

# Differences in total protein concentration, nucleated cell count, and red blood cell count among sequential samples of cerebrospinal fluid from horses

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**Objective**—To examine total protein concentration and cell counts of sequentially collected samples of CSF to determine whether blood contamination decreases in subsequent samples and whether formulas used to correct nucleated cell count and total protein concentration are accurate.

**Design**—Case series.

**Animals**—22 horses.

**Procedure**—For each horse, 3 or 4 sequential 2-ml samples of CSF were collected from the subarachnoid space in the lumbosacral region into separate syringes, and blood was obtained from the jugular vein. Total protein concentration, nucleated cell count, and RBC counts were determined in all samples.

**Results**—Among 3 sequential samples, total protein concentration and RBC count were significantly lower in samples 2 and 3, compared with sample 1. Nucleated cell count was significantly lower in sample 3, compared with sample 1. Among 4 sequential samples, total protein concentration and RBC count were significantly lower in samples 2, 3, and 4, compared with sample 1. Nucleated cell count was significantly lower in samples 3 and 4, compared with sample 1. For 3 correction formulas, significant differences in corrected values for nucleated cell count and total protein concentration were detected between sample 1 and sample 3 or 4.

**Conclusions and Clinical Relevance**—Because iatrogenic blood contamination decreases in sequential CSF samples, a minimum of 3 samples should be collected before submitting the final sample for analysis. Formulas to correct nucleated cell count and total protein concentration are inaccurate and should not be used to correct for blood contamination in CSF samples. (*J Am Vet Med Assoc* 2000;217:54–57)

Analysis of CSF is often an essential procedure for determining a diagnosis or prognosis for patients suspected of having neurologic disease. Several components of the CSF may be evaluated, and routine evaluation usually includes determination of total protein (TP) concentration, nucleated cell count, and RBC count.

For horses, the technique for obtaining CSF from the lumbar subarachnoid space is routinely performed while the horse is standing. Iatrogenic contamination of

CSF with blood is a common problem during collection of samples and causes high RBC counts that are not indicative of CNS disease. Iatrogenic blood contamination of CSF may cause increases in nucleated cell count and TP concentration, which may confound interpretation of results. Blood contamination of CSF has an effect on western blot analysis for detection of antibodies against *Sarcocystis neurona* and on albumin quotient and IgG index in horses.<sup>1</sup> Previous studies<sup>2-7</sup> have examined the effects of iatrogenic blood contamination of CSF in neurologically normal animals and animals suspected to have neurologic disease and found that contamination did not alter TP concentration or nucleated cell count. Formulas have been developed to correct the nucleated cell count for the amount of blood contamination in a CSF sample.<sup>8</sup> Although results of several studies indicate that these formulas are unreliable,<sup>2-4</sup> the use of formulas is still discussed as a means to correct for blood contamination.<sup>9-10</sup>

The initial CSF sample collected during aspiration from the lumbar subarachnoid space is usually analyzed unless there is gross evidence of blood contamination; in those instances, additional CSF samples are collected until a sample appears clear, and that sample is submitted for analysis. Samples that appear clear and colorless may, nevertheless, have microscopic evidence of RBC contamination. Results of 1 study indicated that the range of threshold for human perception of blood in CSF was 700 to 6,020 RBC/ $\mu$ l, with mean threshold of 3,010 RBC/ $\mu$ l.<sup>11</sup> Results of another study indicated that CSF samples with 4 to 1,195 RBC/ $\mu$ l were considered clear, whereas samples with 5,100 to 11,350 RBC/ $\mu$ l were characterized as cloudy.<sup>1</sup> Although it seems likely that when iatrogenic blood contamination occurs, CSF samples collected sequentially contain progressively fewer RBC, this finding has not been documented in horses.

The purpose of the study reported here was to examine TP concentration and cell counts of sequentially collected samples of CSF to determine whether blood contamination decreases in subsequent samples and whether formulas used to correct nucleated cell count and total protein concentration are accurate.

