



HAL
open science

Pigments test strips: A rapid companion test to exclude sub-arachnoid haemorrhage

Julian Boutin, Marie-Lise Bats, Samuel Amintas, Annie Bérard, Sandrine Dabernat, Brigitte Colombies, Benoit Rucheton, Aurélie Bedel

► To cite this version:

Julian Boutin, Marie-Lise Bats, Samuel Amintas, Annie Bérard, Sandrine Dabernat, et al.. Pigments test strips: A rapid companion test to exclude sub-arachnoid haemorrhage. *Clinica Chimica Acta*, 2020, pp.54-59. 10.1016/j.cca.2020.06.052 . hal-03194411

HAL Id: hal-03194411

<https://hal.science/hal-03194411>

Submitted on 18 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Title of Manuscript: Pigments test strips: a rapid companion test to exclude sub-arachnoid haemorrhage

Author List: Julian Boutin MD^{1,4}, Marie-Lise Bats PharmD-PhD^{1,4}, Samuel Amintas PharmD^{3,4}, Annie Bérard PharmD-PhD¹, Sandrine Dabernat PhD^{1,4}, Brigitte Colombies MD¹, Benoit Rucheton PharmD^{2,#} and Aurélie Bedel PharmD-PhD^{1,4,#}.

Author Affiliations:

1 Biochemistry Department, Bordeaux University Hospital, Place Amélie Raba Léon 33076 Bordeaux France

2 Metabolic Biochemistry Department, BioGeM Paris, APHP-Sorbonne Université, Pitié Salpêtrière University Hospital, 42-83 boulevard de l'hôpital 75651 Paris cedex 13, France

3 Tumor biology Department, Bordeaux University Hospital, Place Amélie Raba Léon 33076 Pessac France

4 Université de Bordeaux, France

These authors contributed equally to this work

Corresponding Author: Julian Boutin

Adress : CHU Bordeaux – Hôpital Pellegrin – Service de Biochimie et Biologie moléculaire – Place Amélie Raba Léon 33076 BORDEAUX France

phone number : +33627339930

Mail : julian.boutin@chu-bordeaux.fr

Word Count: 3290

Abbreviations

AU: Absorbance Units

COFRAC: COmité FRançais d' ACcréditation

CSF: CerebroSpinal Fluid

CT: Computed Tomography

Inc: Inconclusive

IQC: Interne Quality Control

ISAT: International Subarachnoid Aneurysm Trial

NBA: Net Bilirubin Absorbance

Neg: Negative

NPV: Negative Predictive Value

NOA: Net Oxyhemoglobin Absorbance

Pos: Positive

PPV: Positive Predictive Value

PTS: Pigments Test Strip-pads (urine test strips Multistix®, Siemens)

PTSb: Pigments Test Strip-pads for bilirubin detection

PTSh: Pigments Test Strip-pads for haemoglobin detection

PTShb: Pigments Test Strips-combined haemoglobin-bilirubin results

RBC: Red Blood Cells

SAH: SubArachnoid Haemorrhage

Se: Sensitivity

SEM: Standard error of the mean

Spe: Specificity

1 **1. INTRODUCTION**

2 Subarachnoid haemorrhage (SAH) accounts for 0.8 to 7% of all strokes [1]. In France, incidence
3 ranges between 2.5 and 7/100 000 individuals [2]. The ISAT (International Subarachnoid
4 Aneurysm Trial) study reported 25% of morbidity/mortality [3], which justifies immediate care
5 and rapid diagnosis of SAH. An incorrect initial diagnosis of SAH worsens the prognosis [4].
6 SAH is a spontaneous arterial bleeding into the subarachnoid space. The main cause is the
7 rupture of aneurysms (80%) [5]. Patients come to the emergency room with a sudden onset of
8 severe headache, nausea, photophobia and neck pain. A high risk of complications exists,
9 including symptomatic vasospasms, hydrocephalus and rebleeding. Early treatment set up of
10 SAH prevents neurologic complications and functional impairments. The cerebral computed
11 tomography (CT) scan is the gold standard method to diagnose SAH with 98-100% sensitivity
12 within the first 6-12 hours [6]. However, 6 hours after the onset of symptoms, the sensitivity
13 drops to about 86-93% [7] and it drops to approximately 50% after one week [8]. A cerebral
14 angiography is usually performed on patients with a positive CT scan to confirm the presence
15 and locate the aneurysm for preventive treatment of re-bleeding. In case of negative CT scan
16 with clinical signs of SAH or beyond 12 hours after the first bleeding, a lumbar puncture of
17 cerebrospinal fluid (CSF) is done to detect the abnormal presence of haem pigments
18 (haemoglobin and bilirubin, a physiological metabolite of haemoglobin breakdown). They
19 generally appear 2-12 hours after the bleeding started [9], and are indirect evidence of the
20 bleeding. Indeed, after haemorrhage, red blood cells (RBC) are lysed and phagocyted by
21 macrophages; they release oxyhaemoglobin further converted into bilirubin.
22 The presence of bilirubin in the CSF, also called xanthochromia, appears to be the most
23 informative biomarker for suspected SAH [10]. By contrast, the presence of oxyhaemoglobin in

