NOTE
Internal Medicine

Serum iron concentration is candidate inflammatory marker for respiratory diseases in beef cows

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ABSTRACT. We hypothesized that the serum iron (Fe) concentration in cows with respiratory diseases is a satisfactory substitute for major inflammatory markers such as haptoglobin (HPT) and serum amyloid A (SAA). Twenty Japanese Black cows aged 279.6 ± 120.0 days were enrolled, and divided into respiratory diseases and control groups based on the presence of clinical findings of respiratory diseases. As a result, area under the receiver operating characteristic curves for plasma HPT, SAA and serum Fe concentrations for respiratory disease-associated systemic inflammation were excellent, at 1.00, 0.96 and 0.97, respectively. Therefore we confirmed that the serum Fe concentration is a satisfactory substitute for HPT and SAA in beef cows with respiratory diseases.

KEY WORDS: beef cattle, haptoglobin, iron, respiratory disease, serum amyloid A

Bovine respiratory disease (BRD) is a major cause of economic loss in the beef cow industry [25, 29]. These economic losses are not only due to mortality [8] and morbidity [25], but also to costs associated with labor and medicine [30]. Thus, decreased production and increased medical costs associated with BRD are serious problems in the beef cow industry. As such, accurate evaluation of the severity is important to determine the appropriate treatment for BRD and to reduce production loss. Eckersall and Bell [5] reported the usefulness of an inflammatory marker that reflects the severity of systemic inflammation due to BRD. Haptoglobin (HPT) and serum amyloid A (SAA) are the major positive acute-phase proteins (APP), and a 100-fold increase in their circulating level in response to stimuli is observed in ruminants [18]. Therefore, the levels of HPT [6, 14, 16, 17] and SAA [6, 16, 20] are widely accepted as an inflammatory marker for BRD. However, due to the difficulty in their measurement and economical problems, the application of HPT and SAA assays to veterinary diagnosis is not widespread.

On the other hand, iron (Fe) plays many roles in enzymatic activity, and is an essential trace element for the host and pathogen [19]. The Fe concentration decreases rapidly in response to inflammation, which can be explained as a host defense mechanism in horse [4]. Decreases in the blood Fe concentration were reported to be useful as an inflammatory marker of acute mastitis [7, 24], endotoxemia [27] and acute traumatic reticuloperitonitis [3], and after minor surgery with inflammation such as dehorning in cattle [28]. As the serum concentration of Fe can be easily measured for a low cost, if it is a satisfactory substitute for HPT and SAA, veterinary practitioners can actively use serum Fe concentrations as an inflammatory marker in clinical cases. However, no study has compared the accuracy of these values as an inflammatory marker in BRD.

The aim of present study was to evaluate the precision of plasma HPT, SAA and serum Fe concentrations as an inflammatory markers related to respiratory diseases in cows. We hypothesized that the serum Fe concentration in cows is a satisfactory substitute for major inflammatory markers in cows with not only mastitis [26] but also BRD.

All procedures were performed in accordance with the Good for the Care and Use of Laboratory Animals of the School of Veterinary Medicine at Rakuno Gakuen University (Approval#: VH18C10). Twenty Japanese Black cows (9 steers and 11 heifers) aged 279.6 ± 120.0 days were enrolled in this case-control study. Nine of the 20 cows were diagnosed with respiratory diseases (BRD group; 4 steers and 5 heifers) based on the clinical findings by veterinary practitioners. The remaining 11 healthy cows (5 steers and 6 heifers) without respiratory diseases were assigned to the control group.

Venous blood samples were collected by jugular venipuncture from all cows during the first medical examination. Blood samples were stored in heparin-coated vacuum tubes and plain tubes, and then centrifuged for 15 min at 1,500 × *g* using a standardized procedure to harvest plasma and serum. Plasma HPT concentrations were measured by ELISA using a commercial

