Practical Session 1- Biochemistry

Learning Outcomes

- **Process and scan CSF sample to distinguish different pigments by their different absorption of light.**
- **Interpretation of scans to determine if Subarachnoid haemorrhage (SAH) has taken place.**

Reagents and materials

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Spectrophotometer	λ 350 nm to 600 nm
2 x CSF samples	Representing samples taking following suspected SAH
Graph paper	For plotting scans
Report algorithm	

Exercise 1- perform CSF scan

It is crucially important to detect subarachnoid haemorrhage (SAH) in all patients in whom it has occurred to select patients for angiography and preventative surgery. A computerized tomography (CT) scan is positive in up to 98% of patients with SAH presenting within 12 hours, but is positive in only 50% of those presenting within one week.

Clearly therefore some patients with a sudden onset headache and a normal CT brain scan may still have had an SAH, particularly if the scan is delayed.

Detecting these cases is important because a missed aneurysmal SAH can be fatal if a recurrent bleed occurs. The standard follow-on test after a negative CT is evidence of blood in the cerebrospinal fluid (CSF). The word 'Xanthochromia' is derived from the Greek meaning yellow.

Normal CSF   Xanthochromic CSF
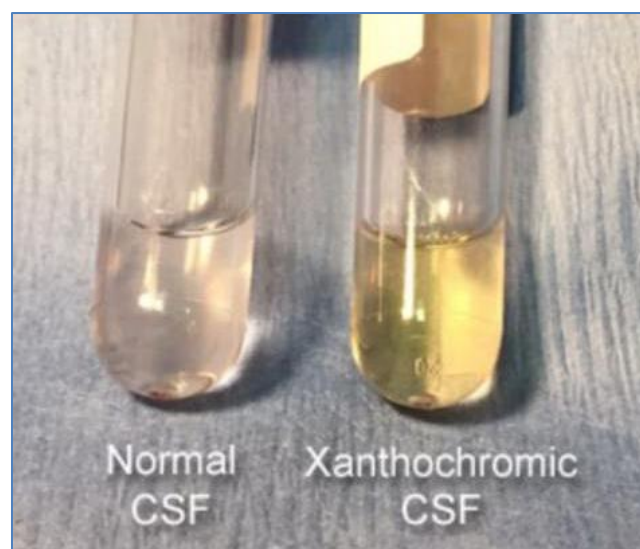
There are two ways to detect CSF Xanthochromia. Clear, yellow fluid can often be seen on visual inspection (see image above). However, spectrophotometry increases the sensitivity of the test, detecting 'yellow' that is not discernible to the human eye, and it can distinguish different pigments by their different absorption of light.
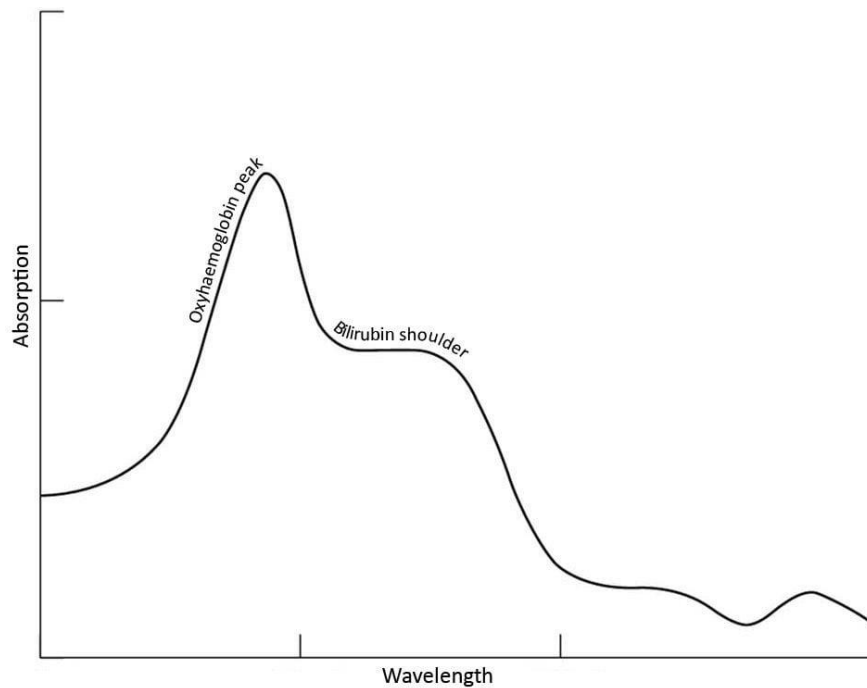
When red blood cells in the CSF haemolyse, they release oxyhaemoglobin, which can be detected within 4–10 h of a bleed. It is important that CSF analysis takes place 12 hours or more after suspected SAH. Various enzymes then convert Oxyhaemoglobin into bilirubin, detected at 450–460 nm.

