Diagnostics in patients presenting to the emergency room with headache

Alons, I.M.E.

Chapter 5

OPTIMIZING BLOOD PIGMENT ANALYSIS IN CEREBROSPINAL FLUID FOR THE DIAGNOSIS OF SUBARACHNOID HEMORRHAGE – A PRACTICAL APPROACH

I.M.E. Alons MD
R.J. Verheul PhD
G.A.E. Ponjee PhD
K. Jellema MD PhD

ABSTRACT

Introduction

Patients presenting with sudden severe headache may have a subarachnoid hemorrhage, (SAH). After a normal head Computer Tomography (CT) a lumbar puncture (LP) is routinely performed to rule out SAH. Photospectrometry is then used to detect bilirubin in cerebrospinal fluid (CSF). Photospectometric analysis of CSF reaches a high sensitivity but a low specificity for SAH. This low specificity necessitates extensive additional research to rule out cerebral aneurysm accompanied by high costs and risk of complications.

Objective

The objective of this study was to retrospectively evaluate two different CSF interpretation methods using photospectrometry in patients presenting with acute headache. The first of these is the Leiden method, an iterative model using a standard calculation. The second is the UK NEQAS guideline, which uses the original spectrum in combination with a decision tree. Our goal was to obtain retrospective data on patients screened with both methods to improve specificity of CSF research.

Results

We included 361 patients in this study, 47 of these had a raised bilirubin concentration in the CSF according to the Leiden method. In only 9 of these 47 patients an aneurysm was found, in the other patients the Leiden test was positive due to other reasons (viral meningitis, hyperbilirubinemia e.a.) Out of the 47 patients with raised bilirubin, 24 could be re-evaluated using the UK NEQAS. Of these 24 patients, 5 had an aneurysm. There were no aneurysms found in patients with a negative result according to the UK NEQAS guideline.

Conclusion

Our data show that a raised bilirubin calculated using the Leiden method seems to have a lower specificity than the UK NEQAS guideline. For practical reasons, it seems advantageous to use the Leiden method as a screening method, and use the UK NEQAS guideline if a positive result is found.

INTRODUCTION

Acute headache is a frequently encountered complaint in the emergency room and can be a presenting sign of subarachnoid haemorrhage (SAH). It is common practice to first perform a Computerized Tomography (CT) of the brain to exclude haemorrhage. The sensitivity of CT is high, almost 100%, in the first 12 hours but this declines to 58% after 5 days [1;2]. In a recent study third generation CT was evaluated with a sensitivity of 100% within the first 6 hours and 85.7% after 6 hours [3]. When there is a high suspicion of SAH it is common practice to subsequently perform a lumbar puncture (LP). Although this practice has been questioned, recent research shows that a LP is still obligatory to exclude an SAH, especially if the patient presents several days after the headache [4;5].

Haemolysis of erythrocytes in the cerebrospinal fluid (CSF) leads to release of oxyhaemoglobin, which is subsequently degraded to bilirubin by the mononuclear phagocyte system. Oxyhaemoglobin (and methaemoglobin) can also be introduced into the CSF by a traumatic puncture and thus may not be conclusive [6]. In contrast, bilirubin levels rise to detectable amounts in the course of approximately 12 hours after an SAH by in vivo conversion of haemoglobin and remains detectable in the CSF for 2-4 weeks after haemorrhage [7]. The presence of the metabolites of erythrocytes in CSF can often be detected by the yellowish appearance of the CSF, called xanthochromia. However, visual inspection of CSF for xanthochromia lacks adequate sensitivity and specificity [8;9;10], so analysis of CSF with UV/VIS-spectrophotometry is often used to qualitively and/or quantitively measure levels of oxy-, methaemoglobin and bilirubin. Currently, in the Netherlands, either an iterative calculation model (Leiden method) [10] or the revised British national guidelines using a decision tree (UK NEQAS guideline) [11] are used to improve the interpretation of the UV/VIS-spectrum of blood pigments in CSF. Sensitivity in CSF-spectrometry methods has been reported to be 100%, however, published specificities of 75 and 83% leave room for improvement [8;12].

