IJIRR

International Journal of Information Research and Review
Vol. 03, Issue, 11, pp. 2971-2975, November, 2016

Research Article

SPLEEN BENIGN AND MALIGNANT NEOPLASTIC DISEASE AND IT INFLUENCE ON RED BLOOD CELLS, MEAN CORPUSCULAR VOLUME, AND PLATELET COUNT VARIATIONS IN DOGS SUBMITTED TO TOTAL SPLENECTOMY

[1,2,3,4*]Miguel Carreira, L., [3]Andreia Marques,[3]Rita Ramalho,[3]Rita Sousa and [3]Pedro Azevedo

[1]Faculty of Veterinary Medicine, University of Lisbon (FMV/ULisboa), Portugal
[2]Interdisciplinary Centre of Research in Animal Health (CIISA), FMV/ULisboa, Portugal
[3]Anjos of Assis Veterinary Medicine Centre (CMVAA), Barreiro, Portugal
[4]Private Practice in Human Dentistry, Lisbon, Portugal

ARTICLE INFO

Article History:

Received 28th August, 2016
Received in revised form
22nd September, 2016
Accepted 15th October, 2016
Published online November, 30th 2016

Keywords:

Dog,
Splenectomy,
Red blood cells,
Mean corpuscular volume,
Platelets.

ABSTRACT

The effects of the different type of spleen neoplastic disease on red blood cells (RBCs), mean corpuscular volume (MCV), and platelet count (PLT) are unknown. We examined 88 dogs (n=88) with benign (GB) or malignant (GM) spleen neoplasia and examined variations in RBCs, MCV, and PLT before(M1), 48 hours after(M2), and 8 days after total splenectomy surgery(M3). Overall, between M1 and M2, RBCs decreased by 12.7%, MCV increased by 0.7%, and PLT increased by 74.3%; between M1 and M3, RBCs decreased by 4.11%, MCV increased by 2.04%, and PLT increased by 129.82%. Variations across M1-M3 for GB and GM patients were, respectively, a 7.14% increase and 15.28% decrease in RBCs, 2.12% and 1.87% increases in MCV, and increases of 248.16% and 103.15% in PLT. We concluded that the type of spleen neoplastic disease does not influence in a statistically significant manner the RBC, MCV, and PLT variations in dogs that underwent total splenectomy.

INTRODUCTION

The spleen is a falciform, long, and narrow organ integrated in the immune system, usually located at the left cranial abdominal quadrant below the costal arch (Dyce *et al*., 1997; Fossum, 2007; Tillon, 2003). The spleen consists of a white pulp with lymph follicles presenting normal phagocytic and lymphogenic properties, intimately related with the splenic arteries, a red pulp composed of the tissue that fills the inter-vessel spaces and venous sinusoids, which are closely associated with the veins (Fossum, 2007; Ellenport, 1986). Among other functions, the spleen is an important blood reservoir which may contract to increase blood and cellular volume, particularly for the erythrocytes and platelets, releasing blood with high hematocrit (with values of around 80% to 90%) (Dellman and Brown, 1976; Dernell, 2005; Otero *et al*., 2004; Carneiro and Donald, 1977; Christopher, 2003; Young, 2007).

Corresponding author: Miguel Carreira, L.,
[1]Faculty of Veterinary Medicine, University of Lisbon (FMV/ULisboa), Portugal
[2]Interdisciplinary Centre of Research in Animal Health (CIISA), FMV/ULisboa, Portugal
[3]Anjos of Assis Veterinary Medicine Centre (CMVAA), Barreiro, Portugal
[4]Private Practice in Human Dentistry, Lisbon, Portugal.

The spleen presents a high prevalence of benign and malignant primary tumours, and is subject, to a lesser extent, metastasis processes, which manifest mostly as nodule formation. In dogs, 43% to 75% of splenomegaly cases are associated with neoplastic diseases, requiring partial or total splenectomy (Fry and McGavin, 2007; McGavin *et al*., 2001; Dobson, 2003; Morris and Dobson, 2001). Following removal of the total spleen, changes in the patient's blood are commonly noted, involving the red blood cells (RBCs) and the platelets (PLTs) (Couto, 1998; Autran de Morais and O'Brien,2005; Schilling *et al*., 2008). The study aimed to: 1) evaluated the RBC, corpuscular mean volume (MCV), and PLT variations over 3 different time points: M1 (before surgery), M2 (48 hours after surgery), and M3 (8 days after surgery) in total splenectomised patients; and 2) to determine if benignancy or malignancy of the tumour influenced these parameters.

