

Interpretation of cerebrospinal fluid analysis from recumbent cows using different thresholds of red blood cell count

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Abstract

Background: Hemodilution of the cerebrospinal fluid (CSF) could confound interpretation of results. Accurately predicting total nucleated cells count (TNCC) and total protein concentration (TPC) attributable to hemodilution is difficult.

Objective: To determine the effects of hemodilution on TPC and TNCC in bovine CSF.

Methods: Retrospective review of CSF analysis results of downer dairy cows treated at Centre hospitalier universitaire vétérinaire between January 2006 and December 2014. Descriptive statistics were performed using 3 scenarios.

Results: Among the 235 samples included, red blood cell (RBC) count (RBCC) ranged from 0 to 869 220 RBC/ μ L (median = 6.6), TPC ranged from 0.04 to 6.51 g/L (median = 0.27), and TNCC ranged from 0 to 7500 cell/ μ L (median = 1.1). Among the 157 samples that had <30 RBC/ μ L (a threshold used in other species), TPC and TNCC varied between 0.13 and 1.06 g/L (median = 0.27) and between 0 and 31.4 cell/ μ L (median = 0.6), respectively. Eighty-four samples had TPC <0.25 g/L and TNCC \leq 4.5 cell/ μ L. Among those 84 samples, RBCC varied between 0 and 1290 RBC/ μ L (median = 4.7). In 20 samples, TNCC was 0 with a variation in RBCC between 0 and 840 RBC/ μ L (median = 3.9). No strong correlations between RBCC and TNCC and TPC were found.

Conclusions: A cutoff around 200 RBC/ μ L is proposed as clinically meaningful in bovine CSF. Results between 200 and 1290 RBC/ μ L are equivocal.

KEYWORDS

cattle, CSF analysis, neurology, nonambulatory, recumbent, ruminant

Abbreviations: CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; RBC, red blood cells; RBCC, red blood cell count; TNCC, total nucleated cell count; TPC, total protein.

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1 | INTRODUCTION

Downer cow syndrome is a common problem in bovine practice. Multiple causes can result in a situation where an adult cow cannot get up or stand on its own. Spinal cord lesions might cause recumbency in adult dairy cattle and are most often associated with a poor prognosis. Neurologic examination is essential for localizing the anatomic area affected.¹

Cerebrospinal fluid (CSF) analysis is a useful test to establish a diagnosis and prognosis in recumbent cows.² Cerebrospinal fluid analysis includes evaluation of total protein concentration (TPC), red blood cell (RBC) count (RBCC), total nucleated cell count (TNCC), and cytologic examination. Cerebrospinal fluid analysis is a useful diagnostic tool for identifying spinal cord lesions in downer dairy cows.² A CSF TNCC >4.5 cells/ μ L, TPC >0.39 g/L or a combination of both yielded a specificity of 100% for the presence of a spinal cord lesion in downer dairy cows. In the same study, all downer dairy cows with TNCC \leq 4.5 cells/ μ L and a TPC <0.25 g/L were free of spinal lesions.²

Red blood cells are not a normal component of CSF. The presence of erythrocytes in a sample is most often of iatrogenic origin (contamination during collection) but could also be because of subarachnoid or ventricular hemorrhage. Iatrogenic hemodilution of the CSF could confound results interpretation since both TNCC and TPC could be falsely elevated.

In other species, some strategies (formulas) have been suggested to correct the TPC and TNCC in a hemodiluted sample but are inaccurate.³⁻⁵ Current literature provides conflicting data on whether hemodilution has a predictable effect on the CSF analysis. Iatrogenic blood contamination decreases in sequential CSF samples in horses⁴ and, in cows, there is no statistically significant relationship between neutrophil or lymphocyte concentrations and RBCC.⁶ In cats, a CSF concentration of RBC >30 cells/ μ L affects the total and differential number of cells.⁷ In dogs, blood contamination significantly affects TPC.⁸ The number of RBC is not significantly associated with nucleated cell or protein concentrations in the CSF of clinically normal dogs or in dogs with signs of neurological disease.⁹ Given the lack of correlation between TNCC or protein concentrations and RBC counts, an increase in cell counts and protein concentrations in CSF samples with blood contamination greater than 13 200 RBC/ μ L should be considered clinically relevant.³ In humans, there are different thresholds to classify a sample as contaminated by blood.^{7,10,11} Greater than 10 RBC/ μ L, 30 RBC/ μ L or 500 RBC/ μ L are suggested as possible thresholds for interpretation.

