NOTE *Internal Medicine*

Evaluation of S100B in cerebrospinal fluid as a potential biomarker for neurological diseases in calves

Yuka KOJIMA[1)], Shiori CHIBA[2)], Noriyuki HORIUCHI[2)] Yoshiyasu KOBAYASHI[2)] and Hisashi INOKUMA[1)]*

[1)]*Department of Clinical Veterinary Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080–8555, Japan*
[2)]*Department of Basic Veterinary Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080–8555, Japan*

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ABSTRACT. S100B in cerebrospinal fluid (CSF-S100B) was measured in calves with 20 neurologic and 21 non-neurologic diseases to clarify its utility as a biomarker for neurologic diseases. The median CSF-S100B value in the neurologic disease group (43.0 *n*g/m*l*) was significantly higher than that in the non-neurologic disease group (10.2 *n*g/m*l*). As CSF-S100B levels in calves with neurologic diseases widely differed, the utility of CSF-S100B as a diagnostic marker for neurologic diseases in cattle remains inconclusive.
KEY WORDS: calf, cerebrospinal fluid, S100B

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Neurologic diseases in cattle are challenging to diagnose, because sophisticated modalities, such as magnetic resonance imaging and computed tomography, are not readily available for this purpose. Clinical examination, including evaluation of sensorium, gait, postural reactions, spinal reflexes and cranial nerve, is typically how bovine neurologic diseases are diagnosed [1]. Thus, alternative methods for accurate diagnosis and prognosis are desirable. S100B is related to the activation of astrocytes in the central nervous system (CNS) and has been used as a biomarker for brain damage in humans and rats [2, 3, 5]. Indeed, increased S100B in cerebrospinal fluid (CSF-S100B) is observed in various neurologic disorders in humans, such as Alzheimer's disease, epilepsy and cerebral infarction [6–8].

Although CSF-S100B levels have been measured in cattle [4, 9], little has been reported on its significance in relation to neurologic diseases. In one study, CSF-S100B was quantified in only two clinical cases with neurologic diseases, with no difference in levels found relative to other diseases [9]. Thus, the utility of CSF-S100B as a biomarker for bovine neurologic diseases has yet to be established. Accordingly, the present study aimed to assess the utility of CSF-S100B in cattle with neurologic diseases.

A total of 41 CSF samples were collected from 20 calves with neurologic diseases (NDs) and 21 calves without neurologic diseases (non-neurologic diseases; non-NDs). Because age-related changes in CSF-S100B have been reported in humans [10], only calves aged less than 3 months were used in order to exclude the influence of age-related factors. All calves were examined at the Animal Teaching Hospital, Obihiro University of Agriculture and Veterinary Science. CSF was collected by lumber puncture using a 23G or 21G needle under xylazine sedation. CSF was kept frozen at −30°C until analysis. After clinical evaluation, definitive diagnoses were made by autopsy and histopathological examination. NDs included cerebral vasculitis, brain malformation, myelin hypoplasia, hydrocephalus, cerebellar hypoplasia, cerebellar herniation, meningocele, degeneration of the visual pathway and brain tumors (medulloblastoma) (Table 1). Non-NDs included pneumonia, arthritis, laryngitis, renal failure, congenital heart diseases, fractures, arthrogryposis, anal atresia, hypospadias and hemoplasmosis (Table 1). CSF from three healthy calves was also included in the non-ND group.

CSF-S100B was measured by sandwich enzyme-linked immunosorbent assay (ELISA), as described by Green and Thompson [5]. Briefly, a mouse anti-bovine S100B monoclonal antibody (Sigma-Aldrich, St. Louis, MO, U.S.A.) was coated onto 96 well-immuno plates (Nunc, Roskilde, Denmark) with 0.05 M carbonate buffer. After washing and blocking, 50 μl of diluted CSF sample or S100B standards (5.00, 2.50, 1.25, 0.06 and 0.0 *n*g/m*l*; Sigma-Aldrich) were added to the wells. Following this, a rabbit anti-bovine S100B polyclonal antibody (Sigma-Aldrich) and peroxidase-conjugated goat anti-rabbit IgG polyclonal antibody (Rockland, Limerick, PA, U.S.A.) were used as the first and second antibodies, respectively. Measurements were performed in duplicate for each sample at 450 nm. CSF-S100B levels in the two groups were compared by the Mann-Whitney test (Excel Statistics). The level of significance was set at 5%.

An ELISA standard curve is shown in Fig. 1. As linear relationship between S100B and OD value was observed between 0.0 and 2.5 *n*g/m*l*, a working range of standards was set at these range. CSF samples were diluted at 1:1, 1:10, 1:100 or 1:1,000 by phosphate buffered saline before measurement.

CSF-S100B levels for each case are shown in Table 1. Each data point is also plotted in Fig. 2. The highest CSF-

*CORRESPONDENCE TO: INOKUMA, H., Department of Clinical Veterinary Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080–8555, Japan.
e-mail: inokuma@obihiro.ac.jp

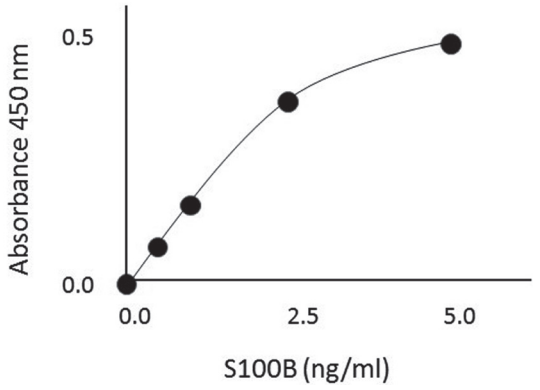

Fig. 1. A ELISA standard curve for S100B. 0.0, 0.6, 1.3, 2.5 and 5.0 *n*g/m*l* standard was prepared using bovine S100B.