MATERIALS AND METHODS

The study was developed in a sample of 88 *Canis familiaris* adults (n=88) of both genders (54.5% females and 45.5% males), with an average age of 10 years evaluated at Anjos of Assis Veterinary Medicine Centre (CMVAA), with a diagnosis

of spleen pathology, confirmed with ultrasound image and fine-needle aspirative puncture. All patients were submitted to total splenectomy intra-abdominal surgery, with histopathology results dividing the sample into two different groups, according to the type of neoplastic disease: benign spleen neoplastic disease (GB) and malignant spleen neoplastic disease (GM). Inclusion criteria were: specimens were up-to-date for vaccinations and internal and external deworming, and negative for haemoparasites.

Three different time points were considered: M1, M2, and M3, to evaluate the RBC, MCV, and PLT variations. Pre-surgical therapeutic protocol comprised the use of amoxicillin/clavulanic acid (10 mg/kg), metronidazol (10 mg/kg), buprenorphine (0.02 mg/kg), and intra-surgery methylprednisolone succinate (1 mg/kg). Anaesthesia induction used propofol (4mg/Kg), and maintenance was achieved with isoflurane. Data were collected and we used SPSS Statistics software for statistical analyses. Because normality was not always present, we used the non-parametric Friedman and the Mann-Whitney tests to compare RBC, CVM, and PLT variations between the 3 time points. Results were considered significant for p-values <0.05.

RESULTS

Statistical analyses for sample characterisation and RBC, MCV, and PLT variations over M1, M2, and M3, and between GB and GM, are presented in Table 1. GB represented 25.0% of the processes, mainly represented by follicular hyperplasia, and GM represented 75.0% of the cases, primarily lymphoma (Figure 1). No significant statistical differences were observed for the RBC, MCV, and PLT between M1, M2, and M3, and no differences were registered between their variations in GB and GM (Figure 2, 3, 4).

DISCUSSION

According to the results, the most representative breed was the Boxer (27.3%), followed by the Poodle, then the Belgian Shepherd and Golden Retriever (18.2% for both), different from the studies of Spangler and Culbertson (Spangler and Culbertson, 1992) and Spangler *et al*. (1994), in which the German Shepherd was the breed with the highest incidence of spleen pathology and the second position was occupied by the Golden Retriever. The average age of the patients with splenic disease was 10 years old, in accordance with the Gamlem *et al*.(2008) study, which mentions that this type of disease is more frequent in patients more than 6 years old.

Contrary to the Gamlem *et al*. (2008) study, females (54.5%) were more affected than males (59.4%). Spangler and Kass (1997) classified spleen diseases into non-neoplastic and neoplastic, and the latter was classified as benign or malignant. According to the results, a higher prevalence of malignant neoplasia (75.0%) was found, compared to the benign neoplasia (25.0%), in agreement with the results of the Spangler and Kass (1997) and Gamlem *et al*. (2008), with values of 89.0% and 84.54% for malignant neoplastic diseases, and 11.0% and 15.46% for benign neoplasia, respectively. Concerning the process characterization, the study results are in line with those developed by Culbertson and Spangler (1992) and Spangler and Kass (1997).

Malignancy showed a higher prevalence, led by lymphoma, with a value of 27.3%, which can be directly related to the fact that the Boxer was the most common breed in this study and this breed presents a greater prevalence of this disease at all ages (from under 6 months of age until up to 15 years) (Spangler and Klass, 1997; Ettinger, 2003; Gavazza, *et al*., 2009; Smith, 2003; Sankhauser *et al*., 2004).

