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Thymidine Kinase Type 1 and C-Reactive Protein Concentrations in Dogs with Spontaneously Occurring Cancer

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Background: Serum thymidine kinase type 1 (TK1) and canine C-Reactive Protein (cCRP) might be useful in detecting dogs with cancer. Algorithms combining biomarkers are sometimes more accurate than results of individual tests.

Objectives: The aim of this study was to compare serum TK1 and cCRP and Neoplasia Index (NI) in healthy and tumor-bearing dogs.

Animals: Client-owned dogs with (n = 253) and without (n = 156) cancer.

Methods: Retrospective case–control study. Dogs with cancer were identified after submission of samples for commercial assay and case details were retrospectively collected. Healthy dogs (control) were identified through breed groups and health status was confirmed by health questionnaire for a minimum of 6 months. Serum TK1 activity was measured using a quantitative chemiluminescent assay and serum cCRP was measured using a quantitative ELISA assay.

Results: TK1 activity in the cancer (n = 253) and control group (n = 156) were 7.0 μ/L (median, range <0.5 to >100) and 1.8 μ/L (median, range 0.4 to 55.3), respectively ($P < .001$). cCRP concentrations in the cancer and control group were 6.0 mg/L (median, range <0.5 to >50) and 1.6 mg/L (median, range 0.09 to >50), respectively ($P < .001$). The NI in the cancer and control group were 6.4 (median, range 0–9.9) and 0.9 (median, range 0–7.6), respectively ($P < .001$). ROC AUCs of the NI and TK1 for all cancers were greater than 0.8, highest for lymphoma and histiocytic sarcoma.

Conclusions and Clinical Importance: Increased concentrations of TK1 and cCRP, when present in dogs with cancer, might be useful in confirming a diagnosis and monitoring response to treatment.

Key words: Biomarker; Inflammation; Monitoring; Screening.

Interest in TK1 as a tumor marker began in the 1970s and centers around the increased serum activity or concentration of this compound during dysregulated proliferation. Cancer cell division is frequently incomplete resulting in necrosis and release of DNA. The salvage pathway, using TK1, recaptures exogenous thymidine from the re-utilization of DNA. As a result of active cancer proliferation and a dysregulated cell cycle, TK1 activity increases substantially and is released into the bloodstream. Furthermore, the level of TK1 activity correlates with the tumor grade and treatment. The progression of the disease increases TK1 activity and TK1 activity can decrease or return to normal with successful treatment.[1–4]

Abbreviations:

cCRP	canine C-reactive protein
NI	neoplasia index
TK1	thymidine kinase

There is high TK1 activity in non-Hodgkins lymphoma and leukemia in people.[4,5] Increases in TK1 activity occur in a wide range of hematologic and solid tumors including lymphoma, leukemia, multiple myeloma, breast cancer, prostate cancer, and small cell lung cancer in people.[4–10]

Increased plasma and serum TK1 activity occurs in dogs with lymphoma and higher activity correlates with advanced stage of disease as well as poorer prognosis.[11–16] TK1 has also been investigated in dogs with hemoabdomen and correlates with the presence of hemangiosarcoma.[17] TK1, in conjunction with measurement of serum canine-specific C-reactive protein (cCRP), has been useful when screening clinically healthy dogs for occult cancer.[18] Although these studies refer to enzymatic activity, recent studies have also evaluated protein concentration.[19,20] It is not known which test is more accurate as protein concentration and enzymatic activity have not been directly compared. TK1 enzymatic activity reflects only functional intact enzyme which might be relevant to certain cancers. Protein concentration allows for measurement of TK1 as a dimer or fragment, which might be more relevant to certain solid tumors, and these assays might have less impact from variations in sample handling compared to assays of activity.[21,22] It remains to be determined what constitutes a true positive and true negative test, and if these definitions for statistics are contextual.

cCRP is an acute phase protein used as a marker of inflammation.[21,23] Increases in cCRP have been

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Samples were submitted from 11 practices nationwide with 126 cases from Dr Husbands's practice in Minnesota, all assays and initial statistics were run at VDI in California, and all data was collated at the University of Missouri. This research was supported by the Veterinary Diagnostics Institute, Inc. and was presented at the Annual Conference of the Veterinary Cancer Society in Minneapolis, MN, October 2013.

