2022

THYMIDINE KINASE

GROWING BODY OF EVIDENCE

VDI LABORATORY LLC

The Right Tests for the
Right Decisions™

SIMI VALLEY, CA

WWW.VDILAB.COM

805.577.6742

1ST EDITION

A growing body of evidence…

There is a growing body of evidence that thymidine kinase, type 1, is an effective biomarker to diagnose & monitor cancer in dogs, cats, and horses:

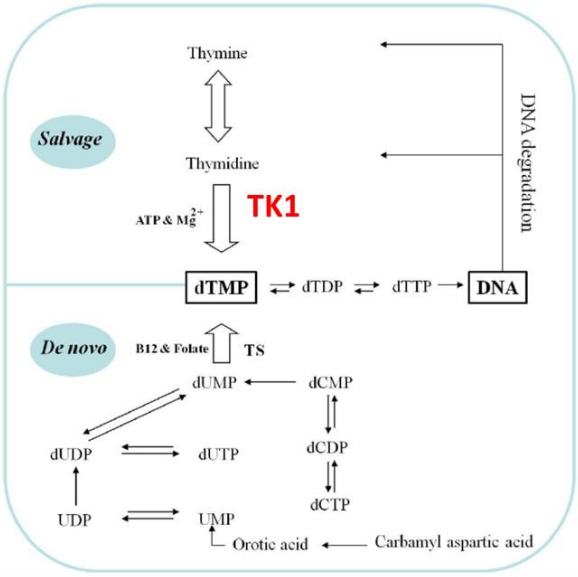
- **Lymphoma diagnosis**
  - **Dogs (1,2,3,4,7,10)**
  - **Cats (6)**
  - **Horses (9)**
- **Solid tumor diagnosis**
  - **Dogs (5,7,10)**
- **Neoplasia Index™ (7,10)**
- **Therapeutic monitoring (1,2,7,13)**
- **Pericardial effusion tumor diagnosis (12)**
- **IMHA in dogs (11)**

Testing Information:

VDI Laboratory offers routine cancer testing in dogs, cats, & horses. For more information please call 805.577.6742 or visit www.vdilab.com

Thymidine Kinase, type 1 (TK1) is a DNA proliferation enzyme. TK1 makes the nucleotide thymine via a salvage pathway from degraded DNA. Normal dividing cells primarily use *de novo* synthesis; however cancer cells have a dysregulated cell cycle resulting in cell death and necrosis. Neighboring cancer cells utilize this DNA via the salvage pathway and upregulate TK1. The cytosolic enzyme leaks into circulation and is detected via a serum test. Studies show TK1 to be increased in lymphoma and solid tumors in both humans and companion animals. Since TK1 is a proliferation enzyme, its level is a function of both tumor proliferation and overall tumor mass. Benign tumors produce little TK1 being normally dividing cells whereas malignant cells produce heightened concentrations. TK1 elevation is more pronounced in poorly differentiated, high grade tumors.

Transient elevations of TK1 can occur in situations where there is significant cell damage or necrosis. Since cancer is an inflammatory disease, coupling TK1 with an inflammatory marker such as C-reactive protein (CRP) or haptoglobin (HPT) improves overall sensitivity and specificity. The union of TK1 and CRP/HPT into an index, termed Neoplasia Index™, aids in result interpretation.



Two pathways to make DNA; *de novo* and salvage. TK1 is part of salvage pathway. As name implies TK1 salvages or reclaims DNA from damaged cells.

Therapeutic monitoring using TK1 is possible since TK1 is a true DNA proliferation enzyme. Following surgery and/or chemotherapy TK1 will rapidly decline if therapy is effective. Conversely, TK1 concentration will increase in disease recurrence.

Ver.1 (2022)

Canine Cancer Applications

Lymphoma

LSA produces substantial amounts of TK1 – up to 1,000U/L. While FNA of enlarged nodes may be sufficient for a diagnosis, biopsy results may yield equivocal results, insufficient cellularity, or just not able to biopsy easily.

- **The VDI TK Cancer Panel is useful to rule-in/out lymphoma in difficult cases.**

Abdominal Mass

Upon physical exam or imaging a mass is detected such as a liver nodule, or splenic mass. Is it cancer? Fine needle aspirates for cellular morphology can have insufficient cellularity, miss the point of interest, or in the case of a splenic mass, cause a rupture.

- **The VDI TK Cancer Panel is useful to differentiate benign vs malignant masses.**

Hypercalcemia of Malignancy

On routine blood work a high calcium is detected for unknown reasons. Some cancers can produce high serum calcium values such as LSA or anal sac adenocarcinoma. High calciums can also be due to endocrine disorders.

- **The VDI TK Cancer Panel can determine if the high calcium is due to cancer. If negative, reflexing to the VDI Calcemia Panel will differentiate hypercalcemia of malignancy from primary hyperparathyroidism.**

ADR (Ain't Doing Right)

Pet owners detect problems in their pets such as not eating, always sleeping, not playful, etc. Upon taking them to the vet, the vet does a physical exam and routine blood work – all normal. Such patients are termed ADR. Is there occult cancer or other inflammatory disease?

- **The VDI TK Cancer Panel is useful to detect cancer or other inflammatory disease in ADR patients.**

Stem Cell Therapy

Stem cells, derived from adipose tissue, are used for canine patients with osteoarthritis and other degenerative joint diseases. Stem cells, being undifferentiated cells, will become new cells within the joint when injected into the affected area. However, as easily as they can differentiate into new joint cells, they can inflame cancer if it is present. It is important prior to stem cell therapy to rule-out cancer.

- **The VDI TK Cancer Panel is useful to rule-out cancer in pre-stem cell therapy patients.**

Metastatic Disease

Surgical removal of solid tumors may or may not have clear margins leaving open the question of metastatic disease.