Iatrogenic hemodilution could confound CSF results interpretation since TNCC and TPC could be falsely elevated. One could hypothesize that part of the TNCC and TPC measured originate from the hemodilution of CSF. Therefore, accurately predicting TNCC and TPC attributable to iatrogenic hemodilution remains a challenge. The question to answer is at which cutoff should we declare a sample as too hemodiluted to be usefully interpretable?

The primary aim of this study is to determine the effects of iatrogenic hemodilution on TPC and TNCC in CSF samples collected from recumbent dairy cows admitted to the Centre hospitalier universitaire vétérinaire. Our goal is to propose a threshold under which samples can still be interpreted. In order to compare our data with reported studies in

cattle and other species, and to investigate the optimal RBCC cutoff in recumbent adult dairy cows, 3 scenarios were explored. We hypothesized that hemodilution above a certain threshold increases the TNCC and the TPC, confounding interpretation of results.

2 | MATERIALS AND METHODS

The medical records of downer dairy cows for which a CSF analysis was performed between January 2006 and December 2014 were reviewed. If >1 CSF sample was submitted during hospitalization, only the first sample's result was included in the study. Inclusion criteria were recumbency (cows that were referred or that became recumbent during their hospitalization), female, dairy breeds, and a minimum age of 2 years, or a heifer that had already calved once. All CSF samples were aseptically collected at the lumbosacral intervertebral space and placed in an ethylenediaminetetraacetic acid (EDTA) vacutainer for clinicopathologic analysis. Excess EDTA in the vacutainer was removed by shaking the tube upside down before fluid collection. All CSF samples were processed by a laboratory technician at the Service de diagnostic de la Faculté de médecine vétérinaire. Briefly, the CSF's TNCC, and RBCC values were determined by directly applying CSF to a Bright Line Neubauer Hemacytometer (Hausser Scientific, Horsham, Pennsylvania, USA). Leukocytes were counted in all 9 large squares in both sides. The mean of both counts was multiplied by 10 and divided by 9 to obtain the number of cells/ μ L. The CSF's TPC was measured using a Unicel Dx C 600 automated chemistry analyzer (Beckman Coulter, Indianapolis, Indiana, USA). One to 4 slides were prepared for cytological evaluation in each case by centrifugation of 50 to 200 μ L aliquot of CSF using a Cytospin III cytocentrifuge (Shandon, Pittsburgh, Pennsylvania, USA) set at 950 RPM, 3 min and stained with Wright-Giemsa stain using a Aerospray HematologySTAT automatic stainer (Elitechgroup Manufacturing and Distributors, Puteaux, France) to be examined by a board-certified clinical pathologist at the Service de diagnostic de la Faculté de médecine vétérinaire. Data recorded included CSF's TNCC, TPC, and RBCC values.

Descriptive statistics (maximum, minimum, mean, and median) and visual assessment of TNCC, TPC, and RBCC distributions were performed on the entire dataset. Then, the same descriptive statistics and assessment were performed on subgroups of data according to 3 different scenarios of classification.

In the first scenario, data were explored using a cutoff used in human and feline medicine to declare samples as contaminated^{7,12} (samples with <30 RBC/ μ L were included). The second scenario included our CSF results considered normal using the interpretation guidelines reported by Achard et al (samples with TNCC \leq 4.5 cell/ μ L and TPC <0.25 g/L).² The assumption is that those CSF results considered normal could not have been hemodiluted to a clinically relevant level. The third scenario investigated all samples without any nucleated cells (TNCC = 0 cell/ μ L). The assumption being that, since TNCC was 0, blood did not considerably affect the TNCC. The distribution of RBCC, TNCC, and TPC for each scenario are presented. Total nucleated cell count and TPC with RBCC were statistically

