All Wales Clinical Biochemistry Audit Group

Standards for Analysis of Cerebrospinal Fluid (CSF) for Xanthochromia

INTRODUCTION

Subarachnoid haemorrhage (SAH) is spontaneous arterial bleeding into the subarachnoid space, usually from a cerebral aneurysm. Up to 50% of patients with SAH have a further episode of bleeding, which can be catastrophic, but is potentially preventable by surgery. Angiography is required to locate the site of bleeding, but it is crucially important to establish a diagnosis of SAH before proceeding to this invasive procedure.

In experienced hands 95% of cases of SAH are detected by CT scanning when performed within 24 hours of bleeding. However this sensitivity declines to 50% after 1 week.[1] For CT negative patients with suspected SAH, analysis of CSF is therefore important.

Following haemorrhage into the CSF, red blood cells are cleared by lysis and phagocytosis; the liberated oxyhaemoglobin is subsequently converted to bilirubin. Thus oxyhaemoglobin and/or bilirubin contribute to xanthochromia and may be detected in CSF following SAH.[2]

In suspected SAH, CSF analysis should aim to detect the presence of both oxyhaemoblobin and bilirubin, but the term xanthochromia (presence of a yellow to reddish-brown discoloration of CSF supernatant) should be used ideally only when the presence of bilirubin is identified.

False negative results are a missed opportunity to effectively intervene in a condition with high morbidity and mortality, whereas false positive results may mean patients proceeding to angiography with its danger of inducing a stroke.

Findings from a survey of Welsh laboratories, presented at an audit meeting in April 2000, showed that many laboratories rely upon visual inspection to identify CSF xanthochromia. Spectrophotometric examination of CSF has been recommended,[3] as visual inspection is neither sufficiently specific nor sensitive for detecting oxyhaemoglobin and/or bilirubin in CSF. The following recommended standards take into account guidelines published by the UK NEQAS working group on CSF analysis.[4]

STANDARDS

1. Sample Collection

a) Sufficient CSF should be collected for all required microbiology and biochemistry tests; usually 4 tubes are needed (3 plain sterile, 1 fluoride oxalate for glucose). For xanthochromia testing, at least 1 mL CSF is preferred; the analysis should **not** be done on the sample taken into the first tube, because of possible red cell contamination from a traumatic tap.

b) It is recommended that blood is collected at the same time for serum total protein and bilirubin analysis, which may be needed to aid interpretation.

c) The date and time of the clinical event and of sample collection should be stated on the request form. It is recommended that CSF for analysis for xanthochromia is not collected until at least 12 hours after the clinical event.

d) Samples should be kept in the dark and delivered promptly to the laboratory, to arrive within 30 minutes of collection. It is recommended that samples are not transported by pneumatic tube (as this may cause lysis of red blood cells) and that prior notice is given to the laboratory so that staff are available to centrifuge the sample on receipt.

e) The CSF sample for xanthochromia analysis should be centrifuged immediately on receipt in the laboratory, at 2000 rpm for 5 minutes. Store the CSF supernatant at 4°C in the dark.

2. Analysis

a) The appearance of the CSF should be recorded before and after centrifugation.

b) A spectrophotometric scan should be performed (ideally without dilution) between 350 and 650 nm, using a 1 cm cuvette and an absorbance sensitivity of 0.2 A full-scale deflection. Record whether peaks are present at 407-8 nm (methaemoglobin), 415 nm (oxyhaemoglobin) and/or 450-460 nm (bilirubin) and determine the net bilirubin absorbance (NBA) at 476 nm.[4]

c) The total protein concentration should also be measured on the same CSF sample.

3. Guidance on Interpretation and Reporting of Results

a) A normal scan, reported as "No haem pigments detected", is the best laboratory evidence that a patient has not had a SAH, but the certainty of this evidence decreases with increasing time between the clinical event and lumbar puncture.

b) The presence of oxyhaemoglobin alone is an equivocal finding, most likely to be due to a traumatic tap, but possibly occurring without bilirubin in a recent SAH.

c) The unequivocal presence of bilirubin (NBA >0.007) is a positive finding and may support the diagnosis of SAH. If NBA >0.007 and serum bilirubin >20 µmol/L, calculate an adjusted NBA,[4] provided that the CSF total protein does not exceed 1 g/L, according to the following formula:
Measured NBA – [Serum bilirubin (µmol/L) x 0.042 AU x CSF protein (g/L)/Serum protein (g/L)]

d) The presence or absence of oxyhaemoglobin and bilirubin should be clearly stated on the final report. However, if analysed "out of hours" and there is uncertainty as to whether bilirubin is present, a provisional report should be issued stating "Presence of haem pigments equivocal - further interpretation to follow"; a definitive report should be issued the next day.

e) The spectrophotometric scan should be interpreted and reported in the context of other relevant clinical and analytical information, including:
   (i) Time of sample collection after the clinical event; oxyhaemoglobin may first be observed 4-10 hours after a bleed, but bilirubin typically does not appear in CSF until 10 hours, or more usually 1-4 days, following a bleed.
   (ii) Whether there has been a recent previous lumbar puncture.
   (iii) Incorrect handling of the sample (e.g. delay in centrifugation).
   (iv) Results of red cell count and microbiological analyses.
   (v) CSF total protein concentration; interpretation is difficult if this exceeds 1 g/L.

4. Service Provision

a) The appropriateness of providing an analytical service locally should be assessed in the context of clinical need, available laboratory expertise and number of requests.

b) It is recommended that laboratories that provide "in house" CSF xanthochromia analysis participate in an accredited external quality assessment scheme (EQAS).

c) It is recommended that the analytical service is provided "round the clock". However, if this is not feasible, samples should be processed within 30 minutes; the CSF supernatant should be stored at 4°C in the dark, analysed the next day and a full report issued then.

ACKNOWLEDGEMENTS

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REFERENCES

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2. Barrows LJ, Hunter FT, Banker BQ. The nature and clinical significance of pigments in the cerebrospinal fluid. Brain 1955; **78**: 59-80.
3. Beetham R, Fahie-Wilson MN, Park D. What is the role of CSF spectrophotometry in the diagnosis of subarachnoid haemorrhage? Ann Clin Biochem 1998; **35**: 1-4.
4. UK National External Quality Assessment Scheme for Immunochemistry Working Group. National guidelines for analysis of cerebrospinal fluid for bilirubin in suspected subarachnoid haemorrhage. Ann Clin Biochem 2003; **40**: 481-488.

VERSION: 1, dated 20th May 2005.

APPENDIX Calendar of audit process for standards for laboratory Investigation of PCOS

April 2000	Findings of survey on CSF xanthochromia testing by Welsh laboratories, undertaken by Mr P Thomas (Newport), presented at an audit meeting held at Ysbyty Glan Clwyd.
Oct. 2000	Initial draft standards prepared by Mr P Thomas, considered by the audit group committee and presented at an audit meeting held at Royal Glamorgan Hospital.
2001-2004	Further consultation with clinical biochemists and neurosurgeons within Wales.
May 2005	Standards ratified at All Wales Clinical Biochemistry Audit Group committee meeting on 19th May 2005 by Dr K Griffiths (chairman).