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associated with cancer, pancreatitis, infections, IMHA, parvovirus infection, SIRS, polyarthritis, renal disease, and other inflammatory diseases in dogs,[21–29] and in a recent study correlated with all-cause mortality.[18] TK1 and cCRP were combined in an algorithm to generate a Neoplasia Index (NI) which was higher in clinically normal dogs that were diagnosed with cancer within 3–6 months of sampling.[18] This parallels similar findings in 2 large-scale human trials in which residents participating in a cancer screening programs were tested for TK1 and those with increased concentrations had a higher chance of being diagnosed with cancer.[14,30]

The complex relationship between inflammation and cancer underscores the rationale for combining these 2 biomarkers. Our goal was to explore TK1 and cCRP concentrations in dogs with a wide variety of cancers, such that future investigations can focus on both diagnostic applications for early detection or to support other definitive diagnostics and the use of serial monitoring to improve therapy and treatment decisions for relapsed disease.

Materials and Methods

Dogs presenting for evaluation for newly diagnosed cancer, and undergoing routine evaluation and staging, had serum collected at the time of evaluation. Any dog with a confirmed diagnosis of cancer was eligible, and included cases were deemed to have been sampled before treatment based upon the relative dates of sampling and reported treatment. After a large number of samples had been submitted, information regarding signalment and diagnosis was collected retrospectively from submission forms. In 117 cases, the submission information was not clear as to whether treatment was started before or after the sample so statistical analyses were performed considering those cases both with and without treatment. Control samples were collected and banked from dogs deemed healthy based on physical examination and history. Bloodwork or other analysis was not required for inclusion. Dogs' health was followed by owner questionnaire for a minimum of 6 months to ensure that dogs remained healthy. To determine whether differences between groups were attributable to age-related changes in general health (such as a greater incidence of chronic inflammatory conditions), a subgroup was analyzed within the control group comparing an older subgroup with the same median age and sex/neuter status to the dogs with cancer.

Specimen Handling

All specimens were drawn using a serum separator tube, separated within 1 hour and then frozen at −20°C. Specimens were then transported with an ice pack to the laboratory by express/overnight service and run within 1 hour of thawing. This procedure was validated for both TK1 and cCRP by examining tube type, time to centrifuge, and impact of storage temperature. Both red top plain and serum separator tubes were evaluated to determine how both time and proximity to clot affect results, using random samples from dogs separate from this study that were prospectively handled in various conditions.

TK1 Assay. The TK1 assay is an indirect, modified 2-step, competitive chemiluminesence immunoassay (CLIA)[a] for the quantitative determination of TK1 in serum, previously validated for use in dogs.[18,31–33] The assay utilizes AZT as substrate and an isoluminol-AZTMP (3′-azido-3′-deoxythymidine monophosphate) conjugate.

cCRP Assay. The cCRP assay is a canine-specific sandwich enzyme linked immunosorbent assay[b] (ELISA) for the quantitative determination of CRP in canine serum, previously validated for use in dogs.[18]

Neoplasia Index

The Neoplasia Index was calculated using a proprietary algorithm for which the 2 biomarkers were combined using weighting factors that prevent either marker from having an inappropriately dominant effect on the result. This preserves the ability to integrate both proliferation and inflammation into a laboratory value that represents a complex physiologic process. A previously reported cohort was used to group TK1 and CRP into ranges that optimized separation between normal, benign, and cancer and then categorized by unitless discrete values (0, 1, 2, etc.) to prevent high values of 1 biomarker overly influencing the value of another.[23] Receiver operating characteristic (ROC) analysis was used to determine limits that optimize specificity. This resulted in discrete cut points for both TK1 and CRP. Because TK1 is viewed as the primary biomarker in this algorithm, no weighting was applied to cCRP when TK1 was below 1.8 μ/L. The goal was to improve overall specificity. Logistic regression was then performed on the discretized data with resulting weighting coefficients for each biomarker. As a result, the Neoplasia Index is a unitless number ranging from 0 to 9.9; 0 being a healthy dog, and 9.9 the best model fit for a dog with cancer.