The objective of this study was to evaluate and compare the Leiden and UK NEQAS methods in clinical practice and to present an optimal approach for blood pigment analysis to reduce the need for potential hazardous and expensive diagnostic tools such as angiography and contrast MRI.

MATERIALS AND METHODS

Design

Here we describe a retrospective study researching the clinical outcomes of patients presenting in the emergency room from January 2008 through May 2011 with severe, acute, non-traumatic headache, in correspondence to their LP analysis. Patients with pathological findings on standard head CT, such as SAH or subdural hematoma were excluded. If available, neuro-imaging

results of CT-angiogram, angiography and MRI were reviewed. In accordance to the hospital's protocol, LP's were performed at least 12 hours after the onset of symptoms and CSF protein and glucose concentrations were determined. Collected samples were transported to the in-house laboratory constantly shaded from light. Cell-counts for erythrocytes and leucocytes were carried out routinely. The Leiden method was used clinically for CSF analysis. There were no specific ethical concerns or need for informed consent as patients were examined and treated according to current clinical practice.

Assays

Automated assays

Total protein in CSF was measured turbidimetrically after the addition of benzethoniumchloride. Glucose in CSF was determined with the hexokinase-method. Plasma total bilirubin was measured photometrically after reacting with diazonium. All automated assays were performed on a Modular analyser (Roche, Almere, The Netherlands).

CSF sample handling and UV-spectrum measurement

CSF samples obtained from the LP were directly taken to the in-house clinical laboratory. The least blood-stained specimens were used for analysis. After a visual inspection and cell-count, samples were centrifuged for 5 minutes at 1250 g, within 1 hour after LP. Then 100 µl of 0.25 M phosphate buffer pH 6.6 was added to 900 µl of CSF to rule out pH-influences on the UV-absorption characteristics [10]. The sample was transferred into a cuvette with a 1 cm path length. Subsequently, absorbance spectra were made from 350 to 700 nm on a Thermo Evolution 300 using Thermo Vision software (Thermo Scientific, Breda, The Netherlands) using demineralised water as a blank reference.

Interpretation methods

Iterative calculation model ('Leiden method')

To calculate the concentrations of methemoglobin, oxyhemoglobin and bilirubin using an iterative model the method described by Duiser et al. [10] was used. This method requires the Duisersoft Microsoft Excell program (obtained from dr. Duiser). The absorption values at 360, 405, 414 and 455 nm were transferred into this program, which instantly produces a model-spectrum and calculates the concentrations of the various blood pigments. As recommended, bilirubin CSF concentration > 0.2 µmol/L were considered higher than the upper reference value [12] indicating the possibility of an SAH. Values ≤ 0.2 µmol/L were interpreted as 'negative'.

Net bilirubin absorbance ('UK NEQAS guideline')

As an alternative to the iterative model, the spectra of the 118 most recent specimens were also retrospectively interpreted according to the revised UK NEQAS guideline [12]. In contrast to the guideline, the samples were diluted to a small extent due to the addition of the phosphate buffer used in the Leiden method. However, the implications for a correct estimation of the net bilirubin absorbance (NBA) were considered insignificant since the dilution was minimal

and equally present in all samples. A predicted baseline was drawn in the original spectrum and the NBA was measured at 476 nm. At the same time, the net oxyhemoglobin absorbance (NOA) was determined using the predicted baseline and the absorbance maximum between 410-418 nm. Subsequently, the interpretation of the specimens was done using the decision tree as described in the guideline, taking into account the NBA, NOA and serum bilirubin and CSF total protein concentrations. Interpretations were categorized into: 'inconclusive' (due to high NOA), 'no evidence to support SAH', 'consistent with SAH' and 'interpret with caution' (due to CSF protein ≥1.0 g/L).

Patient diagnosis

All digital patient files were searched retrospectively to determine clinical diagnosis. When performed, Computer Tomography Angiogram (CT-A), Magnetic Resonance Imaging (MRI) and Angiography (DSA) were reviewed to establish their contribution to the diagnosis. At least four months after the inclusion patient statuses were revised for missed SAHs or altered diagnosis.