Method

1.   The specimen designated for spectrophotometry should be centrifuged at 2000 rpm for 5 min as soon as possible after receipt in the laboratory and in any case within 1 h of collection. The supernatant should be stored in the dark at 4°C until analysis.

2.   Perform a zero-order spectrophotometric scan on the supernatant between 350 and 600 nm using a spectrophotometer and a cuvette with a 1 cm path length. The specimen should not be diluted (use graph paper provided to plot scans) and a minimum of 2ml of CSF sample added to each cuvette.

3.   Inspect the scan and identify and record the presence of the following haem pigments:

- Oxyhaemoglobin: absorbance maximum between 410 and 418 nm.
- Bilirubin: either a broad peak in the range 450–460 nm or a shoulder adjacent to an Oxyhaemoglobin peak if present.

4. Determine the net bilirubin absorbance (NBA) and net Oxyhaemoglobin absorbance (NOA)

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- Draw a predicted baseline, which forms a tangent to the scan between 350 and 400 nm and again between 430 and 530 nm.
- Measure the absorbance of the scan above this predicted baseline at 476 nm; this is the NBA.
- Also measure the absorbance of any Oxyhaemoglobin peak above this predicted baseline; this is the net Oxyhaemoglobin absorbance (NOA).

Reporting and interpretation

For each case, the final interpretation should take into account the available clinical information:

Positive sample for SAH

NBA = _____   NOA = _____

CSF Total Protein = **0.37g/L** (Normal range <0.45 g/L)

CSF Glucose =   **4.1mmol/L** (Normal range 2.8 – 4.2 mmol/L)

