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## Use of feline TK1 as a biomarker in disease monitoring

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Katt TK1 som biomarkör i klinisk diagnostik

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## SUMMARY

Serum thymidine kinase 1 (TK1) activity is used as a tumor marker in disease monitoring in veterinary and human medicine. TK1, an intracellular enzyme, is involved in a salvage pathway of DNA precursor synthesis.

TK1 is used in DNA precursor production by catalyzing the transfer of the gamma-phosphate-group from a phosphate-donor to the 5' - hydroxyl-group of thymidine forming thymidine-monophosphate. Nucleoside monophosphates are finally converted into thymidine-triphosphates. TK1 activity significantly rises in the G1 and the S phase of the cell cycle. Thus, TK1 is found in dividing cells, and in the blood as an extracellular form of the enzyme.

Two different TK isoforms have been identified in animal cells, one in the cytosol (TK1) and the other in mitochondria (TK2). TK1 plays a key role in cell-division, as a step in the reaction chain to introduce thymidine in to DNA. Thymidine is degraded into smaller molecules and secreted. It is found in body fluids, as a result.

Due to the noticeable proliferative activity in tumor cells, TK1 enzyme activity is increased in this type of cells. Increased TK1 activity is a reflection of a high proportion of cells in S-phase of the cell-cycle. Since increased TK1 activity can be measured in serum, TK1 is a useful marker in diagnostics of neoplastic diseases. TK1 activity provides valuable information regarding disease prognosis and treatment results.

Increase in TK1 levels are considered to be a sensitive and valuable marker for not only cell proliferation but also malignancy monitoring in clinical medicine.

In addition, there are viruses able to induce TK production and elevated serum TK levels can be found in a variety of neoplasias as reported in clinical studies. Most of these studies concerned hematologic malignancies. E.g. TK1 is a valuable marker in non-Hodgkin's lymphoma. TK reflects clinical staging and provides noteworthy prognostic information.

In both veterinary and human medicine a radioactive based test (Thymidine kinase radio-enzymatic assay: TK-REA) is commercially available to assess TK1 serum activity. For example, it is used to determine TK1 serum activity in dogs. However, TK-REA requires handling of radioactive material and costs 500 kr (€ 58) per test, which limits its use in veterinary clinical diagnostics and disease monitoring. Antibodies have been used to measure the TK1 serum concentration. Analysis of this type has been successful in canine- and human-medicine and would be valuable to evaluate in feline-medicine.

Since TK1 serum has proven useful in canine- and human- medicine the hypothesis was that feline would be able to serve as a valuable biomarker in feline clinical medicine.

The aim of the study was to evaluate whether feline TK1 has the potential to become a valuable biomarker in feline clinical practice. In order to evaluate TK1 as a biomarker, the enzyme's activity in serum was measured with [<sup>3</sup>H]-dThd phosphorylation assay and with the commercially available TK-REA assay. The results were compared. The study is a first step towards better understanding the feline TK1's role in disease diagnostics, disease monitoring,

and as a proliferation biomarker in neoplastic diseases. Significant correlation was observed between the [<sup>3</sup>H]-dThd phosphorylation assay results and the commercially available TK-REA assay results. A correlation of 0.97 was found between the assay and the commercially available TK-REA measured activity. The [<sup>3</sup>H]-dThd phosphorylation assay showed a mean value of 2.04 pmol/min/mL in sera from cats with diseases, with a standard deviation of 2.07. The level of TK1 activity for healthy cats was not detectable, using a cut of value at 0.8 pmol/min/mL

Both assays show a significant increase in TK activity in cats with lymphoma, leukemia and cystitis versus clinically healthy cats, signifying that TK may serve as a future diagnostic tool in clinical practice. However, the discrepancy in activity level between a cat with lymphoma and a healthy cat was not as significant as that between a dog with lymphoma and a healthy dog.

In conclusion, a high TK serum activity level is greatly indicative of a hematopoietic neoplasia diagnosis, while there is a low predictability for cats with low TK serum activity level. The findings show that feline TK1 has the potential to become a valuable biomarker in clinical practice. These finding in combination with the overview of the lymphoma diagnostic challenges indicate that TK1 has the potential to become a valuable biomarker in clinical practice.