RESULTS

Between January 2008 and May 2011 the CSFs of 361 patients presenting with acute headache were analysed for blood pigments. This cohort was comprised of 234 women (64.5 %) and 127 men, with a mean age of 43.5 years. According to our hospital protocol CSF samples were originally evaluated with the Leiden method. Of all patients included in the study, 47 had a bilirubin concentration of 0.3 µmol/L or higher (13%) in their CSF and 314 were 'negative' according to the Leiden method. Of the 47 patients found to be 'positive', 25 received a CT angiogram, which lead tot the diagnosis of aneurysm in seven patients and sinus thrombosis in two patients The lowest CSF bilirubin concentration in a patient with a confirmed SAH was 0.3 µmol/L. 15 CT angiograms were normal, although two patients with a normal CT angiogram were later diagnosed with an aneurysm after MRI and angiography. Three more patients had an MRI, of which one was normal, one showed hydrocephalus and one showed a sinus thrombosis. Of the 19 patients with a positive bilirubin who did not get a CT angiogram or MRI, six had a viral meningitis, one had had a traumatic LP three days prior showing 1030 erytrocytes and a normal bilirubin count, one had hyperbilirubinemia and 11 did not receive additional neuro-imaging. According to digital patient files after 4 months follow-up none of our patients with a negative CSF result has had a SAH. We cannot however exclude the possibility that patients might have been seen in another neurosurgical centre. The reason for lack of further diagnostics in these cases could not be determined, but in most cases was likely due to low clinical suspicion of SAH.

Of all 361 patients 117 could be re-evaluated using the UK NEQAS guideline. Seven patients were 'consistent with SAH' (6%), 99 spectra were interpreted as 'no evidence to support SAH'. Three patients had an incorrect spectrum recording and one had an inconclusive spectrum due to a severe traumatic puncture. Seven patients were classified as 'interpret with caution' due to

high CSF protein concentrations (>1 g/l). The high protein concentrations could be attributed to (bacterial) meningitis (five patients) and severe traumatic punctures (two patients with erythrocyte counts >100000).

Of the 47 patients that were considered 'positive' according to the Leiden method it was possible to re-evaluate 23 with the UK NEQAS guideline. Six of these patients were also positive using the UK NEQAS guideline (Tabel 1).

Of these 'double positive' patients one did not receive a CT angiogram, but of the other five that did, four had an aneurysm on the CT angiogram, and one an aneurysm after MRI. All the patients that were negative with the Leiden method were also negative using the UK NEQAS guideline.

There were no patients that were positive with the UK NEQAS guideline that were diagnosed with another underlying disease such as viral meningitis, sinus thrombosis or otherwise. One patient with a 'no evidence to support SAH' result following the UK NEQAS guideline was classified as having an aneurysm, however this aneurysm had already been discovered earlier and had been coiled. This patient presented because of a second episode of acute headache.

DISCUSSION

In this study we set out to compare the UK NEQAS guideline and the Leiden method for the interpretation of blood pigment analysis in CSF in patients with acute headache and a normal CT. Our results show that less patients are considered 'positive' using the UK NEQAS guideline (13% vs. 6%). Confounders in the Leiden method are viral and bacterial meningitis and sinus thrombosis, but incorrect spectrum recordings were recorded. This last confounder can be explained by high baseline levels recorded with the Leiden method, caused by spurious UV-absorption (e.g. non-centrifuged samples) resulting in falsely elevated bilirubin levels in the iterative calculation model. In five patients that were positive with the Leiden method (up to 0.6 µmol/L) the UK NEQAS guideline resulted in a negative result without a clear reason, indicating that the cut-off value of 0.007 AU in the UK NEQAS guideline is more conservative. However, as one patient had a confirmed SAH with a CSF bilirubin concentration of 0.3 µmol/L, a higher cut-off value for the Leiden method does not seem feasible.

In earlier studies a high sensitivity has been reported for both the Leiden method and the UK NEQAS guideline of up to 100% [8;12], but, due to a lack of a gold standard for SAH diagnosis we state that it is impossible to calculate a reliable sensitivity and specificity. A previously published prospective study performed a follow-up after 'normal' CT and CSF results and found no patients with SAH. However, in that study one patient was diagnosed with an aneurysm after a MRI [4]. We find similar results; in our study a SAH was discovered with MRI and angiography in two

Table 1. Overview of patients with a positive Leiden guideline reviewed with UK NEQAS Guideline.