Laboratory Diagnosis:
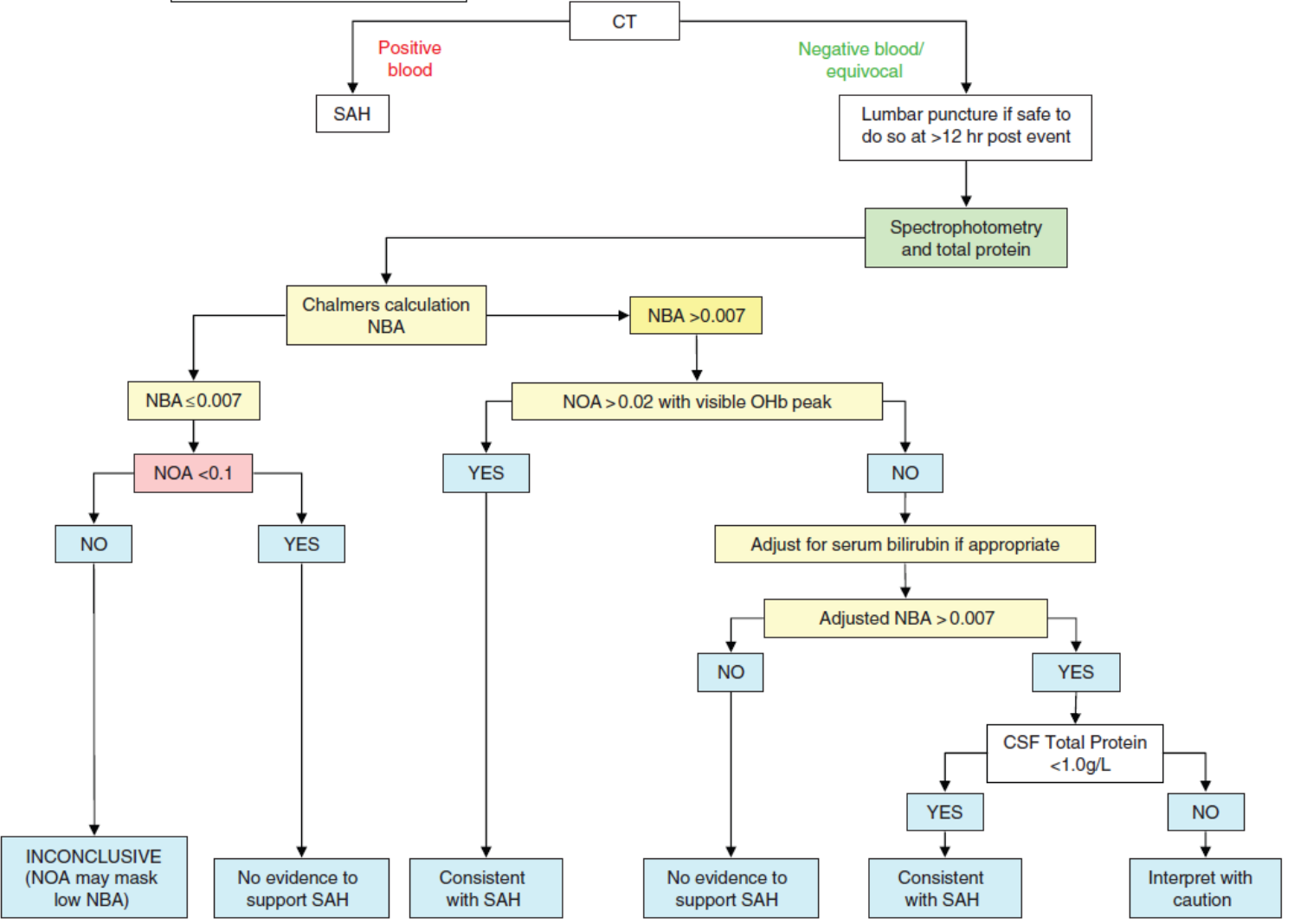
Negative sample for SAH

NBA = _____   NOA = _____

CSF Total Protein = **1.28 g/L** (Normal range <0.45 g/L)

CSF Glucose =   **1.9 mmol/L** (Normal range 2.8 – 4.2 mmol/L)

Laboratory Diagnosis:

NBA = Net Bilirubin Absorbance
NOA = Net Oxyhaemoglobin Absorbance
SAH = Subarachnoid haemorrhage

CT

Positive blood

Negative blood/ equivocal

SAH

Lumbar puncture if safe to do so at >12 hr post event

Spectrophotometry and total protein

Chalmers calculation NBA

NBA >0.007

NBA ≤0.007

NOA >0.02 with visible OHb peak

NOA <0.1

YES

NO

NO

YES

Adjust for serum bilirubin if appropriate

Adjusted NBA > 0.007

NO

YES

CSF Total Protein <1.0g/L

YES

NO

INCONCLUSIVE (NOA may mask low NBA)

No evidence to support SAH

Consistent with SAH

No evidence to support SAH

Consistent with SAH

Interpret with caution

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Spectrophotometer Data:

Abs	Pos	Neg
350	0.339	0.014
360	0.321	0.015
370	0.349	0.016
380	0.334	0.017
390	0.442	0.018
400	0.426	0.02
410	0.51	0.02
420	0.41	0.017
430	0.357	0.016
440	0.346	0.012
450	0.347	0.01
460	0.348	0.01
470	0.35	0.01
480	0.329	0.01
490	0.263	0.008
500	0.199	0.008
510	0.183	0.008
520	0.121	0.008
530	0.087	0.006
540	0.079	0.006
550	0.078	0.006
560	0.062	0.006
570	0.05	0.006
580	0.042	0.005
590	0.041	0.005
600	0.04	0.005

Grams stain results:

Positive sample for SAH	Negative sample for SAH
Lab no. 001	Lab no. 002

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