Visual inspection versus spectrophotometry in detecting bilirubin in cerebrospinal fluid

F H H Linn, H A M Voorbij, G J E Rinkel, A Algra, J van Gijn

Objectives: To compare the diagnostic accuracy of visual inspection and spectrophotometry for identifying the presence of bilirubin in the cerebrospinal fluid (CSF).

Methods: Clinicians and students assessed CSF specimens with seven degrees of extinction between 0.00 and 0.09 at 450–460 nm as “yellow,” “doubtful,” or “colourless” after random presentation under standard conditions. The assessments were compared with spectrophotometry, with 0.05 being taken as the cut-off level for the presence of bilirubin. Results were compared between the two groups and explored by means of receiver operating characteristic (ROC) curves.

Results: All 51 clinicians and 50 of 51 students scored the tubes with extinction of 0.06 or higher as “yellow” or “doubtful.” Tubes without any bilirubin were scored as “yellow” by three of the students only. The ROC curves confirmed that the diagnostic properties of the visual inspection versus spectrophotometry were slightly better for the clinicians than for the students.

Conclusions: If CSF is considered colourless, the extinction of bilirubin is too low to be compatible with a diagnosis of recent subarachnoid haemorrhage. If CSF is not considered colourless, spectrophotometry should be carried out to determine the level of extinction of bilirubin.

In the great majority of patients with subarachnoid haemorrhage (SAH), the presence of extravasated blood can be confirmed on early computed tomography (CT) of the brain. But if CT is normal in patients suspected of SAH, lumbar puncture is indicated to examine the cerebrospinal fluid (CSF) for blood pigments. These pigments (oxyhaemoglobin and bilirubin) are formed by lysis of erythrocytes. Bilirubin gives the CSF a yellow colour (xanthochromia) which can be found from six to 12 hours after SAH until two weeks after the haemorrhage. In the laboratory, CSF is spun down immediately after puncture for visual inspection of the supernatant. Next, spectrophotometry is recommended, because it is a less subjective and supposedly more sensitive method for detecting blood pigments than visual inspection.

The extinction of the supernatant is determined at wavelengths of 415 nm (oxyhaemoglobin) and 450–460 nm (bilirubin).

Visual inspection and spectrophotometry were compared in the 1970s in a few small studies which suggested that visual inspection alone is not reliable. Despite the lack of evidence on the diagnostic properties of visual inspection for detecting bilirubin in CSF, more than half the clinical laboratories in the USA base their results on visual inspection alone. In the United Kingdom, the use of spectrophotometry appears to be limited as well.

Our aim in this study was to compare visual inspection with spectrophotometry in a series of CSF samples with different extinctions of bilirubin. We included the influence of clinical experience as a variable in this experiment.

Methods

Human CSF was used and bilirubin was added in different concentrations. A series of seven tubes with extinctions of 0.00, 0.01, 0.02, 0.04, 0.06, 0.07, and 0.09 at 450–460 nm was prepared with a Beckman DU 650 spectrophotometer (Beckman Coulter, Netherlands). Specimens were stored at 4°C, and wrapped in aluminium foil for protection against the influence of ultraviolet light. Clinicians from the departments of neurology and neurosurgery, and medical students assessed the colour of each tube in the series as “yellow,” “colourless,” or “doubtful.” Assessment was in random order and under standard conditions with bright light, a background of a white paper or white coat, and with a similar tube filled with tap water as reference.

Judgements were classified as “correct” and “not correct” for each extinction, with spectrophotometry as the gold standard: the extinction above 0.05 was used as cut-off for the presence of bilirubin. The extinction values in the test tubes were chosen accordingly. We had two pairs of analyses—a first with three categories assessed separately: “colourless,” “yellow,” and “doubtful”; and a second with two categories: “colourless” versus “doubtful” or “yellow,” because in practice spectrophotometry will be done in case of doubt about the presence of a yellow colour of CSF. We calculated proportions with corresponding 95% confidence intervals (CI) and sensitivity and specificity for clinicians and students who assessed the colour differently from the gold standard. Results were compared between the two groups by means of receiver operating characteristic (ROC) curves. The area under the ROC curve (AUC) can be interpreted as a measure of the diagnostic value of the test.

