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Detection of subarachnoid haemorrhage with spectrophotometry of cerebrospinal fluid – a comparison of two methods

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Abstract

Objectives: Spectrophotometric absorption curve analysis of cerebrospinal fluid (CSF) for oxyhaemoglobin and bilirubin is necessary to accurately diagnose subarachnoid haemorrhage (SAH) in patients with typical symptoms but with negative findings on X-ray examinations. In this study, we evaluated the performance of two methods for interpreting absorption curves; one method from the United Kingdom National External Quality Assessment Service (UK-NEQAS) and the other from the national quality assurance programme in Sweden (Equalis).
Methods: Consecutive absorbance curves (n=336) were interpreted with two different methods, and their performance was compared to the diagnosis as stated in the patient records.
Results: The UK-NEQAS method displayed equal sensitivity to the Equalis method, but the specificity of the UK-NEQAS method was significantly higher than the Equalis method resulting in fewer false positive results. For UK-NEQAS, a positive predictive value (PPV) of 84.6% and a negative predictive value (NPV) of 99.7% were observed, whereas the Equalis method had a PPV of 27.5% and an NPV of 99.7%.
Conclusions: The semi-automated method based on the guidelines from UK-NEQAS provides an efficient and correct interpretation of absorbance curves with short turnaround times. We propose using this method for the routine interpretation of CSF spectrophotometric curves.

Keywords: absorptiometry; cerebrospinal fluid; spectrophotometry; subarachnoid haemorrhage.

Introduction

Subarachnoid haemorrhage (SAH) is a spontaneous arterial bleeding into the subarachnoid space [1]. The routine investigation of a patient with suspected SAH usually involves a computed tomography (CT) scan of the brain [2]. If a CT scan is performed within 6 h from initiation of symptoms, a sensitivity approaching 100 per cent is achieved, which decreases with time to about 90 per cent after 24 h [3]. Hence, in patients with characteristic symptoms of SAH where a CT scan is performed later than 6 h from onset of symptoms with negative or inconclusive results, a lumbar puncture (LP) is made to extract cerebrospinal fluid (CSF) for spectrophotometric analysis to rule out SAH [4]. On the other hand, with a positive spectrophotometry result for SAH, the standard procedure is to perform an angiographic CT of the brain [5].

When blood enters the CSF during SAH, erythrocytes undergo haemolysis, and free oxyhaemoglobin is released [6]. Following haemolysis, oxyhaemoglobin is enzymatically degraded to bilirubin by macrophages. The presence of oxyhaemoglobin and bilirubin in CSF can be detected spectrophotometrically [7, 8]. Subsequent studies have demonstrated the correlation of xanthochromia, i.e. the yellow colour denoting the presence of bilirubin, and a peak in the spectrophotometric curve at a wavelength around 455 nm [7]. Usually, the spectrophotometric assay result is presented as an absorbance curve which needs to be interpreted for the presence of haemoglobin or bilirubin. At the moment, different methods for interpretation exist, and there is no consensus among laboratories in Sweden. Visual inspection alone for xanthochromia is considered insufficient, and a spectrophotometric curve provides enhanced

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sensitivity [9, 10]. However, some spectrophotometric definitions will lead to low specificity, resulting in unnecessary follow-ups with angiographic CT examinations [11]. The aim of this study was to compare the method for interpreting absorbance curves recommended by the United Kingdom National External Quality Assessment Services (UK-NEQAS) to the method recommended by the national quality assurance programme in Sweden (Equalis) and evaluate their performance.

Materials and methods

Patient samples

Consecutive CSF samples from all patients that underwent LP at the hospitals in the county of Kronoberg analysed with spectrophotometry at the Clinical Chemistry Laboratory in Växjö from December 2015 to June 2021 were included in the study (n=354). 18 patients were excluded. Excluded patients were; patients not residing in the area serviced by the laboratory (n=15) and samples where laboratory guidelines for sample handling were not followed (n=3). The sampling of CSF followed standard clinical protocol for LP, i.e. collection of CSF at least 12 h after onset of symptoms of SAH and centrifugation within 30 min at 1,300 *g* for 5 min [12]. The final diagnosis of SAH following LP was confirmed with the presence of aneurysms on angiographic CT examinations or the occurrence of SAH within six months following admission for LP.

