Should Spectrophotometry Be Used to Identify Xanthochromia in the Cerebrospinal Fluid of Alert Patients Suspected of Having Subarachnoid Hemorrhage?

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Background and Purpose—The absence of xanthochromia in the cerebrospinal fluid (CSF) is often used to exclude subarachnoid hemorrhage (SAH). Authorities advocate spectrophotometry to measure xanthochromia, but most North American hospitals use visual inspection. We studied the diagnostic accuracy of spectrophotometry for SAH, and its potential impact on current practice.

Methods—This was a prospective cohort study comparing the diagnostic accuracy of tests. The study was set in 3 university-affiliated tertiary care emergency departments. We enrolled consecutive neurologically intact adults with nontraumatic headache undergoing lumbar puncture (LP) to rule out SAH. CSF was centrifuged, frozen and analyzed later in batch. SAH was defined by (1) subarachnoid blood on CT, (2) $>5 \times 10^5$ red blood cells/L in the final CSF tube and positive angiography, or (3) visible xanthochromia in CSF and positive angiography. All subjects lacking a normal CT and LP were telephoned at 30 days.

Results—We enrolled 220 patients (mean age 42±16 years; CT rate 87.7%; angiography rate 5.9%). Two SAHs were identified: 1 with red blood cells without xanthochromia in the CSF and 1 with visibly xanthochromic CSF. The specificity of xanthochromia was 97% (95% CI: 92% to 99%) for visual inspection, but as low as 29% (95% CI: 23% to 35%) for 2 of the spectrophotometric definitions. Introducing spectrophotometry could lead to angiography in as many as 11% to 71% of patients undergoing LP.

Conclusions—Spectrophotometric definitions of xanthochromia have only moderate to low specificity for SAH. Using spectrophotometry could increase angiography rates, thereby identifying more incidental aneurysms, increasing patient anxiety and exposing patients to unnecessary surgical or investigational complications without benefit. (Stroke. 2006; 37:2467-2472.)

Key Words: cerebrospinal fluid ■ spectrophotometry ■ subarachnoid hemorrhage

Headache is a common symptom representing up to 4.5% of presenting complaints in the emergency department (ED).1 Although subarachnoid hemorrhage (SAH) accounts for only 1% of these headaches,2 emergency physicians endeavor to rule out this potentially catastrophic diagnosis. These efforts are particularly important in alert, neurologically intact patients because these patients benefit most from prompt diagnosis and account for up to one half of all SAH cases.3 It is precisely in these patients, however, that the diagnosis is most challenging.4–7

Currently, most authorities recommend nonenhanced head CT, followed by lumbar puncture (LP) if the CT is normal. This practice is predicated on the belief that CT misses up to 10% of SAHs, and presumably a disproportionately greater number among alert, neurologically intact patients.8 However, there is no consensus regarding what constitutes a positive LP. Because the red blood cell count can be falsely elevated by a traumatic tap, the presence of xanthochromia attributable to hemoglobin oxidation products several hours after the headache onset is considered the sine qua non of SAH. Some authorities claim that spectrophotometry is superior to visual inspection for the identification of xanthochromia.9,10 These recommendations are problematic given that >99% of North American hospitals do not have ready access to spectrophotometry and currently use visual inspection alone, the presence of referral bias in studies used to support these recommendations, and inconsistent definitions of what represents a positive spectrophotometric test.9,11–14

During the course of a larger study to derive a clinical decision rule for acute headache patients, we performed...
spectrophotometry on the cerebrospinal fluid (CSF) obtained from a cohort of patients undergoing LP to rule out SAH. The objective was to characterize the diagnostic accuracy of spectrophotometry for SAH, and to estimate the impact of introducing spectrophotometry into current practice.

Methods

This study was a planned substudy of an ongoing multicenter study at 6 Canadian tertiary care EDs of alert, neurologically intact acute headache patients. Three of the 6 sites with a combined annual census of 160,000 visits, participated in this substudy. For this study, we collected CSF samples from consecutive patients undergoing LP to rule out SAH from July 2002 to January 2004.