24 CSF may result from SAH or from *in vitro* haemolysis of red blood cells contaminating the
25 sample due to traumatic puncture. Oxyhaemoglobin and bilirubin are usually detected by either
26 visual inspection or spectrophotometry. Visual inspection is not reliable and should not be used
27 because it only detects massive xanthochromia. [11]. Spectrophotometric methods are more
28 sensitive and can detect the presence of oxyhaemoglobin and/or bilirubin by the measure of
29 sample absorbances at known wavelengths. In the absence of French recommendations, we use
30 the UK guidelines [10], recommending bilirubin rather than haemoglobin as the best biomarker
31 for detecting SAH 12 hours after haemorrhage onset. Authors also proposed a helpful software to
32 support the interpretation of CSF haem pigment analysis based on the UK guidelines [12]. They
33 also describe the strict preanalytic conditions of sampling, including a large volume and rapid
34 moderate centrifugation, and a fast light-protected transport to avoid misinterpretation.

35 The urine test strips (Multistix®) could be a quick and easy-to-use alternative to
36 spectrophotometry to detect the presence of haemoglobin and bilirubin in CSF for laboratories
37 that do not have a spectrophotometer or as a bedside diagnosis test. Interestingly, urine test strips
38 require a small volume of sample, are available in most biochemistry laboratories for urine
39 analysis, and present a very short turnaround time. Previous studies have already compared the
40 performances of the Multistix® urine test strips for haemoglobin detection in CSF to the
41 oxyhaemoglobin detection by spectrophotometry (at 415nm) or to RBC counts [13, 14].
42 However, the evaluation of bilirubin detection, the main haem pigment for SAH, was left out.

43 To help clinical management of SAH, the aim of this study was to compare the performance of
44 the Multistix® urine test strips (named PTS in the manuscript) for haem pigments (bilirubin and
45 haemoglobin) detection in CSF to the reference measure by spectrophotometry and to the
46 definitive clinical SAH diagnosis.

47 **2. MATERIALS AND METHODS**

48 **2.1. Study Design and Setting**

49 This retrospective pilot study took place between 2014 and 2017 at Bordeaux university hospital.

50 This work conforms in terms of the protection of personal health data and the protection of
51 privacy to the framework provided by Article 65-2 of the Data Protection Act as amended and
52 the General Regulation on the protection of personal data.

53 Data were collected from patient CSFs sent to the biochemistry laboratory for haem pigments
54 detection (suspected SAH) by spectrophotometry. Net bilirubin absorbance (NBA) and net
55 oxyhaemoglobin absorbance (NOA) were calculated as recommended by the UK guidelines. We
56 systematically added the detection test of haem pigments by PTS and the determination CSF
57 total protein concentration, in real time. Only the spectrophotometry results were communicated
58 to the clinician. SAH suspicion according to the biomarkers presence was correlated to the final
59 clinical SAH diagnosis (SAH was confirmed by CT imaging or cerebral angiography or
60 excluded if there was another obvious cause or no evidence of SAH).

61

62 **2.2 Methods**

63 Pre-analytic conditions: samples were drawn at least 12 hours after the onset of symptoms, light-
64 protected, and contained 15-20 drops. We performed systematic analysis of the last tube (the
65 fourth). We centrifuged samples at 1500 g for 5 min. After analysis, they were stored at 4°C in
66 the dark.

67 First, a drop of the centrifuged CSF sample was analysed on Multistix® urine test strips for
68 bilirubin and haemoglobin search by a qualified laboratory technician who was blinded to the
69 spectrophotometry test result. Bilirubin detection threshold is 0.4 mg/dL (7µmol/L) and

70 haemoglobin is positive when its concentration is above 0.015 mg/dL haemoglobin or when >5
71 RBC/ μ L are present. The Multistix® urine test strips semi-quantitative method was validated
72 according to COFRAC (Comité Français d'accréditation) and ISO15189 norm requirements for
73 urine matrix. The repeatability was conform for blood and bilirubin with 10 replicates on 3
74 different samples (100% sensitivity and specificity). We obtained a perfect reproducibility
75 (100% sensitivity and specificity) for haemoglobin and bilirubin on 92 internal quality controls
76 (including 1 negative and 1 positive control for each series) over a period of one and a half
77 month. The exactitude has been assessed based on 4 different extern quality controls and was
78 conform. We are registered with 2 External Quality Assessment Services: Probioqual (France)
79 and RfB (Germany). We used 5 different Multistix® urine test strips lots for performances
80 evaluation.