CSF spectrophotometry

The CSF samples were analysed on an Agilent Cary 60 UV–Vis Spectrophotometer (Agilent, CA, USA), covering wavelengths from 350 to 700 nm. From the spectrophotometer curve, net bilirubin absorbance (NBA) and net oxyhaemoglobin absorbance (NOA) were calculated using the software Cary WinUV (Agilent, CA, USA). This calculation requires manual insertion of a tangent to the absorbance curve between 350–400 nm and 430–530 nm without intersecting the curve.

Samples prior to 13/04/2016 (n=19) were analysed for P-bilirubin and P-protein on an Olympus AU2700 or AU400 instrument (Beckman Coulter, CA, USA) and CSF-protein on a Dimension Xpand (Siemens Healthcare, Erlangen, Germany) according to the manufacturers' instructions. Reagent kits OSR6212 and OSR6132 were used for measuring P-bilirubin and P-protein, respectively (both from Olympus Optical, CA, USA) and UCFP Flex reagent for CSF-protein (Siemens Healthcare, Erlangen, Germany). Samples post 13/04/2016 were analysed on a Cobas c701 (Roche Diagnostics, Basel, Switzerland). For Cobas c701, analysis was performed according to the manufacturer's instructions. Reagent kits BILT3, TPUC3, TP2 were used for measuring P-bilirubin, CSF-protein and P-protein, respectively (all from Roche Diagnostics, Mannheim, Germany). All automated systems are part of our laboratories' routine operations and are subject to accepted quality assurance procedures.

All spectrograms were routinely interpreted according to the guidelines from UK-NEQAS [12]. This was done automatically using an algorithm in the laboratory information system FlexLab (Tieto Corporation, Helsinki, Finland). In brief, NBA is used in conjunction with NOA to determine the probability of SAH using cut-off values. High concentrations of CSF-protein and/or S-bilirubin are compensated for, thus taking into consideration a dysfunction in the blood-brain-barrier and/or hyperbilirubinemia. Samples classified as "Consistent with SAH" were interpreted as positive, whereas samples classified as "Inconclusive", "No evidence to support SAH", and "Interpret with caution" as negative. A sensitivity analysis was performed by recalculating all samples analysed on the Olympus and Dimension Xpand instruments using the verification data (not presented) from the laboratory when replacing these instruments with Cobas. The absorbance curves corresponding to these samples were reanalysed according to the algorithm of UK-NEQAS.

For comparison, we reinterpreted the curves with the method recommended by Equalis [13]. The absorbance was measured at 415 nm. If it exceeded 0.025 AU, the absorbance curve was analysed in the spectra of 350–600 nm; otherwise, it was considered negative. The morphology of the curve was manually interpreted, and special consideration was taken to peaks at 415 and 455 nm corresponding to oxyhaemoglobin and bilirubin. All curves exceeding 0.025 AU at 415 nm were analysed independently by two experienced physicians. The presence of either oxyhaemoglobin, bilirubin, or both was considered positive for SAH. In the case of diverging opinions, a consensus was reached. The physicians were unaware of the results from the automatic interpretation and the diagnosis in the patient records.

Evaluation of outcome

Patient records were checked for a diagnosis describing the event of a subarachnoid haemorrhage within six months following the time of lumbar puncture. SAH was diagnosed based on clinical findings and results from CT examinations.

Statistical analysis

Analysis of data was performed using SPSS (Version 26, IBM Corp., Armonk NY, USA) and R (version 4.0.5; http://www.r-project.org/) using packages *dplyr* and *tidyverse*. Patients were divided into positive and negative cohorts based on diagnosis in the patient record. Statistical significance for biomarkers was tested using the Mann–Whitney U test, and a p-value<0.05 was considered statistically significant. Statistical significance for sex differences was tested with a Pearson chi-square test and for sensitivity and specificity with a McNemar test. All statistical tests were two-sided.