Table 1. Characterization of red blood cells (RBC) mean corpuscular volume (MCV), and platelets (PLT) variations over the 3 considered time points M1, M2, M3

Variations of the parameters over time points M1, M2, M3											
Parameter	N	Test	TP	$\overline{x}$ ± SD	min	max	df	χ^2	Asymp. Sig	p-value	U-value
RBC	88	-	M1	4.36 ±1.96	2	9	-	-	-	< 0.00	-
(x10^6/µl)	88	-	M2	3.64 ±1.43	2	6	-	-	-	< 0.00	-
	88	-	M3	3.73 ±1.56	1	6	-	-	-	< 0.00	-
	88	Friedman	-	-	-	-	2	11.14¥	0.62		
	88	Mann-	M1 vs M2	-	-	-	-	-	-	0.69	89.0*
	88	Whitney	M1 vs M3	-	-	-	-	-	-	0.72	90.0*
	88		M2 vs M3	-	-	-	-	-	-	0.77	91.5*
MCV	88	-	M1	62 ±6.51	49	72	-	-	-	< 0.00	-
(fL)	88	-	M2	62.36 ±5.99	50	71	-	-	-	< 0.00	-
	88	-	M3	63.18 ±6.39	50	72	-	-	-	< 0.00	-
	88	Friedman	-	-	-	-	2	4.71¥	0.03	-	-
	88	Mann-	M1 vs M2	-	-	-	-	-	-	0.23	66.0*
	88	Whitney	M1 vs M3	-	-	-	-	-	-	0.94	89.0*
	88		M2 vs M3	-	-	-	-	-	-	0.35	77.5*
PLT (x10^3/	88	-	M1	230.82±198.62	38	600	-	-	-	0.31	-
µl)	88	-	M2	278.91±183.73	83	630	-	-	-	0.04	-
	88	-	M3	337.18±209.18	160	811	-	-	-	0.02	-
	88	Friedman	-	-	-	-	2	12.28¥	0.70	-	-
	88	Mann-	M1 vs M2	-	-	-	-	-	-	0.24	72.0*
	88	Whitney	M1 vs M3	-	-	-	-	-	-	0.28	74.5*
	88		M2 vs M3	-	-	-	-	-	-	0.80	92.0*

Sample (N); Data mean ($\overline{x}$) and dispersion (SD) measures obtained in a 95% confidence interval, presenting the minimum (min) and maximum (max) values in the 3 surgical time points (TP) considered: M1 (before surgery), M2 (48 hours after surgery), and M3 (8 days after surgery). Degrees of freedom (df)

¥ The critical value of χ^2 at $p< 0.05$ is < 5.99

*The critical value of U at $p< 0.05$ is 55.

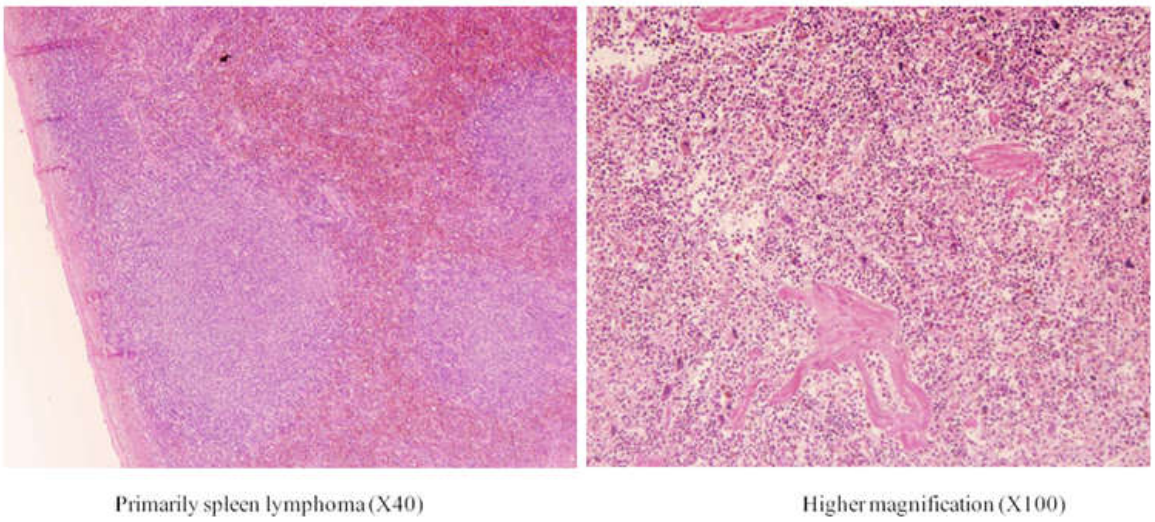

Figure 1- Primarily spleen lymphoma. It is possible to verify the infiltration of the spleen structure by large number of neoplastic lymphoid cells (H&E, X40). Higher magnification of the previous picture, being possible to observe the infiltrated lymphoid population homogeneity, and a large number of megakaryoblasts indicating extramedullary hematopoiesis (H&E, X 100)

