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RESEARCH SUBMISSION

Visual inspection versus spectrophotometry for xanthochromia detection in patients with sudden onset severe headache—A diagnostic accuracy study

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Abstract

Objective: There is still disagreement about whether to routinely use spectrophotometry to detect xanthochromia in cerebrospinal fluid (CSF) or whether visual inspection is adequate. We aimed to evaluate the diagnostic accuracy of these methods in detecting an aneurysmal subarachnoid hemorrhage in patients with sudden onset severe headache.

Background: When a patient presents to the emergency department with a headache for which there is suspicion of a subarachnoid hemorrhage, the gold standard to rule this out is to perform a CSF analysis for xanthochromia with or without spectrophotometry if the cranial non-contrast computed tomography (CT) upon admission is negative.

Methods: Having applied the gold standard, we retrospectively included patients with acute headache who underwent both CT scan and CSF spectrophotometry at our hospital in the period 2002–2020. Patients were excluded if the cranial CT was interpreted as positive, there was a bloody CSF, or if visual assessment data of the CSF was unavailable. We scrutinized the patients' medical records and evaluated the benefit of spectrophotometry compared to visual inspection. The net bilirubin absorbance cut-off for support of subarachnoid hemorrhage was set at >0.007 absorbance units. The spectrophotometry was also considered positive if the net bilirubin absorbance was ≤0.007 and net oxyhemoglobin absorbance was ≥0.1 absorbance units. We calculated and compared the sensitivity and specificity of CSF spectrophotometry and visual inspection of the CSF.

Results: In total, 769 patients, with a mean age of 42.3 ± (standard deviation [SD] = 17.3) years, were included. The headache onset was classified as a thunderclap headache in 41.5%, and 4.7% had a sudden loss of consciousness. Fifteen patients (2%) were finally diagnosed with a subarachnoid hemorrhage, six (0.8%) had an aneurysmal subarachnoid hemorrhage, seven (0.9%) had a perimesencephalic hemorrhage, one (0.1%) had a cortical cerebral sinus venous thrombosis, and one

Abbreviations: aSAH, aneurysmal subarachnoid hemorrhage; AU, absorbance unit; CSF, cerebrospinal fluid; CT, computed tomography; CTA, computed tomography angiography; ED, emergency department; LP, lumbar puncture; NBA, net bilirubin absorbance; NOA, net oxyhemoglobin absorbance; oxyHb, oxyhemoglobin; PMH, perimesencephalic hemorrhage; SAH, subarachnoid hemorrhage; SD, standard deviation; SP, spectrophotometry; UNN, University Hospital of North Norway.

(0.1%) had a spinal epidural hematoma. Four patients (0.5%) had a subarachnoid hemorrhage that was not detected by visual inspection, and two were caused by an aneurysmal rupture. One of these two patients died just before intervention, and the other underwent coiling for an anterior communicating aneurysm. The number needed for lumbar puncture to detect a subarachnoid hemorrhage was 51, but 128 to detect an aneurysmal hemorrhage. The corresponding numbers needed for CSF spectrophotometric analysis were 192 and 385, respectively. Spectrophotometry was positive in 31 patients (4.0%), of whom 18 (2.3%) also had visually detected xanthochromia (11 true positive). The mean net bilirubin absorbance in the 13 samples with visually clear CSF was 0.0111 ± (SD = 0.0103) absorbance units, compared to 0.0017 ± (SD = 0.0013) in the CSF with negative spectrophotometry. The corresponding figures for net oxyhemoglobin absorbance were 0.0391 ± (SD = 0.0522) versus 0.0057 ± (SD = 0.0081). The sensitivity of spectrophotometric xanthochromia detection was 100% (95% confidence interval [CI], 78–100), compared to 73% (95% CI, 45–92) for visual xanthochromia detection. The specificity of spectrophotometric xanthochromia detection was 98% (95% CI, 97–99) compared to 99% (95% CI, 98–100) for visual xanthochromia detection. Both methods had high negative predictive values: 100% (95% CI, 99.5–100) versus 99.5% (95% CI, 98.6–99.9), respectively.

Conclusions: Both visual inspection and spectrophotometry have high diagnostic accuracy for detecting CSF xanthochromia, but the lower sensitivity of visual assessment makes it unreliable, and we recommend the use of spectrophotometry in clinical practice.

Plain Language Summary

Sudden onset severe headache may indicate a life-threatening stroke, a so-called subarachnoid bleed. If a brain scan does not reveal such, a yellowish cerebrospinal fluid will still strongly support that a bleed has occurred. Many consider visual inspection of the cerebrospinal fluid sufficient, but based on the results from the present study, in which we also applied spectrophotometry, one potentially treatable case per 400 assessments may be overlooked.