81 The sensitivity threshold of bilirubin detection in CSF with Multistix® PTS was assessed by
82 determining of the limit of blank according to Clinical and Laboratory Standards Institute
83 guidelines protocol EP17-2A. Three bilirubin-positive CSF samples were serially diluted to
84 identify the limit of blank of PTS. Serial dilutions (from 0.10 AU to 0.02 AU) of 1 "home-made"
85 internal CSF control quality tested 10 fold by PTS for each dilution were prepared. This PTS
86 manual evaluation was carried out. A negative-bilirubin CSF sample (NBA = 0 AU) was
87 performed simultaneously to facilitate the PTS reading. Each dilution value (NBA AU) was
88 checked by the reference spectrophotometry. The spectrophotometer semi-quantitative method
89 was validated according to COFRAC (French Quality committee) requirements for CSF
90 biological matrix.

91 Then, we used a spectrophotometer Uvikon® Secomam in order to perform a spectrophotometric
92 scan on the supernatant ranging from 350 to 600 nm by a qualified medical doctor blinded to

93 PTS results. Absorbance between 410 and 418 nm detects oxyhemoglobin and absorbance
94 between 450 and 460 nm detects bilirubin [15]. The NBA (bilirubin) was calculated according to
95 Chalmers' modification [16] of the original method [15]. Briefly, a tangent to the scan was drawn
96 between 350 nm and 600 nm. NOA (oxyhaemoglobin) peak was measured from the predicted
97 baseline around 415 nm and NBA (bilirubin) peak was measured from the predicted baseline
98 around 476nm. When no oxyhaemoglobin peak was visible, NBA was adjusted to serum
99 bilirubin and CSF total protein was determined. Our interpretation of the scans was based on
100 NBA and NOA values expressed as absorbance units (AU) and according to UK Revised
101 national guidelines [10]. Accordingly, a $NBA > 0.007$ AU associated with a $NOA > 0.02$ AU with
102 a visible oxyhaemoglobin peak or an adjusted $NBA > 0.007$ AU with CSF total protein $< 1\text{g/L}$,
103 were consistent with SAH (bilirubin + haemoglobin = positive) (10). If $NBA \leq 0.007$ AU with a
104 $NOA < 0.1$ AU or $NBA > 0.007$ AU was associated with a $NOA < 0.02$ AU with an adjusted
105 $NBA < 0.007$ AU, did not support SAH (bilirubin and haemoglobin undetectable = negative).
106 Other cases were considered as inconclusive (bilirubin or haemoglobin alone). Qualified
107 laboratory technicians and medical doctors were blinded to the study hypothesis. To ensure the
108 quality of our results, 1 quality control (corresponding to a combined bilirubin-haemoglobin
109 positive CSF) was analysed by spectrophotometry with each series of samples. Furthermore, our
110 laboratory participated in the external quality evaluation program CSF haem pigments
111 (UKNEQAS).

112 The CSF total protein levels were determined by a colorimetric method (Pyrogallol red) and the
113 serum total bilirubin was determined by a colorimetric method (Diazonium salt) on Chemistry
114 Analysers AU5400 (Beckman Coulter®).

115

116 **2.3. Data Analysis**

117 Sensitivity, specificity, positive predict value (PPV) and negative predictive value (NPV) of
118 Multistix® urine test strips to support SAH were calculated using haemoglobin and bilirubin
119 detection by spectrophotometry as the reference method (UK guidelines). Lab conclusions were
120 compared to the final clinical SAH diagnosis.

121 Graph Pad software was used for statistical analysis. Descriptive data were expressed as mean+/-
122 SEM (Standard Error of the Mean) and mean of differences with 95% confidence intervals.
123 D'agostino & Pearson normality test determined normal distribution of the values. The two-
124 tailed unpaired t-test were performed to compare means of two groups and calculate p values. p
125 values less than 0.05 were considered statistically significant.

126

127 **3.RESULTS**

128 **3.1 Study population**

129 We analysed 136 CSF samples received in our laboratory for haem pigments analysis by
130 spectrophotometry (with SAH suspicion). Of 136 CSF, 31 were excluded for absence of PTS
131 results and 5 for absence of CSF total proteins concentration (Fig.1). Thus, 100 patients were
132 included in the study. The analysed population included 42 males and 58 females, and patients
133 were mainly from the emergency room. Age group distribution reflected that SAH suspicion
134 were more frequent in 36-50 years old patients. There were 19/100 biological confirmation of
135 SAH suspicion using the UK algorithm and 13/100 were clinically diagnosed in our global
136 population (N=100).

137

138

139 **3.2 Pigments test strips biological performance for haem pigments detection**

140 First, we evaluated the sensitivity, specificity, NPV and PPV of haemoglobin or bilirubin by PTS
141 *versus* spectrophotometry detection in 100 CSF samples. Indeed, spectrophotometry is the
142 current reference method to detect haem pigments in CSF. The main objective for this biological
143 test was to eliminate a SAH. Haem pigments detection required high sensitivity and high NPV to
144 avoid false negative results.

