Review Article

Cerebrospinal fluid spectrophotometric scanning for suspected subarachnoid haemorrhage: a narrative review

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Background and Objective: SAH refers to bleeding into the subarachnoid space resulting from aneurysmal rupture or traumatic head injury. Spectrophotometric scanning refers to the detection of haemoglobin breakdown products, oxyhaemoglobin and bilirubin, in the CSF using a spectrophotometer. This aim of this review was to summarise the existing knowledge and current best practice on the spectrophotometric scanning of cerebrospinal fluid (CSF) for suspected subarachnoid haemorrhage (SAH).

Methods: A literature search was carried out through PubMed, Google Scholar, and the Cochrane database (up to 18th Jun 2021) using the search terms CSF spectroscopy, CSF xanthochromia, CSF bilirubin, CSF scanning, SAH and xanthochromia. Only articles in English were included. This yielded more than 100 articles, which were all reviewed.

Key Content and Findings: One This narrative review summarises the current best practice guidelines including key pre-analytical and analytical considerations and main sources of error. One of the main pitfalls of relying on haemoglobin breakdown products is that false-positive results can occur following a traumatic lumbar puncture (LP). There are currently no universally accepted methods to distinguish SAH from traumatic LP. It is also acknowledged that even in the absence of traumatic LP, oxyhaemoglobin and biliverdin can be present in sufficient concentrations to impair the reliable detection of bilirubin in CSF. Elevated CSF total protein also leads to unreliable interpretation of elevated CSF bilirubin. Alternative methods have been described and show promise but are not currently in routine use.

Conclusions: The UK NEQAS SAG *revised national guidelines for analysis of cerebrospinal fluid for bilirubin in suspected subarachnoid haemorrhage* still represent best practice for CSF spectrophotometric scanning for SAH although there are limitations and it is accepted that the evidence base for some recommendations in these guidelines is limited.

Keywords: Cerebrospinal fluid (CSF); xanthochromia; subarachnoid haemorrhage (SAH); spectrophotometry; bilirubin

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Introduction

Subarachnoid haemorrhage (SAH) refers to bleeding into the subarachnoid space between the arachnoid membrane and the pia mater that surrounds the brain, resulting from aneurysmal rupture or traumatic head injury. The global incidence of SAH is approximately 6.1 per 100,000 person-years (1). The most common cause of non-traumatic SAH is spontaneous rupture of a saccular aneurysm. The mortality of untreated spontaneous SAH is reported to be up to 65% (2). SAH is, therefore, considered a neurological emergency, and prompt diagnosis and management are essential to reduce mortality and optimise patient outcomes. The typical presentation includes severe, sudden onset headache or thunderclap headache, loss of consciousness, vomiting, focal neurology, neck stiffness and retinal haemorrhages (3). However, 40–50% of patients will not have a neurological deficit and of these only 8–12% will present with thunderclap headache (4). A non-contrast computed tomography (CT) of the brain is widely accepted as the initial test of choice and is reported to be 100% sensitive for SAH if performed within 6 h of symptom onset. Sensitivity however, rapidly diminishes with time (5), and it is reported that although the CT scan will be positive in over 95% of patients presenting within 24 h, sensitivity decreases to <85% after just a further 6 h (6). A recent systematic review and meta-analysis of the diagnostic specificity of CT to exclude aneurysmal SAH also confirmed that a negative brain CT scan within 6 h of headache onset is extremely sensitive provided the CT scan is technically adequate and is interpreted by an experienced radiologist (7). It is still, however, thought that a negative CT scan does not always exclude SAH, and further investigations are therefore required. Catheter angiography is the most sensitive procedure although it carries a small but significant risk of stroke, aneurysm re-rupture and access-site haematoma (8-10). Cerebral angiography detects aneurysms but does not identify whether they have ruptured. There is therefore a need to detect or exclude SAH in CT-negative patients without the need for angiography. Historically guidelines have acknowledged the risk of equivocal CT findings or false negative CT scans in patients who present several days after the suspected bleed (11), and therefore a lumbar puncture (LP) with cerebrospinal fluid (CSF) spectrophotometry is typically performed (12).