- **The VDI TK Cancer Panel is useful for post-surgical surveillance of metastatic disease.**

Therapeutic Monitoring

Is therapy effective? Should rescue therapy be initiated? Monitoring remission/disease recurrence in a simple way.

- **The VDI TK Cancer Panel is useful to monitor therapy and disease recurrence.**

Feline Cancer Applications

GI Lymphoma

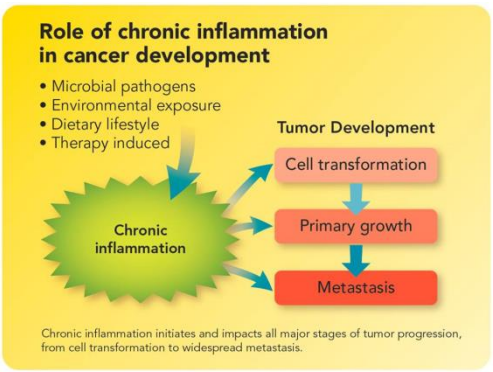
The #1 reason pet owners bring cats to see the vet is GI related problems (eg, diarrhea, vomiting). Chronic IBD is a major problem in older cats (>7yrs). Left untreated chronic IBD can lead to intestinal LSA. Current standard is intestinal biopsy but often not done due to cost and stress to patient. Often empirical use of corticosteroids is used and if unresponsive, attention is drawn to intestinal LSA. Having a simple blood test to differentiate chronic IBD vs intestinal LSA would be valuable.

- **The VDI TK Cancer Panel is useful to differentiate chronic IBD from intestinal LSA.**

A growing body of evidence…

Neoplasia Index™

Inflammation is the cornerstone to cancer initiation and advancement. Chronic inflammation can occur due to an infectious assault, environmental exposure to irritants or toxins, poor dietary lifestyle, or as a side-effect to a therapy. Regardless, left unresolved, chronic inflammation can lead to DNA damage and cell transformation. If the immune system cannot resolve, the transformation leads to tumor growth. Tumor metastasis is further propagated due to the chronic inflammatory process.

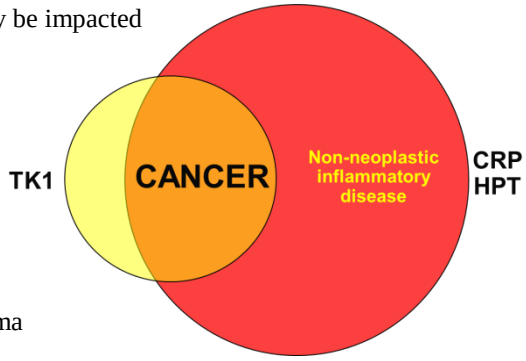


In dogs the major acute phase protein is C-reactive protein (CRP); in cats it's haptoglobin (HPT). These proteins are elevated in a wide range of inflammatory conditions including cancer.

In a recent study (10), it was shown that TK1 and CRP are elevated in both lymphoma and solid tumors. The unification of TK1 and CRP (or HPT) into an index provides improved sensitivity and specificity and is termed **Neoplasia Index™**.

The Neoplasia Index™ has high specificity however performance may be impacted by medication. It is best to test an **untreated patient**, especially if corticosteriods (CS) are used. CS being anti-inflammatory and anti-proliferative will affect both TK1 and CRP concentrations. The panel works best on these tumor types:

- Lymphoma (all types)
- Hemangiosarcoma
- Histiocytic Sarcoma
- Grade II Mast Cell
- Anal Sac Adnenosarcoma
- Leukemia
- Metastatic processes
- Non-cutaneous carcinoma/sarcoma

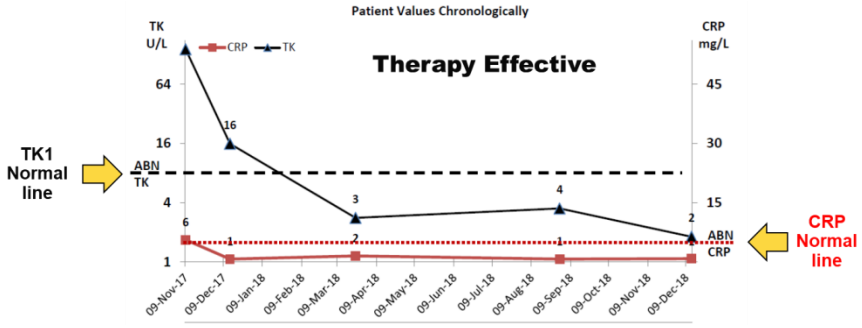


Canines	TK1 (U/L)	CRP (mg/L)	Neoplasia Index™ (a)	Comments
Healthy	<3	<4	<2.0	
Lymphoma	9 - >100	4 - >50	>6.4	LSA can produce substantial amounts of TK1 with values as high as 1,000 U/L.
Solid tumors	9 - 25	4 - 20	>6.4	TK1 values will correspond to both tumor proliferation and grade.
Benign tumors	3 - 8	<4	<4.0	Benign tumors comprising of normally dividing cells will have low TK1/CRP values.
IMHA/ITP	25 - 40	25 - >50	9.9	Autoimmune distruction of hemopoietic cells will significantly elevate TK1. Uncontrolled IMHA will have high CRP; controlled low CRP.
Sepsis	3 - 8	>50	6.4	Infectious assault damages tissues and increases TK1 slightly. CRP is extremely high.
Severe B12 deficiency	9 - 20	<4	5.3	*de novo* thymine synthesis is impaired resulting in dysregulated cell replication and increasing TK1.
Chronic vector-borne disease	9 - 20	<4	5.3	Tick borne diseases are obligatory parasites and use host to replicate. Upregulates TK1.
Other inflammatory diseases	<3	4 - >50	<4.0	Non-cancerous inflammatory diseases (eg, kidney, liver, heart, etc) will generate an elevated CRP without increasing TK1.