145 Pigments test strip-pads for haemoglobin detection (PTSh) displayed a perfect sensitivity (1.00,
146 95% confidence interval (CI) 0.88 to 1.00) and a low specificity (0.47, 95% CI 0.36 to 0.58)
147 (Table 1). The perfect NPV (1.00, 95% CI 0.90 to 1.00) of PTSh was technically interesting. To
148 confirm this high performance, we compared the NOA performance within the haemoglobin
149 negative and positive PTSh. NOA mean in the haemoglobin positive PTSh samples
150 (0.114 ± 0.028 AU) was greatly higher than in the haemoglobin negative PTSh samples
151 (undetectable AU, mean of differences 0.114 AU, 95% CI 0.037 to 0.191, $p=0.004$, Fig.2A).

152 For bilirubin detection, recommended as the best biomarker by the UK Revised national
153 guidelines, pigments test strip-pads for bilirubin detection (PTSb) showed a modest sensitivity
154 (0.54, 95% CI 0.35 to 0.72) but a good specificity (0.97, 95% CI 0.91 to 1.00) (Table 2). PTSb
155 was less sensitive than spectrophotometry (lower NPV). PTSb threshold was probably higher
156 than that of spectrophotometry. To test this hypothesis, we compared the NBA performance
157 within the bilirubin negative and positive PTSb. NBA mean in the bilirubin positive PTSb
158 samples (mean 0.131 ± 0.025 AU) was 5-fold higher than in the bilirubin negative PTSb samples
159 (mean 0.025 ± 0.004 AU, mean of differences 0.106 AU, 95% CI 0.054 to 0.157, $p=0.0003$,
160 Fig.2B). The lowest AU value for positive PTS was 0.03, suggesting that the threshold positive
161 value for PTSb was around 0.03. To confirm this result, we diluted 3 bilirubin positive CSF

162 samples and found a positivity threshold at 0.034 ± 0.006 AU (Spectrophotometry reproducibility
163 coefficient of variation =17%) for PTSb (Fig.2C) whereas the expected AU by
164 spectrophotometry was 0.007. In accordance with international guidelines, the cut-off of PTSb
165 was confirmed by the internal quality CSF control, which identified the limit of blank at 0.03 AU
166 and a positive threshold (limit of detection) of the PTSb at 0.04 AU (Fig 2C). This result
167 explained the lack of sensitivity of PTSb.

168 Together, these data suggest that PTSh was more sensitive than spectrophotometry and that
169 spectrophotometry was more sensitive than PTSb.

170 Because both bilirubin and haemoglobin presences are required to ascertain biological SAH as to
171 UK algorithm guidelines by spectrum, we next considered positive pigments test strip-combined
172 haemoglobin-bilirubin results (PTShb) when bilirubin and haemoglobin were detected. The
173 PTShb was negative when haemoglobin and bilirubin were undetectable. The PTShb was
174 reported as inconclusive when only haemoglobin or bilirubin was present. So, we interpreted the
175 haem pigments results between PTShb and spectrophotometry using the UK guidelines. Then, as
176 our main goal was to evaluate the ability of the PTShb result to rule out SAH (high sensitivity
177 and NPV), we defined those inconclusive results as positive for the 2 tests (PTShb and
178 spectrophotometry). This resulted in high sensitivity (0.97, 95% CI 0.83 to 1.00) and modest
179 specificity (0.46 95% CI 0.35 to 0.57) (Table 3). These results suggest that the combined
180 presence of haemoglobin and bilirubin increases PTShb sensitivity.

181

182 **3.3 Pigments test strips performance for SAH diagnosis exclusion**

183 As PTS, spectrophotometry detects biomarkers as a sign of potential SAH. To fully evaluate

184 PTShb, it was necessary to reposition the results in light of the final clinical SAH diagnosis. To

185 evaluate the clinical performance of our test to rule out SAH diagnosis, we analysed the
186 concordance between the negative PTShb (undetectable bilirubin and haemoglobin) or the
187 negative spectra using the UK guidelines and the final clinical SAH diagnosis. Here again, we
188 considered detectable haemoglobin and/or bilirubin as positive. Thirteen out 100 patients were
189 clinically confirmed for SAH. PTShb was less specific than the spectrum. Interestingly, all
190 clinical SAH had a positive or inconclusive PTShb. However, PPV was low due to many false
191 positive or inconclusive results (54/67 patients, Table 4) versus 18/31 patients with spectrum,
192 indicating that PTShb, like spectrum, was not a good test for SAH biological diagnosis. When
193 PTShb was negative for haem pigments, none of the patients (0/33) presented confirmed clinical
194 SAH (PTShb sensitivity = 1.00, 95% CI 0.77 to 1.00, Table 4) while there was 1/70 false
195 negative for spectrum analysis (spectrum sensitivity = 0.92, 95% CI 0.67 to 1.00, Table 4).
196 Finally, although less specific, PTShb sensitivity was at least as good as that of the spectrum,
197 with a perfect NPV.
198 Taken together, these results suggest that an undetectable bilirubin and haemoglobin by PTShb
199 could probably rule out clinical SAH when spectrophotometry was not available.