HPT ELISA kit (Bovine Haptoglobin ELISA, Immunology Consultants Laboratory, Inc., Portland, OR, USA). In this study, the SAA concentration was measured using an automated LATIA assay kit [21], which is a modified version of the human SAA assay kit (LZ test 'Eiken' SAA, Eiken Chemical Co., Tokyo, Japan), and automated clinical chemical analyzer (Hitachi 7170S, Hitachi Ltd., Tokyo, Japan). The serum Fe concentrations were measured by the 2-nitroso-5-(N-propyl-N-sulphopropylamino)phenol method [12] using an auto-analyzer (LABOSPEC 003, Hitachi Medical, Co., Tokyo, Japan) at an OD of 753 nm with a commercial kit (N-assay L Fe-H Nittobo, Nitto Boseki, Co., Ltd., Tokyo, Japan). In addition, serum aspartate aminotransferase (AST) and γ-glutamyl transpeptidase (GGT) values were measured using an automatic biochemical analyzer (DRI-CHEM 3500V, FUJIFILM Corp., Tokyo, Japan).

Statistical analysis was conducted using Excel Toukei 2010 (SSRI, Osaka, Japan). Data are expressed as the median and range. The Mann-Whitney U-test after the F-test was employed for comparisons of blood data between groups. In addition, the relationships between the serum Fe concentration and plasma HPT, and SAA concentration were evaluated by Spearman's rank test. Furthermore, receiver operating characteristic (ROC) curves were used to characterize the sensitivity and specificity of each parameter for respiratory disease-associated changes. The optimal cut-off point for a test was calculated by the Youden index [2]. The significance level was set at $P<0.05$.

The rectal temperature and respiratory rate in the BRD group [40.8 (39.6–41.9°C) and 54.0 (40.0–94.0 breaths/min)] were higher than that in the control group [38.9 (38.3–39.2°C) and 30.0 (18.0–32.0 breaths/min)], respectively ($P<0.01$). In this study, the BRD was diagnosed clinically based on the results of the clinical examination, such as coughing, nasal discharge and pulmonary adventitious breath sounds, and these cows had no concurrent disorders. In BRD group, all cows recovered with antibiotic and nonsteroidal anti-inflammatory drug treatment.

The plasma HPT, SAA and serum Fe levels, and other biochemical parameters (AST and GGT) in cows with respiratory diseases are summarized in Table 1. In the present study, the plasma HPT concentrations in BRD and control groups were 173.6 (120.1–300.9) and 0.1 (0.0–79.3) μg/ml, respectively. The plasma HPT concentration in the BRD group was higher than that in the control group ($P<0.01$). Similarly, significant differences in SAA concentrations were observed between groups: the BRD group [33.6 (22.1–67.1 mg/l)] had higher SAA concentrations than the control group [2.4 (0.6–26.5 mg/l), $P<0.01$]. In contrast, the concentration of serum Fe in the BRD group [29.0 (18.0–116.0 μg/ml)] was significantly lower than that in the control group [180.0 (59.0–238.0 μg/ml), $P<0.01$]. In addition, negative correlations were observed between serum Fe concentration and the concentration of plasma HPT (r= −0.82, $P<0.01$), and SAA (r= −0.73, $P<0.01$) in this study. In cows with BRD, our results demonstrated that serum Fe concentration decreased and strong negative correlation was observed between concentrations of serum Fe and plasma HPT, and SAA. In short, these results indicated that the serum Fe concentration in cows could be a satisfactory substitute for major inflammatory markers in cows with BRD.

In this study, ROC curves were used to characterize the sensitivity and specificity of plasma HPT, SAA and serum Fe concentrations for the respiratory disease-associated changes (Fig. 1). First, the area under the ROC curves (AUC) for plasma HPT concentrations was 1.00 (Fig. 1A). The proposed cut-off point for the plasma concentration of HPT in cows with respiratory diseases based on analysis of the ROC curves was >120.1 μg/ml. The sensitivity and specificity of the proposed diagnostic cut-off point for the plasma concentration of HPT were 100.0% and 100.0%, respectively. The AUC for SAA concentrations was 0.96 (Fig. 1B). The proposed cut-off point for the SAA concentration in cows with respiratory diseases based on analysis of the ROC curves was >22.1 mg/l. The sensitivity and specificity of the proposed diagnostic cut-off point for the SAA concentration were 100.0% and 81.8%, respectively. Lastly, the AUC for serum Fe concentrations was 0.97 (Fig. 1C). The proposed cut-off point for the serum Fe concentration in cows with respiratory diseases based on analysis of the ROC curves was <44.0 μg/dl. The sensitivity and specificity of the proposed diagnostic cut-off point for the serum Fe concentration were 88.9% and 100.0%, respectively. ROC curves are useful for evaluating the clinical utility of an inflammatory marker and for comparing effectiveness among different marker. It is well known that a higher AUC represents a stronger diagnostic ability. In this study, the AUC for plasma HPT, SAA and serum Fe concentrations were excellent, at 1.00, 0.96 and 0.97, respectively. These values exhibited a high diagnostic accuracy for inflammation associated with respiratory diseases in beef cows. Thus, our results indicated that serum Fe concentration is candidate inflammatory marker for respiratory diseases in beef cows.