KEYWORDS

cerebrospinal fluid, lumbar puncture, spectrophotometry, subarachnoid hemorrhage, thunderclap headache, xanthochromia

INTRODUCTION

Subarachnoid hemorrhage (SAH) is the cause of 3%–5% of all strokes and is life threatening. Approximately 80% of instances are secondary to a ruptured saccular aneurysm.[1] The overall global incidence of aneurysmal SAH (aSAH) is 7.9 per 100,000 person-years.[2]

When a patient presents to the emergency department (ED) with an acute non-traumatic headache, there are multiple differential diagnoses.[3] Thunderclap headache, defined as a hyperacute headache reaching its pain maximum within 1 min, is the cardinal feature of SAH.

There is no unified international consensus on a definite diagnostic algorithm to exclude an SAH, but a non-contrast cranial computed tomography (CT) is used to diagnose SAH or to help rule it out. If the CT scan is negative and SAH is still suspected, it is recommended to perform a lumbar puncture (LP), preferably 12 h after ictus, to look for xanthochromia.[1,4,5]

After subarachnoid bleeding, erythrocytes start to leak hemoglobin, which rapidly oxidizes to oxyhemoglobin (oxyHb) in the cerebrospinal fluid (CSF). Over a period of hours, oxyHb will be enzymatically degraded into bilirubin, leading to the yellowish color of the CSF, called xanthochromia. If xanthochromic CSF is

confirmed, a head CT angiography should be obtained to identify an arterial source of the bleeding. In the United States, visual inspection of the CSF supernatant is the primary method to detect xanthochromia; however, this method may lack the sensitivity necessary to reliably exclude the diagnosis of SAH.[6] In Norway, spectrophotometry analysis of CSF is part of routine investigation, and it is used according to national guidelines in the United Kingdom. The guidelines were proposed in 2003 and revised in 2008.[5]

Spectrophotometry (SP) measures the quality or quantity of a substance based on how much light is absorbed by colored compounds.[7] By using a spectrophotometer to measure the compounds in the visible region of light (350–600 nm), spectrophotometric analyses can be applied to ensure a zero risk of xanthochromia; however, a 100% sensitivity comes at the cost of lower specificity and a potentially very low positive predictive value.

In this study, we wanted to evaluate the benefit of SP compared to visual inspection of the CSF in patients admitted with an acute headache to a district teaching hospital in Norway.

METHODS

Design and settings

This was a single-center diagnostic accuracy study conducted at Nordland Hospital Trust. The study was approved by the institutional data protection official (PVO, #28-18). It was classified as a quality improvement project, and no patient consent was required from the regional ethics committee in northern Norway (REK nord ref. 88990). All work was carried out in compliance with the Ethical Principles for Medical Research outlined in the Declaration of Helsinki.

Nordland Hospital Trust consists of three somatic hospitals located in Nordland County, Norway, and serves a population of approximately 242,000 people. Nordland Hospital in Bodø is the largest. A patient arriving at an ED due to sudden onset severe headache will immediately be scanned with a non-contrast cranial CT. If the CT is negative, it will be followed by an LP no earlier than 12 h after the onset of the headache. The physician on call will evaluate the visual appearance of the CSF, and it is subsequently sent for CSF-SP analysis by the central laboratory in Bodø. Since 2005, this has been a standard procedure. The use of CSF-SP began in 2002, but between 2003 and 2007, there were only 16 patients registered with SP. Complete records of the procedure in the central laboratory were not in place until 2011. From 2008, however, the results were also interpreted by the on-call neurologist, and we have thus obtained complete records from then. On average, 55.7 ± (standard deviation [SD] = 16.0) spectrophotometric analyses were performed annually between 2008 and 2020. Every patient who had their CSF analyzed with SP during 2002 through 2020 was included in the study, but the patients who did not have a CT scan, had a positive CT scan, had a bloody CSF, or did not have a visually described CSF were excluded.

The visual inspection was the index test, and the SP was the reference test for xanthochromia in the CSF.[5] The index test was performed in the last CSF tube, documented by the neurologist who performed the LP, but the result plotted was that from the laboratory technician who interpreted the color of the CSF after centrifugation. The reference test was performed by a laboratory technician, preliminarily interpreted by the on-call neurologist if performed outside office hours, but always evaluated by a medical doctor in clinical chemistry trained in CSF-SP. The individuals conducting the index test did not have any knowledge of the reference test result when the visual inspection of CSF was performed. Neither had individuals conducting the reference test knowledge of the index test results nor the clinical information (besides acute headache).

When an aSAH is detected at Nordland Hospital Trust, the patient is always transferred to the Department of Neurosurgery at the University Hospital of North Norway (UNN) for intervention or, eventually, further investigations. Via our electronic medical record system (DIPS), all medical information (records, charts, images, etc.) at UNN is available for the physicians at Nordland Hospital Trust.

Case ascertainment

The medical records (also at UNN when applicable) for each patient were scrutinized for clinical and paraclinical data, and detailed data on clinical characteristics were collected.

The primary analyses of these data are reported here.