Statistics

Statistical analysis was performed by 1 co-author (RR) using commercially available software.[c] A Kruskal-Wallis one-way ANOVA on ranks or a Mann-Whitney rank-sum test was used to compare continuous data. A receiver operating characteristic (ROC) curve was used to determine the area under the curve (AUC) and select the optimum cutoff value that maximized the Yuden's J statistic (sensitivity +specificity−1) for sensitivity and specificity reporting. Significance was set at $P < .05$.

To ensure an unbiased assessment, statistical analysis was repeated by a statistician unfamiliar with the study, study subjects, and commercial company (PP). Descriptive statistics were calculated by cross-tabulations (for categorical variables), and ANOVA (for continuous and categorical variable comparisons). Chi-square, Fishers exact, *F*- and *t*-tests were used to assess differences between the distribution of categorical and continuous measures. Multiple pairwise comparisons of the means across categorical groups were performed using Tukey's honest significance test.

To determine the power of TK, CRP, and NI for discriminating any cancer (yes/no), ROC curves based on percentile value (PV) calculations were generated as described.[34] The null hypothesis that TK, CRP, and NI were no different in their predictive ability with respect to their areas under the ROC curve (AUC) was also assessed and bootstrap standard errors and bias corrected confidence intervals for AUC and marker differences were calculated.[34]

Results

There were 156 control and 253 tumor-bearing dogs (cancer group). Signalment data are summarized in Table 1. Because at-risk breeds contributed the greatest number of samples for the control group and because these were collected through breed groups, there were significantly more sexually intact dogs in the control group ($P < .001$). Male castrated dogs (n = 133 cancer, n = 32 control) and female spayed dogs (n = 104

cancer, n = 47 control) constituted the largest group with male intact (n = 8 cancer, n = 41 control) and female intact (n = 8 cancer, n = 35 control) occurring less frequently. Large breed dogs such as Golden Retriever, German Shepherds, and Labradors, represented the largest group. The mean age of the control group (6.5 years) was significantly less than the cancer group (9.5 years, $P < .001$). Signalment data and comparisons were similar with both statistical analyses. When the age- and sex-matched subgroup was compared (normal dogs with the same median age and sex distribution compared to rest of control population), there were no clinically relevant differences in TK1 or cCRP and there were 5 outlying values. Overall, using pairwise comparisons of means with equal variances, TK1 increased by 0.88 units with each year of age ($P = .03$). However, using least squares regression and ANOVA, TK1 was not significant (0.3 units/year, $P = .13$).

Table 1. Signalment data for control and cancer groups. Although both included a wide range in age, the dogs in the control group were significantly younger ($P < .001$), and there were more sexually intact dogs ($P < .001$) than in the test group which reflects a selection bias based on targeting at-risk breeds.

	Control	Cancer
N	156	253
Median age in years (range)	6.5 (0.4–10.9)	9.5 (1.8–16)
Sex (n)	F(35), FS(47), M(42), MN(32)	F(8), FS(104), M(8), MN(133)
Breed (n)		Giant (5)
	Large (156)	Large (137)
	[GSD n = 58]	[Lab/mix n = 47]
	[Golden Retriever n = 97]	[Golden Ret/mix n = 29]
		Medium (31)
		Small (61)
		[Beagle n = 13]
		Unknown (19)

Table 2. Tumor type and number of dogs within the group of dogs with cancer.

Tumor Type	Number (n)
Carcinoma	53
Histiocytic sarcoma	9
Hemangiosarcoma	7
Lymphoma	83
Mast cell	28
Melanoma	8
Osteosarcoma	16
Others	13
Sarcoma	36
Hematopoeitic	87
Solid	166

The cancer group was categorized into tumor type as depicted in Table 2 and also grouped as hematopoietic or solid tumors. The largest tumor group was lymphoma (n = 83) for which cell type was known for 13 patients (small cell n = 3, large cell n = 10). Immunophenotype was not recorded for most cases. Other hematopoietic tumors were multiple myeloma (n = 1), plasmacytoma (n = 1), and lymphocytic leukemia (n = 2). Solid tumors included carcinoma (n = 53), sarcoma (n = 36), mast cell tumor (n = 28), histiocytic sarcoma (n = 9), melanoma (n = 8), and hemangiosarcoma (n = 7).