Results

Background characteristics for the positive and negative cases of SAH are summarised in Table 1. Statistically significant elevations of NOA, NBA and CSF-protein were observed in the group positive of SAH, compared with the negative group.

When samples analysed for P-bilirubin, CSF-protein, and P-protein prior to 13/04/2016 were recalculated to

Table 1: Background characteristics of the studied population.

	All patients	Positive for SAH	Negative for SAH	p-Value
n	336	12	324	NA
Age, years	46.0 (32.0–61.8)	64.5 (51.8–80.3)	45 (31.3–60)	0.001
Sex				
Female	177	3	174	0.051
Male	159	9	150	
NOA, AU	0.00 (0.00–0.00)	0.065 (0.03–0.33)	0.00 (0.00–0.00)	<0.001
NBA, AU	0.001 (0.001–0.002)	0.037 (0.011–0.161)	0.001 (0.000–0.002)	<0.001
P-bilirubin, µmol/L	7.59 (5.36–10.24)	6.57 (4.94–10.40)	7.61 (5.36–10.24)	0.74
P-protein, g/L	72 (68–75)	70 (68–75)	72 (68–75)	0.40
CSF-protein, g/L	0.38 (0.28–0.52)	1.65 (0.66–3.75)	0.38 (0.28–0.51)	<0.001

Data are presented as median with the 25th and 75th percentile in brackets. For statistical significance, a Mann–Whitney U test was used for all variables except sex, where the Pearson chi-square test was used. AU, absorbance unit; CSF, cerebrospinal fluid; NA, not applicable; NOA, net oxyhaemoglobin absorbance; NBA, net bilirubin absorbance; P, plasma; SAH, subarachnoid haemorrhage.

match the values on the Cobas instrument, no differences in outcome were observed (data not shown).

The performance characteristics of the UK-NEQAS and the Equalis methods are summarised in Table 2. The UK-NEQAS method showed higher positive and similar negative predictive values compared to the Equalis method (Table 2). Both methods showed identical sensitivity and detected 11 patients where the diagnosis SAH was stated in the patient record. All patients with bleeding at the time of LP were detected with both methods. During follow-up, one patient presented with SAH but had no evidence for SAH at the time of the initial LP, when red blood cells (RBCs) were 28×10^6/L and spectrophotometric findings were negative. The UK-NEQAS method showed a significantly higher specificity, thus leading to few false positive results. In contrast, the method currently recommended by the national quality assurance programme in Sweden results in about a 15-fold increase of false positive results (9.0 vs. 0.61%).

Two patients were diagnosed as false positive for SAH with the UK-NEQAS method. Both of whom were diagnosed with meningitis, treated accordingly and clinically improved.

Table 2: Performance of methods for detection of SAH.

	UK-NEQAS	Equalis	p-Value
Sensitivity, %	91.7	91.7	1.0
Specificity, %	99.4	91.0	<0.001
Positive predictive value, %	84.6	27.5	NA
Negative predictive value, %	99.7	99.7	NA

For the UK-NEQAS method: true positive: 11; false negative: 1; false positive: 2; true negative: 322 and for the Equalis method: true positive: 11; false negative: 1; false positive: 29; true negative: 295. NA, not applicable; SAH, subarachnoid haemorrhage.

Six patients were deceased during the follow-up period. One patient was deceased from SAH following a ruptured aneurysm. The spectrogram from this patient was consistent with SAH for both methods. The remaining five patients died from causes other than SAH: cardiac arrest, thromboembolism in the lungs, bacterial pneumonia, meningitis, and acute myeloid leukaemia.