The patient's headache was classified according to time from ictus to maximal intensity: (1) maximal in a split second, (2) maximal within 1 min, (3) gradual over minutes, or (4) woke up with the headache. We also registered a sudden loss of consciousness. Only patients in categories 1 and 2 were considered to have a definitive thunderclap headache. Time from ictus to LP and CT was documented. We also registered whether the patient had reported a thunderclap headache before or after this admission. Any information about aneurysms (number, location, size, source of bleeding, etc.) and interventions was plotted, as well as final diagnoses at discharge, follow-ups, and drugs prescribed. Information on LP complications, including post-dural puncture headache, was also collected. Other variables registered were headache intensity and location; precipitant factors; associated symptoms; neck pain; focal neurological symptoms or signs; atypical presentation; meningism; fever; increased blood pressure or not; Glasgow coma scale; cell count in the CSF; CSF pressure; CSF-SP; visual xanthochromia; net bilirubin absorbance (NBA); net oxyhemoglobin absorbance (NOA); the result of CT-, magnetic resonance, or digital subtraction angiography; migraine or heredity for migraine; treatment; and rebleeding or whether there had been a warning leak.

If a ruptured intracranial aneurysm caused the SAH, it was categorized as an aSAH. If no bleeding source was identified, it was categorized as an SAH.

Cranial CT

All included patients underwent a modern CT scanning of the brain. The CT was defined as "positive" when the radiologist described something abnormal, including possible SAH.

LP

The LP included measuring the opening pressure, routine CSF analyses, and visual inspection of the supernatant's xanthochromia. Descriptions of spectrophotometric scans, absorbance units (AU), and the reported interpretation of this (see below) were also registered.

Spectrophotometric procedures

All SP samples were protected from sunlight. None of the samples were sent via a pneumatic tube system. The sample used for analysis was the least blood-stained test tube with CSF. Samples were protected from light using aluminum foil. The CSF sample was centrifuged at 100 × *g* for 15 min, and the supernatant was transferred to another tube protected from light using aluminum foil. The absorbance spectrum from 350 to 600 nm of the CSF samples was recorded using a Shimadzu UV-1700 spectrophotometer using distilled water in the reference cuvette.

The measurement of bilirubin and oxyHb was calculated using the net absorbance of each, NBA and NOA. The laboratory participated in an external quality control program for oxyHb and bilirubin analysis in CSF and interpretation of results from UK NEQAS (Sheffield, UK). The analysis used in our laboratory was based on the version of the guidelines provided by Cruickshank et al.[5] Table 1 shows the template for reporting and interpretation.

In this study, we have separated the results into two categories: CSF-SP positive or negative. This means that those patients with more uncertain SP results (category three or six) have been classified as positive.

Sample size rationale and statistical analysis

We hypothesized that using visual inspection and spectrophotometric analyses to detect xanthochromia is equal for detecting SAH. If the alternative hypothesis is true, detecting one more potential lethal hemorrhage among a reasonable number of patients using SP, the null hypothesis must be rejected. Weighing pros and cons, a "reasonable number" can be discussed, but the cost of SP is low, both when it comes to monetary and non-monetary resource use. Based on previous studies, the number needed to analyze with SP to detect an aSAH has been estimated to be between 265[8] and 706,[9] thus implying that we could expect to find at least one in our material. The statistical analysis was performed using SPSS version 27. All authors had access to the data files. We used descriptive statistics with

TABLE 1 Reporting and interpretation of results from spectrophotometry of cerebrospinal fluid.[5]

	Reporting and interpretation	Conclusion
1	Bilirubin and oxyhemoglobin not increased (NBA ≤ 0.007 AU, NOA ≤ 0.02 AU)	No evidence to support SAH
2	Bilirubin not increased, a small amount of oxyhemoglobin. No evidence to support SAH (NBA ≤ 0.007 AU and NOA > 0.02 AU but < 0.1 AU)	No evidence to support SAH
3	Oxyhemoglobin is present in sufficient concentration to impair the ability to detect bilirubin (NBA ≤ 0.007 AU and NOA ≥ 0.1 AU)	SAH not excluded
4	Increased CSF bilirubin (NBA > 0.007 AU and NOA ≤ 0.02 AU or NOA > 0.02 AU but with no visible oxyhemoglobin peak)	Positive (unusual pattern within the first week after ictus)
5	Increased bilirubin, but probably because of increased s-bilirubin (s-bilirubin > 20 μmol/L and CSF protein ≤ 1.0 g/L). Formula to calculate adjusted NBA (NBA then ≤ 0.007 AU) according to UK Guidelines	Probably not SAH
6	Increased CSF bilirubin. May be consistent with SAH. An increased bilirubin accompanies the increased CSF protein or other sources of CSF blood (CSF protein > 1.0 g/L, whatever the serum bilirubin)	Interpret the result with caution regarding SAH, especially within the first week of the event
7	Increased bilirubin and oxyhemoglobin (NBA > 0.007 AU and NOA > 0.02 AU with visible oxyhemoglobin peak)	Positive

Abbreviations: AU, absorbance units; CSF, cerebrospinal fluid; NBA, net bilirubin absorbance; NOA, net oxyhemoglobin absorbance; SAH, subarachnoid hemorrhage.

measures of frequency (%), central tendency (median and mean), and variation (SD, percentiles, and standard error) to describe our data. Data distribution was checked for normality using histograms and Q–Q plots. For the results of the two diagnostic tests, we applied 2 ×2 tables. No direct statistical comparison between the two tests was performed.