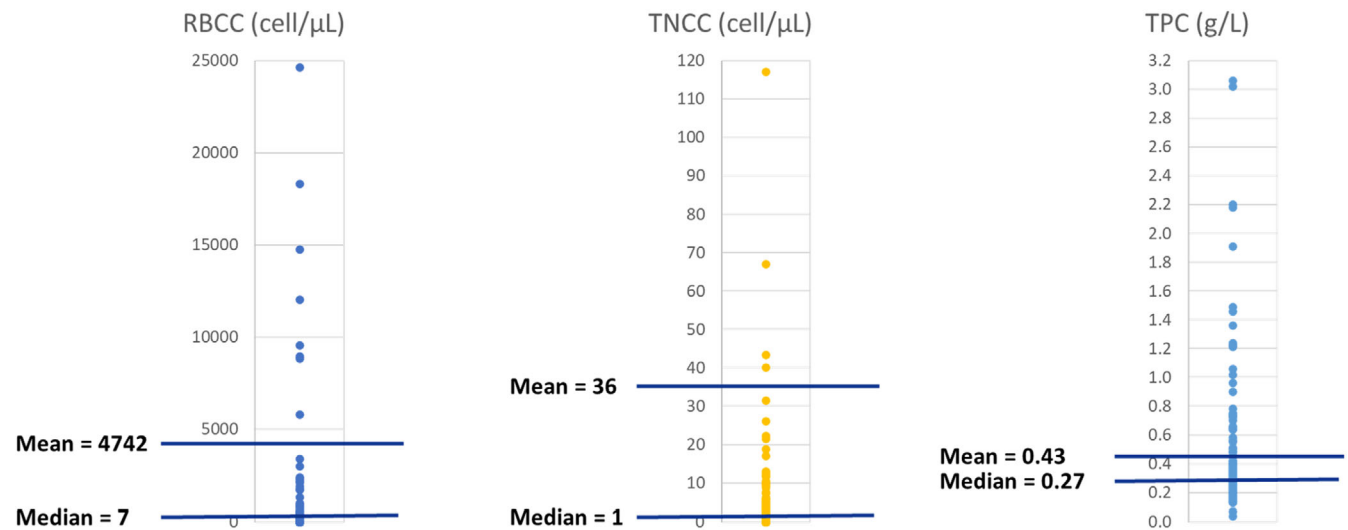


FIGURE 1 Red blood cell count/ μL , total nucleated cell count/ μL , and total protein concentration distribution (g/L) of 235 cerebrospinal fluid samples collected from downer dairy cows referred to the Centre hospitalier universitaire vétérinaire. To better appreciate the distribution of data, extreme values of RBCC $>100\,000$ cells/ μL ($n = 2$), TNCC >5000 cell/ μL ($n = 1$), and TPC >6.0 g/L ($n = 2$) are not included in this figure. RBCC, red blood cell count/ μL ; TNCC, total nucleated cell count/ μL ; TPC, total protein concentration (g/L)

compared in (a) all clinical samples, (b) samples with <30 RBC/ μL , (c) samples with TNCC ≤ 4.5 cell/ μL and TPC <0.25 g/L, and (d) samples with TNCC = 0 cell/ μL using Spearman's rank correlation coefficient. Correlation coefficient values (ρ) ≥ 0.80 were interpreted to reflect strong correlations.¹³ All statistical analyses were performed using Stata Statistical Software (Release 15. College Station, Texas: StataCorpLP).

3 | RESULTS

A total of 235 records were included. Two samples were considered as having extreme RBCC values (RBCC $>100\,000$ RBC/ μL). The other 233 samples had RBCC $\leq 24\,620$ RBC/ μL . One sample was considered as having an extreme value of TNCC (7500 cell/ μL). The other 234 samples had TNCC ≤ 117 cell/ μL . Two samples had extreme values of TPC (>6 g/L). The other 234 samples had TPC ≤ 3.06 g/L. These extreme values corresponded to 3 samples out of the 235 samples included as follows: sample 1: RBCC = 105 890 RBC/ μL , TNCC = 43.4 cells/ μL , TPC = 2.18 g/L; sample 2: RBCC = 869 220 RBC/ μL , TNCC = 7500 cells/ μL , TPC = 6.51 g/L; sample 3: RBCC = 2110 RBC/ μL , TNCC = 4.2 cells/ μL , TPC = 6.42 g/L. The distribution of RBCC, TPC, and TNCC is presented in Figure 1. A low correlation was observed between the values of RBCC and TNCC ($\rho = 0.43$, $P \leq .01$), and RBCC and TPC ($\rho = 0.23$, $P \leq .01$) in all samples.