We included alert patients at least 15 years of age with a chief complaint of nontraumatic acute headache or syncope associated with a headache. Alert was defined as a Glasgow Coma Scale score of 15. Nontraumatic was defined as the absence of falls or direct trauma to the head in the previous 7 days. Acute was defined as an interval of $<1$ hour from headache onset to peak intensity, and an interval of $<14$ days from headache onset to presentation.

The following patients were excluded: (1) history of 3 or more recurrent headaches of the same character and intensity as the presenting headache over a period of $>6$ months, (2) referred from other centers with a confirmed SAH by either CT or LP, (3) returned for reassessment of the same headache if already investigated with both CT and LP, (4) papilledema, (5) new focal neurological deficits, (6) previous diagnosis of cerebral aneurysm or SAH, (7) previous diagnosis of a brain neoplasm, or (8) known hydrocephalus.

After routine analysis for cell count and visible xanthochromia, any remaining CSF in the final tube was immediately centrifuged at 3400 g for 10 minutes to remove cells and debris, and frozen in glass tubes at $-70^\circ$C for later spectrophotometry. If $<1$ mL of fluid remained in the final tube, additional fluid from the penultimate tube was added before centrifugation.

Spectrophotometry was performed by a single investigator (J.R.) using a Milton Roy Spectronic 1001plus. Samples were thawed at room temperature, and recently refrigerated as needed. Absorbances were measured across a 1-cm light path at 360 nm, 415 nm, 440 nm, 476 nm and 530 nm relative to a saline blank. To test sample stability over time and across the freeze-thaw cycle, a convenience sample of freshly collected CSF was analyzed spectrophotometrically by a second investigator (M.L.A.S.) before freezing, and compared with absorbances obtained after 1 or 2 freeze-thaw cycles up to 10 months later. To test a wide range of cell counts and the possible effects of ex vivo hemolysis, several of these fresh samples were also obtained from the first or second tube collected after LP, and included CSF specimens with visible blood sediment after centrifugation.

Four different definitions of positive spectrophotometry were selected a priori: (1) Traditional: an optical density $>0.023$ at a wavelength of 415 nm; (2) Chalmers and Kiley: net bilirubin absorption $>0.015$ positive, 0.010 to 0.015 borderline using absorbances at 415 nm and 440 nm relative to a baseline joining absorbances at 530 nm and 360 nm; (3) Chalmers revised: an optical density $>0.014$ at 476 nm; (4) United Kingdom National External Quality Assurance Service (UK NEQAS) based on net bilirubin and oxyhemoglobin absorbances at 476 nm and 415 nm, respectively, relative to a baseline joining the 530 nm and 360 nm absorbances.

Cases were deemed positive for SAH for the purposes of this study by the presence of any one of the following: (1) subarachnoid blood on CT, (2) $>5 \times 10^6$ red blood cells (agreed a priori by consensus of 5 emergency physicians and 1 neurosurgeon) in the third or fourth tube of CSF and positive cerebral angiography, or (3) visible xanthochromia and positive cerebral angiography. The spectrophotometry results were not available to treating physicians, thereby avoiding any incorporation bias.

Patients were considered missed if there was insufficient quantity of CSF collected to run the spectrophotometry tests, if the laboratory did not keep the sample of CSF or no xanthochromia requisition was sent with the CSF sample.

Results

Figure 1 shows the study flow. From the 840 headache patients seen at the 3 study sites during our enrollment period, there were 407 eligible patients of which we successfully enrolled 220 patients (54%).

Table 1 displays the patient characteristics. The patients were mostly female and relatively young. The cerebral angiography rate (CT angiography, MRI angiography or traditional) was 5.9% in this cohort. The interval from headache onset to LP was $>12$ hours in 55% of the cohort. Benign headaches (tension, cluster headache, neuralgias, etc) were the most common diagnoses followed by migraine, and together accounted for over 80% of subjects. The characteristics of the missed patients were very similar to the enrolled patients (mean age 42.3 years, 62% female, mean systolic blood pressure 140 mm Hg, mean diastolic blood pressure 80 mm Hg, mean heart rate 81 beats per minute, 1 SAH). Sixteen percent of the patients who were eligible but not enrolled were excluded because of an insufficient quantity of CSF remaining for spectrophotometry. The remaining patients did not have a study requisition completed by the treating physician. Only 1 patient who underwent LP but was missed because of the absence of a study requisition was ultimately diagnosed with SAH. This patient presented 6 days after the onset of headache, had a normal CT, $17,000 \times 10^6$ red blood cells/L in tube one, 6750 $\times 10^6$ red blood cells/L in tube 4, with xanthochromia visible in both samples and a 4-mm aneurysm, which was surgically treated.