In this study, 1 of BRD group had no abnormality in serum Fe concentration was observed. Her values of serum Fe, plasma HPT and SAA were 116.0 μg/dl, 170.1 μg/ml and 42.8 mg/l, respectively. It means that plasma HPT and SAA levels could assess

Table 1. The levels of haptoglobin, serum amyloid A and iron, and values of liver function in cows with respiratory diseases

Measurement	Unit	Control (n=11)	Respiratory diseases (n=9)	P-value
HPT	(μg/ml)	0.1 (0.0–79.3)	173.6 (120.1–300.9)	<0.01
SAA	(mg/l)	2.4 (0.6–26.5)	33.6 (22.1–67.1)	<0.01
Fe	(μg/dl)	180.0 (59.0–238.0)	29.0 (18.0–116.0)	<0.01
AST	(U/l)	70.0 (52.0–99.0)	51.0 (35.0–129.0)	NS
GGT	(U/l)	19.0 (14.0–27.0)	20.0 (15.0–29.0)	NS

AST, aspartate aminotransferase and; GGT, γ-glutamyl transpeptidase. NS: not significant.
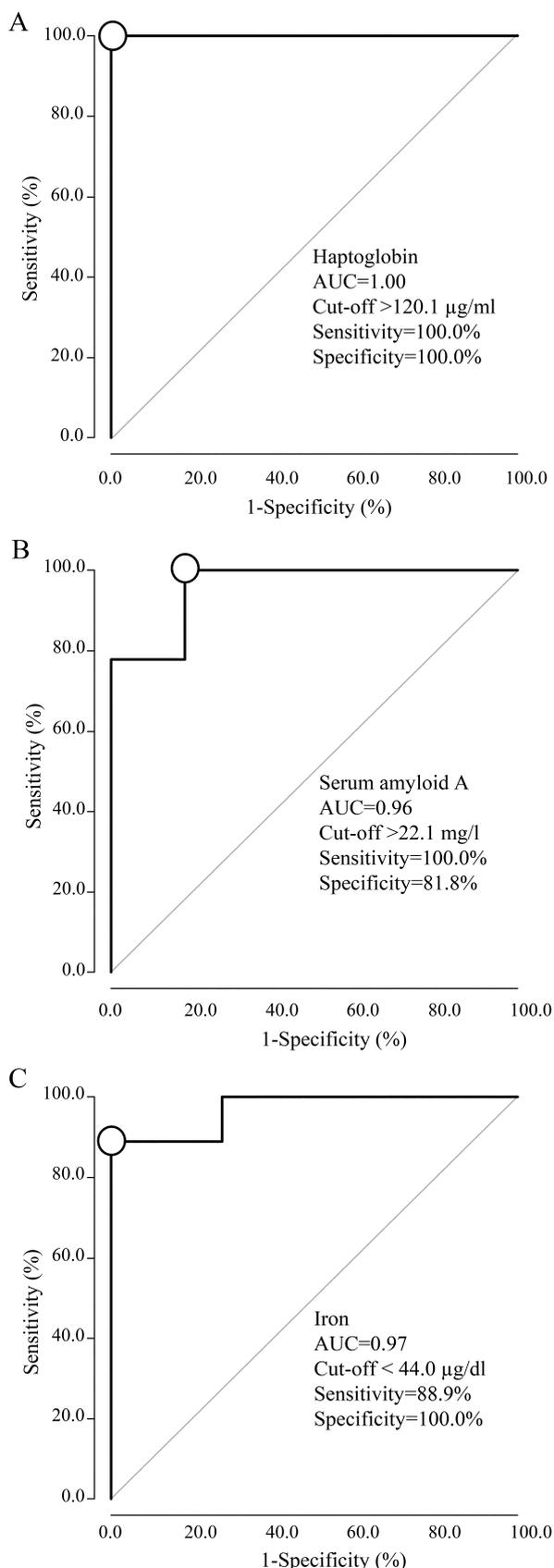
Fig. 1. Receiver operating characteristic (ROC) curves for plasma haptoglobin (A), serum amyloid A (B) and serum iron (C) concentrations. The optimal cut-off points were calculated by the Youden index. Open Circle: Cut-off point. AUC: area under the ROC curves.