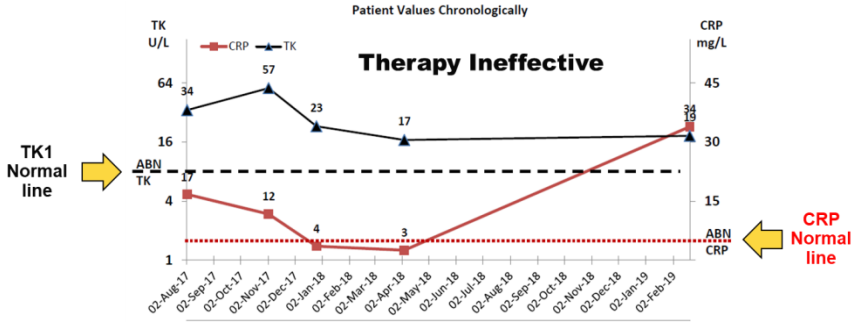
(a) Neoplasia Index™: Positive for cancer >5.5, Equivocal = 5.3

A growing body of evidence…

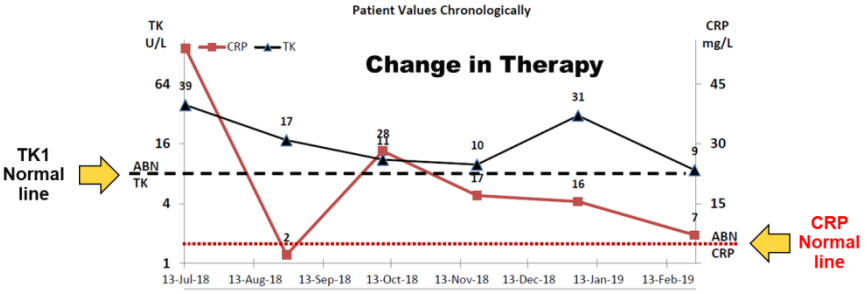
Therapeutic Monitoring



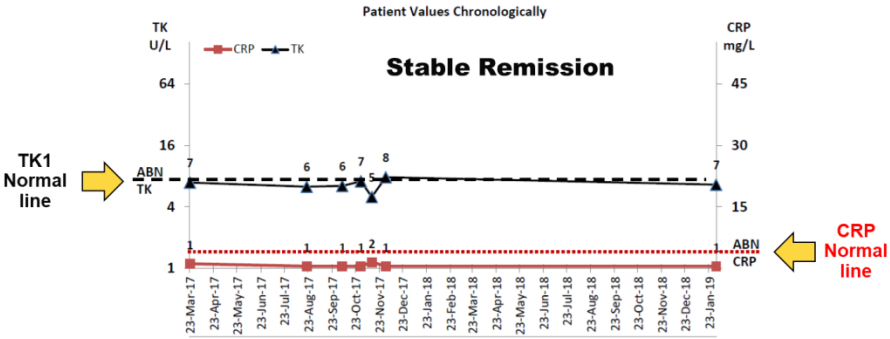
Following both TK1 and CRP (or HPT) during therapy documents **effectiveness**. In the case of chemotherapy TK1 will gradually decline as tumor burden declines. In cases of surgical tumor removal, TK1 will decline immediately. In both cases, CRP (or HPT) will decline if therapy is effective.



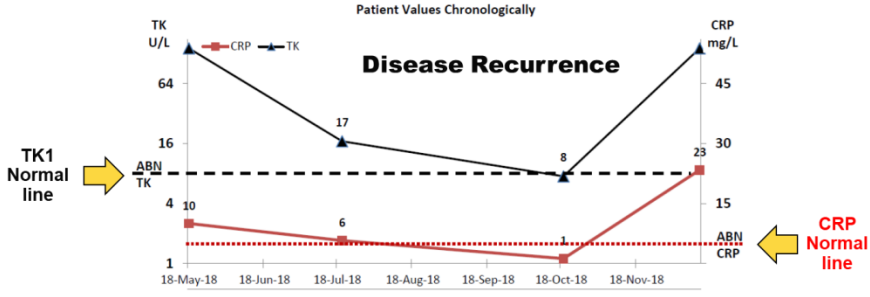
If therapy is **ineffective**, TK1 will not fully decline. Partial reduction may occur and CRP (or HPT) will initially decline and then rise when tumor processes resume.



A **change in therapy** may be warranted when TK1 levels rise. If the new therapy is effective both TK1 and CRP (or HPT) will immediately decline.



Monitoring patients over months and years ensures that the patient is in **stable remission**. Both TK1 and CRP (or HPT) will remain suppressed.

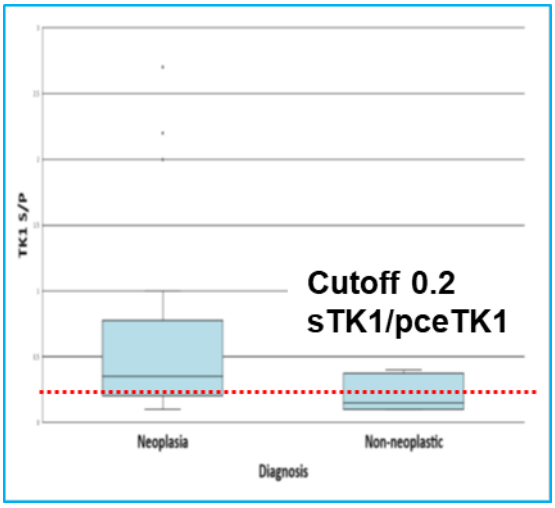


Disease recurrence is documented by the concurrent rise in both TK1 and CRP (or HPT).