The use of standard red top tubes increased TK1 from 5 to 16.4 μ/L when serum was allowed to remain in contact with the clot for periods greater than 1 hour. When a serum separator tube was used and the serum was removed within 2.5 hours, there was no increase in TK1 values. Regardless of tube type, there was no effect on cCRP results (Table 3). Serum was tested at room temperature, 4°C, and −20°C. Room temperature and 4°C storage resulted in an average loss of TK1 activity within 24 hours of 18% and 12%, respectively. There was no significant loss of TK1 activity when specimens were frozen at −20°C. cCRP was unaffected at all storage conditions. Up to 2 freeze/thaw had negligible effect on both TK1 and cCRP (Table 4).

Data were not normally distributed so nonparametric statistical methods were used for data analysis. The

Table 3. Effect of tube type and time on clot on TK1 (μ/L) and cCRP (mg/L).

	Specimen 1		Specimen 2		Specimen 3		Specimen 4		Specimen 5	
	TK1	cCRP	TK1	cCRP	TK1	cCRP	TK1	cCRP	TK1	cCRP
SST1	4.2	4.3	7.4	13.6	3.4	9.6	1.4	1.2	3.2	5.6
SST2	3.4	5.5	8.0	13.8	3.0	9.3	1.6	1.3	3.4	5.6
RT1	4.4	4.4	8.8	14.6	3.0	8.8	1.2	1.1	5.0	5.6
RT2	3.8	4.4	11.0	13.9	12.2	9.7	6.2	1.2	18.2	6.0
RT3	13.4	4.4	20.4	14.5	6.2	9.8	14.0	1.2	21.4	5.6

All samples were allowed to clot for 20 minutes before processing. SST1 = serum separator tube; immediately centrifuged; serum removed within 1 hour. SST2 = serum separator tube; immediately centrifuged; serum removed within 2.5 hours. RT1 = red top tube; immediately centrifuged; serum separated within 1 hour. RT2 = red top tube; immediately centrifuged; serum separated within 2.5 hours. RT3 = red top tube; sat for 1 hour; centrifuged and serum separated. As a group, specimens processed without a gel barrier and delayed clot separation (RT2 and RT3 – shaded) had significantly higher TK1 concentrations than SST1 processed samples ($P < .01$). This difference in processing resulted in an average increase in TK1 of 4 times. Processing had no significant effect on cCRP concentrations.

overall median, mean and range of TK1 activity in the cancer group versus the control group were 7.0 μ/L, 20.1 μ/L (range <0.5 to >100) and 1.8 μ/L, 3.2 μ/L (range 0.4 to 55.3), respectively. Median values, 25–75% percentile, and range by tumor type are shown in the box-and-whisker plot in Figure 1A. The overall median, mean and range of cCRP concentrations in the cancer group versus the control group was 6.0 mg/L, 15.3 mg/L (range <0.5 to >50) and 1.6 mg/L, 3.3 mg/L (range 0.09 to >50). Median values, 25–75% percentile, and range by tumor type are shown in the box-and-whisker plot in Figure 1B. The overall median, mean and range of Neoplasia Index in the cancer group versus the control group was 6.4, 6.3 (range 0–9.9) and 0.9, 1.6 (range 0–7.6). Median values, 25–75% percentile, and range by tumor type are shown in the box-and-whisker plot in Figure 1C.

When tumors were grouped as hematopoietic or solid, TK1 median, mean and range was 19.1 μ/L, 40.3 μ/L (range 1.5 to >100), and 5.9 μ/L, 9.3 (range 0.4 to >100), respectively; cCRP median, mean and range was 13.3 mg/L, 20.7 mg/L (range 1.2 to >50), and 4.1 mg/L, 12.5 mg/L (range 0.4 to >50), respectively; Neoplasia

Table 4. Effect of temperature on TK1 activity and cCRP concentration in serum.

