Platelet Abnormalities in a Dog Suffering from Gangrenous Mastitis by *Staphylococcus aureus* Infection

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ABSTRACT. Severe gangrenous mastitis due to *Staphylococcus aureus* infection was diagnosed in a 7 year-old intact female beagle which was presented with swelling of mammary glands after dystocia. Leukocytosis (25,200–48,600/ μ l), decreased platelets (107,000–179,000/ μ l), and abnormal platelet pattern continued during the critical condition. Consistent with platelet pattern, large platelets were observed in the blood smear. The number of leukocytes and platelets rapidly returned to normal during treatment, and the platelet pattern was also restored. The number and pattern of platelet may provide a clue for the evaluation of the clinical condition and/or severity of the lesions in the dog with mastitis.—**KEY WORDS:** dog, gangrenous mastitis, platelet pattern.

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Mastitis is one of the commonest diseases in cows. Bacteremia and septicemia play critical role in the pathogenesis and clinical manifestation of coliform mastitis [4, 13]. Septicemia induces vasoconstriction of artery, platelet adhesion to endothelial cells, and development of hemostatic plugs [3, 7, 10]. Consumption coagulopathy, therefore, is frequently observed in coliform mastitis. Musa *et al.* [10] reported that hemorrhage, thrombosis, and thrombocytopenia were found in calves experimentally treated with *E. coli* endotoxin or *Pasteurella multocida*. Smith [13] recommended heparin therapy in gram-negative septicemia to prevent disseminated intravascular coagulation (DIC) and further platelet aggregation. These reports suggest that platelets also participate in the pathogenesis of mastitis infected with gram-negative bacteria in cows. However, there is little information on the interaction of platelets with mastitis associated with gram-positive bacteria. Additionally, platelet abnormalities in the mastitis of small animals have not been reported. Clinical signs and laboratory findings including platelets abnormalities in a dog suffering from severe gangrenous mastitis by staphylococci are described in this paper.

A 7 year-old intact female beagle weighing 11 kg was presented to the Veterinary Hospital, Faculty of Agriculture, Miyazaki University, with anorexia and swelling of mammary glands 36 hrs after the fourth parturition. All puppies died after birth, because the dog refused to nurse them. At the first admission, slight pale mucous membrane and elevated rectal temperature (40.0°C) were detected. The third right and second left mammary glands were discolored, firm, swollen (right: 6 × 4 × 4 cm, left: 5 × 3 × 3 cm), hot, and painful (Fig. 1). From the laboratory examination, abnormal findings included leukocytosis (36,300/ μ l), a marked increase of α , β , and γ -globulin, mild anemia, slight increase of plasma alkaline phosphatase and creatine phosphokinase levels, mild hyponatremia, hypokalemia, and positive reaction of limulus test (Table 1). Abnormal pattern of platelets was also detected by automatic microcellcounter (Sysmex F-800, Toa, Hyogo, Japan) (Fig. 2A). Large platelets were observed in the blood smear (Fig. 2B). Hemostatic screening tests such as bleeding time, activated partial

thromboplastin time (APTT), plasma prothrombin time, and clot retraction was not done because of the normal hemostasis. An amount of 30 ml of brown viscous milk was taken from the third right gland by aspiration. This milk contained rods and cocci, polymorphonuclear leukocytes, and macrophages. The affected milk was cultured in order to isolate bacteria. Only *Staphylococcus aureus* (*S. aureus*) was isolated. Antibiotic sensitivity test revealed that the organism was susceptible to penicillin, ampicillin, fosfomycin, tetracycline, oxytetracycline, and cephalixin.

The dog was hospitalized, and treated systemically and topically with antibiotics. The dog also received fluid therapy. The clinical course of the dog is summarized in Fig. 3. Since the third right gland was punctured on the night of the admission, capillary drainage and bandage

Fig. 1. Ventral view of the dog with severe gangrenous mastitis. Note the swollen and discolored mammary glands (arrows).

Table 1. Laboratory findings on the first admission

PCV (%)	28	ALP (U/l)	193	T.P. (g/dl)	10.5
RBC ($\times 10^6/\mu$ l)	4.58	ALT (U/l)	12	Albumin (%)	18.3
Hb (g/dl)	10.0	AST (U/l)	34	α_1 -globulin (%)	1.6
WBC ($\times 10^3/\mu$ l)	363	LDH (U/l)	107	α_2 -globulin (%)	19.6
Band (%)	14	BUN (mg/dl)	6.6	β -globulin (%)	21.5
Seg. (%)	84	Cr. (mg/dl)	0.4	γ -globulin (%)	32.9
Lympho. (%)	2	CPK (U/l)	201		
Eosino. (%)	1	Na (mEq/l)	150	Limulus test	Positive
Baso. (%)	0	K (mEq/l)	3.4		
PLT ($\times 10^4/\mu$ l)	21.7	Cl (mEq/l)	110

