Vitamin D and acute phase protein expression in dogs with multicentric lymphoma: association with clinicopathological parameters

•M.C.P. Rocha*, T.K.Garnica[2], H. Fukumasu[2] R.A.R. Uscategui[3], J.M. Pazzini[4] , F.A.R. Sueiro[5], I. Amorim[6], A.B. DeNardi*

*• *FCAV-UNESP-Paulista State University Julio de Mesquita Filho, [2]Department of veterinary medicine, Faculty of Animal Science and Food Engineering - University of São Paulo, Pirassununga, BR, [3]INCA-CES research group, Facultad de Medicina Veterinaria y Zootecnia, Universidad CES, Medellín, CO, [4]Department of General Surgery and Oncology, Clinic my pet -UniSALESIANO, Araçatuba, BR, [5]CEO (Pathology and Molecular Biology) Employment, VETPAT Campinas, São Paulo, Brazil, [6]ICBAS School of Medicine and Biomedical Sciences, Porto University, Porto, PT.*

INTRODUCTION

Lymphomas are a group of neoplasms that originate in lymphocytes witch represents 8.8% of all cancers in dogs. This study aimed to evaluate the serum concentration of vitamin D (25-Hydroxyvitamin D) and acute phase proteins (APPs; alpha1 acid glycoprotein, haptoglobin, transferrin, ceruloplasmin, albumin, IgA, IgG and alpha-1 - antitrypsin) as potential biomarkers for prognostic and therapy response in dogs with multicentric lymphoma submitted to CHOP (Cyclophosphamide, Doxorubicin, Vincristine and Prednisone) chemotherapeutic protocol. Serum vitamin D (vit D) concentrations and acute phase protein (APF) levels have been identified as prognostic and predictive factors with high sensitivity in several neoplasms, including lymphomas, in both humans and animals Conversely, APFs have already been listed as possible markers for multicentric lymphoma in dogs, including in cases of recurrence, demonstrating early changes in their concentrations.

MATERIALS AND METHODS

Thirteen dogs diagnosed with high grade multicentric lymphoma were included in the treatment group (LG), while ten healthy dogs were included in the control group (CG). Diagnosis was performed for all the LG dogs, based in cytopathology, histopathology and immunohistochemistry, to test the expression of anti-CD3, anti-PAX-5 and KI67, of the affected lymph node. In addition, staging was performed using chest radiographs, abdominal ultrasound, and myelogram. Serum was collected a single time in the animals of CG, while in the animals of LG, serum was collected at weeks T0, T5 and T10 of CHOP chemotherapy. All the collected samples were evaluated for the APPs and vitamin D concentrations through electrophoresis and chemiluminescence methods, respectively.

RESULTS

Of the 13 dogs analyzed, 9 achieved a complete response and 4 a partial response to the adjuvant treatment. The results demonstrated that serum concentrations of IgA, haptoglobin and α1-acid glycoprotein were significantly higher in the LG group and also between the different chemotherapy periods analyzed (p< 0,05).

Clinical data of patients

Figure 1. Photomicrograph of canine lymph node immunostaining. **A:** T-cell lymphoma, demonstrating diffuse cytoplasmic immunostaining for CD3, obj.40x., with CD3 antibody. **B:** B-cell lymphoma, demonstrating intense nuclear immunostaining for Pax5, obj.40x.

Table 1. Histopathological and immunohistochemical evaluation, therapeutic response, staging and sub-staging of patients in the GL group

Pacient	Histological grade	Time to relapse in days	Immunophenotype	Ki67	Staging and sub-staging
A1	Large cell diffuse lymphoma	200	B	40%	IV b
A2	Immunoblastic lymphoma	121	B	70%	IV b
A3	T lymphoblastic lymphoma	28	T	70%	IV b
A4	Peripheral T-cell lymphoma unspecified	77	T	20%	V b
A5	Large cell diffuse lymphoma	137	B	65%	V b
A6	Large cell diffuse lymphoma	65	B	45%	IV b
A7	T lymphoblastic lymphoma	353	T	90%	IV b
A8	Large cell diffuse lymphoma	461	B	85%	IV a
A9	Large cell diffuse lymphoma	Norelapse	B	15%	III a
A10	Unspecified peripheral T-cell lymphoma	No relapse	T	85%	IV b
A11	Immunoblastic lymphoma	No relapse	B	70%	IV b
A12	Large cell diffuse lymphoma	84	B	80%	IV b
A13	Large cell diffuse lymphoma	294	B	70%	IV b