There were 2 enrolled patients satisfying the study definition of SAH. Both cases were identified with current practice. One was a 77-year-old male with a sudden severe headache peaking within 5 seconds who was assessed 8 hours after the...
onset of headache. He had an aneurysm but no blood identified on plain CT. LP performed 13 hours after headache onset demonstrated 53,500×10^6/L red blood cells and visible xanthochromia in the fourth tube of CSF. CT angiography followed by traditional angiography both demonstrated an 11×8-mm aneurysm right posterior communicating artery for which he underwent craniotomy and aneurysm clipping. The second patient was a 49-year-old female with a sudden onset headache peaking within 10 seconds and a normal CT who was assessed 7 days after headache onset. Only the fourth tube of CSF was examined, and it contained 41×10^6/L red blood cells with no visual xanthochromia. This patient underwent CT angiography which demonstrated a 5-mm aneurysm of the right middle cerebral artery bifurcation. The treating neurosurgeon blinded to the conduct of the study diagnosed this to be an incidental aneurysm with traumatic tap, and elected to follow the patient without surgical intervention. She remained well 1 year later. None of the remaining patients contacted by telephone have any subsequent diagnosis of SAH. There were 4 patients who could not be contacted by telephone. None had any repeat hospital visits to a study site, which constitute the only neurosurgical centers in a geographically continuous area with a catchment population of ~1.6 million people. The 1 patient who had died on follow-up had experienced an ischemic stroke not attributed to SAH, confirmed with delayed CT scan demonstrating right frontal infarct.

Table 2 reports sensitivity, specificity and predicted impact on angiography rates for each definition of xanthochromia. With only 2 positive aneurysm cases, 1 of which was deemed not to have bled, the sensitivity estimates are somewhat unstable. Notably, however, the original Chalmers and Kiley definition missed the one true SAH. The 3 remaining spectrophotometric definitions of xanthochromia had specificities ranging from 29% to 83%. If the presence of visible xanthochromia were the only indication for cerebral angiography, the angiography rate would be reduced by 85% from current usage. On the other hand, using any 1 of the 3 sensitive spectrophotometric definitions of xanthochromia would result in an increase in angiography rates from 254% to 1208% compared with current practice.

No cases of SAH were missed using current practice and the study definitions. One patient did experience a perimesencephalic SAH but did not meet our study definition of SAH attributable to the absence of aneurysm. He was a 57-year-old male with a sudden severe headache peaking within 1 second and a normal CT who was assessed 7 days after headache onset. However, 2 tubes of CSF were visibly xanthochromic and contained 6500×10^6/L and 7500×10^6/L red blood cells. After negative cerebral angiography, he was diagnosed with a perimesencephalic hemorrhage, which is a low-pressure bleed associated with a favorable prognosis. He required no interventions and was in good health 2 years after this ED visit. This patient’s spectrophotometer results were positive by all definitions except UK NEQAS, which classified the CSF as inconclusive.

Both discrete absorbance values and the classification as negative, borderline or positive were consistent when fresh samples were compared with frozen samples. Based on data from 15 samples analyzed both immediately and again after freezing for up to 10 months with fresh absorbances ranging from −0.003 to 0.267, absorbances were on average about 0.010 U higher after freezing at absorbances near 0, but agree closely for absorbances above 0.030 (fitted line by least squares for all absorbances combined FROZEN=0.885×FRESH+0.0095, $r^2=0.94$; Figure 2). The calculated value for net oxyhemoglobin absorbance were extremely stable (eg, UK NEQAS NOA FROZEN=0.912×FRESH−0.001, $r^2=0.99$, fresh range −0.004 to 0.204), whereas net bilirubin absorbance showed more scatter attributable to the narrower range at baseline inflating the scatter (UK NEQAS NBA FROZEN=0.380×FRESH−0.002, $r^2=0.19$, range −0.013 to 0.006). With regards to the classification as negative, inconclusive/borderline, or positive, there was perfect agreement (15/15) using the UK NEQAS criteria, moderate agreement using Chalmers and Kiley (13/15 with 2 fresh samples which were tested as “borderline” becoming “negative” after freezing) and traditional based on absorbance at 415 nm (11/15 with all discordant pairs again becoming “negative” after freezing), but poor agreement with the revised Chalmers at 476 (4/15 with all discordant pairs again becoming false-positives after freezing).