## SAMMANFATTNING

Mätning av aktivitet av serum thymidinkinas 1 (TK-1) används som biomarkör inom human- och veterinär- medicinen. Det intracellulära enzymet TK-1 ingår i cellens DNA-syntes "The Salvage Pathway" och uttrycks under cellcykeln. TK-1 aktiviteten är som högst i cellen då DNA syntesen är aktivast mellan G1- och S-fasen av cellcykeln. I normala celler avtar enzymets aktivitet när cellen påbörjar G2-fasen av cellcykeln. Vid neoplastiska och inflammatoriska sjukdomar är TK-aktivitet i cellen högre då cell-proliferationen och DNA-syntesen är intensiv. Den förhöjda TK1 aktiviteten i serum går att mäta vilket gör TK-1 till en brukbar biomarkör för diagnostik av inflammatoriska och neoplastiska sjukdomar. TK1 som biomarkör kan användas som hjälpmedel för att ställa diagnos, bedömning av prognos och för att utvärdera insatt behandling.

I både veterinär- och human-medicin finns ett radioaktivt test tillgängligt för att mäta TK1 aktivitet i serum: TK-REA. TK-REA används tex för att mäta TK1 aktivitet i hundar med misstänkt neoplastiska sjukdomar. Dock kräver TK-REA radioaktiva ämnen och kostar 500 kr per prov, vilket begränsar dess användning i veterinär kliniska sammanhang. Antikroppar har används för att mäta serum nivåer av TK1. Denna typ av analys har varit framgångsrik som biomarkör inom human- och hund- medicin och skulle med stor sannolikhet vara användbar även inom katt-medicin.

Eftersom TK1 aktivitet i serum har visat sig vara en användbar biomarkör i både human- och hund-medicin var hypotesen att TK1 aktivitet i serum skulle visa sig ha potentialen att kunna bli en brukbar biomarkör inom katt-medicin.

Målet med denna studie var att utvärdera katt TK1 som en användbar biomarkör i klinisk diagnostik. För att kunna utvärdera katt TK1 som biomarkör mättes kattserum aktivitet med [<sup>3</sup>H]-dThd fosforylering testet och TK-REA testet för att sedan kunna jämföra resultaten. Studien, är ett första steg mot att utvärdera katt TK1 som biomarkör för att diagnostisera neoplastiska och inflammatoriska sjukdomar, följa upp insatt behandling och bedöma prognosen.

Resultaten mellan [<sup>3</sup>H]-dThd fosforylering testet och TK-REA testet hade en korrelation på 0.97. [<sup>3</sup>H]-dThd fosforylering testet hade en genomsnittlig aktivitet på 2,04 pmol/min/mL med en genomsnittlig standardavvikelse på 2.07 hos prover från sjuka katter. De friska kattarnas TK1 serum aktivitet låg under detektionsgränsen på 0.8 pmol/min/mL.

Båda testerna visar förhöjd TK1 serum aktivitet hos katter med lymfom, leukemi och cystit jämfört med friska katter, vilket indikerar att TK1 har potential att kunna bli en användbar biomarkör inom kattmedicin. Dock så är skillnaden i aktivitet mellan en katt med lymfom och en frisk katt inte lika markant som hos hund.

Konklusion, en hög TK1 serum aktivitet är indikativ för en neoplastisk diagnos, medan diagnosen är osäker vid låg aktivitet. Resultatet från testerna i kombination med

sammanställningen av de diagnostiska utmaningarna visar att TK1 har potential att bli en brukbar biomarkör i klinisk medicin.

## INTRODUCTION

### DNA Replication and the role of thymidine kinase

Thymidine kinase (TK) is one among several enzymes involved in the synthesis of DNA precursors. TK catalyzes the phosphorylation of thymidine (deoxythymidine/ dTdR) to thymidine 5'-phosphate (dTMP) (Uzman *et al.*, 2012). dTMP is incorporated into DNA after further phosphorylation. TK activity can be measured by using radioactive labelled thymidine.

Deoxyribonucleic acid (DNA) precursor synthesis in cells follows *de novo*-pathway or salvage pathway. In the *de novo*-pathway, pyrimidine and purine ribonucleotides are reduced to deoxyribonucleotides by the enzyme ribonucleotide reductase. If the *de novo*-pathway is obstructed, the cells utilize the *salvage pathway* in order to stay alive. For deoxy- pyrimidine synthesis, the *salvage pathway* forms thymidine monophosphate from thymidine using thymidine kinase. The cells die if both pathways are being obstructed.

Purine (bases A and G) and pyrimidine (bases C and T) deoxyribonucleotides, making up the two groups of nitrogenous bases (Calladine *et al.*, 2004). Purine and pyrimidine, two aromatic organic compounds, are synthesized by *de novo pathway*. Salvage pathways recover nucleosides and bases that are made during the degradation of nucleic acids. Some tissues cannot undergo *de novo* synthesis why the *salvage pathway* plays an essential role. The salvaged nucleosides and bases can be converted back into nucleotides. The *salvage pathway* uses substrates for free purines from the hydrolysis of nucleotides, or thymidylate (dTMP) produced by thymidine kinase from thymidine, instead of the typical path where deoxyuridylate (dUMP) is methylated using thymidylatesynthetase into thymidylate (dTMP).