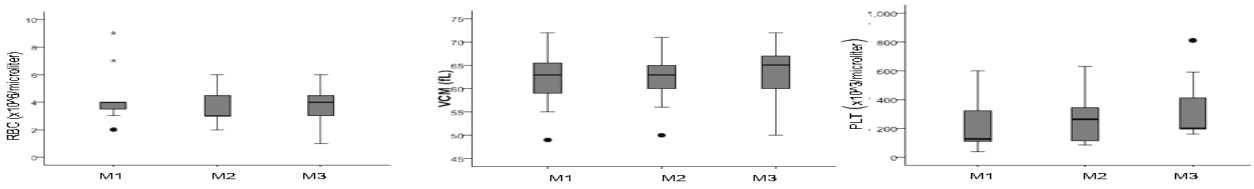

Figure 2- Distribution of red blood cells, mean corpuscular volume, and platelets on the 3 considered time points: M1 (pre-surgery), M2 (48 hours after surgery), and M3 (8 days after surgery)

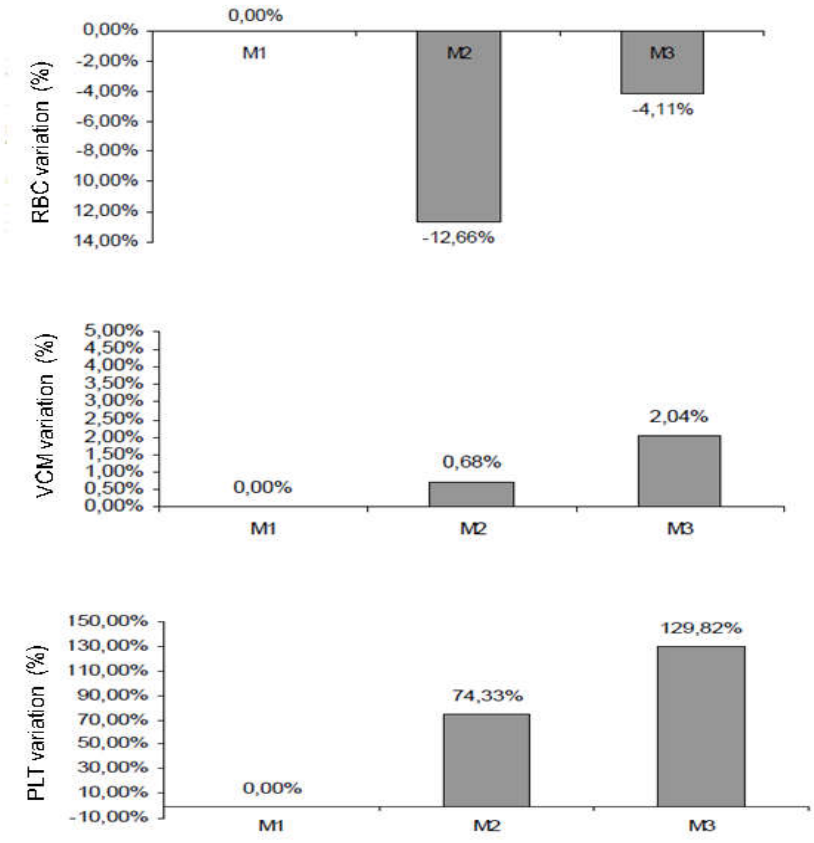

Figure 3- Red blood cells, mean corpuscular volume, and platelets mean percentage variations in time points M2 (48 hours after splenectomy) and M3 (8 days after splenectomy) respect to M1 (pre-operative) values