200

201 **4. DISCUSSION**

202 In this study, we evaluated the Multistix® urine test strips as a rapid diagnosis tool in clinical
203 suspicion of SAH.

204 Although haem pigments analysis by Multistix® urine test strips was not recommended by the
205 UK published guidelines [10], our results showed good performance to exclude SAH.
206 Spectrophotometers are not always available and many laboratories currently use PTS instead for
207 SAH-derived biomarkers detection in CSF samples when SAH is suspected. Multistix® urine

208 test strips are available in most laboratories, easy to run as a bedside diagnosis test, need low
209 volumes of CSF and give quick results. As Multistix® urine test strips are already used and
210 could be helpful for SAH clinical management, their performance evaluation was required.

211 We systematically blindly analysed the presence of pigments in CSFs by both Multistix® urine
212 test strips and spectrophotometry.

213 First, we analysed PTSh and PTSb separately, as compared to spectrophotometry. We confirmed
214 that PTSh was very sensitive [13,14] but PTSb was not suitable for bilirubin detection because
215 the colour change remained difficult to assess and PTSb detected only macroscopic
216 xanthochromia. Lack of sensitivity led to false negative results for bilirubin detection when the
217 NBA was below 0.04 AU. According to the UK guidelines, the critical threshold for probable
218 SAH is a NBA > 0.007 AU (almost 5 times less). So, despite its simplicity PTSb, did not seem to
219 be reliable. However, when we considered PTShb as compared to the spectrophotometry
220 algorithm, we obtained a high sensitivity but a modest specificity. These results supported that
221 PTShb was nearly as sensitive as spectrophotometry using the UK guidelines to rule out SAH.
222 Finally, and importantly, when we compared PTShb to clinical diagnosis, we found that PTShb
223 biological performance had a perfect sensitivity (no false negatives) for SAH exclusion.
224 However, positive PTShb could not affirm SAH because of the numerous false positives (54/67,
225 PPV = 0.19). Noticeably, spectrophotometry also could not be used, with 18/30 false positives
226 (PPV = 0.40). Indeed, this low PPV was also described by Birch *et al* [17] where only 0.38 of
227 positive spectra had a clinically confirmed SAH. These results may explain the discordance
228 between PTShb performance compared to the recommended spectrophotometry and final clinical
229 SAH diagnosis. So, the PTShb alone could rule out SAH but should not be used to confirm SAH.
230 Others studies were interested in SAH diagnosis exclusion by alternative biological tools. Birch

231 K *et al* [17] have already highlighted that SAH is associated with higher CSF total protein
232 concentrations and concluded that no single CSF total protein value could be used to rule out
233 SAH. Other biomarkers such as RBC counts had a theoretical lower limit of detection than that
234 of the Multistix® urine test strips threshold (1 RBC *vs* 5 RBC/ μ L). However, it was imperfect
235 because this approach did not differentiate SAH diagnosis from traumatic lumbar punctures in
236 first tube. An absolute RBC count < 500 cells in tube 4 (manually measured) could exclude SAH
237 [18]. The major limit was that, in our hands, the collection of 4 tubes, numbered in the order of
238 sampling, is rare. Finally, other biological tools were tested such as CSF siderophages count,
239 CSF ferritin concentration and CSF methaemoglobin, but seemed to lack sensitivity for SAH
240 diagnostic [19]. Because lumbar punctures are invasive, peripheral blood biomarkers were tested.
241 High levels of TNF-alpha receptor 1 (TNFR1) in venous blood were associated with the presence
242 of aneurysms in case of SAH [20]. Similarly, combination of 3 microRNAs was upregulated
243 during the SAH in peripheral blood and could help monitoring the different phases of SAH [21].
244 However, these markers are not routinely determined in medical laboratories.

245 None of these biomarkers could easily exclude SAH and pigments detection in CSF was still the
246 best biomarker.

247 Our study demonstrates that Multistix® urine test strips could be an alternative to
248 spectrophotometry. However, our study presents some limitations. It is a descriptive study where
249 haem pigments detection was usually prescribed to exclude SAH diagnosis, but in a few cases, it
250 was used for SAH patients follow-up. Moreover, haem detection analysis is a second-line test
251 when CT-scan is negative after 12 hours SAH symptoms. This is a strict condition to use haem
252 pigments presence in CSF. Another limitation was that the urine tests strips were read by

253 operators, which may deteriorate the performance. Automated reading should be preferred to
254 limit this important source of fluctuations.

255 Finally, this monocentric study included a limited number of patients since it needed Multistix®
256 (Siemens) urine test strips limited the generalizability of the results. Bigger cohorts are needed to
257 confirm this promising result. Other urine test strip tools should also be used to check if they
258 present similar performance.