### Biomarkers

Definition of a biomarker: “A biomarker is a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention” (Puntmann , 2009).

In oncology trials, serum analysis constitutes the most used source of biomarkers (Goulart *et al.*, 2007). Safety, patient acceptance, easy accessibility, and the fact that it is relatively inexpensive, are contributing determining factors of a practical biomarker. Actual tissue of a tumor is the second leading biomarker. Biopsies from normal tissue can also represent useful biomarker sources as they include sites more accessible than actual tumors while providing cells and tissues with modulatory effects of a drug.

In veterinary medicine, diagnosis of diseases and appropriate therapeutic treatment are clinical challenges. In order to assist in diagnostics, an ideal biomarker for the corresponding disease should have appropriate sensitivity and specificity to differentiate between healthy and sick patients. Thus, provide reliable information about the disease progression, how it responds to therapy, and predict the recurrence of the disease.

## Serum TK1 as a biomarker

Extreme and unrestrained cell-proliferation is a hallmark of cancer. Several types of cell-proliferation markers have been used in diagnosis, prognosis, monitoring of therapy outcome and follow-up in clinical oncology. Few tumor markers are useful in all cases, with the S phase-specific TK1 being an exception. TK1 expression is closely correlated to the overall level of proliferation. Several commercial serum TK1 activity assays have proven valuable in clinical practice (Von Euler & Eriksson, 2011).

Blood biomarkers include infectious disease biomarkers used when inflammatory mediators are released into the blood at the infection site, and cell proliferation biomarkers such as tumor markers (Sharif, 2012). Tumor markers are any substance found in the body when malignant cells are present.

The activity of TK1 in late the G1- and the early S -phase of dividing cells clarifies the elevated enzyme levels in various tumors, as they typically contain fewer quiescent cells (cells in a resting phase G0) than do regular tissue (Hallek *et al.*, 1992). Hence, serum elevations of TK1 levels have been detected in several malignancies.

A tumor is an abnormal mass of body tissue that lacks body function. Tumors are typically classified as benign or malignant. A benign tumor is not classified as cancer. It grows slowly, does not spread, nor does it invade surrounding tissue. Once a benign tumor is entirely removed, it typically does not recur. A malignant tumor, on the contrary, is cancer that has the capability to spread through metastases. Some "benign" tumors may later transform into malignant tumors, due to further genetic changes in subpopulations of neoplastic cells of the tumor. Hence, early cancer diagnosis allows for early treatment and greater survival rate.. Appropriate tumor markers can considerably reduce the diagnostic lead time, and serve as a screening tool for the prevalence of malignancy in populations at risk and apparently healthy populations. Good markers will allow for early detection of tumors and thus a better chance for appropriate therapy. These markers may also be used to evaluate the therapy and predict any possible disease recurrence.

In a number of studies, serum TK changes have been found to parallel and/ or anticipate the clinical course, i.e. the response to treatment and possible relapse, of low-grade non-Hodgkin's lymphoma in longitudinal individual patient observations (Hallek *et al.*, 1992)

A health screening study including more than 35,000 people confirms that serum TK1 concentration can be a valuable biomarker for detecting future potential malignancies (Chen *et al.*, 2011). Serum TK1 assay can distinguish between sick people with tumor, people with non-malignant proliferation diseases, and healthy individuals.

TK-REA is an established tool for tumor diagnosis and prognosis in human medicine that has been used for several decades (Von Euler & Eriksson, 2011). More recently, it has also been

used in dogs with malignant lymphoma. Serum TK1 activity differs noticeably between healthy dogs and dogs with lymphoma.

High levels of TK1 can be detected in the sera of animals and humans with tumors; thus, TK1 activity in serum is used as a prognostic marker for several types of tumors, foremost leukemia and lymphoma (Sharif & von Euler *et al.*, 2012).

Serum TK1 protein level assessments in tumors provide accurate information about the cancer patient's prognosis (He *et al.*, 2010). TK1 protein level assessment can also be used for monitoring tumor therapy and as an early detector of diseases such as cancer. Serum TK1 protein values correlate to remission after chemotherapy and surgery. The TK1 intracellular expression in human breast tumors is used to predict the survival rate of patients with breast cancer.

The cytoplasmatic enzyme TK1 is a candidate for the early detection of tumor cell division and proliferation (Aufderklamm *et al.*, 2011). The use of TK1 antibodies has been investigated in different studies including different types of solid tumors. This makes TK1 feasible for monitoring of therapeutic outcome and efficacy especially in the post-surgery phase of treatment.