**Discussion**

Our study found that it is relatively rare to diagnose SAH on LP, as most cases are identified on CT even in alert, neurologically intact patients. Moreover, no cases were missed using current practice methods over an 18-month enrollment period at 3 large urban EDs. Each of the 4 spectrophotometric definitions had only moderate to poor specificity and thus unacceptably large false-positive rates. As a result, in addition to the direct costs of

<table>
<thead>
<tr>
<th>Eligible for Acute Headache Study</th>
<th>N=2050</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excluded – Outside CSF Study Enrollment Period</td>
<td>N=1681</td>
</tr>
<tr>
<td>Excluded – Site Not Participating in CSF Sub-Study</td>
<td>N=429</td>
</tr>
<tr>
<td>Possible SAH ED Headache Patient</td>
<td>N=840</td>
</tr>
<tr>
<td>Excluded – No LP</td>
<td>N=433</td>
</tr>
<tr>
<td>Missed Eligible Patients</td>
<td>N=187</td>
</tr>
<tr>
<td>Enrolled Patients</td>
<td>N=220</td>
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</tbody>
</table>

**Figure 1. Study Flow.**
the test itself, introducing spectrophotometry into current practice would be expected to result in substantial increases in angiography rates. This in turn is expected to identify many benign incidental aneurysms, estimated to occur in 1% to 5% of the general population, further complicating clinical decision-making and perhaps leading to unnecessary surgeries with the associated increased patient morbidity and mortality.

This study did not find spectrophotometry to be useful in diagnosing the elusive case of SAH despite normal CT and visibly clear CSF. Studies demonstrating missed cases of SAH are generally older studies, or are in more rural or remote areas where access to CT scanners is not readily available and LP rates may be suboptimal. The introduction of spectrophotometry into these rural areas is not only impractical, but also unlikely to address these underlying deficiencies.

Two recent studies, one by Sidman and colleagues and another by Petzold and colleagues, performed laboratory studies where they lysed red blood cells artificially spiked into samples of CSF. Sidman concluded that visual detection of xanthochromia was only 27% sensitive and 98% specific for xanthochromia using spectrophotometry as the gold standard with the traditional definition of absorbance >0.023 at 415 nm. Unfortunately, although this study demonstrated that oxyhemoglobin can be produced in vitro and that the instrument is more sensitive to traces of pigment than the naked eye, their model is more relevant to erythrocytes introduced into CSF at the time of traumatic LP, and cannot estimate the diagnostic accuracy of spectrophotometry for SAH. Petzold added hemolysed blood and bilirubin to samples of CSF. Petzold concluded that spectrophotometry is superior to the visual inspection given that most CSF samples are contaminated with oxyhemoglobin or only contain low levels of bilirubin. Neither study was conducted on CSF samples of patients suspected of having SAH. Another recent laboratory study by Linn and colleagues tested human CSF samples which had bilirubin added to them to determine the sensitivity of physicians and medical students for visually detecting xanthochromia. They used spectrophotometry with an absorbance >0.05 at 450 to 460 nm as their gold standard. They found that visual detection of xanthochromia was very sensitive, with sensitivities of 100% for physicians (n=51) and 99% for medical students (n=51). They concluded that there was likely minimal benefit of spectrophotometry over visual inspection of CSF for detecting xanthochromia. Our findings further demonstrate that the traditional definition of xanthochromia, or any of the proposed alternative spectrophotometric definitions, are not adequately specific for clinical practice. Because CT-negative patients undergoing LP have a low prevalence of SAH, using spectrophotometry to identify xanthochromia results in an unacceptably high angiography rate. Introducing spectrophotometry into our population of “rule out SAH” patients can be expected to increase cost and patient complications with little if any increase in diagnostic yield.