		24 hours		48 hours		−20°C	
	Initial	RT	4C	RT	4C	1× FT	2× FT
TK1 (U/L)							
Specimen 1	16.0	14.6	15.9	11.9	12.0	16.8	15.7
Specimen 2	31.5	23.5	31.9	19.2	24.4	32.4	31.1
Specimen 3	7.3	6.3	6.8	4.4	5.8	7.1	7.6
cCRP (mg/L)							
Specimen 1	5.6	5.4	5.7	5.5	5.6	5.3	5.7
Specimen 2	12.9	13.3	12.8	12.5	12.7	12.7	13.2
Specimen 3	37.8	37.1	38.0	36.9	37.5	37.4	38.3

RT, room temperature; 4°C, 4° celcius refigeration; −20°C, minus 20° celcius; FT, freeze/thaw.

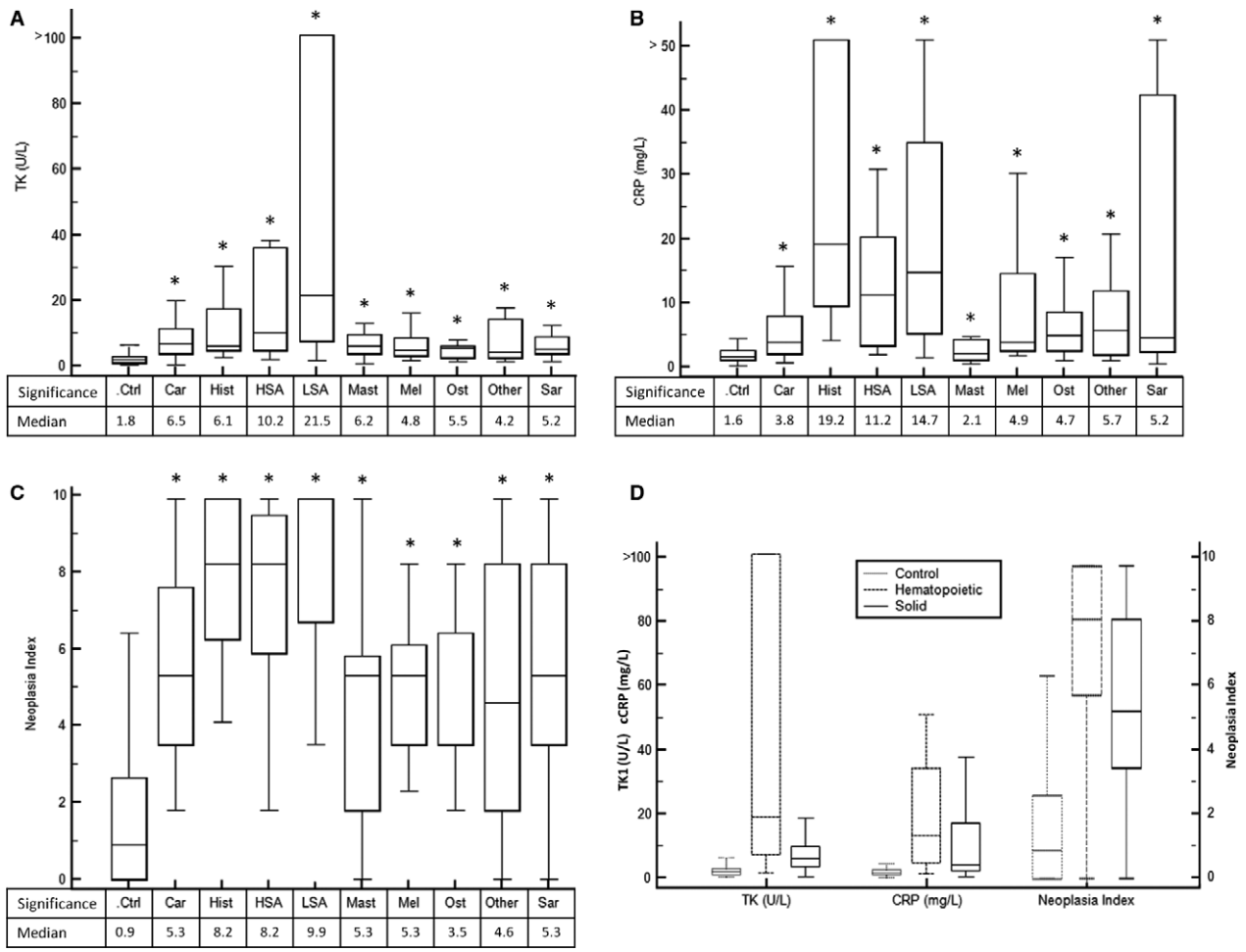

Fig 1. (**A**) Box and whiskers plot of serum TK1 activity for dogs in control and cancer groups. Groups that were statistically different ($P < .05$) than the control group are indicated by *. (**B**) Box and whiskers plot of serum cCRP for dogs in control and cancer groups. Groups that were statistically different ($P < .05$) than the control group are indicated by *. (**C**) Box and whiskers plot of Neoplasia Index for dogs in control and cancer groups. Groups that were statistically different ($P < .05$) than the control group are indicated by *. (**D**) Box and whiskers plot of TK1, cCRP, and Neoplasia Index for control, hematopoietic, and solid tumors.

Index median, mean, and range was 8.2, 7.7 (range 0–9.9) and 5.3, 5.5 (range 0–9.9), respectively. Median values, 25–75% percentile, and range by tumor type are shown in the box-and-whisker plot in Figure 1D.

TK1, cCRP, and Neoplasia Index were evaluated by tumor grade. When available, across all diagnoses, tumor grade was recorded as low/grade 1, moderate/grade 2, or high/grade 3. Biomarker values and their variability both increased with grade as depicted in Figure 2. Despite the overlap of values, the difference in TK1 between high and low or moderate grade tumors, was statistically significant ($P < .001$). The difference in NI among grades was significant only between high and low tumors ($P = .003$).