As TK1 is expressed in proliferating cells during the DNA synthesis, it can be detected in both malignant and non-malignant cells (Chen *et al.*, 2011). TK1 serum concentration and its activity are elevated in the case of cancer patients, while TK1 serum concentration and its activity in healthy people are low or undetectable. Though, in the case of human patients with acute sickness such as inflammation or infection, or experiencing other physiological changes (menstruation, surgery, or blood-donation), TK1 can increase momentarily. The reasons for differences in STK1 levels of sick people and healthy people are still not entirely known. A likely explanation is related to cell death. Regular healthy cells die during the G1/G0 stage of the cell cycle, when the intra-cellular levels of TK1 are low. Tumor cells can die in the S/G2 stage of the cell cycle, when TK1 levels are high, causing more serum TK1. Another explanation is a factor in serum, which acts differently in healthy people compared to sick people, affecting the stability of serum TK1. Since serum TK1 increases when people have infections or inflammations as a result of the activation of the immune system, it may be possible that a part of the increased serum TK1 levels of cancer patients results from the stimulated immune system and not only from the cancer cells. Hence, when interpreting the implications of an increase of STK1 as a malignancies development risk, reasons for the short term increase in STK1 levels other than possible malignancies should be taken into account. Thus, the medical history of the patient is essential.

### **Aim of the study**

Distinguishing between the diagnosis lymphoma, IBD and allergies can be a challenge. It is important to arrive at the accurate diagnosis since the therapy varies for different pathological conditions. Thus, the outcome of the clinical treatment is dependent on an accurate diagnosis and the biomarkers involved. TK1 activity in serum has proven to be a valuable diagnostic

tool in human and canine medicine, which may also be the case for feline medicine. The aim of the study was to evaluate whether feline TK1 serum activity has the potential to become a valuable biomarker in feline clinical practice and to summarize the clinical diagnostic challenges of lymphoma versus IBD.

A significant correlation was found between TK-REA and [<sup>3</sup>H]-dThd assay when measuring serum TK1 activity in dogs with hematological tumors (Sharif & von Euler *et al.*, 2012).

The study is a first step towards defining the role of TK1 in feline disease diagnostics, disease monitoring, and as a proliferation biomarker in neoplastic diseases. TK1 activity in serum was measured with the [<sup>3</sup>H]-dThd phosphorylation assay and compared with the commercially available feline TK-REA assay results. Temperature, pH, and time were altered in an attempt to optimize the [<sup>3</sup>H]-dThd phosphorylation assay.

## **BACKGROUND**

### **Feline Cancer**

Cancer is a one of the major causes of mortality and sickness in domestic animals (Argyle *et al.*, 2008). Current reports indicate prevalence increase of cancer in cases of dogs and cats, partly due to the increased life span of domestic animals. Life span has increased due to infectious disease control, vaccination, and improved nutrition. Thus, there is an increased expectation of the veterinary practitioner, to be able to diagnose and treat patients with cancer in general practice.

Compared to dogs and other domestic animals, cats have higher cancer rates (Eldredge *et al.*, 2008). Feline cancers typically occur in middle-aged to older cats at the age of 10 to 15 years. However, lymphoma being an exception occurs most often in young cats. Besides skin and breast tumors, most feline cancers are not visible by outward inspection. Skin and breast neoplasms can be detected by inspection and palpation. Feline leukemia virus and the feline immunodeficiency virus are assumed to be a contributing factor to the high cancer rate in cats. Common involvement sites are lymph nodes (i.e. lymphoma) and circulating blood cells (i.e. leukemia). Any organ or body tissue of the cat can be affected. The leukemia virus of cats (FeLV) most likely accounts for fifty percent of all internal cancers, lymphoma being the majority (Eldredge *et al.*, 2008). Low immunity level associated with feline leukemia virus infection certainly contributes to the high prevalence of secondary diseases. The feline leukemia virus is associated with other serious cat diseases such as toxoplasmosis, spinal cord cancers, glomerulonephritis, feline infectious peritonitis, and anemia.

### **FELINE CANCER FACTS**

- Hemopoietic tumors are the most commonly diagnosed feline neoplasm type, accounting for about 35% of all diagnosed tumors and is directly related to Feline Leukemia Virus (Argyle *et al.*, 2008).
- 32% of cats over 10 years old will die from cancer.

- Approximately 0,025% of female cats will develop mammary cancer
- 17 % of feline cancers are mammary related.
- Feline lymphoma will affect 0,002% of all cats.
- FeLV positive cats have a 60% higher risk than healthy cats to be affected by lymphoma.
- 10% of feline tumors are located in the mouth.
- Vaccination related sarcomas has a prevalence between in 0,1% to 0,01% in cats
- 25 % of all cancers in cat are cancers of the skin, with approximately 50-65 % being malignant (Eldredge *et al.*, 2008).

