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Cerebrospinal fluid spectrophotometry. Do we need to hurry?

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SUMMARY
Introduction: Cerebrospinal fluid (CSF) spectrophotometry (SPFM) is a laboratory assessment used in diagnostics of subarachnoid hemorrhage, mainly in cases when it is not proven by computed tomography (CT), but clinical manifestations still persists. Sample of CSF for SPFM examination should be delivered to the laboratory and analyzed as soon as possible, at least within 1 hour. In delay, the superanatant should be stored at 4°C in dark. The aim of the study was to investigate the consequence of delayed processing of CSF samples in SPFM assessment.
Methodology: A total of 48 samples delivered to the laboratory until 30 minutes from lumbar puncture. Collection were enrolled in the study. If a sufficient amount of CSF was obtained, the sample was divided into two aliquots (A and B). In the first aliquot (A), routine biochemical and cytological examination, including erythrocyte counting, was performed. SPFM examination was immediately performed on the centrifuged aliquot. Values of net oxyhemoglobin absorbance (NOA) and net bilirubin absorbance (NBA) were calculated according to the UK recommendation. Aliquot "B" was stored at room temperature for 60 minutes in the light, then centrifuged and SPFM determination was performed as in aliquot "A". A Wilcoxon non-parametric test (paired version) was used for comparisons between groups (group A and B). Results were considered statistically significant at $P < 0.05$. Upon NOA and NOB, samples were classified as "positive", "negative" and "inconclusive". MedCalc software was used for statistic evaluation.
Results: Median erythrocyte count was 16/μL (0–119 460). In 27 (56%) samples, there was a detectable amount of oxyhemoglobin and/or bilirubin. Changes in NOA and NBA levels in samples A in comparison with samples B did not reach statistical significance ($P = 0.68$ and 0.21, respectively). We found one case classified as "positive" when processed immediately, but an aliquot that was processed after a delay was classified as "inconclusive". The samples with median (min–max) of erythrocyte counting 16/μl (0–119 460) were used. Changes in values of NOA in aliquots analyzed immediately compare to values of samples analyzed after 60 minutes did were not statistically significant (values of median [min–max]): NOA (0 min) = 0.001 (0.001–0.863), NOA (60 min) = 0.001 (0.001–0.898), p=0.67. In case of NBA aliquots with 60 min delay there also were no statistically significant changes: NBA (0 min) = 0.001 (0.001–0.599), NBA (60 min) = 0.001 (0.001–0.601), p=0.12. In classification of samples to groups „positive", „negative" and „inconclusive" there was, in samples with 60 min delay, the change in classification from group „positive" to „inconclusive" in one sample with limit value of NBA.
Conclusion: Significant differences in values NOA and NBA were not detected in samples analyzed with 60 min delay compare to those analyzed immediately after collection. In case of limit values of NOA and NBA there is necessary to pay attention in situations of delay in delivery for the sample to laboratory more than 60 minutes.
Keywords: cerebrospinal fluid, spectrophotometry, preanalytical phase, subarachnoid haemorrhage.

SOUHRN
Brož P., Rajdl D., Racek J., Ženková J., Petříková V.: Spektrofotometrie cerebrospinální tekutiny. Musíme spěchat?
Úvod: Spektrofotometrie mozkomíšního moku (SPFM) je vyšetření používané k diagnostice subarachnoidálního krvácení, zejména v případech, kdy nativní CT hlavy krvácení neprokazuje, avšak nadále přetrvávají klinické příznaky. Vzorek mozkomíšního moku by měl být doručen do laboratoře a analyzován co nejrychleji, nejdéle však do jedné hodiny od odběru. V případech, kdy vzorek není zpracován nejdéle do jedné hodiny od odběru, by měl být supernatant skladován ve tmě při 4°C. Cílem studie bylo zjistit vliv časové prodlevy zpracování mozkomíšního moku na výsledek spektrofotometrického vyšetření.
Metodika: Do studie bylo zařazeno 48 vzorků doručených do laboratoře do 30 minut od provedení lumbální punkce. Současně s provedením základního biochemického a cytologického vyšetření, zahrnujícího stanovení počtu erytrocytů, byly vytvořeny dva alikvoty (A a B). Alikvot „A" byl ihned po doručení vzorku zcentrifugován a bylo provedeno SPFM vyšetření dle britských doporučení se stanovením net oxyhemoglobin absorbance (NOA) a net bilirubin absorbance (NBA). Alikvot „B" byl ponechán při pokojové teplotě na denním světle 60 minut, poté centrifugován a následně bylo provedeno SPFM vyšetření jako v případě alikvotu „A". Pro statistické porovnání hodnot NOA a NBA ve skupině „A" a ve skupině „B" byla užita párová verze Wilcoxonova testu. Za statisticky významnou byla považována hodnota $p < 0,05$. Jednotlivé vzorky byly dle hodnot NOA a NBA klasifikovány na „positive", „negative" a „inconclusive". Pro statistické zhodnocení byl užit MedCalc software (MedCalc Software, version 16.4.1, MedCalc Software bvba, Ostend, Belgium).
Výsledky: Do studie byly zařazeny vzorky s mediánem (min–max) počtu erytrocytů 16/μl (0–119 460). Změny v hodnotách NOA v alikvotech zpracovaných ihned v porovnání s hodnotami ve vzorcích zpracovaných s 60 minutovou časovou prodlevou nedosáhly statisticky významných změn (hodnoty uvedeny jako medián [min–max]): NOA (0 min) = 0,001 (0,001–0,863), NOA (60 min) = 0,001 (0,001–0,898), p=0,67. V případě NBA u alikvotů zpracovaných s 60 minutovým odstupem v porovnání s alikvoty zpracovanými ihned rovněž nedošlo ke statisticky významným změnám: NBA (0 min) = 0,001 (0,001–0,599), NBA (60 min) = 0,001 (0,001–0,601), p=0,12. Při klasifikaci vzorků do skupin „positive", „negative" a „inconclusive" došlo u vzorků zpracovaných s časovou prodlevou 60 minut ke změně v klasifikaci u jednoho vzorku s hraniční hodnotou NBA ze