The number needed to screen is the number of people that need to be screened for a given duration to prevent death or one adverse event.[10] In this study, we calculated the number needed to LP, NN_{LP}, to reveal an SAH when the initial cranial CT was negative. Similarly, we calculated the number of CSF samples that needed to be analyzed with SP to detect xanthochromia when visual inspection of the CSF supernatant was negative, $NN_{spectrophotometry}$.

RESULTS

In total, 801 patients with a mean age of 42.3±(SD=17.4) were identified in the laboratory database, of which 32 were excluded from participation (see Figure 1). Table 2 shows the demographics and time from headache onset to CT and LP of the 769 included patients.

Table 3 shows the final diagnostic outcome for patients with or without definitive thunderclap headache.

The headache onset was described as or presumed to be thunderclap headache type in 41.5% of the patients; 7.4% experienced a gradual onset of headache, 12.9% woke up with a headache, and 4.7% experienced a sudden loss of consciousness. In the remaining 33.5%, the headache was classified as "acute headache." The reported values of bilirubin and oxyHb were based on the specific values from 427 patients. Of the remaining 342 missing values, 15 were not obtainable, and the others were reported as: "negative" (77), "normal" (244), or "positive."[6] Of the latter, one was a false positive (a patient with meningitis and CSF protein >2 g/L). Time from ictus to CT and LP were missing in 62 and 63 patients, respectively. In 65 patients, time to LP was <12 h.

Eleven did not undergo a cranial CT, primarily because of pregnancy, and all of these were excluded. All patients who did not undergo a CT had clear CSF and a negative SP. Nine patients were excluded due to a positive CT.

TABLE 2 Demographics and time from headache onset to CT and LP.

Patients, *n* = 769	
Age, mean years ± SD	42.3 ± 17.3
Sex, males/females, *n* (%)	316 (41)/453 (59)
Time to CT, hours, mean ± SD (range)	48.9 ± 87.9 (1–672)
Time to LP, hours, mean ± SD (range)	60.6 ± 100.1 (3–864)

Abbreviations: CT, computed tomography; LP, lumbar puncture; SD, standard deviation.