In this clinical context, spectrophotometric scanning refers to the detection of haemoglobin breakdown products, oxyhaemoglobin and bilirubin, in the CSF using a spectrophotometer. Xanthochromia refers to the yellow-orange discoloration of the CSF as a result of the presence of these pigments (13).

A thorough review of published literature was performed in order to establish the current best practices for CSF spectrophotometric scanning for SAH. The literature search was carried out through PubMed, Google Scholar, and the Cochrane database (up to 18th Jun 2021). The search terms used were CSF spectroscopy, CSF xanthochromia, CSF bilirubin, CSF scanning, SAH and xanthochromia. The searches yielded more than 100 articles which were all reviewed.

We present the following article in accordance with the Narrative Review reporting checklist (available at https://jlpm.amegroups.com/article/view/10.21037/jlpm-21-62/rc).

Methods

A thorough review of published literature was performed in order to establish the current best practices for CSF spectrophotometric scanning for SAH. The literature search was carried out through PubMed, Google Scholar, and the Cochrane database (up to 18th Jun 2021). The search terms used were CSF spectroscopy, CSF xanthochromia, CSF bilirubin, CSF scanning, SAH and xanthochromia. The searches yielded more than 100 articles which were all reviewed (*Table 1*).

Current best practice guidelines

An ad hoc Specialist Advisory Group (SAG) to the United Kingdom National External Quality Assessment Service (UK NEQAS) for CSF Proteins and Biochemistry was established in 2000 and formalised in 2004. The group set out to describe the best practice of spectrophotometry and how the findings should be interpreted. The group used expert consensus and available evidence, guideline production, dissemination and review and the measurement of practice against them. In 2003 the first national guidelines for the spectrophotometric analysis of CSF bilirubin in suspected SAH were published (14). Following release of these guidelines, a UK national audit concluded that some interpretative comments were ambiguous and could lead to diagnostic confusion among clinicians (15). The guidelines were subsequently revised in 2008 (16). A study by Griffiths *et al.* retrospectively re-interpreted 93 spectrophotometric CSF scans using the amended

Table 1 Search strategy summary

Item	Specification
Date of search	18th June 2021
Databases and other sources searched	PubMed, Google Scholar, Cochrane database
Search terms	CSF spectroscopy, CSF xanthochromia, CSF bilirubin, CSF scanning, SAH and xanthochromia
Timeframe	Not limited- up to 18th June 2021
Inclusion and Exclusion criteria	Limited to articles in English
Selection process	Search performed by primary author, consensus on included articles agree by all authors
Additional considerations	None

guidelines and showed the revised national guidelines for the analysis of CSF in suspected SAH offered greater clarity than previous guidelines (17). The revised guidelines state the key pre-analytical and analytical considerations as well as providing a standardised algorithm for the interpretation of results. The guidelines state that the LP should not be undertaken before 12 h post symptoms. The rationale for this is that this is the time taken bilirubin to form. When red blood cells enter the CSF, in the absence of the plasma proteins that stabilise their membrane they are rapidly lysed, and oxyhaemoglobin appears within a few hours. The haem portion then induces a haem oxygenase in the brain to form bilirubin, and it is generally accepted that this occurs within 12 h, although the evidence base for this is limited (16).

Pre-analytical considerations

The oxyhaemoglobin, which appears following SAH, may also be formed from the *in vitro* from lysis of red cells in the CSF obtained following puncture, or from the trauma of the puncture itself. To minimise interference, CSF for spectrophotometry should be collected into a separate container to those in which the first few mL of fluid are placed, and ideally the last or fourth sample should be analysed. It is claimed that transport of CSF samples by pneumatic tube results in excess haemolysis, and it is for this reason that the 2008 revised guidelines state that transport by pneumatic tube is not recommended. Although subsequent studies have refuted this claim (18,19), samples are still typically transported to the laboratory by hand.