About two thirds of samples had <30 RBC/ μL ($n = 157$). Among them, TPC varied between 0.13 and 1.06 g/L (mean = 0.29, median = 0.27) while TNCC varied between 0 and 31 cell/ μL (mean = 2, median = 1). The distribution of RBCC, TNCC, and TPC are presented in Figure 2. A weak correlation was observed between

the values of RBCC and TNCC ($\rho = 0.26$, $P \leq .01$), and RBCC and TPC ($\rho = -0.02$, $P = .8$) of these samples.

In 84 samples, TNCC and TPC were ≤ 4.5 cell/ μL and <0.25 g/L, respectively. Among them, RBCC varied between 0 and 1290 RBC/ μL (mean = 62, median = 5). Distribution is shown in Figure 3. All but 2 samples had a RBCC under 500 RBC/ μL (Figure 3). No significant correlation was observed between the values of RBCC and TNCC ($\rho = 0.15$, $P = .2$), and RBCC and TPC ($\rho = -0.08$, $P = .5$) of these samples.

A total of 20 samples had no nucleated cells (TNCC = 0 cell/ μL). Among them, RBCC varied between 0 and 840 cell/ μL (mean = 65.8, median = 3.9). Total protein concentration varied between 0.13 and 0.24 g/L. Distribution is shown in Figure 4. All samples but 1 had a RBCC under 200 RBC/ μL (Figure 4). There was no evidence of a correlation between TPC and RBCC ($\rho = -0.25$, $P = .3$) of these samples.

4 | DISCUSSION

This study describes the variation of TPC, TNCC, and RBCC in CSF samples collected from recumbent dairy cows admitted to a referral hospital and found that up to 200 RBC/ μL can be present without affecting interpretation of bovine CSF.

The 20 samples from this study with RBCC <840 RBC/ μL and no nucleated cells (Scenario 3), suggest that this degree of blood did not appreciably increase the TNCC. Furthermore, TPC also remained normal (<0.25 g/L). The number of RBC in 19 of 20 of these samples suggests that a threshold around 200 RBC/ μL would be a conservative estimate to declare a sample as hemodiluted and non-interpretable.

