**Thrombocytopenia in dogs with babesiosis – the role of extracellular histones**

Barić Rafaj Renata1 \*PhD, Tumpa Andrea1 Mag.Med.Biochem, Bilić Petra2 Mag.Mol.Biol., Gotić Jelena2 PhD, Mrljak Vladimir2 PhD

1Department of Chemistry and Biochemistry and 2Clinic for Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb, Croatia

Corresponding author\*: Renata Barić Rafaj, Department of Chemistry and Biochemistry, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia; tel ++385 1 2390 301, e-mail: rrafaj@vef.hr

**Introduction**: Babesiosis is a tick-borne malaria-like illness, zoonotic infection caused by species of the intraerythrocytic protozoan Babesia, thought to be the second-most common blood [parasite](https://en.wikipedia.org/wiki/Parasite" \o "Parasite) of mammals. The most frequent hematological abnormalities in babesiosis are anemia and thrombocytopenia. There is emerging evidence that platelets are major contributors to inflammatory processes through the cooperation with innate immune cells, in which neutrophils can release DNA fibers with histones to form extracellular traps (NETs) (*Kapur and Semple, 2016*). Parasites bind to NETs and are killed by the antimicrobial actions of histones (*Caudrillier et al., 2012*). Since platelets also bind to extracellular histones, we reasoned that this pathophysiological mechanism could be one of the causes of thrombocytopenia in babesiosis.

**Material and methods**: 18 dogs with acute babesiosis (B) and 18 healthy control dogs (C) participated in the study. CBC was performed using ABC Vethematologyanalyzer (ABX Diagnostics). Histone (H2A) concentration was measured with Canine ELISA kit (MyBioSource, San Diego, CA). To The significance was determined using Student’s *t* test. *P* values of less than 0.05 were determined to be significant.

**Results:** Table 1: Concentration of circulated histones and number of blood

cells in dogs with babesiosis compared with healthy dogs

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| --- | --- | --- | --- |
|  | **C-control (n=18)**  mean±SD | **B-babesiosis (n=18)**  mean±SD | ***p* (t- test**)  **\****p*<0,05 |
| **H2A** (ng/ml) | 23,9±12,2 | 37,5±24,8 | 0,04**\*** |
| **PLT** (x109/L) | 273±76,3 | 40±18,9 | 0,0000001**\*** |
| **RBC** (x1012L) | 6,7±0,8 | 5,1±0,9 | 0,000003**\*** |
| **WBC** (x109/L) | 8,4±2,4 | 5,9±2,2 | 0,002**\*** |

**Discussion**: Babesiosis is characterized by increased circulated histones, anemia, leucopenia and thrombocytopenia. Extracellular histones have been shown to play an important pathogenic role in many diseases, primarily through their cytotoxicity towards nucleated cells and their ability to promote platelet activation (*Kordbacheh et al., 2017*). Moreover, the binding of platelets to histones may lead to platelet activation and propagation of platelet-platelet binding around NETs (*Caudrillier et al., 2012*). Histones induce *in vivo* anemia, thrombocytopenia and leukopenia within a few minutes (*Kordbacheh et al., 2017*). Histone release have recently been described in critically ill humans, where histone-associated thrombocytopenia is described as a new cause of this condition (*Thachil and Warkentin, 2017*). To date, the mechanisms of thrombocytopenia in canine babesiosis include disseminated intravascular coagulation, sequestration in the spleen, increased body temperature and immune-mediated lysis. Circulating histones might also be responsible for thrombocytopenia, as well as for the anemia and leucopenia, and should be taken into account in the approach to the thrombocytopenic patients, where targeting NET formation may be a promising new direction for the treatment of cytopenias in inflammatory diseases.

**References:**

**1**. Kapur R, Semple JW . (2016): The nonhemostatic immune functions of platelets. Semin Hematol. 53 (Suppl 1):S2-S6. **2.**[Caudrillier A,](javascript:void(0);) [Kessenbrock K, Gilliss B.M., Nguyen J.X.,](javascript:void(0);) [Marisa B. Marques M.B.,](javascript:void(0);) [Monestier M.,](javascript:void(0);)  [Toy P., Werb Z, Looney M.R.](javascript:void(0);) (2012): Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. J Clin Invest. 122(7):2661–2671. doi:10.1172/JCI61303.

**3.**  Kordbacheh F, O'Meara C.H., Coupland L.A,, Lelliott P.M., Parish C.R. (2017): Extracellular histones induce erythrocyte fragility and anemia. Blood 2017 :blood-2017-06-790519; doi: <https://doi.org/10.1182/blood-2017;06-790519> **4.** Thachil, J. and Warkentin, T.E. (2017): How do we approach thrombocytopenia in critically ill patients? Br J Haematol. 177(1):27-38. doi: 10.1111/bjh.14482.