It is widely acknowledged that bilirubin is susceptible to photodegradation, and it is recommended that the sample is protected from light between collection and analysis. The UK national guidelines state that CSF bilirubin degradation occurs at a rate of at least 0.005 AU/h when stored in a plastic tube and exposed to spring daylight through a north-facing window (16). However, Foroughi *et al.* found that bilirubin degradation occurred at a mean rate of 0.002 AU/h in normal room light conditions (20). The guidelines also state that samples should be stored in the dark at 4 ℃ until analysis, however Foroughi *et al.* also found that even in dark conditions, bilirubin degradation can lead to false-negative results (20).

Analytical considerations and interpretation

The UK NEQAS SAG recommends a zero-order spectrophotometric scan between 350 and 600 nm. The scan should incorporate the background subtraction method of Chalmers in which a tangent to the curve is drawn between 350–400 nm and again between 430–530 nm (12). This tangent can be drawn manually but the use of fully automatic data analysis software reduces subjectivity. The tangent is used as a baseline, and despite a lambda max of 460 nm, the estimation of the net bilirubin absorbance (NBA) is measured at 476 nm to reduce the interference from oxyhaemoglobin. The net oxyhaemoglobin absorbance (NOA) is the height of any discernible oxyhaemoglobin peak at 413–415 nm above the baseline. Methaemoglobin is haemoglobin in which the haem group contains iron in the ferric (Fe^{3+}) instead of ferrous (Fe^{2+}) form. Its presence, between 403–410 nm, is believed to be due to the artefactual conversion from oxyhaemoglobin but should also be recorded (16). A clear interpretive guide is presented in the revised national guidelines

for analysis of CSF for bilirubin in suspected SAH, but in summary the CSF NBA that indicates a clinically significant bleed is >0.007 AU. This is based on a single study in which 16 control patients who underwent CT myelography had NBAs ranging from 0–0.007 AU (21). Validation of interpretative cut-offs has been performed in a multicentre observational case note study of 463 CT negative patients, with suspected SAH who underwent CSF spectrophotometry (22). It is generally accepted that the presence of oxyhaemoglobin in isolation is likely a result of *in vitro* formation as opposed to SAH. Similarly, the presence of bilirubin in isolation within the first few days is unexpected but the likelihood of SAH with this finding increase with time (16). Once the NOA reaches 0.1 AU it is sufficient to impair the ability to detect bilirubin. In addition, CSF protein >1 g/L can increase NBA and should be interpreted with caution if the NBA is >0.007 AU (16).

Sources of error

A major pitfall of relying on haemoglobin breakdown products is that false-positive results can occur following a traumatic LP. A large Canadian study showed the rate of traumatic LP to be >36% (23). A traumatic LP simply describes the presence of red blood cells in the CSF, although there is no standardised definition of what constitutes a traumatic LP. A traumatic tap introduces erythrocytes into the subarachnoid space resulting in the presence of oxyhaemoglobin in the CSF in sufficient concentrations to impede the reliable detection of bilirubin. The presence of serum bilirubin, biliverdin and other proteins in serum introduced into the CSF as a result of a traumatic LP can also cause false positive results. False positive CSF scans may also be seen with repeated LPs as the blood introduced into the subarachnoid space leads to increased oxyhaemoglobin and bilirubin (24). Serum bilirubin concentrations ≥20 μmol/L may result in protein-bound bilirubin in the CSF. Therefore, the NBA in CSF is adjusted for the serum bilirubin concentration and the degree of contamination estimated using the ratio of the protein concentrations in CSF and serum. The correction described in the 2008 guidelines (16) reduced the number of equivocal results obtained with the original guidelines published in 2003 (17). However, a combination of traumatic LP and either hyperbilirubinaemia or raised CSF protein may still lead to false positive scans for SAH (24). And it has also been argued that incorporating 4 measured parameters into the adjustment calculation compounds the imprecision (25).