Results

The assessments of 51 clinicians and 51 students are presented in table 1. In the analysis of the three categories taken separately, at least 88% of clinicians and students scored any tube with extinction above the cut-off point of 0.05 as yellow. None of the clinicians and only one student assessed a tube with an extinction above 0.05 as colourless (95% CI for clinicians 0 to 2.4% and for students 0 to 3.6%). Tubes without any trace of bilirubin were assessed as “yellow” by three of 51 students. When the categories “colourless” versus “yellow” and “doubtful” combined were analysed, for all samples together the sensitivity (spectrophotometry above 0.05 used as true positive) was 100% in the clinicians and 99% in the students. The specificity above 0.05 was consistently lower in the clinicians than in the students.

Abbreviations: AUC, area under the curve; ROC, receiver operating characteristic; SAH, subarachnoid haemorrhage.
One student (2%) rated a tube without a trace of bilirubin as doubtful.

The ROC curves (fig 1) showed that the AUC was slightly larger for clinicians than for students. The figure also showed that the ROC curves were very informative, with a high sensitivity above the cut off point 0.05 extinction for bilirubin, and a high specificity below.

**Discussion**

The clinicians attained 100% sensitivity in visually detecting the yellow colour of CSF from in vivo specimens when the extinction was above 0.05, the cut off value for “positive” spectrophotometry in our laboratory, including the assessments in which they were doubtful. In practical terms, our findings imply that on the basis of visual inspection alone, no patient with a subarachnoid haemorrhage would have been sent home. Only one patient (2%) would have been sent home by the students because of false negative visual inspection of the CSF. If an experienced clinician judges the colour of CSF to be crystal clear and colourless, the chance of SAH is negligible.

Our results should be seen against the background of the small but important additional value of CSF investigations after negative CT in patients with sudden headache. If a patient with a ruptured aneurysm is investigated within 12 hours, CT of the brain shows blood in the basal cisterns in at least 97%. After two or three weeks, magnetic resonance imaging shows blood in up to 100%, but in these studies CSF investigations (without specifications) were used as the gold standard.

We do not recommend a change in the guidelines for methods of examining the CSF. Spectrophotometry is the preferred method for detecting blood pigments in CSF in cases of suspected SAH. However, if meticulous inspection of the CSF under standard conditions shows that it is crystal clear and colourless, the chance of SAH is negligible. If an experienced clinician judges the colour of CSF to be yellow or doubtful, spectrophotometry is necessary to determine the level of extinction. All institutions relying on clinical visual inspection should monitor the reliability of visual and spectrophotometric interpretations and should emphasis the standard conditions in which the visual inspection of CSF has to be undertaken (in bright light, under standard conditions).
background of white paper or a white coat, comparison with a tube of tap water).

ACKNOWLEDGEMENTS
We thank all clinicians and medical students for their cooperation in the study. We thank Mrs A Peterusma for preparing the samples and quality control.

Authors’ affiliations
F H H Linn, G J E Rinkel, J van Gijn, Department of Neurology, University Medical Centre Utrecht, Utrecht, Netherlands
H A M Voorbij, Department of Clinical Chemistry, University Medical Centre Utrecht
A Algra, Julius Centre for Clinical Sciences and Primary Care, University Medical Centre Utrecht

Competing interests: none declared

Correspondence to: Dr F H H Linn, Department of Neurology, University Medical Centre/Central Military Hospital, Heidelberglaan 100, 3584 CX Utrecht, Netherlands; f.h.h.linn@neuro.azu.nl

Received 12 August 2004
In revised form 30 January 2005
Accepted 9 February 2005

REFERENCES
Visual inspection versus spectrophotometry in detecting bilirubin in cerebrospinal fluid

F H H Linn, H A M Voorbij, G J E Rinkel, A Algra and J van Gijn

J Neurol Neurosurg Psychiatry 2005 76: 1452-1454
doi: 10.1136/jnnp.2004.051318