Fig. 2. A) Platelet pattern on the first admission. Distribution of platelets is abnormal. Note increased large platelets including irregular type. Solid line expresses the pattern of platelets counted by automatic microcellcounter. Size of platelets represents as a volume, fl, in the system of this equipment. B) Large platelets containing irregular type are observed in the smear on the first admission. Abnormal pattern of platelets is consistent with the findings of blood smear. ($\times 250$). C) Normal platelet pattern on day 21. There is no difference on the distribution of platelets between the case and healthy dogs. D) Blood smear showing normal pattern of platelets on day 21. There is no abnormal platelets in this smear. ($\times 250$).

were carried out after washing with saline containing antibiotics. The number of circulating leukocytes temporarily decreased ($26,000/\mu\text{l}$) on day 1. The second left gland was undulant on day 2. Increased peripheral leukocytes ($48,600/\mu\text{l}$), decreased circulating platelets ($179,000/\mu\text{l}$), abnormal pattern of platelets, and bad physical conditions were seen on the same day. An amount of 10 ml of affected brown milk was taken from the left gland. Leukocytes decreased on day 3, though decreased circulating platelets ($107,000/\mu\text{l}$), marked abnormal pattern of platelets, and necrosis of the left gland were found. The left gland was surgically cut and washed with saline containing antibiotics. Necrotized acini from the right and left gland were noted on day 1 to 5 and day 3 to 8, respectively. Circulating platelets increased from day 4 to day 6. On day 7, increased leukocytes and a decreased platelets were observed. However, there were no abnormal findings including pattern of platelets after that, except for a rebound of platelets on day 8 (Figs. 2C and 2D). Hepatozoon sp. in the leukocyte could be seen on the peripheral blood smear inspected on day 11. Although

discharges were observed for a long while in both glands, the dog was healed of the mastitis on day 25.

Limulus test using patient plasma was positive, suggesting that some clinical manifestations were induced by endotoxemia [3, 7, 10, 13, 15]. Gram-negative bacteria were found on the smear in the microbiological examination at the first admission. However, gram-negative bacteria were not predominant in the presented case, because *S. aureus* was purely isolated in the microbiological examination. Severe inflammation may be attributed to many factors such as the organism, time of infection, stasis of milk, and host resistance [2, 5, 8, 9, 14]. This inflammatory response plays an important role in the induction of the abnormal findings of platelets. Platelets participate in the clearance of bacteria, amplification and modulation of inflammation, and tissue damage [3, 7]. In addition, platelet aggregation is stimulated by many inflammatory agents, such as adenosine diphosphate, thromboxane A_2 , platelet activating factor, and platelet-bound immunoglobulin G [3]. Enhanced platelet aggregation and vasoconstriction assist in the induction of micro

Fig. 3. Clinical course of the dog.

thrombus or DIC [3, 7]. Test of fibrinogen degradation products was not done, though these phenomena might be present in this case. Hence, there may be great consumption of platelets in the gangrenous mastitis associated with *S. aureus*. This phenomenon induces decreased platelets and abnormal sizes (pattern) of platelets [7].

On the other hand, infection of *S. aureus* is one of the key events in the induction of platelet abnormalities. Ikejima *et al.* [6] demonstrated 20,000–30,000 dalton toxin produced by *S. aureus* isolated from patients with toxic shock syndrome. This toxin induced production of interleukin 1 (IL-1). Circulating IL-1 and tumor necrosis factor (TNF) were observed in rabbits experimentally treated with heat-killed *S. epidermidis* [15]. IL-1 and TNF act on the vascular endothelial cells to induce procoagulant activity and plasminogen activator inhibitor [1, 11, 12]. Anticoagulant protein C pathway is suppressed by TNF [12]. These reactions promote DIC and platelet aggregation, thereby leading to further consumption of platelets. Toxin produced by *S. aureus* may contribute to the induction of platelet abnormalities found in this case through production of inflammatory cytokines such as IL-1 and TNF.

Even if normal hemostasis is detected, systemic examinations for the blood coagulation should be done in small animals with severe infection to demonstrate the correlation between platelet abnormalities and coagulopathy. But these tests are usually less readily available and much more costly to perform. While, platelet count and platelet pattern can be routinely examined everywhere. Platelet abnormalities were associated with the clinical signs, lesions, and number of circulating leukocytes in this case (Fig. 3). The number and pattern of platelet may provide a clue for the evaluation of the clinical condition

and/or severity of the diseases.

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