## Materials and Methods

**Sample collection**—Cerebrospinal fluid was collected from the lumbosacral subarachnoid space by use of a routine collection technique<sup>12</sup> in 22 horses evaluated for neurologic disease on 41 occasions. Nineteen horses had signs of spinal cord ataxia, whereas 3 horses did not have neurologic signs. Horses were standing in stocks during the collection procedure. Sedation was used in 16 horses and included use of

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detomidine alone (26 occasions), detomidine followed some time later by xylazine (2 occasions), xylazine alone (1 occasion), and xylazine and butorphenol (1 occasion). After removal of the hair by clipping, the skin of the lumbosacral area was aseptically prepared. Subcutaneous tissues were infiltrated with approximately 3.0 ml of 2% lidocaine. An 18-gauge, 6-in (15.3-cm) spinal needle was inserted into the lumbosacral cistern, and CSF was aspirated gently into a 10-ml syringe with the seal already broken. Following aspiration of approximately 2 ml of CSF, the syringe was changed, and another 2-ml sample was collected into a different syringe. This procedure was repeated once or twice, so that 3 or 4 samples were collected sequentially on each occasion. Visual assessment of the appearance of the sample was done before submission to the laboratory. A sample of venous blood was obtained from the jugular vein at the time of CSF sampling on all but 3 occasions.

**Laboratory analysis**—Cerebrospinal fluid analysis included determination of nucleated cell and RBC counts and TP concentration. Nucleated cell and RBC counts were determined, using a hemocytometer, within 1 hour after collection of CSF samples. Total protein concentration was measured by use of an automated chemistry analyzer, using a method<sup>4</sup> based on a modification of the biuret reaction. Results were expressed as mg of protein per dl of CSF. Venous blood samples were analyzed for nucleated cell and RBC counts and TP concentration. The RBC and nucleated cell counts were determined electronically. The TP concentration was determined by direct measurement of the plasma refractive index, using a hand-held refractometer.

A formula that uses the ratio of venous RBC count to venous TP concentration and WBC count was used to correct values obtained from CSF contaminated with blood,<sup>8</sup> as follows:

$$X_c = X_{CSF} - (X_{blood} \times RBC_{CSF} / RBC_{blood})$$

where  $X_c$  is the corrected value for nucleated cell count or TP concentration in CSF;  $X_{CSF}$  is the observed nucleated cell count or TP concentration in CSF;  $X_{blood}$  is the WBC count or TP concentration in venous blood;  $RBC_{CSF}$  is the observed RBC count in CSF; and  $RBC_{blood}$  is the RBC count in venous blood.

Two other simplified formulas<sup>7</sup> were also used: CSF TP concentration was increased 1 mg/dl for every 1,000 RBC/ $\mu$ l of CSF, and CSF nucleated cell count was increased 1 nucleated cell/dl for every 500 RBC/ $\mu$ l of CSF.

If sample 1 had RBC count > 10 cells/ $\mu$ l, its corrected values for TP concentration and nucleated cell count were

compared with corrected values in the last sequential sample (sample 3 or 4).

**Statistical analyses**—Repeated measures ANOVA was used to determine whether there was variation in TP concentration and nucleated cell and RBC counts among samples 1 through 4. When significant differences were found, the Tukey-Kramer multiple-comparison test was used to compare differences among groups. A paired *t*-test was used to determine significant differences between corrected TP concentration or nucleated cell counts in samples with RBC counts > 10 cells/ $\mu$ l, compared with a sequential sample with no blood contamination or less blood contamination from the same horse on the same occasion. The Pearson correlation coefficient was used to determine associations between CSF TP concentration or nucleated cell counts and RBC counts in samples with blood contamination and subsequent uncontaminated or less contaminated samples. The null hypothesis was rejected at  $P < 0.05$ .