Several methods to differentiate a traumatic LP from SAH, including CSF red blood cell clearance, CSF opening pressure, CSF D-dimer, bilirubin, and ferritin, have been evaluated, none however have proved conclusive largely due to small sample sizes (26-29). It has recently been suggested that a red blood cell count <2,000×10^6/L with no visual xanthochromia is indicative of a traumatic tap and can reasonably exclude a diagnosis of aneurysmal SAH (23). The number of positive SAH samples in this study, however, was small (n=15) and strong reliance on subjective visual inspection, with no objective record of the examination procedure is not desirable.

Rodríguez claims that the method of Chalmers *et al.* underestimates NBA and may therefore lead to false negative results. He describes a method purported to be free of oxyhaemoglobin and biliverdin interference by utilising the secondary peaks of the oxyhaemoglobin spectrum at 540–577 nm as a reference for the absorbance due to oxyhaemoglobin at 456 nm. In addition, presence of high concentrations of proteins do not cause interference because their spectra have a substantially flat background absorbance at wavelengths greater than 400 nm (30). Rodríguez suggests that this new approach can be easily evaluated retrospectively in a very simple way and also suggests that the NBA cut-off could be increased from 0.007 to 0.016, which would also reduce imprecision (31). It is also claimed that absence of interference by oxyhaemoglobin and biliverdin in this method could permit the detection of SAH sooner than 12 h after the onset of the symptoms. Despite the promise of this approach, it has neither been verified by another group or established in routine practice, possibly because many laboratories take advantage of automatic data analysis software in which this method has not yet been validated.

It is not possible to reliably interpret an increased NBA in the presence of CSF total protein concentrations exceeding 1.0 g/L. Indeed, the revised national guidelines recommend the result is interpreted 'with caution in relation to SAH especially if within first week of event' (16). Smith *et al.* describe a multi-wavelength spectral method for the measurement of bilirubin in CSF. Their method is reported to accurately measure CSF bilirubin in the presence of high concentrations of haemoglobin and protein (32). In the cohort studied, 11.5% of samples had haemoglobin concentrations that impeded reliable interpretation based on the UK NEQAS revised guidelines. The multiwavelength method was reported to have a

specificity of 100% at 0.2 mg/L CSF bilirubin. Similarly, to the method of Rodríguez it is suggested that this method has not be introduced into routine practice as it is not currently included in automatic data analysis software.

Conclusions

A thorough review of the current literature revealed that the UK NEQAS SAG *revised national guidelines for analysis of cerebrospinal fluid for bilirubin in suspected subarachnoid haemorrhage* still represent best practice for CSF spectrophotometric scanning for SAH although it is accepted that the evidence base for some recommendations in these guidelines is limited. A major pitfall of relying on haemoglobin breakdown products is that false-positive results can occur following a traumatic LP. It has been suggested that CSF red blood cell clearance, CSF opening pressure, CSF D-dimer, bilirubin, and ferritin, red cell count and ferritin may be of value in distinguishing SAH from traumatic LP although evidence for this is limited and currently there is no standard definition of what constitutes a traumatic LP. It is acknowledged that even in the absence of traumatic LP, oxyhaemoglobin and biliverdin can be present in sufficient concentrations to impair the reliable detection of bilirubin in CSF using Chalmers method. Alternative methods which utilise multi-wavelength analysis to remove protein and haemoglobin interference, or the secondary peaks of the oxyhaemoglobin spectrum as a reference for the absorbance due to oxyhaemoglobin have been described and show promise but are not currently in routine use.

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Footnote

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://jlpm.amegroups.com/article/view/10.21037/jlpm-21-62/rc

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