the presence of her severe inflammation related to BRD, but serum Fe levels could not. While Aich *et al*. [1] also reported that no correlation was observed between serum HPT and Fe concentrations in cows with BRD. Although, present study could not clarify those contradictions, some factors unrelated to inflammation may affect serum Fe concentration. For instance, hemolytic anemia and Fe overload can increase serum Fe concentrations, which could lead to false-negative result [15]. In this study, the serum biochemical values in cows with BRD were also measured, but there were no significant differences in serum AST or GGT related to liver function between the groups. The liver diseases also alter the levels of HPT, SAA and Fe in cows. Liver function in particular affects the induction and regulation of APP synthesis [18]. Similarly, hepcidin, which is essential for systemic Fe homeostasis, is also affected by liver function [23]. Therefore, in this study, it was necessary to examine liver function. However, no significant differences were observed in serum AST or GGT concentrations between the groups (Table1). Therefore, the effects of liver function on the inflammatory markers examined in the present study were minimal.

Because of the high costs involved, routine use of APP analysis to evaluate inflammatory status is not realistic [9]. Generally, it is well known that measurement of serum Fe levels are individually available and easy applicable. Therefore, several researchers have reported that whether serum Fe levels can be used instead of APP as an inflammatory marker [3, 7, 24, 28]. If the serum Fe concentration in cows with BRD is a satisfactory substitute for major inflammatory markers, such as HPT and SAA, veterinary practitioners can use serum Fe concentrations not only in clinical cases but also in monitoring animal health and welfare. The assessment of animal health at the herd level using serum Fe measurements may be more economical than that of APP [9].

The present study has several limitations that should be addressed. First, present study failed to determine the severity of BRD. In order to verify whether concentration of serum Fe can evaluate the severity of cows with BRD, ideally, it was necessary to classify the severity of BRD based on the thoracic ultrasound and/or radiological examinations. Since the thoracic image diagnosis was not used in this study, it is unclear whether the main infection sites are upper respiratory tract inflammation, bronchitis, and pneumonia. Furthermore, the sample size was too small to verify the reliability of serum Fe concentration as an inflammatory marker in cows with BRD. Therefore, to prove that serum Fe concentration is a reliable inflammatory marker of BRD in beef cows, further studies on the relationship between serum Fe level and severity of BRD are required. While, diagnostic tests to identify pathogens involved in cows with BRD also help to understand severity [22]. Serum HPT and SAA response in coinfections with virus and bacteria is greater when compared to pure virus infections, and the magnitude of the response correlated well with the severity of the clinical signs [10, 11]. Horadagoda *et al.* [13] revealed that SAA is a more rapid bovine acute phase protein than serum HPT in its response to infection with *Pasteurella haemolytica*. Namely, previous

reports showed that the magnitude and the duration of the response in APPs as an inflammatory markers related to BRD are differs depending on the pathogen. Thus, future studies need to focus on evaluation whether difference in pathogen related to BRD, such as viral and/or bacteria, affects value of serum Fe as an inflammatory markers.

In conclusion, although the degree of inflammation related to respiratory diseases is generally evaluated using the values of plasma HPT and SAA, which are major inflammatory markers in cows [5], our study confirmed that the serum Fe concentration is a satisfactory substitute for plasma HPT and SAA. Our results indicated that serum Fe concentration is candidate inflammatory marker for respiratory diseases in beef cows.

CONFLICT OF INTEREST DECLARATION. The authors declare no conflict of interest.

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