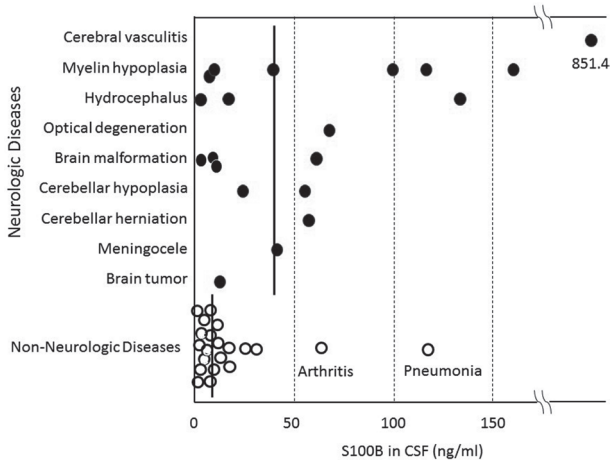

Fig. 2. CSF-S100B levels in calves with neurologic diseases (closed circles) and non-neurologic diseases (open circles). Bars represent the median of each group.

Table 1. CSF-S100B in neurologic diseases and non-neurologic diseases

Diseases	S100B (*n*g/m*l*)
Neurologic diseases (N=20)	
Cerebral vasculitis (N=1)	851.4
Myelin hypoplasia (N=6)	9.8, 11.6, 42.6, 97.7, 121.9, 161.6
Hydrocephalus (N=3)	6.6, 19.3, 135.0
Degeneration of the visual pathway (N=1)	70.4
Brain malformation (N=2)	3.5, 11.6, 12.4, 66.4
Cerebellar herniation (N=1)	55.2
Cerebellar hypoplasia (N=2)	25.5, 54.1
Meningocele (N=1)	43.3
Brain tumor (medulloblastoma) (N=1)	13.9
Non-neurologic diseases (N=21)	
Pneumonia (N=4)	3.0, 3.5, 9.3, 124.4
Arthritis (N=2)	6.6, 64.2
Hemoplasmosis (N=1)	25.0
Hypospadias (N=1)	18.4
Fracture (N=2)	8.3, 18.9
Congenital heart diseases (N=4)	2.9, 3.7, 11.1, 14.5
Arthrogryposis (N=1)	12.9
Renal failure (N=1)	7.0
Laryngitis (N=1)	5.8
Anal atresia (N=1)	1.9
Healthy (N=3)	8.7, 12.3, 29.6

S100B value was recorded in a calf with cerebral vasculitis (851.4 *n*g/m*l*), followed by myelin hypoplasia (161.6 *n*g/m*l*) and hydrocephaly (135.0 *n*g/m*l*). These values were comparatively higher than those reported in the previous studies. A research reported that the mean CSF-S100B from cattle with bovine spongiform encephalitis (BSE) and without BSE was 4.6 and 2.0 *n*g/m*l*, respectively [4]. Another research also reported that mean CSF-S100B from healthy and diseased cattle was 2.9 and 7.0 *n*g/m*l* [9]. ELISA method used in the present study was basically the same as those used in the previous studies. The higher results observed in this study might be related to the lower specificity of anti-bovine S100B antibody or peroxidase-conjugated goat anti-rabbit IgG polyclonal antibody, although the real reason was unclear.

Median CSF-S100B levels in the NDs group were significantly higher than in the non-NDs group (43.0 vs. 10.2 *n*g/m*l*). This suggests that increased CSF-S100B levels may somehow correlate with NDs in calves. We also noted wide variation in CSF-S100B levels in the NDs group (Fig. 1). Even in the same disease, wide variation in CSF-S100B was recorded. For example, the CSF-S100B in 5 calves with my-

elin hypoplasia ranged from 9.8 to 161.6 *n*g/m*l* and 3 calves with hydrocephalus from 6.6 to 135.0 *n*g/m*l*. As S100B has been used as a biomarker for astrocytes damage in human [2, 11], it is easily suspected that CSF-S100B can also be a marker for brain damage in cattle. A wide variation of CSF-100B in NDs suggested that there might be wide range of damages in the CNS. It might also be possible that different stages of disease showed different levels of CSF-100B. A quantitative analysis of brain damage is required to demonstrate the direct evidence between the increased CSF-100B and histological damage in CNS. In the present study, the utility of CSF-S100B as a diagnostic marker for neurologic diseases in cattle remains inconclusive.

In non-NDs, a calf with pneumonia and another with arthritis showed comparatively higher CSF-S100B with 124.4 and 64.2 *n*g/m*l*, respectively. These calves did not show any damage in CNS by necropsy. The reason why these two cases showed such higher CSF-S100B values was unknown. In a previous study, increased S100B levels in the CSF were also observed in cattle with non-neurologic diseases, such as mastitis and locomotive disorders [9]. Systemic inflammation might affect S100B secretion in CNS. Specificity of CSF-S100B as a biomarker for neurologic diseases should also be analyzed in the future studies.

NDs are observed more often in calves than in adult cows, because calves are at a higher risk of developing neurologic diseases, such as hypoxic encephalopathy, congenital diseases and infection. Our findings collectively suggest that CSF-S100B might serve as a potential biomarker for CNS damage in some NDs in calves, although the data are still preliminary and both the sensitivity and specificity of this marker are undetermined. More clinical cases should be analyzed in order to clarify the significance of CSF-S100B as a specific biomarker for some specific diseases.

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