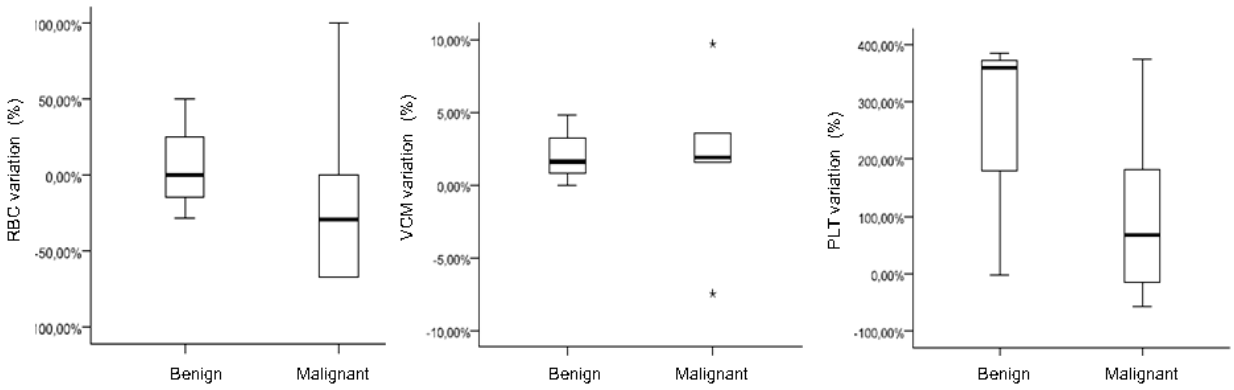

Figure 4- Red blood cells, mean corpuscular volume, and platelets number percentage variation according to the type of spleen neoplastic disease (benign or malignant) presented in M1 (pre-operative), M2 (48 hours after splenectomy), and M3 (8 days after surgery)

Between M1 and M2, we observed a 12.7% decrease in the amount of RBCs, a 0.7% increase of in the MCV, and a 74.3% increase in the number of PLTs, in agreement with studies by Hayes *et al*. (1963), Schalm *et al*. (1975), Athens *et al*. (1993), and Khan *et al*. (2009), resulting from spleen sequestration of a considerable volume of blood during patient anaesthesia, maintaining its important function as a blood reservoir (Athens,1993), and because total splenectomy is associated with a significant loss of blood volume and its figurative elements (Schalm *et al*.,1975; Athens, 1993; Waldmann *et al*., 1960).

Comparing the studied parameters and their variations between M1 and M3, RBCs decreased by 4.11%, MCV increased by 2.04%, and PLTs increased by 129.82%, as in previous studies. These increases may be related to a positive response from the bone marrow to anaemia and the therapeutic protocol. The MCV increase from its baseline in M1 can be explained by the fact that the spleen is responsible for sequestration of the physiological reticulocytes until they reach a mature state, at which its volume reduces. After a total splenectomy, sequestration of RBCs ceases and thus the reticulocytes' volume reduction process no longer occurs, resulting in an increase of MCV and other leptocytic forms. The PLT number increase may extend over 15 weeks after splenectomy, and may persist for a period of months or even years, because splenectomy leads to the development of a reactive thrombocytosis, allowing the spleen to concentrate about 1/3 of the all available PLTs in the body.

The differences occurring in these parameters between GB and GM reflect the evolution from an anaemic state revealed at M1, noting that most of GB individuals showed a positive trend regarding the recovery of the existing anaemia, contrary to what was seen with GM individuals. This may be due to a possible development of a haematologic paraneoplastic syndrome, which complicates the recovery of anaemia in GM, even leading to their deterioration. The development of anaemia is the most common paraneoplastic syndrome present in dogs, with a prevalence of 30.0% to 40.0% in this specie with malignant lymphoma and 69.0% in dogs with hemangiosarcoma, which in GM had a prevalence of 50.0% and 16.7%, respectively (Gaschen and Teske,2005). For the PLTs, the difference between GB and GM may be explained by the presence of the haematologic paraneoplastic syndrome related to thrombocytopenia, due to a marked difficulty in reaching a PLT increase after total splenectomy. The phenomenon of thrombocytopenia occurs, according to Gaschen and Teske (2005), in 50.0% of dogs with lymphoma and is even considered, according to Gould and Sara (2003), as the most frequent laboratory sign of this disease in dogs, which had a prevalence of 50.0% among GM individuals. Although clinically present, variations in the RBCs, MCV, and PLTs between GB and GM, along M1, M2, and M3 were not statistically significant, allowing us to conclude that the type of neoplastic disease present in spleen (benign or malignant) does not influence, in the first 8 days after a total splenectomy, the type of variations that occur in the patients' RBCs, MCV, and PLTs.