A growing body of evidence…

Pericardial Effusion

Pericardial effusion (PCE) represents ~7% of canine cardiac disease and causes significant morbidity and mortality. The most common causes include neoplastic infiltration of the right atrium and/or right auricle (most frequently from hemangiosarcoma), idiopathic pericarditis, and neoplasia at the heart base (ex: chemodectomas, thyroid gland adenocarcinomas, and other carcinomas and sarcomas). Neoplastic causes of PCE, particularly hemangiosarcoma, impart a significantly worse prognosis than nonneoplastic etiologies. Currently, echocardiography is the method of choice for non-invasive antemortem diagnosis of cardiac neoplasia; however, it requires specialized equipment, an experienced echosonographer, and may not detect smaller tumors. A simple blood test would be a valuable alternative.



In a recent study (12), the use of TK1was evaluated to discern malignant from benign causes of pericardial effusion. TK1 analysis of both serum and pericardial effusion was evaluated. While the use of either serum TK1 (sTK1) or pericardial effusion TK1 (pceTK1) alone did not provide sufficient specificity, the **RATIO** of sTK1/pceTK1 was able to rule-out cancer effectively.

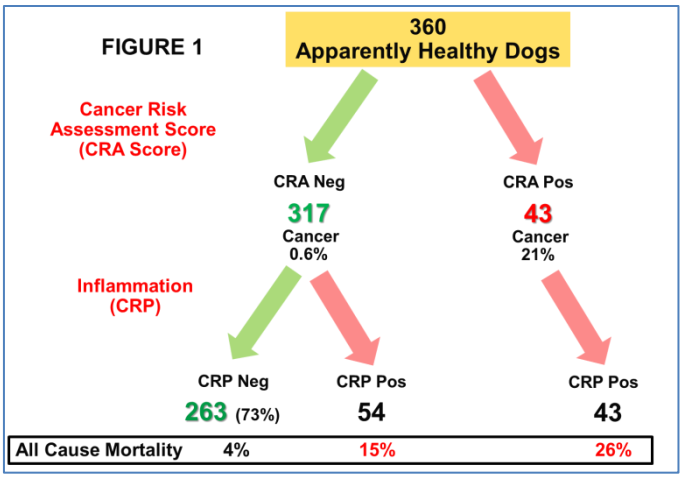
Using a sTK1/pceTK1 ratio cutoff of 0.2, the rule-out of cancer as the cause of pericardial effusion had a specificity of 91%.

Wellness Screening

Routine chemistries such as albumin, creatinine, ALT, evaluate key organ systems however there is significant capacity within these organs to maintain homeostasis. Kidneys have to lose half their function before creatinine levels rise, liver dysfunction has to be advance before liver enzymes rise. The body works very hard to maintain status-quo.

Inflammation on the other hand is the genesis of all the inflammatory diseases. Whether it is cancer, heart disease, or IBD, the inflammatory process begins early.

To evaluate how TK1 and CRP could be used to detect cancer and other serious inflammatory diseases a study (7) was conducted evaluating 360 apparently healthy dogs and following them over a 6-month period. TK1 and CRP measurements were taken initially and grouped according to their Cancer Risk Assessment score (CRA) for cancer risk and then by C-reactive protein for disease risk. Figure 1 details how dogs were grouped.



Through the use of TK1/CRP, 82% of all cancers were detected six months **PRIOR TO** clinical signs. The CRA score effectively concentrated the at-risk dogs. Within this group, overall mortality was 26%.

Within the CRA negative group, the cancer incidence was extremely low (0.6%). Further those that had heightened inflammation levels overall mortality was 15%.

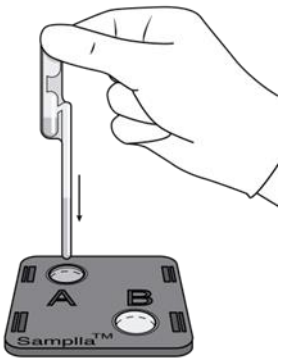
The routine screening of the apparently healthy dog for TK1 and CRP could be valuable in a disease prevention program.

A growing body of evidence…

Transportation and Test Performance

Thymidine kinase, type 1, is a low concentration (pmol), heat liable enzyme. TK1 values will increase if left on the clot for more than 2.5 hours as lysed WBC will release TK1. It is highly recommended serum samples are drawn in a gel barrier tube and serum removed within 1 hour. Frozen samples are stable for 30 days at minus 20ºC.

To aid in the tranportation of specimens, VDI Laboratory developed an innovative new technology termed, Samplla™. Samplla™ technology stabilizes blood components allowing for ambient storage and transportation.

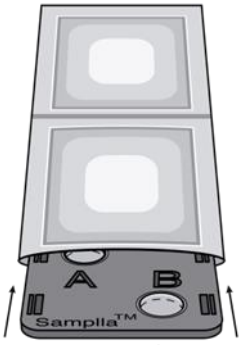


Patent pending

Samplla™ provides:

- 21-day stability at ambient temperatures;
- high degree of analytical sensitivity down to pmol concentration;
- correlates well with neat serum

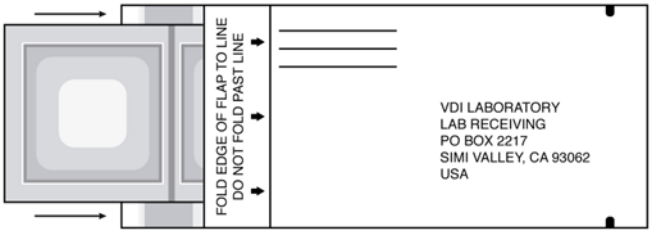
Key Components & Process



Patent pending

Specimen is applied to a custom made device containing two wells. Specimen does not have to be applied with precision – the device auto regulates volume. Air drying is not required with on-board desiccant.