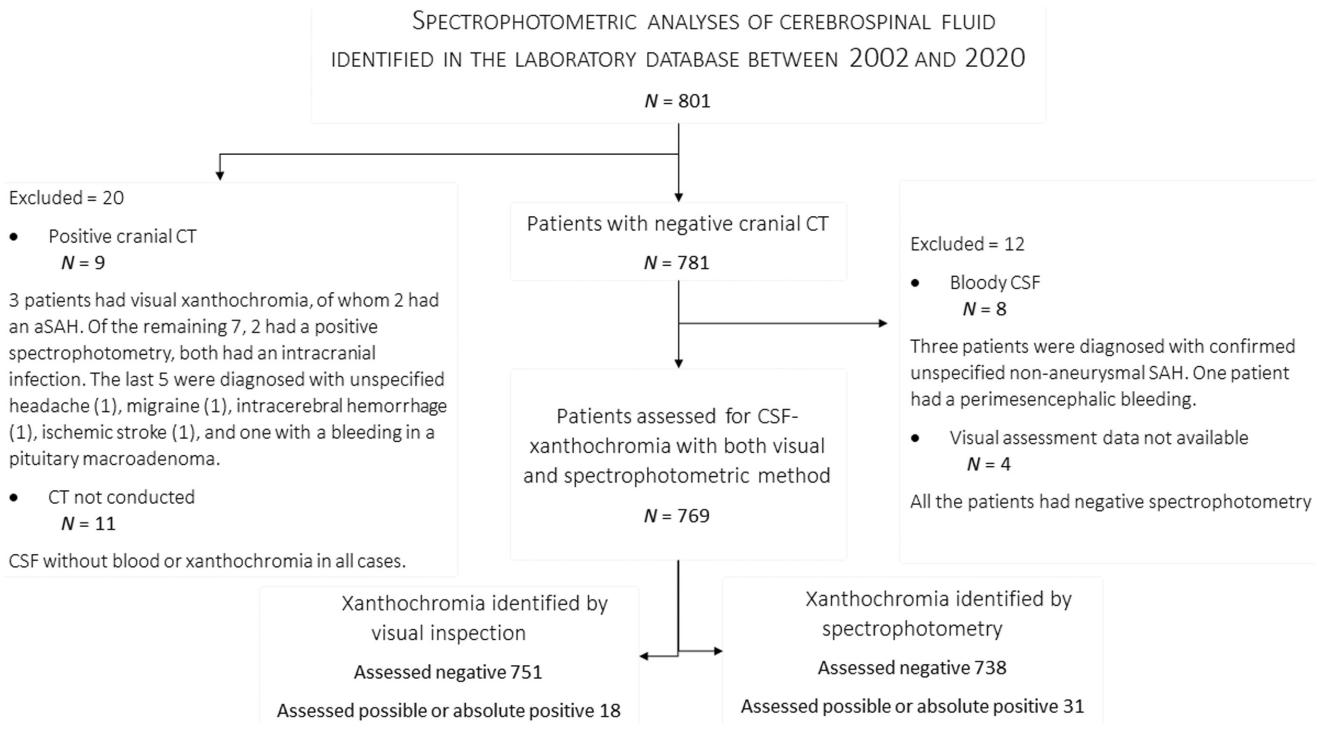

FIGURE 1 STARD flow diagram.[11] The study population for evaluating the accuracy of visual and spectrophotometric assessment of xanthochromia for diagnosis of subarachnoid hemorrhage. CSF, cerebrospinal fluid; CT, computed tomography; STARD, Standards for Reporting of Diagnostic Accuracy Studies.

TABLE 3 Thunderclap headache and diagnostic outcome.

Definitive (category 1 and 2)	Sex, males/females (%)	Age, mean years ± SD	Time to CT, hours ± SD	Time to LP, hours ± SD	SAH, *n*	aSAH, *n*
Yes	125 (31.3)/194 (60.8)	43.0 ± 17	49.1 ± 84.9	55.5 ± 83.5	11	5
No	174 (58)/126 (42)	40.7 ± 17.5	52.9 ± 97.5	52.9 ± 97.5	4	1
Not classifiable	85 (56.7)/65 (43.3)	43.8 ± 17.2	41.5 ± 75.7	55.8 ± 84.3	0	

Abbreviations: aSAH, aneurysmal subarachnoid hemorrhage; CT, computed tomography; LP, lumbar puncture; SAH, subarachnoid hemorrhage; SD, standard deviation; TCH, thunderclap headache.

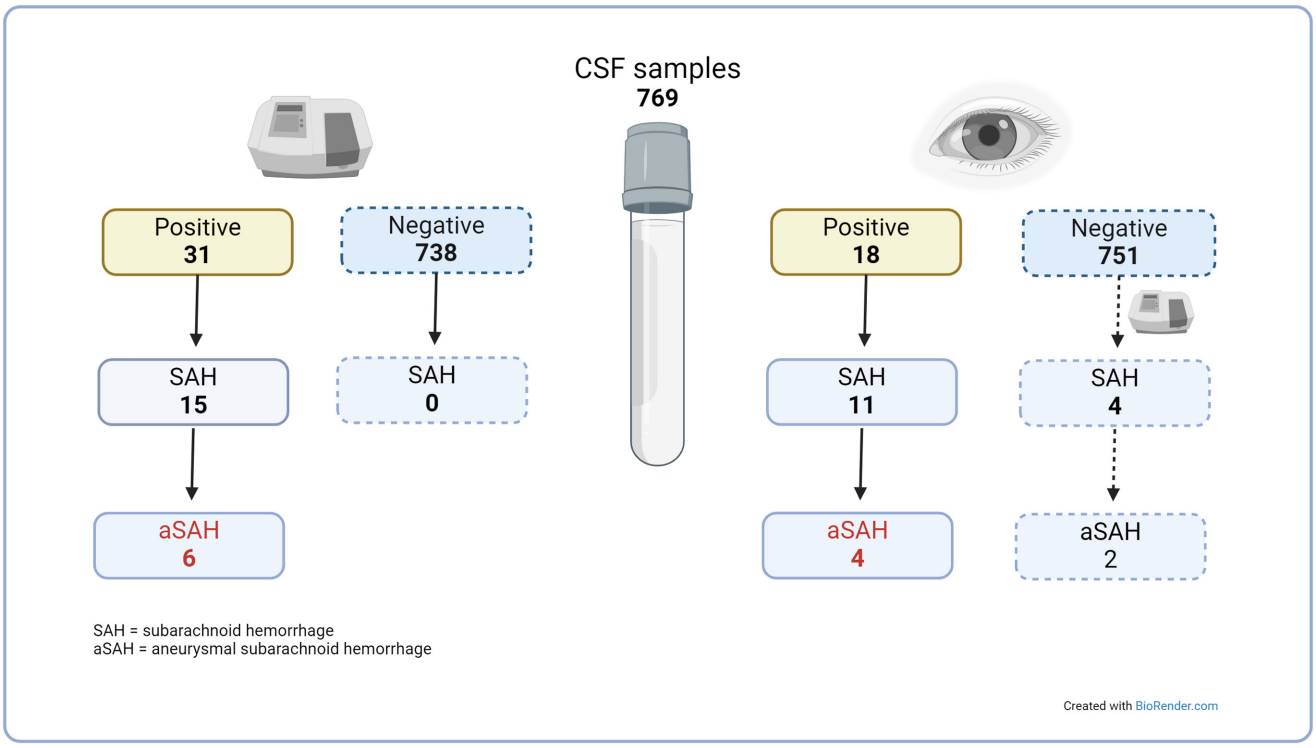

FIGURE 2 Flow diagram of CSF samples with positive and negative tests. aSAH, aneurysmal subarachnoid hemorrhage; CSF, cerebrospinal fluid; SAH, subarachnoid hemorrhage.