Analysis of serum concentrations of APPs

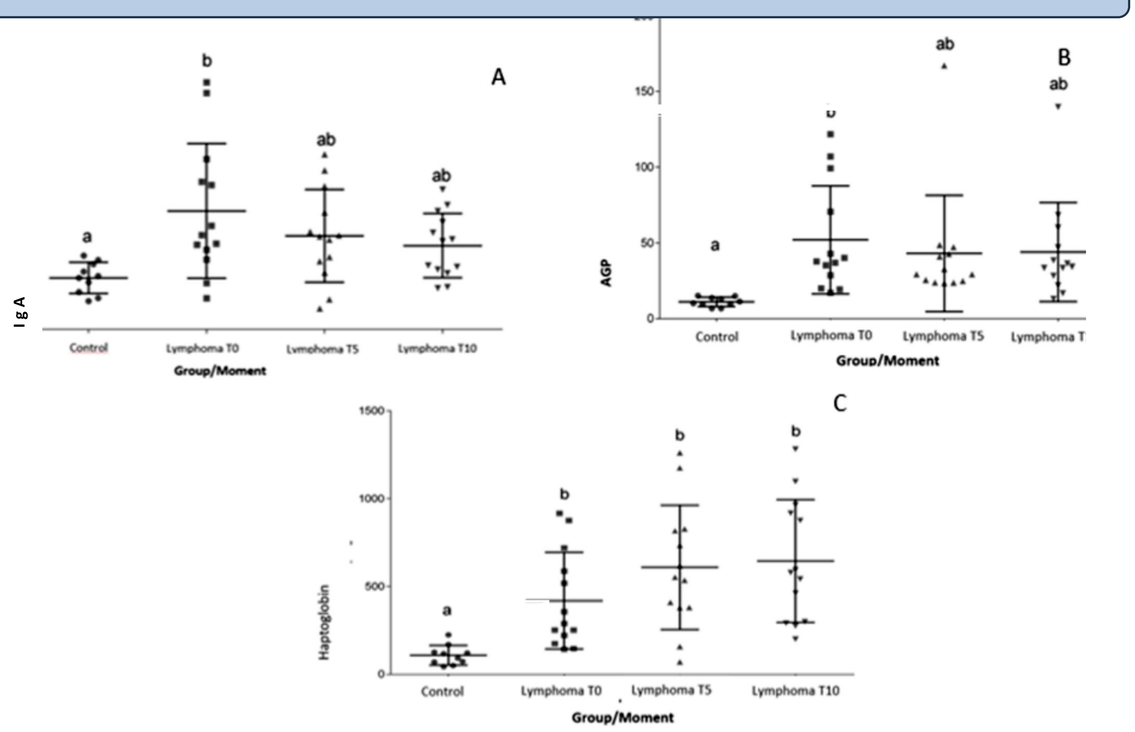

Figure 2. (A): Dispersion between IGA concentration values in mg/dL of dogs with Lymphoma during chemotherapy treatment and in the control group. Comparison of IGA concentrations between the GL at weeks T0, T5 and T10 of chemotherapy treatment and the GC. There was a significant difference by the t test (p = 0.0118). **(B):** Dispersion between the values of haptoglobin concentration in mg/dL of patients with Lymphoma during chemotherapy treatment and in the control group. Comparison between haptoglobin concentrations between the LG at weeks T0, T5 and T10 of chemotherapy treatment and the GC. There was a significant difference by the t test (p=0.0003). **(C):** Comparison between the concentrations of α1-acid glycoprotein during the chemotherapy treatment and between the experimental groups (CG: Control Group and LG: Lymphoma Group). Comparison of AGP concentrations between the GL at weeks T0, T5 and T10 of chemotherapy treatment and the GC. There was a significant difference by the t test (p=0.0247).

Correlation between APPs concentrations and survival time and disease-free time

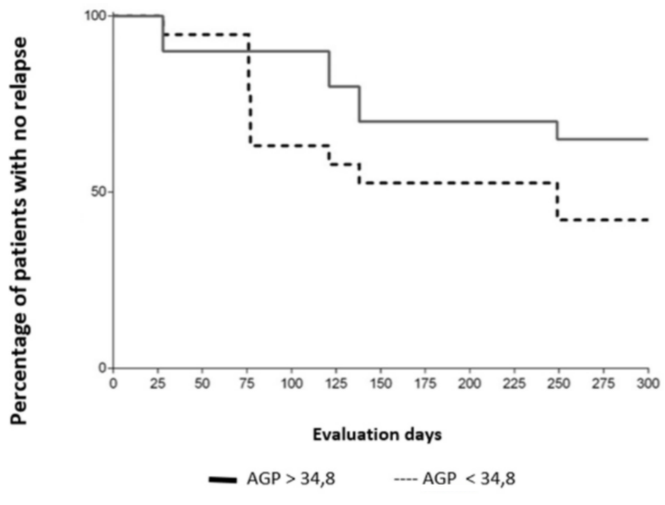

Figure 3. Recurrence curves over time of patients with lymphoma with a serum concentration of α1-acid glycoprotein greater than and less than 34.8 mg/dL. Survival curves of dogs with Lymphoma with "low" alpha-1-acid glycoprotein and "high" alpha-1-acid glycoprotein. There was a significant difference by the analysis of ROC curves and the Kaplan Meier test (p=0.0304).