Using ROC analysis, Table 5 evaluates the area under the curve (AUC) for each tumor type for both the Neoplasia Index and TK1. After MedCalc analysis, in all cases except for mast cell tumor, the ROC AUC was greater using the Neoplasia Index than TK1 alone. The difference was not significant except for histiocytic sarcoma ($P = .004$). When grouped by solid versus hematopoietic there was no statistical difference, however, if mast cell tumors were removed from the solid group the difference became significant ($P = .02$). Using nonparametric analysis, the ROC AUC for TK1 was greater than for cCRP or NI (Table 6). Statistical analysis for individual tumor types was not repeated with pairwise comparisons because of small numbers in some groups. Statistical findings in Table 5 were reviewed by both statisticians and assessed as an appropriate univariate analysis.

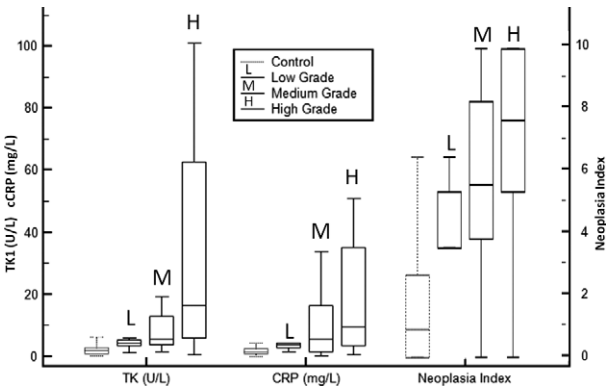

Fig 2. Box-and-whisker plot of TK1, cCRP, and Neoplasia Index for control, and tumor grades.

Table 5 also evaluates likelihood ratios at different values for TK1 and the Neoplasia Index. In all cases, there was a significant improvement in likelihood ratios when NI was ≥7.6, when compared to TK1 as a sole biomarker. Table 7 lists tumor types with NI ≤3.0, tumor types that significantly overlap the control group (ie, false negatives). Although a few represent cancers that are typically aggressive with rapid growth or metastasis (melanoma, hemangiosarcoma, osteosarcoma), included are tumors that typically follow a more indolent course and therefore would be less likely to have a large proliferating fraction (cutaneous lymphoma, sarcomas, and anal sac adenocarcinoma). Tumor types with very high likelihood ratios for the Neoplasia Index to be increased (≥7.6) included those that produced significant increases in both TK1 and cCRP. Common aggressive tumors predominate such as carcinomas and sarcomas of the viscera, histiocytic sarcoma, most of the osteosarcoma cases in this series, lymphoma, and hemangiosarcoma (Table 8).