Patients with assessed positive CSF

Of the 769 included patients, 31 (4.0%) had a positive CSF-SP (Figure 2). Eighteen patients (2.3%) with a positive CSF-SP also had visually detected xanthochromia. Eleven (1.4%) of those with positive CSF-SP and visually detected xanthochromia turned out to have an SAH; six (0.8%) had a perimesencephalic hemorrhage (PMH), four (0.5%) had an aSAH, and one (0.1%) had a spontaneous spinal epidural hematoma. Of the 13 patients with a negative visual inspection of the CSF but a positive CSF-SP, four (0.5%) were finally diagnosed with an SAH, two (0.3%) with an aSAH, one (0.1%) with a PMH, and one (0.1%) with an SAH of unknown source (but suspected bleeding from a superficial sinus vein thrombosis).

The mean NBA in the 13 samples with visually clear CSF was 0.0111 ± (SD = 0.0103) AU (95% confidence interval [CI], 0.0042–0.0180), and the mean NOA was 0.0391 ± (SD = 0.0522) AU (95% CI, 0.0041–0.0742). In the patients who were CSF-SP negative, the mean NBA was 0.0017 ± (SD = 0.0013) AU (95% CI, 0.0016–0.0018), and the mean NOA was 0.0057 ± (SD = 0.0081) AU (95% CI, 0.0050–0.0065).

Table 4 summarizes the characteristics of the 15 patients with true positive SP.

Sensitivity and specificity

The sensitivity of CSF-SP was 100% (95% CI, 78–100), compared to 73% (95% CI, 45–92) of visual xanthochromia detection (Table 4). Both methods have high negative predictive values: 100% for CSF-SP (95% CI, 99.5–100) versus 99.5% (95% CI, 98.6–99.9). No direct statistical comparison between these tests was performed.

Tables 5 and 6 summarize the calculations; the calculations for SP are considered a gold-standard test with 100% sensitivity.

TABLE 4 Characteristics of the 15 patients with true positive spectrophotometry.

Age	Clinical history	VX	Bilirubin (NBA, AU)	Oxyhemoglobin (NOA, AU)	Category (Ref. 5)	Diagnosis
40s	TCH	No	0.013	0.033	7	aSAH, coiled aneurysm
60s	TCH	No	0.039	0.041	7	aSAH, re-bled straight after angiography, died
20s	TCH	No	0.0132	0.0264	7	PMH
50s	TCH	No	NA	Pos	NA	Probable cortical SVT
30s	TCH	Yes	Pos	Pos	NA	aSAH, clipped aneurysm
50s	TCH	Yes	0.064	0.086	7	aSAH, coiled aneurysm
40s	Gradual headache	Yes	Pos	NA	NA	aSAH, clipped aneurysm
50s	TCH	Yes	0.147	0.253	7	aSAH, coiled aneurysm
50s	Gradual headache	Yes	0.031	0.127	7	PMH
50s	TCH	Yes	Pos	Pos	NA	PMH
80s	Gradual headache	Yes	0.0692	0.3508	7	PMH
40s	TCH	Yes	Pos	0.067	NA	PMH
70s	Wake up with	Yes	0.29	0.81	7	PMH
40s	TCH	Yes	0.0168	0.0144	4	PMH
50s	TCH	Yes	0.036	0.0489	7	Epidural hematoma

Abbreviations: aSAH, aneurysmal subarachnoid hemorrhage; AU, absorbance units; NA, not available; NBA, net bilirubin absorbance; NOA, net oxyhemoglobin absorbance; PMH, perimesencephalic hemorrhage; SVT, sinus venous thrombosis; TCH, thunderclap headache; VX, visual xanthochromia.

TABLE 5 Diagnostic features of visual assessment.

Xanthochromia by visual inspection	Value	95% CI
Sensitivity	73.3%	45%–92.2%
Specificity	99.1%	98.1%–99.6%
Positive likelihood ratio	79.0	35.6–175.5
Negative likelihood ratio	0.3	0.1–0.6
Positive predictive value	61.1%	41.4%–77.7%
Negative predictive value	99.5%	98.8%–99.8%
Accuracy	98.6%	97.5%–99.3%

Abbreviation: CI, confidence interval.

TABLE 6 Diagnostic features of spectrophotometry.