259

260 **5. CONCLUSIONS**

261 In conclusion, 2 to 3 percent of SAH have a negative cerebral CT scan within the 12 hours
262 following the start of haemorrhage, and CT-Scan sensitivity decreases after 12 hours. CSF haem
263 pigments absence can help to rule out SAH. The reference method for lab-based SAH exclusion
264 is spectrophotometry. PTShb negativity determined by Multistix® urine test strips appeared to
265 be a good first-line, easy and bedside test to rule out SAH diagnosis. However, spectrum analysis
266 must remain the reference method when available.

Table 1: Pigments test strip vs spectrum for haemoglobin detection

		PTSh (N=100)	
		Negative	Positive
Spectrum	Negative	34	39
oxyhaemoglobin	Positive	0	27
Total		34	66
Sensitivity		1.00	
Negative predictive value		1.00	
Specificity		0.47	
Positive predictive value		0.41	

PTSh: Pigments test strips-pads for haemoglobin detection.

Table 2: Pigments test strip vs spectrum for bilirubin detection

		PTSb (N=100)	
		Negative	Positive
Spectrum bilirubin	Negative	74	2
	Positive	11	13
Total		85	15
Sensitivity		0.54	
Negative predictive value		0.87	
Specificity		0.97	
Positive predictive value		0.87	

PTSb: Pigments test strips-pads for bilirubin detection.

Table 3: Pigments test strip vs spectrum for biological SAH detection

		PTShb (N=100)		
		Negative	Positive	Inconclusive
Biological SAH by spectrum	Negative	32	0	38
	Positive	1	12	9
	Inconclusive	0	2	6
Total		33	14	53
Sensitivity		0.97		
Negative predictive value		0.97		
Specificity		0.46		
Positive predictive value		0.43		

PTShb: Pigments test strips-combined haemoglobin-bilirubin results, SAH: Subarachnoid haemorrhage.

Table 4: Pigments test strip or spectrum vs SAH final clinical diagnosis

		PTShb (N=100)			Biological SAH by spectrum (N=100)		
		Neg	Pos	Inc	Neg	Pos	Inc
Clinical SAH diagnosis	Neg	33	7	47	69	12	6
	Pos	0	7	6	1	7	5
Total		33	14	53	70	19	11
Se		1.00			0.92		
NPV		1.00			0.99		
Spe		0.38			0.79		
PPV		0.19			0.40		

Inc: Inconclusive, Neg: Negative, NPV: Negative predictive value, Pos: Positive, PPV: Positive predictive value, PTShb: Pigments test strips-combined haemoglobin-bilirubin results, SAH: Subarachnoid haemorrhage, Se: Sensitivity, Spe: Specificity.

Acknowledgments: No

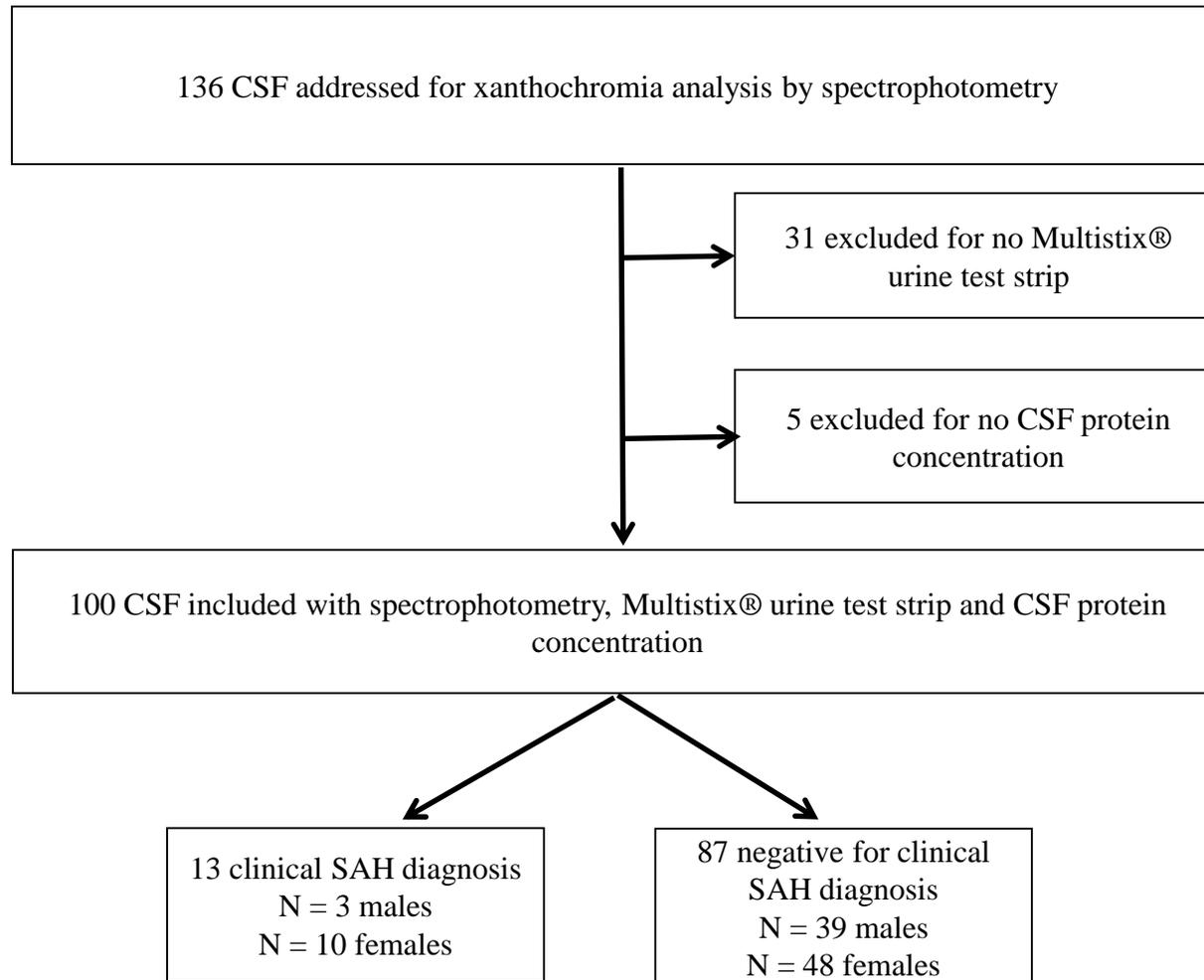
Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