skupiny „positive“ do skupiny „inconclusive“.
Závěr: Nebyly zjištěny statisticky významné rozdíly v hodnotách NOA a NBA u vzorků analyzovaných s hodinovou prodlevou v porovnání se vzorky analyzovanými ihned po přijetí do laboratoře. V případech s hraničními hodnotami NOA a NBA je třeba při hodnocení dbát zvýšené opatrnosti zejména v situacích, kdy připadá v úvahu opožděné doručení vzorku do laboratoře o více jak jednu hodinu.
Klíčová slova: mozkomíšní mok, subarachnoidální krvácení, spektrofotometrie, preanalytická fáze.

Introduction

Cerebrospinal fluid (CSF) spectrophotometry (SPFM) is a laboratory assessment used in diagnostic work up of subarachnoid haemorrhage, mainly in cases when clinical suspicion after negative noncontrast computed tomography (CT) of the brain remains high [1]. Sample of CSF for SPFM examination should be delivered to the laboratory, centrifuged and the supernatant processed as soon as possible, but should be processed within 1 hour of collection [2]. In cases when analysis is not performed immediately, the supernatant should be stored in the dark at approximately 4°C [2]. Our study aimed to investigate the consequence of delayed processing of CSF samples in SPFM assessment.

Materials and Methods

A total of 48 samples delivered to the laboratory until 30 minutes from collection were enrolled in the study. If a sufficient amount of CSF was obtained, the sample was divided into two aliquots (A and B). In the first aliquot (A), routine biochemical and cytological examination, including erythrocyte counting, was performed. SPFM examination was immediately performed on the centrifuged aliquot at 350–700 nm (Cecil Aquarius CE 7500, Cecil Instruments). Values of net oxyhaemoglobin absorbance (NOA) and net bilirubin absorbance (NBA) were calculated according to the UK National External Quality Assessment Service (NEQAS) guidelines [2]. The second aliquot (B) was left in the laboratory at room temperature under daylight for 60 minutes. After 60 minutes, the second sample (B) was centrifuged, and SPFM was performed. A Wilcoxon non-parametric test (paired version) was used for comparisons between groups (group A and B). Results were considered statistically significant at $P < 0.05$. We classified all samples (in both groups A and B) as “positive”, “negative” or ”inconclusive” according to the decision limits of NOA and NBA as recommended in the guidelines. For statistical analysis, MedCalc Software (MedCalc Software, version 16.4.1, MedCalc Software bvba, Ostend, Belgium) was used.

Results

Median erythrocyte count was 16/μL (0–119 460). In 27 (56 %) samples, there was a detectable amount of oxyhaemoglobin and/or bilirubin. Changes in NOA and NBA levels in samples A in comparison with samples B did not reach statistical significance ($P = 0.68$ and 0.21, respectively). We found one case classified as “positive” when processed immediately, but an aliquot that was processed after a delay was classified as “inconclusive”. Results are summarized in Table 1.

Discussion

When samples are stored at room temperature without the influence of factors capable of causing erythrocyte lysis, significant changes in oxyhaemoglobin concentration do not necessarily occur. Additionally, changes in bilirubin occurring at room temperature may not reach statistically significant levels. However, one case was classified as “positive” when processed immediately, but an aliquot that was processed after a delay was classified as “inconclusive” due to a decrease in the bilirubin concentration in the aliquot processed 1 hour later. Immediately after admission to the laboratory, the NBA value in this sample was 0.009, and 60 minutes later, it was 0.003. The decision limit according to the recommendations is 0.007 [2]. Clearly, the 1-hour delay in processing can cause the change in classification only in borderline concentrations. However, in our opinion, all borderline samples should be evaluated carefully.

The limitation of this study is that the experiment was focused only on the preanalytical phase of SPFM examination. SPFM examination should be evaluated

Table 1: NOA and NBA values. Number of positive/negative/inconclusive samples included.

	Sample A (0 minutes)	**Sample B (60 minutes)**	
NOA median (min–max)	0.001 (0.001–0.863)	0.001 (0.001–0.898)	P = 0.67
NBA median (min–max)	0.001 (0.001–0.599)	0.001 (0.001–0.601)	P = 0.12
Sample classification positive/inconclusive/negative	12/0/36	11/1/36	–

with a knowledge of the patient's history. Other examinations can be useful in some cases, e.g. cytological examination with the presence of siderophages [3]. Additionally, according to the UK NEQAS guidelines, some samples can be classified differently with the knowledge of total CSF protein levels or blood bilirubin concentrations [2]. Finally, only experienced staff should interpret the results of CSF spectrophotometry.

Conclusion

No statistically significant changes were found in NOA or NBA values in CSF samples analysed immediately in comparison with samples analysed 60 minutes later. However, in one case, sample A and sample B were classified differently. Therefore, samples with borderline values should be classified carefully, especially when delayed processing is conceivable.

Declaration of conflicting interests

The authors declare that there are no competing interests.

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