Acknowledgment

The authors thank to CIISA - Centre for Interdisciplinary Research in Animal Health, of Faculty of Veterinary Medicine of Lisbon, of the University of Lisbon – Portugal (FMV-ULisboa); to Anjos of Assis Veterinary Medicine Centre (CMVAA), Barreiro – Portugal. This manuscript is dedicated to my friend, colleague and pupil Dr. Ricardo Marques, who will always be with us.This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

REFERENCES

Athens, J.W. 1993. The reticuloendothelial (mononuclear phagocyte) system and the spleen. In: Lee, G.R., Bithell, T.C., Foerster, J., Athens, J.W. and Lukens, J.N. (Eds.) *Wintrobe's Clinical Hematology.* 9th Ed.. 311-325). Philadelphia, London: Lebiger.

Autran de Morais, H. and O'Brien, R.T. 2005. Non-neoplastic diseases of the spleen. In:Ettinger, S.J. and Feldman, E.C. (Eds.), *Textbook of Veterinary Interal Medicine.*6th Ed.1944-1957. St. Louis Missuri: Saunders Elsevier.

Carneiro, J.J. and Donald D.E. 1977. Blood reservoir function of dog spleen, liver and intestine. *American journal of physiology,* 232, 67-72.

Christopher, M.M. 2003. Cytology of the spleen. *The Veterinary Clinics Small AnimalPractice*, 33, 135-152.

Couto, C.G. 1998. Lymphadenopathy and splenomegaly. In: Nelson, R.W. and Couto,C.G. *Small animal internal medicine.*3rd Ed.1204-1209). St. Louis, Missouri: Mosby.

Dellman, E.H. and Brown, E.M. 1976. Sistema linfático. In: Dellman, E.H., Brown and E.M. *Histologia veterinária.*1st Ed.189-199. Zaragoza: Editorial Acriba.

Dernell, W.S. 2005. Hemangiosarcoma [versão electrónica]. In: Proceding of de North American Veterinary Conference, 8-12 Jan., Orlando, Florida, pp. 622-623.

Dobson, J.M. 2003. Tumours of the spleen. In: Dobson, J.M. and Lasceller, B.D.X.*BSAVA Manual of canine and feline oncology.* 2nd Ed. 293-296. Quedgeley, Gloucester: BSAVA.

Dyce, K.M., Sack, W.O. and Wensing, C.J.G. 1997. Abdomen dos carnivoros. In: Sack, K.M.,W.O. and Wensing, C.J.G. (Eds.), *Tratado de Anatomia Veterinária.*2nd ed. 410-411. Rio de Janeiro: Guanabara Koogan S. A.

Ellenport, C.R. 1986. Sistema linfático em geral. In: Getty, R. (Eds.), *Sisson/Grossman Anatomia dos animais domésticos.*5th Ed.,166-167. Rio de Janeiro: Guanabara Koogan S. A.

Ettinger, S.N. 2003. Principles of treatment for canine lymphoma. *Clinical Techniques in Small Animal Practice*, 18, 92-97.

Fankhauser, R., Leroy, B.E., Tarpley, H.L., Bain, P.J., Jonhson, M.A. and Latime, K.S. 2004. Canine Hemangiossarcoma.

Fossum, T.W. 2007. Surgery of the spleen. In: Fossum, T.W., Hedlund, C.S., Johnnson, A.L., Schulz, K.S., Seim III, M.B., Willard, M.D., Bahr, A. and Carroll, G.L. (Eds.), *Small Animal Surgery.* 3rd Ed. 624-634. St. Louis, Missoury:Mosby Elsevier.

Fry M.M. and McGavin M.D. 2007. Bone marrow, blood cells, and lymphatic system. In:McGavin, M.D. and Zachary, J.F. (Eds.), *Pathologic basis of veterinary disease*.4th Ed.751-755. St. Louis Missouri: Mosby Elsevier.