Using pairwise comparisons, round cell tumors had significantly higher TK1 activity ($P < .001$) and NI ($P < .04$) than epithelial and mesenchymal tumors. There was no difference in TK1 activity, cCRP concentration, or NI when the effect of possible treatment was considered for cases (n = 117) for which it was unclear whether treatment had been started before the blood draw ($P > .05$ for all).

Table 5. TK1 and Neoplasia Index interval likelihood ratios based upon number of dogs within each group.

Interval TK1 (U/L)	Car	Hist	HSA	LSA	Mast	Mel	Ost	Othr	Sar	Ctrl	Total Cancer	Likelihood Ratio	Sens.	Spec.
0–1.9	1	0	0	1	2	1	3	3	3	87	14	0.10	1.00	0.00
2.0–5.9	20	4	3	11	11	5	8	4	19	56	85	0.94	0.95	0.56
6.0–9.0	14	2	0	10	6	0	5	2	5	5	44	5.43	0.61	0.92
9.1–19.9	12	1	1	18	8	2	0	3	6	4	51	7.86	0.44	0.95
≥20	6	2	3	43	1	0	0	1	3	4	59	9.10	0.27	0.98
Total	53	9	7	83	28	8	16	13	36	156	253			
ROC AUC	0.866	0.887	0.891	0.948	0.837	0.813	0.783	0.775	0.841		0.876			
Neoplasia index														
0–1.7	0	0	0	1	2	0	0	2	1	78	6	0.05	1.00	0.00
1.8–4.1	21	1	1	9	11	3	10	4	13	62	73	0.73	0.87	0.75
4.2–5.3	11	0	1	7	7	2	0	2	6	8	36	2.78	0.69	0.90
5.4–7.5	4	2	0	4	3	2	3	0	4	7	22	1.94	0.56	0.95
7.6–10	17	6	5	62	5	1	3	5	12	1	116	71.53	0.46	0.99
Total	53	9	7	83	28	8	16	13	36	156	253			
ROC AUC	0.886	0.980	0.935	0.954	0.805	0.895	0.862	0.789	0.873		0.896			

Car, carcinoma; Hist, histiocytic sarcoma; HSA, hemangiosarcoma; LSA, lymphoma; Mast, mast cell; Mel, melanoma; Ost, osteosarcoma; Othr, other; Sar, sarcoma. Likelihood ratios based on total cancer group. Numbers in boxes represent number of dogs in each stratum (except for ROC, Ratio, Sensitivity, and Specificity).

Table 6. Summary of receiver operating characteristics (ROCs) for TK1, cCRP, and NI to discriminate cancer from healthy dogs (all tumor types combined). In this analysis (PP), TK1 performed better than cCRP ($P = .038$) and than NI ($P = .032$). There was no difference between cCRP and NI.

Variable	AUC	Standard Error	95% Confidence Interval
TK1	0.873	0.018	0.838–0.908
cCRP	0.818	0.021	0.776–0.860
NI	0.844	0.019	0.807–0.882

Table 7. Tumor types with Neoplasia Index ≤3.0 (false negative).

Tumor Type	Location
Carcinoma	Prostate
	Anal gland (4)
	Dorsal neck
	Salivary
	Shoulder
Hemangiosarcoma	Spleen
Lymphoma	Cutaneous (4)
	Lymph node
Mast cell	Cutaneous (9)
Melanoma	Oral
Osteosarcoma	Forelimb (3)
Other	Thyroid
	Multiple myeloma
	Plasmacytoma cutaneous (3)
Sarcoma	Fibrosarcoma mandible
	Neurofibrosarcoma medistinal
	Spindle cell oral
	Sarcoma elbow (2)
	Sarcoma abdominal
	Chondrosarcoma chest

Table 8. Tumor types with Neoplasia Index ≥7.6 (high likelihood ratio).