Skin tumors are common in cats (Eldredge *et al.*, 2008). Many feline tumors of the skin are benign; however, the prevalence of skin cancer accounts for 25% of feline cancers. Next in incidence is breast (17 %). Cancer is a condition, characterized by, cell division and tissue growth that transpires at the expense of organ-specific functions. As an example, under the microscope a feline kidney-cancer is biopsied and found to be composed of tissue that only slightly resemblance that of normal cells of the kidney. The tumor kidney mass does not function as kidney tissue, and does not produce any urine. If the cancer is left untreated, it will eventually replace the kidney tissue while spreading to other parts of the body. In time, it will render the cat in a state of death. Cancer is staged according to the malignancy degree. Low-grade tumor grows locally and can grow to a large size. Low-grade tumors extend to remote organs late in the course of the sickness. High-grade tumors spread early in the course of the sickness, while the primary tumor is hardly detectable.

One of the most common forms of malignancies encountered in small animal practice is lymphoma (Argyle *et al.*, 2008). Lymphoma is characterized by lymphoid cell malignant proliferation, which may occur in organs with lymphoid tissue. Lymphoma (also known as malignant lymphoma or lymphosarcoma) is also one of the more common neoplasms of the dog.

### **Feline Lymphoma**

Feline lymphoma is a systemic, i.e. can involve multiple anatomic sites, neoplastic disease of the lymphoid system (Argyle *et al.*, 2008). Etiology is in many cases unknown. Retroviral infection such as FeLV and FIV increase risk of lymphoma. In order to get a reliable prognosis, staging based on the anatomic site is indicated. Organ that can be involved are: extra nodal, gastrointestinal, CNS, nasal, mediastinal, multi-centric or renal. Chemotherapy systemically may be indicated when treating lymphoma that has spread to multiple organs or in case of diffuse disease. Low-grade gastrointestinal lymphoma (i.e. small-cell also called lymphocytic) is an indolent type of neoplasm with a good prognosis with proper therapy compared to high-grade gastrointestinal lymphoma (i.e. large-cell also called lymphoblastic).

Biopsy may be used to differ between these two types of neoplasm. It is important to establish which type it is, as the treatment plan differs between the two types of neoplasm. Lymphoma recurrence versus mitigating therapy is more complicated in feline patients than it is in dogs.

The risk of getting feline lymphoma has been reported to increase till 1.5 years of age, followed by a stable flat risk rate for the rest of the cat's lifespan (Carolyn J. Henry *et al.*, 2010). Lymphoma appears in cats of various breed, age, or sex. Siamese have been reported to be over-represented as a breed. Burmese (risk rate of 3.1) and Manx (risk rate of 4.6) appear to be at higher risk. Intact females (risk rate of 0.7) appear to be at a lower than-expected risk rate. Viral causes of lymphoma in cats are well defined. Simultaneous feline leukemia virus infection and feline immunodeficiency virus infection increase the risk factor 77 fold. Feline leukemia virus infection increases the risk factor 62 fold. Feline immunodeficiency virus infection increases the risk factor six fold. Depending on the duration of exposure, cigarette smoke also increases the risk of getting lymphoma. Chronic gastrointestinal inflammation has also been proposed to be a cause of gastrointestinal lymphoma. Immunosuppressive treatment in association with renal transplantation has also been associated with lymphoma development. Treatment for vaccine-associated sarcoma has also been reported to be associated with the development of lymphoma.

FeLV-related mediastinal lymphoma tends to be more prevalent in younger cats while older cats tend to have non-FeLV-related intestinal lymphoma (Carolyn J. Henry *et al.*, 2010).

Lymphoma cats can often be categorized in two groups; young cats with feline leukemia virus (FeLV)-associated disease (Carolyn J. Henry *et al.*, 2010). The young lymphoma cats often have respiratory signs due to pleural effusion and enlargement of the mediastinal lymph node. Older cats often have extra nodal lymphoma or gastrointestinal lymphoma non-FeLV related. Lymphoma in an extra nodal location can have a variety of clinical signs such as masses that destroy bone or solitary masses in typical locations: e.g., cutaneous. Hypoadrenocorticism and hypercalcemia may be clinical signs of feline lymphoma, but not as common as for dogs. Depending on geographic location the feline lymphoma characteristics and clinical symptoms may vary considerably.

