Haematologic and biochemical changes in bitches with clinical and subclinical mastitis

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Abstract

Mastitis in bitch is in general superficially researched and evaluated. Data regarding blood tests during bitch glandular tissue inflammation is scarcely documented, therefore this paper aims to assess blood response in such cases. A total of 74 blood samples were obtained from lactating dams. These included females with *Lactatio sine graviditate*, with or without overt signs of mammary gland illness and with or without other signs of disease. Routine complete blood count and serum total proteins were evaluated. Complete blood count and total serum protein levels showed great flexibility, thus making it very difficult to establish and describe a clear and common haematological and biochemical pathway during mastitis inflammatory response. Clinicians should not rely only on blood tests whenever dealing with such pathology. Complete blood count and total serum protein levels should only be used as complementary tests, alongside other assays such as milk microbiology or cytology.

Keywords: *bitch, complete blood count, mastitis, serum total protein*

INTRODUCTION

In current veterinary practice, mammary gland inflammation diagnostic is based mainly on history, clinical and microbiological examination (Traschet *et al.*, 2008). There is scarce data regarding blood changes during clinical or subclinical mastitis episodes in this species (Hasegawa *et al.*, 1993). However, there is one experimental study (Ververidis *et al.*, 2007). Where leukocytosis was observed immediately after mammary gland bacterial challenge. At 12 hours, shortly after inoculation, a drop in the leukocyte count was observed, then followed by an immediate increase. On the sixth day, after challenge, leukocytes were in normal range. Neutrophilia was also observed shortly after challenge, but after the 6th day, this parameter dropped in range as well. Severe thrombocytopenia was recorded in the first hours after inoculation, followed by an increase in the 4th day. However, there were no

important changes in total serum protein levels (Ververidis *et al.*, 2007).

The aim of this research was to evaluate whether complete blood count (CBC) and serum total proteins in lactating bitches (*antepartum*, *postpartum* and *Lactatio sine graviditate*), with or without overt signs of mastitis and other concurrent signs of illness, follow the same type of haematological or biochemical blood response.

MATERIALS AND METHODS

In order to evaluate haematological and biochemical changes during bitch mastitis cases, 100 lactating dams were included in a case-controlled study, but because of owners reluctance, we were only able to obtain 74 blood samples.

Dams in this study were divided into two groups. Females with primary mastitis and females with secondary mastitis. During consult,

females with concurrent affections were included in the second category (i.e. secondary mastitis), whereas clinically healthy females or just with clinical or subclinical mastitis, were included in the first category.

Haematology and biochemistry assays were processed on venous blood after a thorough local disinfection with betadine (Betadine 100 ml, Egis Pharmaceuticals Ltd., Budapest, Hungary). After collection, blood samples were rushed as quickly as possible to the laboratory at the University of Agricultural Science and Veterinary Medicine Cluj-Napoca.

Complete blood count was determined with Abacus Junior Vet analyzer (Diatron Messtechnik, Budapest, Hungary) whilst differential blood count was counted on blood film stained with Dia-Quick Panoptic dye kit (Reag-Fix Panoptic, ReagensKft., Budapest Hungary). Biochemical results for total serum protein, were obtained with a Spectrophotometer analyzer UV-VIS Screen Master Touch (Hospitex Diagnostix, Fiorentino, Italy).

Complete blood count and serum proteinogram changes prevalence and its 95% confidence intervals were assessed. The differences were considered significant if p values were lower than 0.05. Data analysis was performed using EpiInfo 7 software (CDC, USA).

RESULTS AND DISCUSSION

In bitches with primary mastitis, 40% (4/10, CI 95%: 6.67-65.25) of cases were recorded with Normocytic hyperchromic followed by Normocytic normochromic and Microcytic hypochromic mild anemia in 20% (2/10, CI 95%: 2.52-55.61) of tested samples. Normocytic normochromic moderate anemia and Normocytic hypochromic mild anemia were also present in 10% (1/10, CI 95%: 0.25-44.50) of the tested cases, each (Tab.1) ($p>0.05$). Presence of mild anemia could have been caused by the loss of blood due to inflamed mammary glands and infections (Kerr, 2002a).

Thrombocytopenia was recorded in 60% (3/5, CI 95%: 14.66-94.73) of cases, whilst 40% (2/5, CI 95%: 5.27-85.34) were recorded with Thrombocytosis ($p>0.05$).

Mild Leucocytosis was encountered in 4 (4/4, CI 95%: 10.00) of the primary mastitis bitches ($p>0.05$).