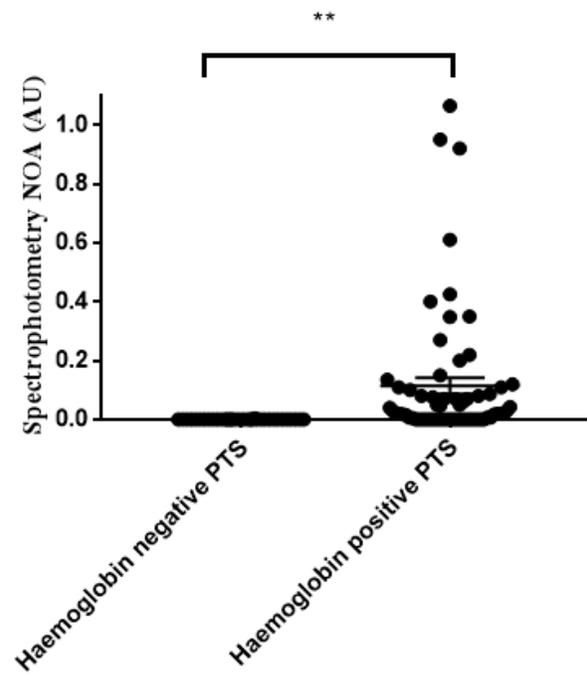
- [1] Feigin VL, Lawes CM, Bennett DA, Anderson CS. Stroke epidemiology: a review of population-based studies of incidence, prevalence, and case-fatality in the late 20th century. *Lancet Neurol* 2003;2:43-53.
- [2] Wolfe CD, Giroud M, Kolominsky-Rabas P, et al. Variations in Stroke Incidence and Survival in 3 areas of Europe. *Stroke* 2000;31:2074-9.
- [3] Molyneux AJ, Kerr RS, Yu LM, et al. International subarachnoid aneurysm trial (ISAT) of neurosurgical clipping versus endovascular coiling in 2143 patients with ruptured intracranial aneurysms: a randomised comparison of effects on survival, dependency, seizures, rebleeding, subgroups, and aneurysm occlusion. *Lancet* 2005;366:809-17.
- [4] Polmear A. Sentinel headaches in aneurysmal subarachnoid haemorrhage: what is the true incidence? A systematic review. *Cephalalgia* 2003;23:935-41.
- [5] Suarez JJ, Tarr RW, Selman WR. Aneurysmal subarachnoid hemorrhage. *N Engl J Med* 2006;354:387-96.
- [6] van der Wee N, Rinkel GJ, Hasan D, Gijn JV. Detection of subarachnoid haemorrhage on early CT: is lumbar puncture still needed after a negative scan? *J Neurol Neurosurg Psychiatry* 1995;58:357-9.