The optimal test for CSF has been the subject of much debate. Previous studies have argued to test the red blood cell count of the final tube of CSF. A study by Macdonald in 1988 found that the red blood cell count was more sensitive than xanthochromia. Vermeulen refuted this by finding that xanthochromia by spectrophotometry was 100% sensitive in a case review of 111 SAH patients. They argued that Macdonald’s study had used visual inspection of the CSF supernatant, accounting for the lack of sensitivity of xanthochromia. However, 2 of these studies were retrospective case series of patients with confirmed SAH rather than of undifferentiated acute headache patients, and would be expected to

### TABLE 1. Characteristics of Subjects

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Enrolled n=220</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age in years (SD)</td>
<td>42.1 (16.1)</td>
</tr>
<tr>
<td>Female</td>
<td>120 (54.6%)</td>
</tr>
<tr>
<td>Mean heart rate (SD)</td>
<td>79.2 (15.5)</td>
</tr>
<tr>
<td>Mean systolic blood pressure (SD)</td>
<td>142.1 (23.5)</td>
</tr>
<tr>
<td>Mean diastolic blood pressure (SD)</td>
<td>82.2 (12.5)</td>
</tr>
</tbody>
</table>

### Diagnostic Imaging

- CT: 87.7%
- Angiography: 5.9%

### Final Diagnosis

- Benign headache: 53.6%
- Migraine headache: 28.2%
- Viral illness: 6.8%
- Postcoital headache: 2.7%
- SAH: 1.8%
- Transient ischemic attack: 0.9%
- Sinusitis: 0.5%
- Bacterial meningitis: 0.5%

### Follow-up

- Follow-up completed: 98%
- Dead on follow-up: 0.5%

### TABLE 2. Sensitivity, Specificity and Change in Angiography Rate of Each Method of Xanthochromia Detection (SAH=2)

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%; 95% CI)</th>
<th>Specificity (%; 95% CI)</th>
<th>Projected Angiography Rate</th>
<th>% Change in Angiography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual inspection</td>
<td>50 (3.0–81)</td>
<td>97 (92–99)</td>
<td>0.9</td>
<td>−85</td>
</tr>
<tr>
<td>Traditional</td>
<td>100 (16–100)</td>
<td>29 (23–35)</td>
<td>71.4</td>
<td>+1208</td>
</tr>
<tr>
<td>Chalmers and Kiley</td>
<td>0.0 (0.0–16)</td>
<td>89 (84–92)</td>
<td>10.9</td>
<td>+185</td>
</tr>
<tr>
<td>Chalmers revised</td>
<td>100 (3.0–100)</td>
<td>29 (23–35)</td>
<td>67.7</td>
<td>+1146</td>
</tr>
<tr>
<td>UK NEQAS</td>
<td>100 (3.0–100)</td>
<td>83 (76–87)</td>
<td>15.0</td>
<td>+254</td>
</tr>
</tbody>
</table>
suffer from workup, referral, spectrum and perhaps other biases. Another retrospective case series by Gunawardena and colleagues reviewed 463 CT-negative neurologically intact patients suspected of having SAH.22 They concluded that the bilirubin (by spectrophotometry) was significant in patients with 21% having an aneurysm. This study did not have follow-up on all patients, and it is not known how many of these aneurysms were incidental versus actual SAHs.

A retrospective case series by Foot and colleagues studied 189 patients with a normal CT head scan and had CSF analysis found that only 1 patient of 60 positive results had an aneurysm.23 It was unclear what the final diagnosis was for many of these patients although their angiograms were normal. This case series corresponds to our experience that very few SAHs in patients were identified in patients with a negative CT head. An additional retrospective case series was reported by Wood and colleagues.24 They reviewed 253 patients suspected of having SAH with a normal CT head. Two of these patients had a SAH (1 aneurysm, 1 arteriovenous malformation). They determined that xanthochromia by spectrophotometry had a sensitivity of 100%, specificity of 75% which corresponded to a positive predictive value of just 3.3% in their patient population.