Table 2. Comparison between patients with multicentric lymphoma who relapsed before 200 days (6/13), with a mean disease-free time of 182 days, and those who did not relapse (7/13) within the study time interval

Variable	Relapse	Mean	SD	P Value	P- Prognosis	Coorte value
IGA	N	30,84	15,84	0,0866	0,9999	
	Y	31,78	17,41			
CERULOPLASMIN	N	43,99	17,69	0,2014	0,1213	
	Y	35,4	23,5			
TRANSFERRIN	N	291,3	119,3	0,0906	0,09103	
	Y	361,5	133			
ALBUMIN	N	4629	1180	0,1764	0,2258	
	Y	4098	1225			
A1 ANTITRIPSIN	N	276,1	101,1	0,9034	0,8658	
	Y	280,5	125,3			
TOTAL IGG	N	1217	711,8	0,5476	0,2599	
	Y	1337	482,2			
HAPTOGLOBIN	N	592,7	315,9	0,0484	0,3981	
	Y	516,1	360,1			
AGP	N	55,94	42,03	0,0232	0,0304	<34,8
	Y	35,15	20,18			
VITAMIN D3	N	29,87	15,07	0,5722	0,5447	

Equal letters: values do not differ from each other. The results are presented as mean ± SD, and the significance was set at 5% (p<0.05) for all tests. SD: Standard deviation: P-value: significance value.

Higher serum concentrations of alpha-1-acid glycoprotein was considered a positive prognostic factor. The sensitivity and specificity of the concentration of α-1-acid glycoprotein in dogs with multicentric lymphoma were 61% and 62%, respectively. The probability of recurrence in patients in a cutoff was 35%, whereas the value of recurrence in patients without a cutoff was 58%, with a P-relapse of 0.139 and a P-value of 0.0232.

Table 3. Means, standard deviations, and significance values of PFA concentrations (mg/dL) in of dogs with multicentric lymphoma and healthy dogs, obtained using SDS-PAGE

Variable	Group	Moment	Mean	SD	P Value	Letter
IgA	Control	T0	16,33	4,971	0,0118	a
	Lymphoma	T0	37,58	21,38		b
CERULOPLASMIN	Control	T0	26,3	15,91	0,166	a
	Lymphoma	T0	35,95	17,66		a
TRANSFERRIN	Control	T0	249,7	63,3	0,3575	a
	Lymphoma	T0	307,4	109,5		a
ALBUMIN	Control	T0	3926	260,8	0,4418	a
	Lymphoma	T0	4372	1356		a
A1 ANTITRYPSIN	Control	T0	185,6	37,11	0,0645	a
	Lymphoma	T0	270,9	128		a
IgG	Control	T0	1320	193,9	0,1909	a
	Lymphoma	T0	1542	737,4		a
HAPTOGLOBIN	Control	T0	108,7	56,15	0,0003	a
	Lymphoma	T0	418,9	275,4		b
AGP	Control	T0	11,14	3,066	0,0247	a
	Lymphoma	T0	52,03	35,67		b

Equal letters indicate that the values do not differ from one another. SD: standard deviation, p-value: significance value.

DISCUSSION AND CONCLUSION

Similar to the present study, the research by Vieira et al. (2010) also noted a substantial increase in AGP concentration in canine patients with multicentric lymphoma compared to the CG, suggesting that these patients had an exacerbated acute phase reaction at the time of serological collection. However, even though the prognostic value of this protein was not evaluated in the afore mentioned study, it was observed that patients with higher levels of this protein had greater survival.

Indicating that these proteins can be considered as sensitive biomarkers for lymphoma in dogs. Furthermore, the α1-acid glycoprotein showed prognostic value for the disease, when compared with the time of survivor, with 63% specificity. Finally, the vitamin D3 level measured in patients undergoing chemotherapy was not an effective prognostic marker in patients with multicentric lymphoma .

Reference: Vieira, M.C., et al. Acute Phase Proteins in Canine Lymp- homa During Antineoplastic Chemotherapy. Brazilian Journal of Veterinary Pathology. 2010, **3**(2), 86-92.