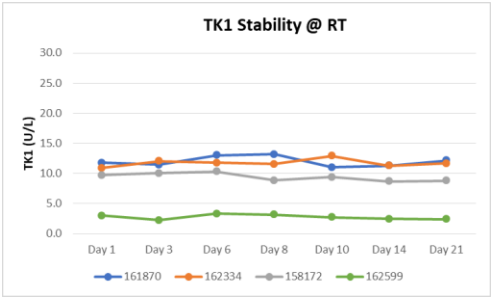
The device is inserted into a foil envelope that develops a special atmosphere stabilizing test components.

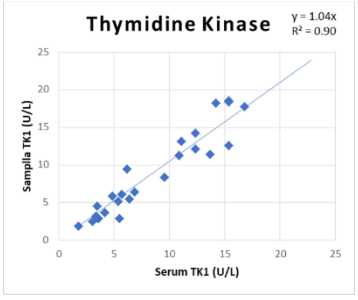


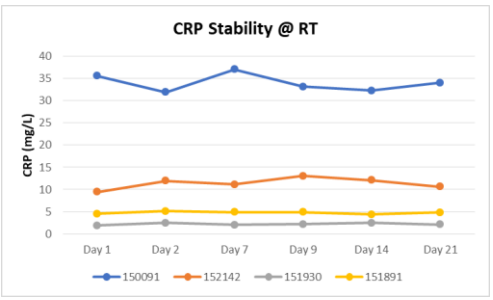
Patent pending

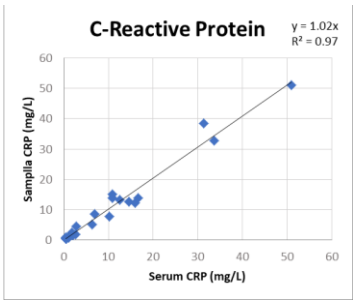
The entire assembly is inserted into a special mailer that maintains the integrity of the package for transit via common carrier, including international. Once received by the lab, the media is removed, and specimen is extracted and run as usual.

Analytical Performance









Canine samples stored at room temperature for 21 days did not demonstrate any loss of signal.

	Samplla TK1		Samplla VitD		Samplla CRP
	6.1		63.3		6.3
	6.4		63.6		6.2
	6.6		64.4		5.8
	6.4		62.9		6.2
	6.2		63.9		6.2
avg	**6.3**	avg	**63.6**	avg	**6.1**
SD	**0.19**	SD	**0.57**	SD	**0.19**
CV	**3%**	CV	**1%**	CV	**3%**
	14.0		30.8		13.3
	13.4		28.5		13.4
	13.2		31.0		12.8
	13.6		30.5		15.3
	14.8		31.4		14.3
avg	**13.8**	avg	**30.4**	avg	**13.8**
SD	**0.63**	SD	**1.13**	SD	**0.99**
CV	**5%**	CV	**4%**	CV	**7%**
	3.5		76.1		22.7
	3.2		75.6		22.0
	3.5		75.9		21.2
	3.7		73.9		24.3
	3.6		78.3		23.3
avg	**3.5**	avg	**76.0**	avg	**22.7**
SD	**0.19**	SD	**1.57**	SD	**1.19**
CV	**5%**	CV	**2%**	CV	**5%**

1. Plasma thymidine kinase activity in dogs with lymphoma and leukemia

Nakamura N, et al, J Vet Med Sci, 1997 **cohort = 42 dogs**

- Dogs with lymphoma or leukemia had significantly (P<0.01) elevated TK1 concentrations (6.8 to 430 U/L) compared to control group (<6U/L). Dogs monitored after remission induction and relapse demonstrated falling and rising TK1 concentrations in relation to clinical status.

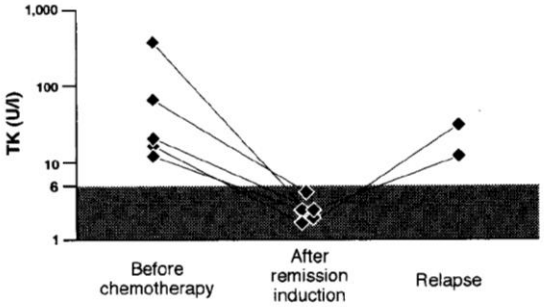


Fig. 3. Changes of plasma TK activity in 5 dogs with lymphoma. TK activity in 5 cases (case 2, 5, 10, 11 and 14) before chemotherapy, after remission induction and at relapse are shown.

2. Serum thymidine kinase activity in dogs with malignant lymphoma: a potent marker for prognosis and monitoring the disease

von Euler, et al, JVIM, 2004 **cohort = 65 dogs**

- Serum TK1 concentrations were significantly higher in dogs with lymphoma than control dogs or dogs with non-cancerous inflammation or benign tumors. TK1 concentrations increased with tumor grade.

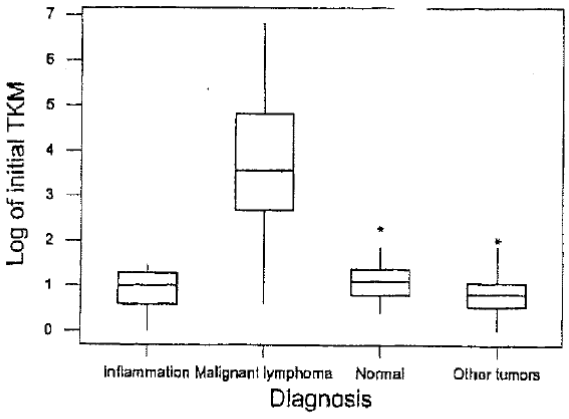


Fig 2. Distribution of serum thymidine kinase (sTK) activity in healthy dogs (n = 21), dogs with inflammatory diseases (n = 7), dogs with ML (n = 52), and dogs with other tumors (n = 25).