The CBC showed that Eosinopenia was present in 21% (4/19, CI 95%: 6.05-45.57) of cases whilst Eosinophilia was present in only 5% (1/19, CI 95%: 0.13-26.03) of the tested females. Monocytopenia with eosinophilia was encountered in 11% (2/19, CI 95%: 1.30-33.14) of the samples, followed by Monocytosis with eosinophilia and by Lymphocytosis with eosinopenia in 5% (1/19, CI 95%: 0.13-26.03) of the cases, each (Tab.1) ($p>0.05$).

In 16% (3/19, CI 95%: 3.38-39.58) of samples, CBC encountered Neutrophilia with left shift along with eosinophilia, 11% (2/19, CI 95%: 1.30-33.14) were with Neutrophilia with left shift along with monocytosis, showing chronic signs of disease ($p>0.05$).

Without correlation with clinical features, 5% (1/19, CI 95%: 0.13-26.03) of the samples presented, Neutrophilia with a left shift with eosinopenia, lymphocytosis and eosinopenia, lymphopenia, monocytopenia and eosinophilia and also with Neutropenia with a right shift along with monocytosis, lymphocytosis and eosinophilia, each (Tab.1) ($p>0.05$).

In 31% (5/16, CI 95%: 11.02-58.66) of tested samples Hyperproteinemia with hyperglobulinemia was encountered, followed by 25% (4/16, CI 95%: 7.27-52.38) of dams with Hyperglobulinemia. In 13% (2/16, CI 95%: 1.55-38.35) of cases presence of Hyperproteinemia with hyperalbuminemia and Hyperproteinemia with hyperalbuminemia and hyperglobulinemia was noticed. In just 6% (1/16, CI 95%: 0.16-30.23) of cases Hyperalbuminemia followed by Hypoproteinemia and Hyperalbuminemia with hyperglobulinemia were encountered (Tab.1) ($p>0.05$).

Usually, elevated levels of total serum proteins, including, albumins and globulins could be caused by dehydration, chronic inflammation or chronic subacute bacterial infection (Kerr, 2002b). These hypothesis can explain the presence of high levels of these parameters in primary mastitis bitches.

In one documented bitch *Mastitis gangrenosa* case report, where biochemical and haematological changes were recorded, results showed presence of severe leukocytosis (36,300/ μ l) with marked elevated α , β and γ -globuline levels, moderate anemia, a slightly increased level of alkaline phosphatase and creatine phosphokinase with moderate hypernatremia and hypokalemia. On the day of admission, the amount of circulating leukocytes decreased temporarily to 26,000/ml,

Tab. 1. Hemoleucogram and biochemical results in both types of bitch mastitis

No.	Diagnostic – Primary mastitis	Percentage	No.	Diagnostic – Secondary mastitis	Percentage
WBC			WBC		
1	Mild leukocytosis	100%	1	Severe leukopenia	14%
			2	Moderate leukocytosis	29%
			3	Severe leukocytosis	29%
			4	Mild leukocytosis	29%
RBC			RBC		
2	Normocytic normochromic moderate anemia	10%	5	Normocytic hypochromic moderate anemia	14%
3	Normocytic hypochromic mild anemia	10%	6	Normocytic hypochromic mild anemia	14%
4	Microcytic hypochromic mild anemia	20%	7	Normocytic normochromic mild anemia	14%
5	Normocytic normochromic mild anemia	20%	8	Normocytic hyperchromic mild anemia	57%
6	Normocytic hyperchromic mild anemia	40%			
PLT			PLT		
8	Thrombocytosis	40%	9	Thrombocytosis	33%
9	Thrombocytopenia	60%	10	Thrombocytopenia	67%
CBC			CBC		
10	Eosinophilia	5%	11	Monocitopenia with eosinophilia	6%
11	Lymphocytosis with eosinopenia	5%	12	Neutrophilia with left shift with lymphocytosis and eosinopenia	6%
12	Monocytosis with eosinophilia	5%	13	Neutrophilia with left shift with lymphopenia	6%
13	Neutrophilia with left shift with eosinopenia	5%	14	Neutrophilia with left shift with lymphopenia, monocytopenia and eosinopenia	6%
14	Neutrophilia with left shift with lymphocytosis and eosinopenia	5%	15	Neutrophilia with left shift with monocytosis	6%
15	Neutrophilia with left shift with lymphopenia	5%	16	Neutropenia with eosinophilia	6%
16	Neutrophilia with left shift with monocytopenia and eosinophilia	5%	17	Neutrophilia with left shift	12%
17	Neutropenia with right shift with monocytosis, lymphocytosis and eosinophilia	5%	18	Neutrophilia with left shift with eosinopenia	12%
18	Monocytopenia with eosinophilia	11%	19	Neutrophilia with left shift with monocytosis and eosinophilia	12%
19	Neutrophilia with left shift with monocytosis	11%	20	Neutrophilia with left shift with lymphopenia and eosinopenia	29%
20	Neutrophilia with left shift with eosinophilia	16%			
21	Eosinopenia	21%			
Total Proteins			Total Proteins		
22	Hyperalbuminemia	6%	21	Hyperalbuminemia	13%
23	Hyperalbuminemia with hyperglobulinemia	6%	22	Hyperglobulinemia	13%
24	Hyporproteinemia	6%	23	Hyperproteinemia with hyperalbuminemia	20%
25	Hyperproteinemia cu hyperalbuminemia	13%	24	Hyperproteinemia with hyperglobulinemia	20%
26	Hyperproteinemia with hyperalbuminemia and hyperglobulinemia	13%	25	Hyperproteinemia with hyperalbuminemia and hyperglobulinemia	33%
27	Hyperglobulinemia	25%			
28	Hyperproteinemya with hyperglobulinemia	31%			