- [7] Perry JJ, Stiell IG, Sivilotti ML, et al. Sensitivity of computed tomography performed within six hours of onset of headache for diagnosis of subarachnoid haemorrhage: prospective cohort study. *BMJ* 2011;18:343:d4277. doi: 10.1136/bmj.d4277.
- [8] van Gijn J, van Dongen KJ. The time course of aneurysmal haemorrhage on computed tomograms. *Neuroradiology* 1982;23:153-6.
- [9] Vermeulen L, van Gijn J. The diagnosis of subarachnoid haemorrhage. *J Neurol Neurosurg Psychiatry* 1990;53:365-372.
- [10] Cruickshank A, Auld P, Beetham R, et al. Revised national guidelines for analysis of cerebrospinal fluid for bilirubin in suspected subarachnoid haemorrhage. *Ann Clin Biochem* 2008;45:238-44. doi: 10.1258/acb.2008.007257.
- [11] Martin SM, ed. Is the naked eye as sensitive as the spectrophotometer for detecting xanthochromia in cerebrospinal fluid? Proceedings of the National Meetings, 30 April-4 May 2001, London: Association of Clinical Biochemists; 2001: 53.
- [12] Collet N, Garcelon N, Robbe V, Lucas-Clerc C, Cuggia M, Bendavid C. Bioinformatic software for cerebrospinal fluid spectrophotometry in suspected subarachnoid haemorrhage. *Ann Clin Biochem* 2012;49:177-83.
- [13] Marshall RA, Hejmanowski C. Urine test strips to exclude cerebral spinal fluid blood. *West J Emerg Med* 2011;12:63-6.
- [14] Reeve SF, Johnson JS, Marshall R. Urine Test Strips as a Bedside Diagnostic Aid to Rule out Blood in CSF: A Validation. *Mil Med* 2016;181(3):199-201.
- [15] Chalmers AH, Kiley M. Detection of xanthochromia in cerebrospinal fluid. *Clin Chem* 1998;44:1740-2.

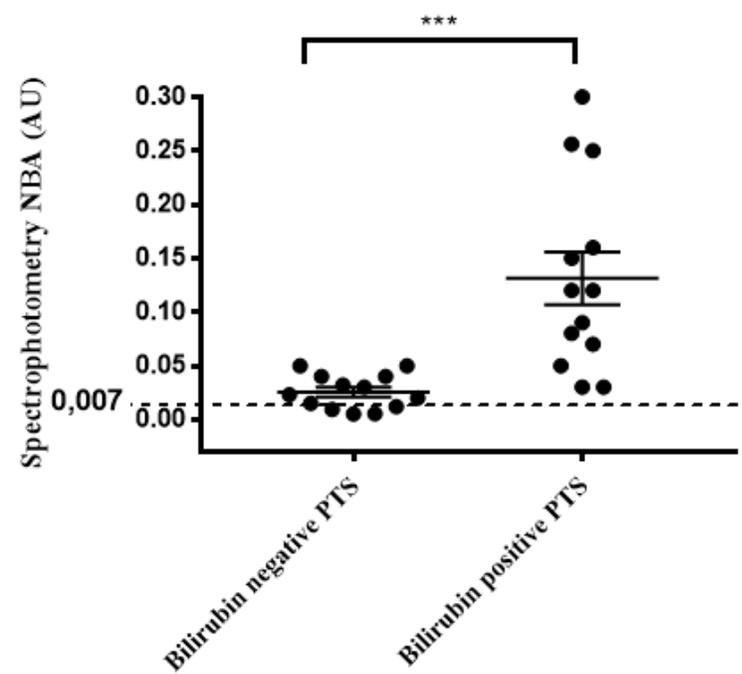
- [16] Chalmers AH. Cerebrospinal fluid xanthochromia testing simplified. *Clin Chem* 2001;47:147-8.
- [17] Birch K, Burrows G, Cruickshank A, et al. Cerebrospinal fluid total protein cannot reliably distinguish true subarachnoid haemorrhage from other causes of raised cerebrospinal fluid net bilirubin and net oxyhaemoglobin absorbances. *Ann Clin Biochem* 2014;51:657-661.
- [18] Gorchynski J, Oman J, Newton T. Interpretation of Traumatic Lumbar Punctures in the Setting of Possible Subarachnoid Hemorrhage: Who Can Be Safely Discharged? *Cal J Emerg Med* 2007;8(1): 3–7.
- [19] Nagy K, Skagervik I, Tumani H, Petzold A, Wick M, Kühn HJ, et al. Cerebrospinal fluid analyses for the diagnosis of subarachnoid haemorrhage and experience from a Swedish study. What method is preferable when diagnosing a subarachnoid haemorrhage? *Clin Chem Lab Med* 2013;51(11):2073-86. doi: 10.1515/cclm-2012-0783.
- [20] de Torres R, Mancha F, Bustamante A, Canhao P, Fragata I, Montaner J. Usefulness of TNFR1 as biomarker of intracranial aneurysm in patients with spontaneous subarachnoid hemorrhage. *Future Sci OA* 2020;6(1): FSO431.
- [21] Ye1 F, Zhou X, Zhang J, Gao H, Jiang J, Liu N. Circulating microRNAs act as fingerprints in patients after acute aneurysmal subarachnoid hemorrhage. *Int J Clin Exp Pathol* 2017;10(6):7154-7160.



A



B



C