Gamlem H, Nordstoga K, Arnesen K. 2008. Canine vascular neoplasia--a population-based clinicopathologic study of 439 tumours and tumour-like lesions in 420 dogs. APMIS Suppl. 2008;(125):41-54.

Gaschen, F.P. and Teske, E. 2005. Paraneoplastic Syndrome. In: Ettinger, S.J. andFeldman, E.C. (Eds.), *Textbook of Veterinary Interal Medicine.*6th Ed.789-795. St. Louis Missuri: Saunders Elsevier.

Gavazza, A., Sacchini, F., Lubas, G., Gugliucci, B. and Valori, E. 2009. Clinical, laboratory, diagnostic and prognostic aspects of canine lymphoma: a retrospective study. *Comparative Clinical Pthology*, 18, 291-299.

Gould and Sara 2003. Paraneoplastic syndromes. In: Dobson, J.M. and Lasceller, B.D.X.,(Eds.), *BSAVA Manual of canine and feline oncology.* 2nd Ed. 293-296. Quedgeley, Gloucester: BSAVA

Hayes, D.M. and Spurr, C.L., Hutaff, L.W. and Sheets, J.A 1963. Post-splenectomy thrombocytosis. *Annals of internal medicine*, 58, 259-267.

Khan, P.N., Nair, R.J., Olivares, J., Tingle, L.E. and Li, Z. 2009. Post-splenectomy reactive thrombocytosis. *Proceedings - Baylor University. Medical Center*,22,9-12.

McGavin, M.D., Carlton, W.W. and Zachary, J.F. 2001. The hemaopoietic system. In: *Thomson's specialveterinary pathology.* 3rd Ed. 373-379. St. Louis Missouri: Mosby.

Morris, J. and Dobson, J. 2001. Miscelaneous tumours. In: Morris, J. and Dobson, J.*Small animal oncology.* 272-275. Ames, Iowa: Blackwell Science Press.

Otero, L.C., Pérez, M.J.B., Villamandos, J.C.G., Martinez, F.J.P., Campilho, J.S. and Alvarez, J.S. 2004. Sistema inmunológico y linfoide. In: Ortiz, A.G. and Rodríguez, A.B. (Eds.), *Tratado de Histologia Veterinária.*1st Ed.230-238. Barcelona: Masson.

Schalm, O.W., Jain, N.C. and Carroll, E.C. 1975. The hematopoietic system. In: *Veterinary Hematology.* 3rd Ed, 327-329. Philadelphia: Lea and Febiger.

Schilling RF, Gangnon RE, Traver MI. Delayed adverse vascular events after splenectomy in hereditary spherocytosis. J Thromb Haemost 2008;6:1289-95

Smith, A.N. 2003. Hemangiossarcoma in dogs and cats. *The Veterinary Clinic of Small Anaimal* Practice, 33, 533-552.

Spangler, W.L. and Culbertson, M.R. 1992. Prevalence, type, and importance of splenic diseases in dogs: 1,480 cases (1985-1989). *Journal of the American VeterinaryMedical Association*, 200, 829-834.

Spangler, W.L. and Kass, P.H. 1997. Pathologic factors affecting postsplenectomy survival in dogs. Journal of Veterinary Internal Medicine, 11, 166-171.

Spangler, W.L., Culbertson, M.R. and Kass, P.H., 1994. Primary mesenchymal (nonangiomatous/ nonlymphomatous) neoplasms occurring in the canine spleen: anatomic classification, immunohistchemistry, and mitotic activity correlated with patient survival. *Veterinary Pathology*, 31, 37-47.

Tillon, M. 2003. Speen. In: Slatter, D. (Eds) *Textbook of Small Animal Surgery.* 3rd Ed. 1046-1062. Philadelphia: Elsevier Science.

Waldmann, T.A., Weissman, S.M. and Berlin, N. 1960. The effect of splenectomy in the dog. *Blood*, 15, 873-883.

Young, K.M. 2007. Cytology of the spleen: basic and advanced cases. In: *Proceedings of the SCIVAC Congress, 1-3 June, Rimini, Italy*, 498-499.

****