### **Feline Lymphoma and FeLV**

Based on viral interference with super infection, FeLV isolates are categorized into 3 groups (Argyle *et al.*, 2008). The groups probably define envelope types using diverse cellular receptor molecules for viral entry in to the host cell. FeLV A can only infect feline cells (ecotropic) and exemplifies the dominant form of the virus. FeLV B can also infect human cells (polytropic) and is common in feline virally induced lymphoma cases. FeLV B are believed to arise from recombination measures between feline endogenous sequences in the genome and FeLV A. FeLV C is believed to arise by mutation of the envelope gene in FeLV A. It is not transmitted in nature and is distinctively linked to feline pure red cell aplasia (PRCA). The main source of infection is persistent viremic individuals. FeLV is transmitted by close social contact and is continuously secreted in the saliva. FeLV is also transmitted vertically from a carrying queen to her kittens (congenitally). During the first weeks after the

viral exposure, the outcome of infection is determined contingent on interaction between the cat's immune system and the FeLV. The potential outcomes consist of latent infection, persistent viral infection, and viral clearance with FeLV-immunity. Persistently viremic individuals may develop virus related diseases such as lymphomas, being the most common tumor of cats, and leukemia. Mechanism of tumorigenesis comprises of insertions of viral-DNA on cellular oncogenes e.g. myc and suppression of the cat's immune-system. Approximately 80 percent thymic lymphoma cats are viremic, 60 percent of alimentary lymphoma cats are viremic, and 30 percent of alimentary lymphoma cats are viremic. The viruses may be part of an initiating event before being vacated by the host's immune-system. FeLV is also associated with immunosuppression, bone marrow failure, and reproductive failure. The pathogenesis of these diseases is yet to be understood.

### **Lymphoma and FIV**

FIV is a lentivirus (Argyle *et al.*, 2008). Lentivirus is a retrovirus that typically causes diseases with a long incubation period. Examples of lentiviruses are HIV, Maedi-Visna, FIV, and equine infectious anemia. FIV has been linked with feline neoplasm, particularly lymphomas. This can generally be explained by the immunosuppression of the host's immune-system caused by FIV.

### **Diagnostics and Staging of Feline Lymphoma**

In order to arrive at the correct diagnosis low-grade alimentary lymphoma (LGAL) requires histological biopsies assessment, whereas intermediate- (IGAL) and high-grade (HGAL) alimentary lymphoma (AL) can be diagnosed by cytology of aspirates from mesenteric or intestinal lymph node (Russell *et al.*, 2012).

With different locations such as mediastinal, gastrointestinal, multicentric, nasal, and extra nodal, each type of lymphoma seems to have clinically diverse development and possibly suitable therapy (Carolyn J. Henry *et al.*, 2010). Each type of lymphoma presents exceptional therapeutic challenges. Diagnosis of mediastinal lymphoma is made of mediastinal mass aspirate cytology or pleural fluid cytology. Biopsy of the mediastinal lymph node is complicated by location within the confines of the thoracic cavity. There is a risk of puncturing a vascular structure or a lung. Extra nodal and multi-centric lymphoma can generally be diagnosed by biopsy or fine-needle aspirate. Immunohistochemistry on histopathology of biopsy specimens of a surgically or endoscopically collected sample is used to diagnose gastrointestinal lymphoma. Nasal lymphoma typically requires biopsy to confirm the diagnosis. Nasal lymphoma may have a characteristic appearance on skull CT. In the case when histopathology is inconclusive, T-cell receptor gene or immunoglobulin gene rearrangement clonality valuation can aid in diagnostics. Once the diagnosis is established, a thorough staging is appropriate in order to apply appropriate treatment and reduce toxicity.

### **Biological Behavior/Metastasis**

Feline lymphoma, just like in dogs, tends to spread systemically (Carolyn J. Henry *et al.*, 2010). Typically, it is the non-Hodgkin's form of lymphoma. However, Hodgkin's lymphoma

with Reed-Sternberg cells has also been reported. Due to the uncommon anatomic symptoms of lymphoma in cats, the WHO standard staging scheme is less prognostic than in the case of canine lymphoma. Immunophenotype may differ by etiology and by location. Among the retrovirus-induced neoplasms, FeLV-associated lymphomas tend to be of T-cell origin, where FIV-associated lymphomas tend to be of B-cell lineage. FIV-related lymphomas tend to involve extra nodal locations whereas FeLV-associated tumors typically involve the mediastinum.

Nasal lymphomas tend to be of B-cell origin, and curable by radiation therapy. Nasal lymphomas may spread systemically (Carolyn J. Henry *et al.*, 2010). Immunophenotype (contrary to dogs), age, gender, weight, FIV status, and tumor-stage are not useful prognostic factors.