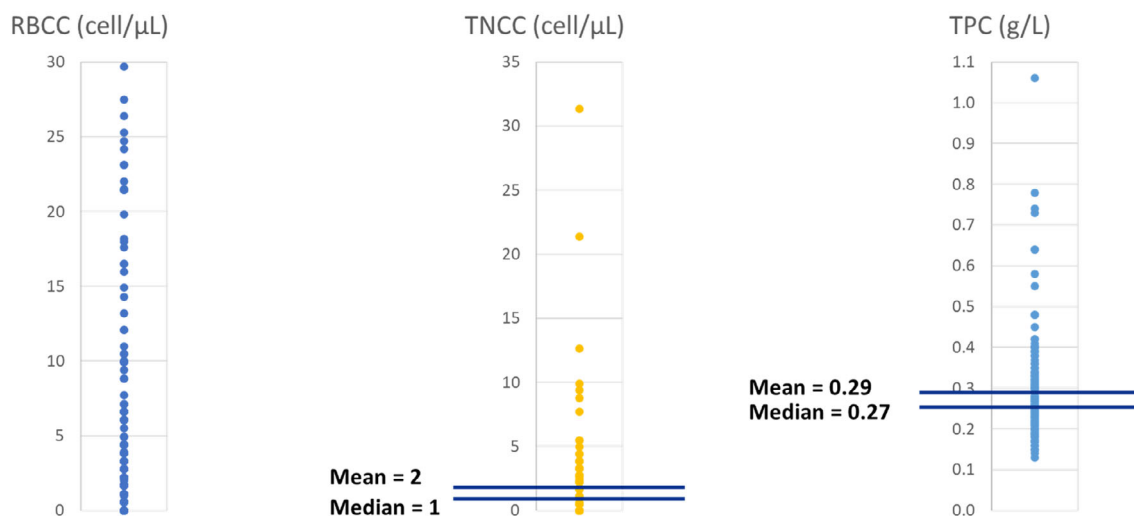


FIGURE 2 Red blood cell count/ μL , total nucleated cell count/ μL , and total protein concentration (g/L) distribution of 157 cerebrospinal fluid analysis with RBCC <30 cell/ μL collected from downer dairy cows referred to the Centre hospitalier universitaire vétérinaire. RBCC, red blood cell count/ μL ; TNCC, total nucleated cell count/ μL ; TPC, total protein concentration (g/L)

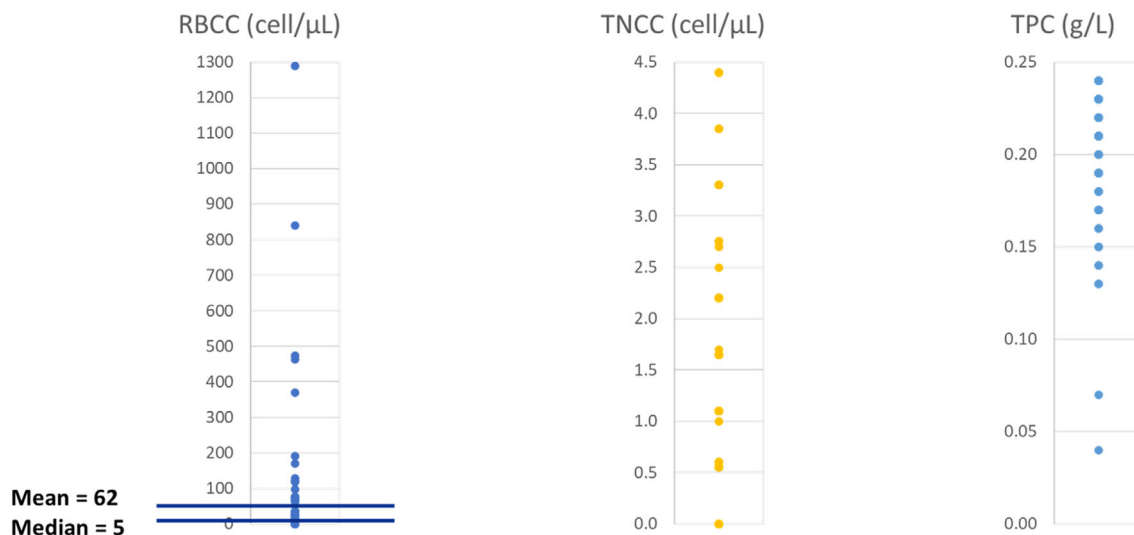


FIGURE 3 Red blood cell count/ μL , total nucleated cell count/ μL , and total protein concentration (g/L) distribution of 84 cerebrospinal fluid samples with TNCC ≤ 4.5 cell/ μL and TPC <0.25 g/L collected from downer dairy cows referred to the Centre hospitalier universitaire vétérinaire. RBCC, red blood cell count/ μL ; TNCC, total nucleated cell count/ μL ; TPC, total protein concentration (g/L)

Up to 1290 RBC/ μL were observed among the 84 cows considered normal according to the criteria described by Achard et al (Scenario 2). The number of RBC in these samples, suggest that a threshold of 500 RBC/ μL is unlikely to interfere with interpretation of CSF results. If TNCC and TPC are considered normal and there is presence of RBC, one could hypothesize that the clinical interpretation is not influenced by the blood contribution to the counts.

There are conflicting reports regarding the effects of blood on cytologic evaluations of CSF.^{3,6-11} There is no statistically significant relationship between the concentration of neutrophils or lymphocytes and the concentration of erythrocytes in CSF of healthy cattle.⁶ However, our findings, confirmed a weak correlation between RBCC and TNCC or TPC.⁹

In human medicine, cutoffs as low as 10, 30 or 50 RBC/ μL are used when CSF biomarkers are measured. In these cases, minor blood contamination would most likely have a major impact on the biomarkers (enzymes and cytokines) measured.^{10,11,14} In equine medicine, blood contamination can introduce serum antibodies against *Sarcocystis neurona* at concentrations sufficient to affect results of Western Blot analysis.¹⁵ However, thresholds as high as 10 000 RBC/ μL are accepted for the diagnosis of *S. neurona*.^{16,17} In bovine medicine, biomarkers are less often measured and CSF analysis in cattle is more often restricted to RBCC, TNCC, TPC, and cytological interpretation.