In a prospective cohort study of emergency patients presenting with the worst headache of their lives, Morgenstern found that of 107 patients, 18 had a SAH. He found that 2 patients were missed by CT and both were identified by high red blood cells in the CSF. There were many false-positive tests for xanthochromia using traditional spectrophotometry.25 Neither of the 2 patients who were diagnosed with SAH by LP who had normal CT scans were found to have aneurysms with repeated cerebral angiograms. Hence, it is unclear if these 2 cases were false-positives or perimesencephalic bleeds. Morgenstern’s study corroborates our findings that there are very few cases of aneurysmal SAH diagnosed by CSF analysis.

Limitations

There were some potential limitations to our study. Firstly, we batch-analyzed previously frozen samples in order to make the study feasible from an analytical perspective. We did measure only a minimal effect on the CSF absorbances attributable to this process in our analysis of fresh samples which were frozen and retested. This approach is also supported by the study by Roost et al. in an animal study which found bilirubin would not form in the CSF in vitro, as a cellular mechanism was necessary for its formation in vivo.26

However, both the Traditional and the Chalmers revised definitions were sensitive to the freeze-thaw cycle with some specimens reclassified as positive on retesting. As such, the poor specificity of these 2 methods may be partly artifactual. Moreover, it is known that specimen transfer via a pneumatic tube transport system can generate falsely positive spectrophotometric results attributable to ex vivo hemolysis.27 Advocates of spectrophotometry state that special precautions must be followed to ensure accurate readings, including rapid transport of the light-shielded sample by hand to the testing site.14 Although we did not explicitly require such precautions, our specimens were handled in the routine fashion including hand transport to the laboratory shortly after collection. As such, our study should reflect the real-world performance of the test if introduced into general practice. Moreover, we are not optimistic that any test which is highly dependent on specimen handling will perform well in the ED environment.

It is important to point out that our spectrophotometry method differs slightly from the guidelines proposed by the UK NEQAS in so far as we measured absorbances at 5 discrete wavelengths, rather than performing a continuous scan from 350 to 600 nm, attributable to the limitations of our instrument. Nevertheless, because our baseline is constructed from the discrete absorbances at 360 and 530 nm, it can only overestimate the actual baseline which should be tangential to the full spectrum scan beyond 500 nm. As such, our calculated values for net bilirubin and oxyhemoglobin absorbances are conservative in nature, and would be expected to underestimate the sensitivity but overestimate the specificity of this method. Accordingly, the true specificity of the method using full spectrum scans can only be lower than what we observed.

Another potential limitation of this study was that 46% of the eligible patients were not enrolled. We made a good faith attempt to obtain CSF in all consecutive patients. Insufficient quantities of CSF were common because of multiple operators needed attributable to the setting of the study, low volumes collected at the time of LP, laboratory processing and additional testing for culture and Gram’s stain, and the curettes selected for spectrophotometry. There was no suggestion of selection bias given that the baseline characteristics.

An additional potential criticism of this study is the lack of standardization for the timing of the LP, given the delay needed for xanthochromia to develop in vivo after the release of erythrocytes into the CSF space. Over half of our subjects underwent LP at least 12 hours after the ictus, the time interval reported to be 100% sensitive for xanthochromia in confirmed SAH patients by traditional spectrophotometry.9 Because our methodology aimed to not alter current practice, we could not prespecify a minimum interval before LP. We do not believe that waiting 12 hours to perform the LP would have meaningfully altered this study’s results. Finally, we did
not attempt a formal cost-benefit economic analysis of spectrophotometry for the diagnosis of aneurysmal SAH.

**Conclusions**

The use of spectrophotometry to identify xanthochromia has only moderate to poor specificity for SAH. If this modality were introduced into current practice, any of the currently proposed spectrophotometric definitions would result in a substantial increase in angiography, and would therefore be expected to identify more incidental aneurysms, increase patient anxiety and expose patients to unnecessary surgical or investigational complications without benefit.

**Acknowledgments**

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**Disclosures**

None.

**References**

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