### **Feline Gastrointestinal Lymphoma**

Feline gastrointestinal lymphoma is characterized by intestinal and/ or gastric infiltration with or without the mesenteric lymph node being involved (Argyle *et al.*, 2008). Gastrointestinal lymphoma is one of the common types of feline lymphoma. Gastrointestinal lymphoma can present itself as a diffuse infiltration of extensive areas of the bowel or as a solitary mass lesion. Clinical signs may be nonspecific, including diarrhea, vomiting, weight loss, and anorexia. Patients that have formerly been diagnosed with lymphoplasmacytic gastroenteritis may end up developing gastrointestinal lymphoma. Most of the alimentary lymphoma cats are FeLV negative. Removing apparent solitary lesions by surgical excision is not to be recommended since the neoplasm frequently spreads beyond the detectable lesion. In many cases, the lymphoma is diffuse. In the case of chemo-resistant obstructive lesions or perforated lesions, surgical excision must be considered.

In some cases it is difficult to distinguish inflammatory bowel disease from low-grade small-cell lymphoma purely on pathological assessment of the affected gut (Argyle *et al.*, 2008). However, a number of groups have described a PCR (polymerase chain reaction) based test that determines the clonality of biopsied tissues by analyzing, for example, the T cell receptor gamma variable gene. Tumor lesions are clonal in origin and therefore have a different PCR pattern to polyclonal inflammatory lesions.

### **Therapy and Prognosis**

For renal-, multi centric-, thymic- and high-grade gastrointestinal- forms of lymphoma, the therapy options are similar to those of canine patients (Argyle *et al.*, 2008). Cyclophosphamide, vincristine, and prednisone (COP regime) provide prolonged remission and prolonged survival times. As for canine patients, protocols that include doxorubicin may give better clinical results. However, this is not widely supported by clinical experience. Doxorubicin can be nephrotoxic to feline patients, why many oncologists use COP-based protocol for treating feline patients with lymphoma. In the case of renal function insufficiencies doxorubicin should not be used in cats. Oral prednisolone and Chlorambucil is the best treatment for low-grade gastrointestinal lymphoma.

The mainstay of treatment for lymphoblastic lymphoma is combined chemotherapy (Carolyn J. Henry *et al.*, 2010). The combined approach of COP may result in complete remission for about 50–70% of treated cats. Doxorubicin as a sole treatment has proven less successful, mostly resulting in complete remission rates of less than 50 percent. Still, the most durable remissions have been associated with doxorubicin. Thus, the COP Dox protocol is used by some clinicians. The most dependable favorable prognostic survival factor, with median survival approaching 1 year, appears to be achieving a complete remission. An Australian study suggested that 25% of cats achieving complete remission might never relapse, which might reflect geographic differences. When complete remission is not achieved, median survival drops to a few months.

Multi-agent chemotherapy is the therapy of choice for high-grade feline gastrointestinal lymphoma patients. For feline patients with small-cell or low-grade lymphoma, a treatment combination of chlorambucil and orally administered prednisolone offers good treatment modality (Argyle *et al.*, 2008).

Cats have been administered Lomustine as a primary or rescue agent at a 45.5 - 60 mg/m<sup>2</sup> dosage (Carolyn J. Henry *et al.*, 2010). The dosage appears to be well tolerated. Renal lymphoma cats run the risk of CNS metastasis, which can be reduced by adding cytosine-arabinoside to a COP therapy protocol called: COAP. Radiation therapy has been used to treat localized lymphoma, which has proven to be effective particularly for nasal lymphoma. Adjunct systemic chemotherapy is typically used to delay or prevent systemic spread of the tumor. Cats treated with radiation therapy for nasal lymphoma often have a survival rate exceeding 12 months. Alimentary lymphoma cats with complete remission with chemotherapy can have a survival time that exceeds 11 months. Lymphoblastic forms of alimentary lymphoma ought to be treated with combination chemotherapy. In a study, 69 percent of cats with alimentary lymphocytic lymphoma and treated with chlorambucil and prednisone reached complete remission, indicating that chlorambucil and prednisone combinations are appropriate in the case of indolent lymphomas.

### Monitoring Therapy

For patients undergoing lymphoma treatment it is necessary to perform serial assessments (Argyle *et al.*, 2008). These assessments will differ depending on the extent of the lymphoma, the original site, clinical status of the patient, and the therapy protocol. Timely re-evaluations should focus on documenting treatment response and make sure the patient is safely administered a scheduled treatment 7–10 days after chemotherapy. Physical examination should take place 7-10 days after the chemotherapy to ensure remission. Vital signs are valuable indicators such as: temperature, heart-rate, respiration-rate, and CBC (complete blood count). If the temperature exceeds 39,4 °C and PMN <2000 admit to hospital for intravenous antibiotics and fluids. If temperature is less than 39,4 °C and PMN <2000 prescribe antibiotics. While at home, the owner should monitor the temperature twice a day and returns if T >39,4 °C.