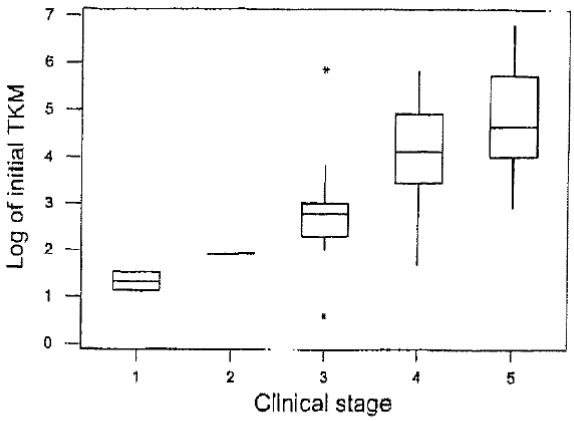


Fig 4. Serum thymidine kinase (sTK) activity versus clinical stage of malignant lymphoma (ML). Error bars show the standard deviation.

A growing body of evidence…

3. A non-radiometric method for measuring serum thymidine kinase activity in malignant lymphoma in dogs

von Euler H, et al, Res Vet Sci, 2006 **cohort = 45 dogs**

- Mean TK1 values in dogs with lymphoma increased with grade. Mean TK1 concentration in grade I/II, III, and IV/V were 12.7, 24.2, and 109.9 U/L, respectively.

4. Monitoring therapy in canine malignant lymphoma and leukemia with serum thymidine kinase 1 activity - evaluation of a new, fully automated non-radiometric assay

von Euler H, et al, Intl J Onco, 2008 **cohort = 213 dogs**

- A high precision, fully automated TK1 method was evaluated with normal dogs and dogs with non-cancerous inflammation, lymphoma, leukemia, remission induction, and disease recurrence.
- Inter-assay precision was within 5%.
- Dogs in complete remission had TK1 values return to normal. Dogs with only partial remission did not reach normal levels. TK1 is useful to monitor disease status.

5. Elevated serum thymidine kinase activity in canine splenic hemangiosarcoma

Thamm D, et al, Vet Comp Onco, 2011 **cohort = 150 dogs**

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- In dogs with splenic hemangiosarcoma TK1 was able to distinguish malignant disease with a PPV of 0.93.

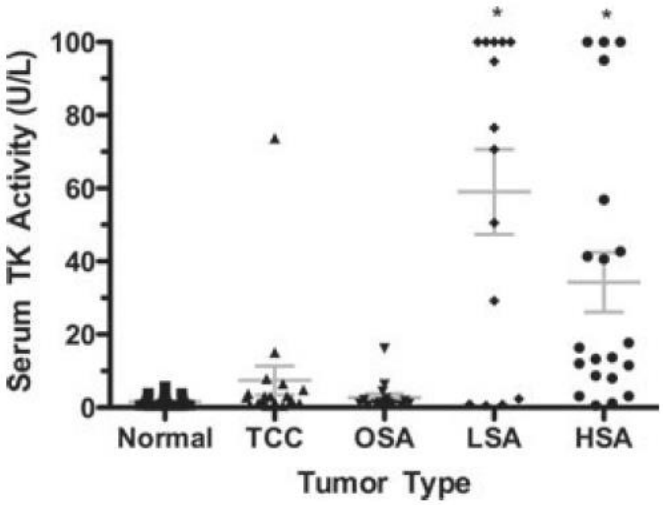

Figure 1. Pilot study: serum thymidine kinase 1 activity in normal dogs and dogs with neoplasia. Error bars indicate standard error. $^{*}P < 0.01$ versus normal.

6. Serum thymidine kinase activity in clinically healthy and diseased cats: a potential biomarker for lymphoma

Taylor S, et al, J Feline Med Surg, 2012 **cohort = 171 cats**

- Cats with lymphoma had significantly higher TK1 concentrations than healthy cats or cats with inflammatory disease (P<0.0002). Clinically healthy cats had a mean TK1 concentration of 2.2 U/L. Cats with lymphoma and other inflammatory disease had mean TK1 concentration of 17.5 and 3.4 U/L, respectively.
- TK1 is a potentially useful biomarker for feline lymphoma.

7. Serum thymidine kinase 1 and C-reactive protein as biomarkers for screening clinically healthy dogs for occult cancer

Selting K, et al, Vet Comp Onco, 2013 **cohort = 360 dogs**

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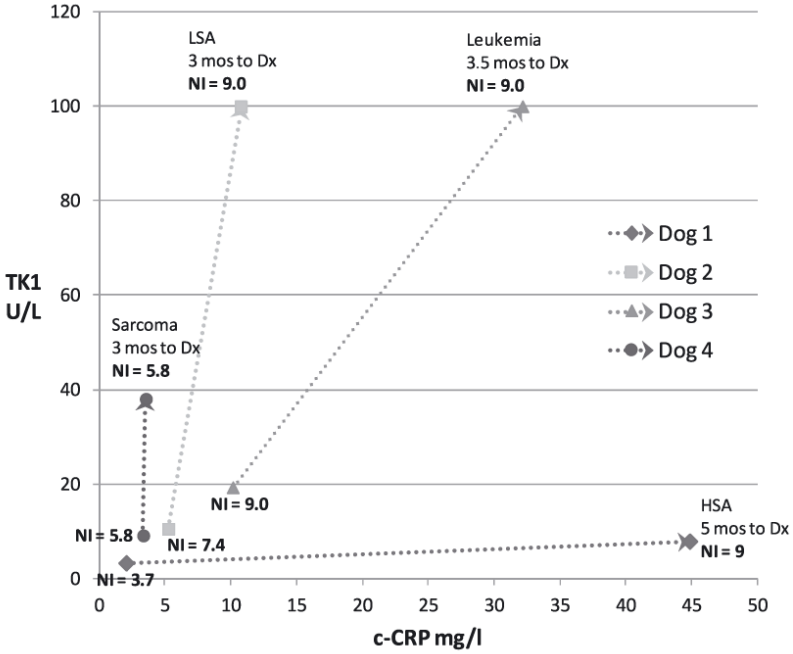
- 360 apparently healthy dogs were tested for TK1/CRP and followed for 6 months. Median age was 7 years. Overall incidence of cancer was 3% within the 6-month followup.
- Abnormal TK1/CRP concentrations, expressed as the Neoplasia Index, were able to detect 100% of the cancers at 4 months and 82% of the cancer at the 6-month interval.