Xanthochromia by spectrophotometry	Value	95% CI
Sensitivity	100.0%	78.2%–100.0%
Specificity	97.9%	96.6%–98.8%
Positive likelihood ratio	47.1	29.0–76.5
Negative likelihood ratio	0.00	
Positive predictive value	48.4%	36.6%–60.4%
Negative predictive value	100.0%	99.5%–100.0%
Accuracy	97.9%	96.6%–98.8%

Abbreviation: CI, confidence interval.

DISCUSSION

Findings

Overall, 4.0% of all the 769 spectrophotometric analyses in our study were positive. Given a negative cranial CT at admission, the NN_{LP} to detect one SAH was 51. The NN_{LP} to avoid missing one aSAH, however, was 128. The corresponding number was 237 in a study by Sayer et al.,[12] 265 in a study by Bakr et al.,[8] and 141 in a study by Gangloff et al.[9] The prevalence of aneurysms in the general population has been reported to be approximately 2%, with a range of 0.9%–9%.[13] The prevalence of an aSAH in the Sayer et al. study was 0.4% of patients, compared to 0.7% in the Gangloff et al. study, 0.4% in the Bakr et al. study, and 0.8% in our study. In 2011, Sandvei et al.[14] found an incidence rate of 10.3 aSAH per 100,000 person years in two large Norwegian population-based cohort studies. The discrepancy in these numbers may be due to different evaluations of the pre-test probability of an SAH and the threshold for performing LP, as well as the time from ictus to CT and LP evaluation. Our study's average time from ictus to CT and LP is relatively high (Table 2), which reflects our population's wide geographical catchment area. Furthermore, omitting LP in patients with a negative cranial CT performed within 6 h after ictus[15] would increase the NN_{LP}. However, a negative CT scan with almost 100% negative predictive value within 6 h of ictus depends on high radiological competence. Studies performed in high-volume centers have also shown that subarachnoid blood on the initial CT scan is often overlooked, especially during out-of-office hours.[16] In our material, the on-call radiologist initially overlooked three cases of aSAH. Notably, these patients

underwent a CT scan >24 h after ictus. We also identified one patient who underwent a CT of the brain 2 h after ictus, for whom the CT was initially interpreted as negative, but a positive CSF-SP resulted in a second opinion and a PMH was detected. In several cases in our study, neurosurgeons requested a LP with CSF-SP when they were in doubt.

The $NN_{spectrophotometry}$ to avoid missing one SAH in our study was 192, and 385 to detect one aSAH. Gangloff et al. found this number to be 706,[9] while Bakr et al., in comparison, found it to be 265.[8] In the Bakr et al. study, the number of CSF samples with visually detected blood or xanthochromia was not given, probably resulting in a far-too-low estimate. Similar studies of patients admitted directly to neurosurgical centers seem to have higher rates of vascular abnormalities than studies of acute headache patients in the ED.[12]

The sensitivity of CSF-SP was presumed to be 100% in our material; at least, no patient with a negative result suffered from an SAH during an average of 10 years of follow-up. This was obtained by reviewing the patients' medical records thoroughly; we found no evidence of a subsequent subarachnoid hemorrhage. This is compared to the 73% sensitivity of visual xanthochromia detection; however, both methods had very high negative predictive values.

False positive spectrophotometries

Of the nine false positive CSF-SPs, there were five central nervous system infections. Four of these patients had high or very high protein in the CSF, probably indicating damage to the blood–brain barrier, which may explain the spectrophotometric result. Intracranial hemorrhage occurs rarely and in less than 1% of the cases with bacterial meningitis.[17] One patient was diagnosed with posterior reversible encephalopathy syndrome, and two were ultimately diagnosed with an unspecific headache (R51). Of these two patients, one had high protein in the CSF (category six), and the other one had such a high concentration of oxyHb (without any definite cause) that the bilirubin concentration was unreliable. One likely explanation for the high oxyHb in some CSF samples may be the rapidly changing pH in the CSF after LP, leading to hemolysis of the red blood cells.[18] The last of these nine patients had a very high level of oxyHb in CSF, possibly due to a traumatic tap or hemolysis of red cells after sampling.[18,19]

Pros and cons of performing LP

LP is one of the most commonly performed invasive procedures in clinical medicine. It is highly available in all hospitals, easy to master, and has low economic costs. It is beneficial in patients whose time from ictus to CT is long, and the subarachnoid blood may no longer be visible on the CT scan. We also know that looking for xanthochromia with the naked eye is quite accurate. Although it is not a procedure without risk, serious complications are infrequent. The most common complication is post-dural puncture headache, and in our material, 80 patients (10%) received this diagnosis. Fourteen of these were treated with a blood patch. Five patients had other minor complications. There were no severe complications.