Our study has limitations. The inclusion of recumbent dairy cows presented to a veterinary teaching hospital for treatment limits

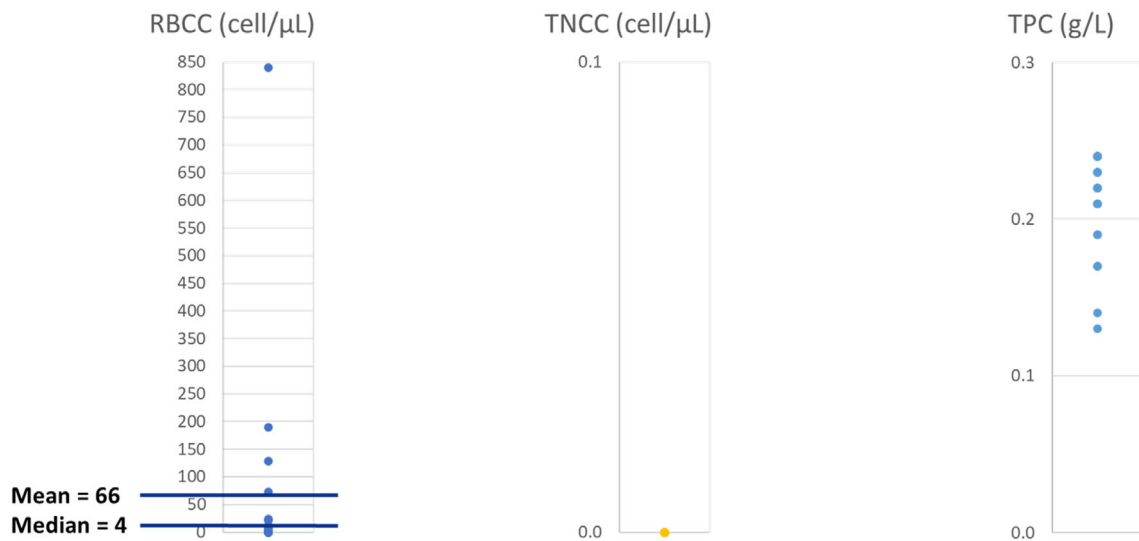


FIGURE 4 Red blood cell count/ μL and total protein concentration (g/L) distributions of 20 cerebrospinal fluid samples without any nucleated cell (total nucleated cell count = 0 cell/ μL) collected from downer dairy cows referred to the Centre hospitalier universitaire vétérinaire. RBCC, red blood cell count/ μL ; TNCC, total nucleated cell count/ μL ; TPC, total protein concentration (g/L)

extrapolation of our findings to other populations (eg, beef cattle). Given that the cell count was performed using hemacytometer, the absence of nucleated cells could be because of the imprecision of the method. The cytology analysis was performed by several pathologists during the study period, making cytological interpretation quite variable and difficult to compile and study. Therefore, cytological interpretation was not included in our analysis, so the association between hemodilution and differential cytological diagnosis was not performed. Without cytology, we cannot exclude the possibility that blood originated from previous hemorrhage rather than contamination during collection. It is likely that alterations in the peripheral circulatory and hematologic status of the cow such as polycythemia, anemia, hyperproteinemia, leukopenia, and leukocytosis alter the way hemodilution affects CSF, TNCC, and TPC. Since hematology results were not included in this study, we could not determine the possible impact of hematologic abnormalities on the correlations between RBCC and TNCC or TPC.

5 | CONCLUSIONS

Based on our findings, we propose a RBC count threshold for accurate interpretation at 200 RBC/ μL . However, results can likely be interpreted, although cautiously, if the RBCC is between 201 and 1290/ μL . Above 1300 RBC/ μL , hemodilution could appreciably affect results of CSF analysis and we suggest being prudent with the interpretation.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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