</td>
<td>↑</td>
<td>erythrocytes,</td>
</tr>
<tr>
<td>haemorrhage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>macrophages,</td>
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<td>=</td>
<td>=</td>
<td>=</td>
<td>=$/$↑</td>
<td>MNC</td>
</tr>
<tr>
<td>Leptomeningeal</td>
<td>↑</td>
<td>=$/$↓</td>
<td>NA</td>
<td>=$/$↑</td>
<td>MNC</td>
</tr>
<tr>
<td>metastases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>malignant cells,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mononuclears</td>
</tr>
</tbody>
</table>

CSF, cerebrospinal fluid; MNC, mononuclear cells; PNC, polymorphonuclear cells. ↑/$/$↓, increased/decreased; =, within normal limits; NA, evidence not available.

$^a$Normal values are given for lumbar CSF in adults.
Table 1.3 Inflammatory diseases of the CNS associated with CSF oligoclonal IgG bands [32].

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Incidence of oligoclonal bands (%)</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple sclerosis</td>
<td>95</td>
<td>Class I*</td>
</tr>
<tr>
<td>Auto-immune</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuro-SLE</td>
<td>50</td>
<td>Class III</td>
</tr>
<tr>
<td>Neuro-Behçet’s</td>
<td>20</td>
<td>Class II</td>
</tr>
<tr>
<td>Neuro-sarcoid</td>
<td>40</td>
<td>Class III</td>
</tr>
<tr>
<td>Harada’s meningitis-uveitis</td>
<td>60</td>
<td>Class III</td>
</tr>
<tr>
<td>Infectious</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute viral encephalitis (&lt;7 days)</td>
<td>&lt;5</td>
<td>Class II</td>
</tr>
<tr>
<td>Acute bacterial meningitis (&lt;7 days)</td>
<td>&lt;5</td>
<td>Class II</td>
</tr>
<tr>
<td>Subacute sclerosing panencephalitis (SSPE)</td>
<td>100</td>
<td>Class I</td>
</tr>
<tr>
<td>Progressive rubella panencephalitis</td>
<td>100</td>
<td>Class I</td>
</tr>
<tr>
<td>Neurosyphilis</td>
<td>95</td>
<td>Class I</td>
</tr>
<tr>
<td>Neuro-AIDS</td>
<td>80</td>
<td>Class II</td>
</tr>
<tr>
<td>Neuro-borreliosis</td>
<td>80</td>
<td>Class I</td>
</tr>
<tr>
<td>Tumour</td>
<td>&lt;5</td>
<td>Class III</td>
</tr>
<tr>
<td>Hereditary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ataxia-telangiectasia</td>
<td>60</td>
<td>Class III</td>
</tr>
<tr>
<td>Adrenoleukodystrophy (encephalitic)</td>
<td>100</td>
<td>Class II</td>
</tr>
</tbody>
</table>

CNS, central nervous system; CSF, cerebrospinal fluid; IgG, immunoglobulin G; SLE, systemic lupus erythematosus.
### Probability of an abnormal screening test result

<table>
<thead>
<tr>
<th>Number of independent tests</th>
<th>Probability of abnormal test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5 percent</td>
</tr>
<tr>
<td>2</td>
<td>10 percent</td>
</tr>
<tr>
<td>4</td>
<td>19 percent</td>
</tr>
<tr>
<td>6</td>
<td>26 percent</td>
</tr>
<tr>
<td>10</td>
<td>40 percent</td>
</tr>
<tr>
<td>20</td>
<td>64 percent</td>
</tr>
<tr>
<td>50</td>
<td>92 percent</td>
</tr>
</tbody>
</table>
Over-testing: Why More Is Not Better

Over-testing is at the root of many of our problems.

There are at least 5 reasons why clinicians over-test:

1) Belief that ordering many tests will help detect subclinical disease
2) Defensive medicine
3) Lack of knowledge or confidence
4) Patients’ expectations
5) Profit

Over-testing is often learned in training,

...
Summary

1. What kind of test is ordered?

2. Collection

3. Identification, Transportation, Preparation

4. “Closing the loop”

5. Conclusions
Lumbar puncture

A lumbar puncture is where a thin needle is inserted between the bones in your lower spine. It shouldn't be painful, but you may have a headache and some back pain for a few days.

It's carried out in hospital by a doctor or specialist nurse.

When a lumbar puncture may be needed

A lumbar puncture may be used to:
- CSF is a very “precious” sample;
- CSF is a very “precious” sample;

- its collection is laborious and usually not “repetable”;
- CSF is a very “precious” sample;

- its collection is laborious and usually not “repetable”;

- sample conservation is still a big issue and we still need markers of sample stability;
- CSF is a very “precious” sample;
- its collection is laborious and usually not “repetable”;
- sample conservation is still a big issue and we still need markers of sample stability;
- all preanalytical aspects need a better understanding;
- CSF is a very “precious” sample;

- its collection is laborious and usually not “repetable”;

- sample conservation is still a big issue and we still need markers of sample stability;

- all preanalytical aspects need a better understanding;

- the clinical value of CSF biomarkers needs to be better studied;
For example:

- compare CSF collected at different time points of the day in the same individuals with several weeks between the LPs;
For example:

- compare CSF collected at different time points of the day in the same individuals with several weeks between the LPs;

- use fresh CSF stored for various times up to 1–2 weeks at RT or 4°C, compared with samples frozen at -20°C or -80°C for the same period;
How many people in the world have some access to electricity:

a. 40 %

b. 50 %

c. 60 %

d. 70 %

e. 80 %

Hans Rosling, “Factfulness”, 2018
Moçambique. Sobreviventes à espera de resgate nos telhados

Imagens: Pierre Markuse

Moçambique antes do ciclone
Moçambique. Sobreviventes à espera de resgate nos telhados

Imagens: Pierre Markuse

5. Conclusions