Tumor Type	Location
Carcinoma	Bladder
	Stomach
	Lung (2)
	Liver (2)
	Upperjaw
	Kidney
	Spleen
	Inguinal
	Anal sac (3)
	Submandibular
	Sinus
	Jugular furrow
Hist Sar	Mediastinum
	Distal humerus
	Chest (2)
	Liver
	Pulmonary
Hemangiosarcoma	Tongue
	Pelvic canal
	Spleen
	Liver (2)
Lymphoma	Lymph node (53)
	Liver (2)
	Periorbital
	Bone marrow
	Epitheliotropic
	Spleen (3)
Mast cell	Cutaneous (5)
Melanoma	Oral
Osteosarcoma	Forelimb (2)
	Ilium
Other	Gastric
	Thyoma (2)
	Blood (leukemia)
	Spleen
Sarcoma	Spleen (3)
	Heart
	Shoulder
	Axilla
	Leg
	Lung
	Liver
	Pelvis
	Prostate
	Forelimb

Discussion

These data demonstrate that TK1 is significantly higher in dogs with a wide range of hematopoietic and solid tumors than in healthy dogs. Dogs with multicentric lymphoma have a median of 6.2 μ/L with 47% above the reference interval compared to our study median of 21.5 μ/L and 86% above the reference interval, which included cases that generate less TK1 activity such as cutaneous and indolent lymphoma.[16] There is no significant TK1 increase in dogs with solid tumors unlike what has been demonstrated in our study. Results will differ between plasma and serum, making comparisons problematic. Also, sample handling and shipment in a cooled container without being frozen until the following day upon arrival at the laboratory, could affect results.[11] We found that the handling of specimens for TK1 testing is important. This could explain the difference in findings between our and other studies. Also with regard to hematopoietic tumors, lymphoma of T cell immunophenotype produces lesser increases in TK1.[16] Unfortunately, most dogs with lymphoma in our series did not have their lymphoma immunophenotyped, which is a limitation of the interpretation of our results. A subset of T-cell lymphoma cases could explain the variability in our results, and could hinder understanding the value of TK1 in monitoring B-cell lymphoma. However, given the usual distribution of cases, it is likely that most cases were of B-cell origin and the highest values of TK1 were from among the lymphoma cases, most of which were very high. Therefore, although low values (primarily from cutaneous lymphoma) might lower the overall value, the greater concern is perhaps an inability to identify how many of the dogs with high TK1 might have had

T-cell lymphoma, thus suggesting some utility in that disease.

Marked increases in serum TK1 were seen in dogs with histiocytic sarcoma, and this tumor type can be very unpredictable in its behavior and difficult to treat. The use of TK1 for monitoring dogs with this disease should be explored.

The higher TK1, cCRP, and NI in dogs with increasing grade of tumor is unsurprising, given that higher grade tumors have consistently been associated with higher markers of proliferation on immunohistochemistry (Ki67, AgNORs, PCNA).[22,35] These biomarkers could be useful in identifying dogs with a higher risk of failure from their disease, and those that could be more likely to benefit from more aggressive local and systemic treatment. Although TK1 was not evaluated in the findings presented here as a prognostic factor, its association with outcome in hematopoietic tumors is well-documented, as is the association of grade with outcome across most tumor types. The use of TK1 to monitor response to treatment should also be explored.

Consistent with previous work by the authors,[18] the Neoplasia Index is able to achieve high likelihood ratios when increased (high specificity [or strong rule-in]) and conversely very low likelihood ratios when decreased (high sensitivity [or strong rule-out]). Therefore, different cutoffs would be used for screening (higher sensitivity) than for monitoring response to treatment (higher specificity). The data presented here are largely descriptive in nature and create a baseline for future studies.

Footnotes

[a] LIAISON, DiaSorin, Stillwater, MN
[b] TECO Medical, Sissach, Switzerland
[c] MedCalc version 15.8, Ostend, Belgium

Acknowledgments

Conflict of Interest Declaration: Dr Selting is a paid consultant to Veterinary Diagnostics Institute (VDI), Inc., and Randy Ringold is an employee of VDI. Neither had direct contact with or knowledge of the cases included in this series and all information on disease diagnosis and treatment status was collected after samples were assayed. Dr Husbands had no role in interpretation of the data and contributed only samples, requested case information, and assisted with preparation of the manuscript. Dr Pithua had no prior knowledge of the study or subjects at the time of statistical analysis, and is not affiliated with VDI in any way.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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