Discussion

This study shows that the semi-automated method based on UK-NEQAS has adequate performance for detecting subarachnoid haemorrhage. The specificity is higher than the Equalis method. A low specificity can lead to unnecessary angiographic examinations [11] since clinical guidelines recommend proceeding with an angiographic CT of the brain when the absorbance curve is positive for SAH [5]. Avoiding unnecessary angiographic CT scans reduces the risk of adverse reactions to contrast media [14], radiation exposure, and more efficient use of healthcare resources. In part, the high number of false positive findings in the Equalis method could be due to interference from oxyhaemoglobin and protein. The UK-NEQAS method partly corrects for an increased protein concentration in the CSF, i.e., impairment of the blood-brain barrier. However, a multi-wavelength analysis has been proposed to reduce the interference of protein and haemoglobin [15].

In this study, we decided on a six-month follow-up time to detect additional subarachnoid haemorrhage events in the patients studied. It is estimated that around 30–50% of all rebleeding incidents occur within 3–6 h after the first symptoms [16]. Indeed, we only observed one event of SAH during the follow-up period, but this occurred around three months from the initial LP at the emergency

department. Therefore, this patient was probably not affected by SAH at the time of LP, which RBCs less than 100×10^6/L also supported [17].

Two patients with suspected meningitis had LP findings that were erroneously misinterpreted as SAH. Indeed, meningitis can result in a spectrophotometric curve positive for SAH [18]. This indicates that not all patients had an LP on an SAH indication and thus a likelihood for lower pre-test-prevalence. However, the included patients represent the selection usually seen at the emergency department and hospital wards. In addition, some of the symptoms of meningitis are similar to those of SAH. Hence, physicians might order laboratory analyses for both conditions to save time to treatment and reduce the need for additional LPs.

The method recommended by UK-NEQAS, in conjunction with our semi-automated assay, provides short turn-around times and answers without the need for a physician to interpret the results. Time to treatment is essential for SAH to improve clinical outcome and avoid rebleeding [19].

Due to the structure of public health care in Sweden, CSF from all patients residing in the county will be handled at our laboratory. Furthermore, they will receive their post-operative care after SAH at the hospitals in the region. This is a strength in our study since it guarantees that patient data will be available through electronic patient records. We identified 11 positive cases of SAH, which accounts for approximately three per cent of the total studied population. This is in line with the assumed number of patients with a ruptured aneurysm following LP 12 h after the onset of typical SAH symptoms [20]. A possible deduction is that both methods identified all patients with SAH at the time of LP. Only 354 patients were included in the study, and it would have been preferable with more participants. However, to our knowledge, this is the largest study performed on patient outcomes in relation to spectrophotometry results interpreted by the UK-NEQAS method. Since the prevalence of SAH is generally low in the population, more comprehensive studies in this field need to further evaluate the method's performance.

A limitation is that we used different analysers for P-protein, P-bilirubin, and CSF-protein during the study since the instruments at the laboratory were replaced. Although our sensitivity analysis demonstrated that this did not affect the result, it still needs to be considered a possible confounder. Furthermore, our system for interpreting absorbance curves is not fully automated. Although laboratory staff is educated and audits are performed, there is a risk for human errors.

Another limitation is that not all patients underwent CT angiography post-LP, and thus patients with minor aneurysmal bleeding might have been misdiagnosed. For example, if no aneurysm ruptured during our follow-up period, such a patient could still have been erroneously deemed negative on LP and negative for SAH. To overcome this problem, a prospective study where all enrolled patients undergo CT angiography after LP analysis would be necessary. However, a CT angiography also risks missing a non-aneurysmal SAH [21]. The clinical follow-up and CT angiography are also cumbersome, highlighting the need to develop an efficient and reliable gold standard method for detecting SAH.

In conclusion, the semi-automated UK-NEQAS method provides an accurate and rapid alternative for interpreting spectrophotometric curves with better specificity than the Equalis method. Therefore, we suggest using the UK-NEQAS method for interpreting CSF spectrophotometry as part of SAH diagnosis in routine clinical practice.

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