## Results

Three sequential CSF samples were collected on all 41 occasions. On 35 of the 41 occasions, a fourth sequential sample was collected. When 3 sequential samples were analyzed, TP concentration ( $P < 0.001$ ) and RBC counts ( $P = 0.01$ ) were significantly lower in samples 2 and 3, compared with sample 1 (Table 1). Nucleated cell count was significantly ( $P = 0.02$ ) lower in sample 3, compared with sample 1. When 4 sequential samples were analyzed, TP concentration ( $P = 0.004$ ) and RBC counts ( $P = 0.006$ ) were significantly lower in samples 2, 3, and 4, compared with sample 1 (Table 2). Nucleated cell count was significantly ( $P = 0.02$ ) lower in samples 3 and 4, compared with sample 1.

For samples obtained on 29 occasions, RBC count of sample 1 was > 10 RBC/ $\mu$ l, and a venous blood sample was available. By use of any of the 3 correction formulas (data not shown), significant differences in corrected values for nucleated cell counts ( $P = 0.03$  to  $P = 0.04$ ) and TP concentrations ( $P = 0.01$ ) were detected between sample 1 and sample 4 (or sample 3 if a fourth sample was not available). Because corrected values for these variables would be similar if the formulas were truly accurate, we concluded that the 3 formulas were not accurate.

Nucleated cell count was significantly associated

Table 1—Results (mean  $\pm$  SD [range]) of analysis for evidence of blood contamination in 3 sequential CSF samples obtained from 22 horses on 41 occasions

Sample No.	RBC count (cells/ $\mu$ l)	Nucleated cell count (cells/ $\mu$ l)	Total protein (mg/dl)
1	3,438 $\pm$ 9,801 <sup>a</sup> (0–48,000)	8 $\pm$ 19 <sup>a</sup> (0–110)	81 $\pm$ 27 <sup>a</sup> (36–141)
2	188 $\pm$ 397 <sup>b</sup> (0–1,700)	2 $\pm$ 3 <sup>a,b</sup> (0–18)	75 $\pm$ 23 <sup>b</sup> (35–138)
3	82 $\pm$ 151 <sup>b</sup> (0–700)	1 $\pm$ 1 <sup>b</sup> (0–6)	73 $\pm$ 20 <sup>b</sup> (35–110)

<sup>a,b</sup>Within a column, different superscripts indicate significant ( $P < 0.05$ ) differences among groups.

Table 2—Results (mean  $\pm$  SD [range]) of analysis for evidence of blood contamination in 4 sequential CSF samples obtained from 22 horses on 35 occasions

Sample No.	RBC count (cells/ $\mu$ l)	Nucleated cell count (cells/ $\mu$ l)	Total protein (mg/dl)
1	3,801 $\pm$ 10,600 <sup>a</sup> (0–48,000)	8 $\pm$ 20 <sup>a</sup> (0–110)	83 $\pm$ 28 <sup>a</sup> (36–141)
2	211 $\pm$ 425 <sup>b</sup> (0–1,700)	3 $\pm$ 4 <sup>a,b</sup> (0–18)	77 $\pm$ 22 <sup>b</sup> (35–138)
3	91 $\pm$ 177 <sup>b</sup> (0–700)	1 $\pm$ 1 <sup>b</sup> (0–6)	74 $\pm$ 20 <sup>b</sup> (35–110)
4	72 $\pm$ 158 <sup>b</sup> (0–750)	1 $\pm$ 1 <sup>b</sup> (0–7)	75 $\pm$ 20 <sup>b</sup> (36–115)

See Table 1 for key.

with RBC count in sample 1 ( $r = 0.653$ ;  $r^2 = 0.43$ ;  $P = 0.01$ ), whereas no such association was found in sample 4 ( $r = 0.013$ ;  $r^2 = 0.0002$ ). Total protein concentration was significantly associated with RBC count in sample 1 ( $r = 0.506$ ;  $r^2 = 0.256$ ;  $P = 0.01$ ), whereas no such association was found in sample 4 ( $r = 0.072$ ;  $r^2 = 0.005$ ).