Nevertheless, waiting for xanthochromia to develop (>12 h after headache onset) implies cost and, in the worst case, a rebleeding from a ruptured aneurysm. Some advocate performing a CT angiography (CTA)[20,21] instead of an LP, as an investigation that can be performed shortly after the patient is admitted. However, as the prevalence of cerebral aneurysms in the general population is relatively high, an LP could still be necessary to demonstrate whether a detected aneurysm is incidental or symptomatic. Incidental aneurysms must be followed up, with all the consequences for the patient and the health system that this entails. A CTA will also cause significant radiation exposure. Nor should one forget that the use of contrast could be associated with toxicity and allergy. Furthermore, the cost of conducting a CSF analysis is much lower than that of a CTA.

In our study, we excluded 32 patients, of whom 9 had a positive CT scan and still had LP. Two of these had an aSAH—in one case, the radiologist was unsure about the result of the CT, and the neurologist recommended doing an LP; the second patient had a huge aneurysm, and the neurosurgeon wanted an LP to decide whether there had been a hemorrhage.

Pros and cons of using SP

The use of SP of the CSF to verify an SAH has the highest sensitivity. We detected subarachnoid blood in the CSF of four patients with acute headaches who had a negative preliminary CT scan of the brain and a visually clear CSF. The spectrophotometric analysis of the CSF was crucial for further follow-up in these patients. Two of these patients had an aSAH—one died because of a massive rebleeding, and the other, a woman in her 40s, survived and had an anterior communicating artery aneurysm coiled. She had no neurological deficits after the procedure.

Performing a SP is more expensive than conducting a visual inspection of the CSF, but the economic costs we have estimated are minimal. Based on the purchase price of our Shimadzu spectrophotometer (which was used during the whole study period), test vials, and reagents (=0, as we use water in the reference vial), and the remuneration for half an hour of work by a bioengineer, the economic expense per test in our hospital has been estimated at NOK 175 (USD16).

The flip side of performing CSF-SP is the caveats involved in interpreting the analysis and obtaining false positive results. A Swedish study found that in patients with a CSF-SP–verified SAH, an underlying vascular pathology was found in only 20% of the patients.[22] This is the same as in the present study, in which we found that there were four false positives for each true positive test. Unnecessary investigations and anxiety are thus the consequences for four out of five with a positive test. On the other hand, both LP and CSF-SP will be useful in finding other potentially life-threatening diagnoses causing the symptoms, such as central nervous system infections. A suspected traumatic tap will also be much easier to interpret with a CSF-SP.

TABLE 7 Advantages and disadvantages of the two xanthochromia detection methods.

Xanthochromia detection method	Advantages	Disadvantages
Spectrophotometry	• 100% sensitivity • Potential operational and interpretational biases • Low economic costs	• False positive cases • Interpretation difficulties
Visual inspection	• High accuracy • No economic costs • Low bias operation	• False negative cases

Table 7 summarizes the pros and cons of spectrophotometric versus visual eye detection of xanthochromia.

Strengths and limitations

The study's retrospective design makes it prone to biases. Our data were incomplete from 2002 to 2008, and we observed a breach of protocol in a few instances. For example, 65 of the patients had the LP performed before 12 h had passed. None of these had an aSAH; one had a PMH and had a LP after 10 h.

We have focused exclusively on the patients who screened negative on CT. However, a false positive CT, including a pseudo subarachnoid hemorrhage, may, in some instances, warrant a LP. The yield of using SP in such cases could not be statistically evaluated in our study.

On the positive side, we conducted a thorough patient review and a detailed overview of past and subsequent medical history, complications, and diagnoses. In addition, the CSF-SP analysis and interpretation were subject to external quality control; however, we did not have a systematic follow-up of the patients after discharge. The external validity is reduced as all the data came from a single center. The reliability of the measurements in our study is considered high. Standard procedures for sampling and measurements have been followed, and the apparatus used for measurements has been regularly calibrated. Our laboratory has also participated in an external quality control program for the analysis and interpretation of SP from UK NEQAS. The amount of missing values for the SP may, however, potentially introduce a diagnostic bias. It is unlikely that the final reports from the analyses are based on false values, and the values per se are not central to answering the main research question.

CONCLUSION

Using SP for detecting xanthochromia in the CSF is, without doubt, superior to visual assessment. The negative predictive value in both methods is very high, and the higher sensitivity of SP comes at the cost of false positive analyses. Almost 400 spectrophotometric analyses of CSF in patients with a negative CT scan must be performed to detect one aSAH.

Given that SP is a low-cost method and has the potential to save lives, we recommend using it as a standard in the algorithm for detecting an aSAH.

AUTHOR CONTRIBUTIONS

Ane Skaare Sjulstad: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; software; validation; visualization; writing – original draft; writing – review and editing. **Karl B. Alstadhaug:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing – review and editing. **Ole-Lars Brekke:** Conceptualization; data curation; investigation; methodology; project administration; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

Ane Skaare Sjulstad, **Ole-Lars Brekke**, and **Karl B. Alstadhaug** declare no conflicts of interest.

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