		Positive		High positive	
Neoplasia index	**ROC AUC**	**Sensitivity at ≥5.8**	**Specificity at ≥5.8**	**Sensitivity at ≥9.0**	**Specificity at ≥9.0**
Confirmed	0.940	0.86	0.90	0.29	0.98
4-month status	0.967	1.00	0.91	0.44	0.98
≥6-month status	0.933	0.82	0.91	0.36	0.98

- 4 dogs with different cancers were monitored at time zero and again at time of cancer diagnosis. In all cases both TK1 and CRP increased reflecting the overall growth of the tumor.



- The use of TK1 and CRP is useful to detect occult cancer in the apparently healthy dog.

8. A comparative proteomic study of plasma in feline pancreatitis and pancreatic carcinoma using 2-dimensional gel electrophoresis to identify diagnostic biomarkers: A pilot study

Meachem M, et al, Canadian J Vet Res, 2015 **cohort = 18 cats**

- Feline haptoglobin, an acute phase protein, is mildly elevated in exocrine pancreatic insufficiency (EPI).
- In intestinal lymphoma, haptoglobin is strongly elevated and the use of haptoglobin assists in identifying EPI in GI cats.

9. Serum thymidine kinase activity in clinically healthy and diseased horses: a potential marker for lymphoma

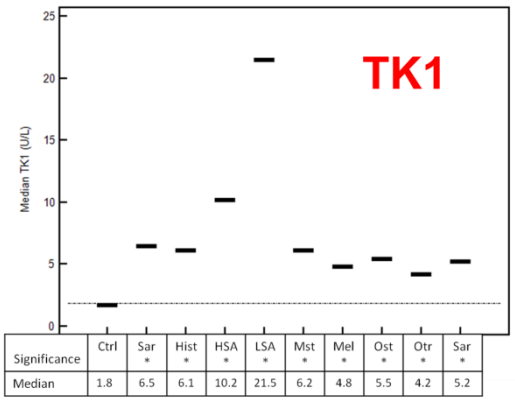
Larsdotter S, et al, Vet J, 2015 **cohort = 83 horses**

- TK1 (mean ± SD) was 26.3 ± 91.5 U/L (range 0.8-443 U/L) for horses with lymphoma, 2.3 ± 1.4 U/L (range 0.6-5.7 U/L) for horses with non-haematopoietic neoplasia and 1.5 ± 0.6 U/L (range 0.6-2.8 U/L) for horses with inflammatory disease.
- Horses with lymphoma had significantly higher TK1 activity than horses without clinical signs of disease ($P < 0.01$), horses with inflammatory disease ($P < 0.01$) and horses with non-haematopoietic neoplasia ($P < 0.05$).
- TK1 activity is a potentially useful biomarker for equine lymphoma.

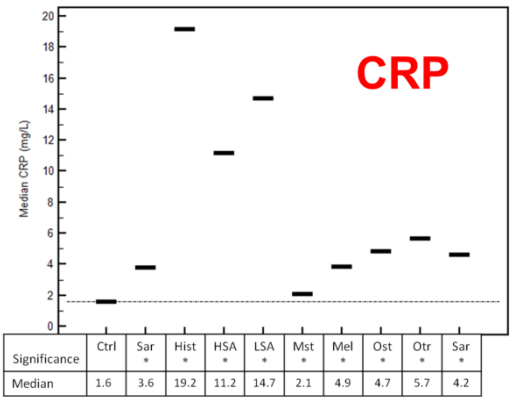
10. Thymidine kinase type 1 and C-reactive protein concentrations in dogs with spontaneously occurring cancer

Selting K, et al, JVIM, 2016 **cohort = 409 dogs**

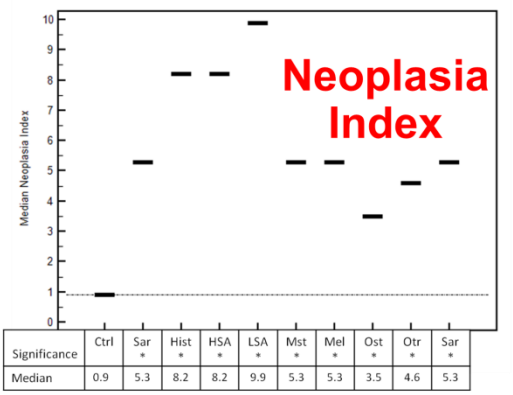
- TK1 activity in the cancer (n = 253) and control group (n = 156) were 7.0 l/L (median, range <0.5 to >100) and 1.8 l/L (median, range 0.4 to 55.3), respectively ($P < .001$).
- CRP concentrations in the cancer and control group were 6.0 mg/L (median, range <0.5 to >50) and 1.6 mg/L (median, range 0.09 to >50), respectively ($P < .001$).
- The Neoplasia Index 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.