On the basis of visual assessment of the 158 CSF samples, 3 samples (range, 18,550 to 48,000 RBC/ $\mu$ l of CSF) were characterized as blood-tinged, 12 samples (range, 1,150 to 5,300 RBC/ $\mu$ l of CSF) were cloudy, and the remaining 143 samples (range, 0 to 1,000 RBC/ $\mu$ l of CSF) were considered clear.

## Discussion

The horses in the study reported here had signs of spinal cord ataxia ( $n = 19$ ) or did not have neurologic signs (3). Although neurologic disease may have altered TP concentration and nucleated and RBC counts in the CSF, values should have been consistent in samples 1 through 4, because these samples were collected within seconds of each other. Nucleated cell count in the final sample (sample 3 or 4) was  $< 3$  cells/ $\mu$ l on 40 of 41 occasions. The TP concentration in the final sample was  $< 100$  mg/ $\mu$ l on 37 of 41 occasions. Because TP concentration and nucleated cell counts of these samples were within reference ranges for horses,<sup>9</sup> neurologic disease did not seem to affect results of this study.

Although most individuals do not submit the first CSF sample collected for analysis if it appears cloudy or bloody, it is not standard practice to remove multiple samples of CSF and submit the last sample collected. The results of our study suggest that a minimum of three 2-ml samples should be collected before submitting the final sample for analysis. Although significant differences between sample 1 and samples 2, 3, and 4 were detected for TP concentration and RBC count, nucleated cell count was not significantly lower in sample 2, compared with sample 1.

The study reported here did not determine whether removing a larger volume of CSF in the initial syringe would have resulted in less blood contamination in sample 2. The advantage of removing small volumes in each syringe is that, should blood contamination become visible, only that sample needs to be discarded; an adequate volume of CSF may have already been obtained, enabling performance of all necessary tests.

Contamination of CSF samples by blood usually results from puncture of meningeal or spinal cord vessels. With few exceptions, these vessels are small arterioles, capillaries, and venules. Correction formulas are applied under the assumption that nucleated cells, protein, or other substances in these small vessels will be carried into the CSF in amounts proportional to RBC concentration in large peripheral veins. On the basis of results of the study reported here and others,<sup>2,7</sup> this assumption does not appear to be correct. Results of our study indicate that leakage of nucleated cells and protein is disproportionate to RBC count in venous blood. If these formulas were accurate, the corrected nucleated cell and TP concentration values from sample 1 would be similar, if not identical, to the correct-

ed values for subsequent samples that, most often, had less blood contamination (judged on the basis of lower CSF RBC count). Statistical differences between these 2 samples indicated that the formulas are inaccurate and should not be used to predict the proportion of nucleated cells or TP in a CSF sample that is attributable to blood contamination.

For the 29 samples with high RBC count in sample 1, nucleated cell count was significantly associated with RBC count. This finding suggests that nucleated cell count was significantly influenced by blood contamination, although only 43% of the variability in the nucleated cell count was attributable to RBC in the CSF ( $r^2 = 0.43$ ). A similar association between nucleated cell count and RBC count was not detected in sample 4. The TP concentration was significantly associated with RBC count (albeit with a low value for  $r^2$ ) in sample 1, but not associated with RBC count in sample 4, which indicated that in many instances the initial CSF sample was contaminated by blood. Others<sup>13</sup> recommend that sequential CSF samples should be collected, and biochemical and serologic studies should be performed on the first sample, bacteriologic studies performed on the second sample, and cytologic examination performed on the third sample. This recommendation is based on the assumptions that bacterial or blood contamination of the first sample should not affect biochemical and serologic assays, bacterial contamination should not affect the second sample, and blood from a traumatic tap should not affect the third sample. Because even a small amount of blood contamination can cause false-positive results for western blot tests for *S. neurona*,<sup>1</sup> we recommend that the sample with the least amount of blood contamination should be used for serologic assays and cytologic examination. Results of the study reported here suggest that the initial CSF sample is not suitable for this purpose, even if gross examination does not reveal blood contamination.

\*Vitros PROT slide, Johnson & Johnson, Rochester, NY.

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