but then increased to 48,600/ml. Along with these parameters, thrombocytopenia (179,000/ml) with giant platelets were also recorded (Hasegawa *et al.*, 1992). These changes are partially confirmed by our study, where mild to severe leukocytosis were recorded (Tab. 1) alongside with a left shift and increase in total protein serum levels (Tab. 1).

In bitch *Mastitis acuta* reports, leukocytosis with or without a left shift was encountered (Lopate, 2012). In a recent bitch *Mastitis acuta* case report, the authors (Vasiu *et al.*, 2015) indicated presence of moderate anemia with leukocytosis with a left shift. Presence of eosinophilia with dehydration along with elevated liver enzymes and renal biomarkers with electrolyte imbalance, had also been reported (Lopate, 2012). Eosinophilia and eosinopenia, alongside various combinations of other types of leukocytes were, as well, recorded in our study.

In secondary mastitis cases, Normocytic hyperchromic mild anemia was diagnosed in 57% (8/14, CI 95%: 28.86-82.34) of cases, alongside of Normocytic hypochromic moderate anemia, Normocytic hypochromic mild anemia and Normocytic normochromic mild anemia in 14% (2/14, CI 95%: 1.78-42.81) of samples, each.

Thrombocytopenia was registered in 67% (4/6, CI 95%: 22.28-95.67) of cases, whilst 33% (2/6, CI 95%: 4.33-77.72) of the samples were with Thrombocytosis (Tab.1).

Test results showed that in secondary mastitis cases, mild, moderate and severe Leukocytosis were encountered in 29% (2/7, CI 95%: 3.67-70.96) of cases, each. Severe Leukopenia was registered only in 14% (1/7, CI 95%: 0.36-57.87) of the tested bitches.

Complete blood count showed presence of Neutrophilia with left shift with lymphopenia and eosinopenia in 29% (5/17, CI 95%: 10.31-55.69) of cases, followed closely by Neutrophilia with left shift with eosinopenia and with monocytosis and eosinophilia in 12% (2/17, CI 95%: 1.46-36.44) of cases, each.

Neutrophilia with left shift with monocytosis, with lymphopenia, monocytopenia and eosinopenia and lymphocytosis and eosinopenia were diagnosed in 6% (1/17, CI 95%: 0.15-28.69) of cases, each.

In 33% (5/15, CI 95%: 11.82-61.62) of evaluated samples, Hyperproteinemia with hyperal-

buminemia and hyperglobulinemia were recorded, followed by Hyperproteinemia with hyperalbuminemia and Hyperproteinemia with hyperglobulinemia in 20% (3/15, CI 95%: 4.33-48.09) of cases, each and Hyperalbuminemia and Hyperglobulinemia in 13% of tested samples (2/15, CI 95%: 1.66-40.46), each (Tab.1).

In secondary mastitis bitches, blood parameters should always be interpreted according to the primary underlying causes.

CONCLUSION

Haematology and biochemical assays proved to be modest and limited. The use of these tests in order to evaluate mammary gland health status in bitch should resume only as complementary assays. However, one should try to evaluate bitch mammary gland health status, alongside clinical and other paraclinical (i.e. microbiological) testing. Clinicians should avoid relaying only on these test whenever treating or diagnosing mastitis in the bitch. In cases where mastitis is secondary, one should always carefully interpret blood test and treat the dam, according to the underlying cause.

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