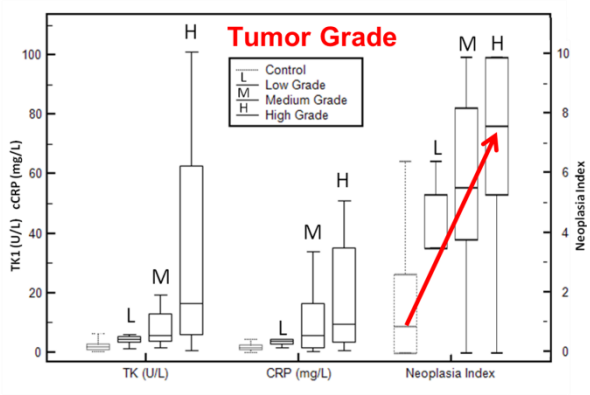
	Ctrl	Sar *	Hist *	HSA *	LSA *	Mst *	Mel *	Ost *	Otr *	Sar *
Significance										
Median	1.8	6.5	6.1	10.2	21.5	6.2	4.8	5.5	4.2	5.2



	Ctrl	Sar *	Hist *	HSA *	LSA *	Mst *	Mel *	Ost *	Otr *	Sar *
Significance										
Median	1.6	3.6	19.2	11.2	14.7	2.1	4.9	4.7	5.7	4.2



	Ctrl	Sar *	Hist *	HSA *	LSA *	Mst *	Mel *	Ost *	Otr *	Sar *
Significance										
Median	0.9	5.3	8.2	8.2	9.9	5.3	5.3	3.5	4.6	5.3



11. Serum Thymidine Kinase 1, Canine-C-Reactive Protein, Haptoglobin, and Vitamin D Concentrations in Dogs with Immune-Mediated Hemolytic Anemia, Thrombocytopenia, and Polyarthropathy

Grobman M, et al, JVIM, 2017 **cohort = 38 dogs**

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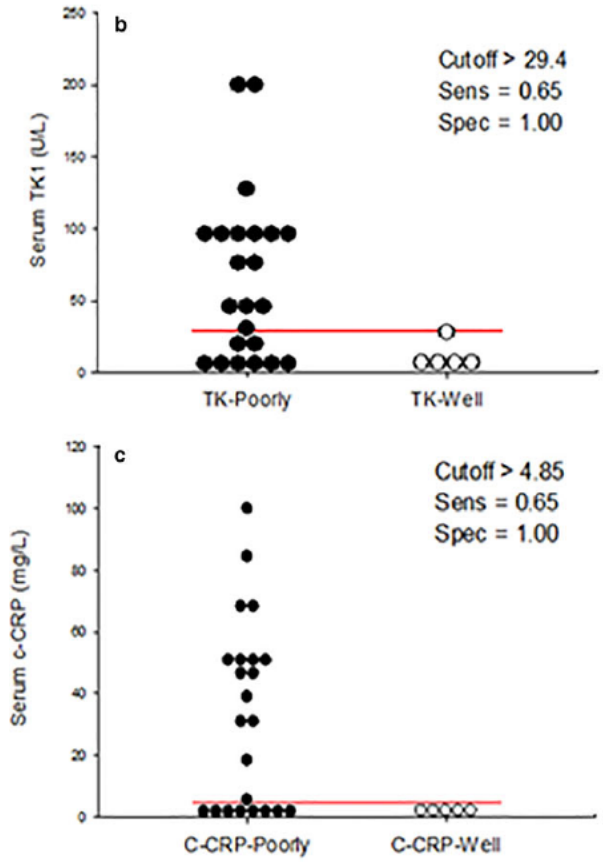
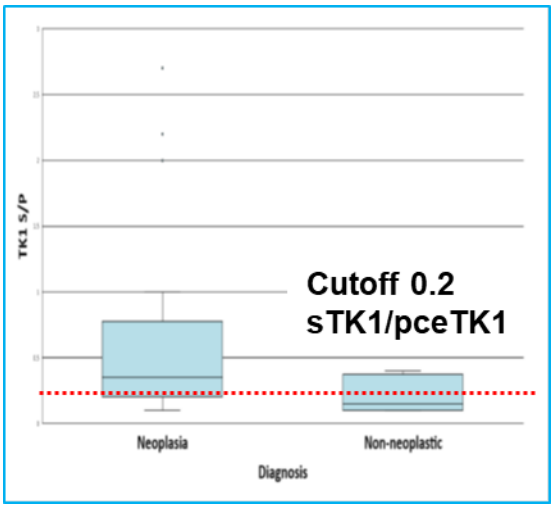
- TK1 and CRP significantly decreased with well versus poorly controlled IMHA (P = 0.047, P = 0.028, P = 0.37).
- Sensitivity and specificity of TK1 and CRP (simultaneously) for detecting dogs with poorly controlled IMHA were 88 and 100%, respectively.



12. Thymidine kinase-1 and C-reactive protein concentrations in dogs with neoplastic and nonneoplastic causes of pericardial effusion

Kong L, et al, ACVIM poster, 2019 **cohort = 94 dogs**

- Using a ratio of serum TK1 to pericardial TK1 (TK1 S/P), distinguishing neoplastic causes from non-neoplastic causes had a ROC AUC of 0.72.
- Using a cut-off of 0.2, a rule-out of neoplastic cause had 91% specificity.



13. Evaluation of serum thymidine kinase 1 activity as a biomarker for treatment effectiveness and prediction of relapse in dogs with non-Hodgkin lymphoma

Boye P, et al, ACVIM, 2019 **cohort = 120 dogs**

- Serum TK1 was significantly (P<0.0001) lower in complete remission (CR) dogs vs partial remission (PR).
- An increase of TK1 activity of 2.7-fold was predictive of relapse.