Serum amyloid A assay using a new reagent is the most useful in the diagnose of mastitis

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Introduction

Serum amyloid A (SAA) is an acute phase protein (APP) that increases in concentration in the serum in the early stages of inflammation. There are many studies that have concluded that SAA is useful to diagnose inflammatory diseases in cattle. However, the SAA assay using an automated latex agglutination turbidimetric immunoassay for humans was less accurate in cattle. Accordingly, the new SAA assay for cattle has developed. The aim of the present study was to clarify usefulness of a new reagent and to evaluate its diagnostic ability in comparison to other APPs, such as C-reactive protein (CRP), haptoglobin (HPT) and α1-acid glycoprotein (AGP).

Materials and methods

Fifty-two Holstein-Friesian milking cattle were enrolled as a control group, and 33 Holstein-Friesian milking cattle were enrolled as a mastitis group in the present study. If there was a significant difference between the control and the mastitis groups, the mastitis group was further subdivided into subclinical (n = 18) and clinical mastitis groups (n = 15) for statistical analysis. Blood samples were withdrawn from the contralateral jugular vein, and stored in both serum separator and heparine-2K-coated tubes. Serum and plasma were harvested after centrifugation at 3,000 rpm at room temperature for 15 min, and stored at -80°C until analyzed. Then, SAA concentrations in serum were measured using a brand-new automated latex agglutination turbidimetric immunoassay on an automated clinical chemical analyzer (Hitachi 7170S, Hitachi Ltd., Tokyo, Japan). HPT and AGP concentrations in plasma were measured using commercial ELISA kits. The data are shown as the means ± SD. Each dependent variable between groups were compared using Tukey HSD test. A proposed cut-off value of SAA concentrations to diagnose mastitis was determined by the point of SAA concentration with maximum sum of sensitivity and specificity, using receiver operating characteristic (ROC) curve. All statistical analyses were performed by IBM SPSS Statistics, v.23 (IBM Co, Somers, NY, USA). The significance level was $P < 0.05$.

Results

The significant difference in plasma CRP concentration were not observed between control group and mastitis cow. There was no significant difference between subclinical and clinical group in plasma HPT and AGP concentrations. However, although significant differences were observed in subclinical (112.7 ± 174.4 μg/mL) and clinical (58.9 ± 40.9 μg/mL) groups compared to the control group (3.1 ± 15.9 μg/mL, $P < 0.001$, respectively), no significant difference was observed between subclinical and clinical groups in plasma HPT concentrations. Likewise, in plasma AGP concentrations, although significant differences were observed in the clinical group (0.44 ± 37.62 mg/mL) compared to the control group (0.35 ± 0.13 mg/mL, $P < 0.05$), no significant difference was observed between subclinical and clinical groups. On the other hand, serum SAA concentrations were significantly higher not only in the subclinical (24.2 ± 25.9 mg/L) and the clinical group (68.8 ± 53.0 mg/L) compared to the control group (3.3 ± 5.48 mg/L, $P < 0.01$, respectively), but also between the subclinical and the clinical group ($P < 0.05$). The proposed diagnostic cut-off points for serum SAA concentrations in order to identify dairy cattle with mastitis based on analyses of ROC curves was set at > 3.65 mg/L. The sensitivity and specificity of the proposed diagnostic cut-offs and the area under the ROC curve for serum SAA concentrations were 84.8%, 86.3% and 0.891 ($P < 0.001$), respectively. The proposed diagnostic cut-off points for serum SAA concentrations in order to identify dairy cattle with mastitis based on analyses of ROC curves was set at > 6.30 mg/L. The sensitivity and specificity of the proposed diagnostic cut-offs and the area under the ROC curve for serum SAA concentrations were 100.0%, 73.9% and 0.923 ($P < 0.001$), respectively.

Significance

In this study, while plasma CRP and AGP concentration was not suitable as a biomarker of mastitis, concentrations of serum SAA and plasma HPT has a validity as a biomarker to diagnose severity of inflammation due to mastitis in milking cow. However, even if HPT was elevated in cattle with mastitis, the ability to differentiate between moderate and acute cases was not recognized. On the other hand, SAA was able to determine clinical mastitis cows with severe inflammation. The SAA assay using new reagent is superior in assessing mastitis severity due to its higher sensitivity and specificity than other inflammatory markers. The severity of inflammation is considered to be an important factor to determine a prognosis of cow with mastitis. Therefore, serum SAA concentrations might be a prognostic tool for determining the outcomes of cattle with mastitis.