Sex, age (years)	Bilirubin (µmol/l)	UK NEQAS guideline	Erytrocyte count (Cells*10^6/l)	Leucocyte count (Cells*10^6/l)	Protein (g/l)	CT angiogram
F, 58	0.8	Consistent with SAH	10443	16	0.4	Not performed
F, 61	6	Consistent with SAH	17000	21	0.66	Normal
F, 45	4.5	Consistent with SAH	20000	209	0.59	Aneurysm
F, 46	1	Consistent with SAH	191000	296	1.15	Aneurysm
M, 53	0.9	Consistent with SAH	40	12	0.34	Aneurysm
F, 69	0.3	Consistent with SAH	78	10	0.6	Aneurysm
F, 52	0.6	No evidence of SAH	830	2	0.3	Normal
M, 53	0.4	No evidence of SAH	25000	19	1.01	Not performed
M, 51	0.3	No evidence of SAH	61	3	0.36	Not performed
M, 39	0.3	No evidence of SAH	23	2	0.48	Not performed
F, 42	0.3	No evidence of SAH	47000	76	0.92	Normal
F, 23	0.3	No evidence of SAH	14933	2	0.78	Normal
M, 48	0.6	TP >1g/l, caution	113000	0	3.18	Normal
F, 57	0.5	TP >1g/l, caution	118000	56	1.36	Normal
F, 57	0.4	TP >1g/l, caution	13	32	1.2	Normal
M, 19	0.3	TP >1g/l, caution	360	280	1.0	Not performed
F, 38	4.2	TP >1g/l, caution	1348	128	1.41	Normal
M, 84 †	0.9	TP >1g/l, caution	352	3200	4.21	Not performed
F, 54	0.3	TP >1g/l, caution	170	1944	1.67	Not performed
M, 44	0.8	NOA >0.1, inconclusive	435000	180	5.35	Tumor mass
M, 16	0.6	'Wrong spectrum'	8	0	0.3	Normal
M, 32	0.3	'Wrong spectrum'	n.d.	n.d.	0.39	Not performed
F, 47	2.2	'Wrong spectrum'	121	2	1.0	Aneurysm

patients after a normal CT angiography. If we were to calculate the sensitivity of the Leiden method and the UK NEQAS guideline this would amount to 100% for both methods in this series. Specificity of the Leiden method was 89%, while specificity of the UK NEQAS method was 99%.

In our study, we evaluated the use of the Leiden method for primary CSF interpretation and, in case of a 'positive' outcome, the use of the UK NEQAS guideline as a secondary method to optimize specificity. This approach was taken since the UK NEQAS guideline, although it appears to have superior specificity, has some practical disadvantages: interpretation has to be performed by an experienced interpreter and CSF protein and serum bilirubin concentrations are mandatory variables [11]. In addition, the time of onset of the symptoms should be taken into account [11;13] and many other variables such as CSF glucose and leucocyte count, recent traumatic punctures and/or light exposure may, although not incorporated in the UK NEQAS guideline, provide crucial information.The Leiden method, on the other hand, has little inter-person variation and a relatively simple workflow [11]. Our data show that by using the Leiden method as a screening tool about 87% of the patients can be diagnosed as 'no evidence to support SAH' as the UK NEQAS guideline and clinical outcome confirmed all these 'negative' patients. In only 13% of the cases an experienced technician or clinical chemist is required for alternative CSF interpretation using the UK NEQAS guideline. As fast CSF interpretation is highly recommended [11], the workflow we propose has practical benefits, especially during out-of-office hours. Moreover, the increase in specificity following this workflow may, due to the low incidence of SAHs in our population (approx. 3%), lead to a significant reduction in potentially harmful and costly imaging techniques

CONCLUSION

Based upon our retrospective evaluation of two CSF interpretation methods in 361 patients with suspicion of a SAH we propose to first perform the Leiden method and in case of a positive result, use the UK NEQAS guideline as a secondary diagnostic method. Using the workflow we propose optimal specificity is achieved with high practicality for clinical laboratories.

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