Response should be measured on resolution of physical examination and abnormalities, biochemical values, resolving clinical signs, and improved hematology (Argyle *et al.*, 2008). Measuring response to therapy is essential and it is a poor prognostic indicator in cats, if the lymphoma fails to respond to the chemotherapy. In most cases, cats with lymphoma should respond after 3–6 weeks of therapy. If no or poor response is evident within 3-6 weeks, rescue therapy or euthanasia should be considered. Not remaining within remission is also a poor prognostic factor defined as progressive lymphoma after remission has been achieved with proper therapy. Advance of the lymphoma after initial remission as well as failure to respond to adequate therapy may be due to chemo-resistance.

### **Inflammatory Bowel Disease (IBD)**

IBD is a common malady among domestic cats (August, 2006). In some veterinary hospitals and specialty clinics, IBD is the most prevailing gastrointestinal feline malady.

Three feline bowel problems characterized by random vomiting, chronic diarrhea, and mal-absorption are classified as inflammatory bowel disease (IBD) (Eldredge *et al.*, 2008). In prolonged cases IBD can lead to weight loss, anemia, and malnutrition. Cats may show recurring clinical signs, while others have constant clinical signs. IBD is immune-mediated reactions from the gastrointestinal tract to bacteria, parasites, and food. These reactions get over dimensioned, with inflammatory type of cells conjugating in the gastrointestinal tract interfering with absorption and digestion. These syndromes are seldom cured but can be managed. Over the long run might lead to cancer or ulcers, such as lymphoma.

The World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization Group uses the following definition: gastrointestinal clinical signs for duration of more than 3 weeks, histologic lesions of mucosal inflammation of taken biopsy, inadequate response to anthelmintics therapy and dietary modulation, and evidence of clinical response to immune-modulating treatment.

### **IBD Diagnostics**

An IBD diagnosis is an exclusion diagnosis that entails careful elimination of differential diagnosis of IBD (Jergens, 2012). After excluding intestinal structural abnormalities, parasitic agents, infectious agents, non-gastrointestinal disorders, exocrine pancreatic insufficiency, the most prevailing intestinal maladies related to chronic small bowel diarrhea include food-associated enteropathy, alimentary lymphoma, and idiopathic FIBD. A strict exclusive diet for a minimum of seven days effectually eliminates adverse food reactions that can cause diarrhea and vomiting in feline patients. The ability to tell the difference between well-differentiated lymphoma and severe feline IBD may be particularly tough in cats. Diagnostic methods including mucosal biopsies, immunohistochemistry for B-cells and T-cells markers, and PCR might help to distinguish alimentary lymphoma from IBD.

An IBD diagnosis is one of exclusions. It entails the elimination of several other diagnoses causing intestinal inflammation (August, 2006). Main differential diagnoses of feline IBD include alimentary lymphosarcoma, dietary sensitivity, systemic diseases, infectious diseases,

and chronic parasitism. A study showed that up to 30 % of cats with idiopathic gastrointestinal signs might be suffering from food sensitivities. Thus, proper diagnostic testing should be used to eliminate other causes of chronic intermittent mucosal inflammation, and histological evidence and clinical signs should be correlated. Hypoallergenic or anthelmintic diets may be appropriate when the patients are suffering from dietary sensitivity or parasites.

## IBD Therapy

The realistic goal of feline IBD is control rather than cure (Eldredge *et al.*, 2008). In many cases, therapy tends to extend for the remainder of the cat's life. Despite the fact that the medication can differ for all three types of IBD, they all typically respond partially or completely to changes in diet. Complementary, immune-modulating drugs such as azathioprine and prednisolone, antioxidants, omega-3 fatty acids, and probiotics (e.g. acidophilus) can be valuable. In order to reduce bacterial count Metronidazole can reduce symptoms. Budesonide, a corticosteroid, is being evaluated in the treatment of IBD. However, it is too early to recommend. More research is needed.

Goals of Treatment are to minimize stimulation of antigenic in the gastrointestinal tract and to modulate the immune response (Jergens, 2012). Diet is an important part of treatment in all three IBD forms. An elimination diet should be easily assimilated, greatly digestible, and low in fat. It should consist of a unique protein source and be corn-, milk-, and wheat-free. Commercially, hydrolyzed diets such as Royal Canine Feline Hypoallergenic diet and Hill's z/d Ultra Allergen Free are available. Cats with IBD of the colon may benefit from a highly digestible fiber e.g. psyllium (Metamucil) 1.7g - 3.4g PO with food q12 to 24h supplement. Of all cats with idiopathic gastrointestinal signs, up to 50 percent respond to elimination diets. Lymphocytic/ plasmacytic IBD cats can attain clinical remission with diet.

Chlorambucil; an alkylating agent effective combined with prednisolone for GI small cell lymphoma and in many cats with IBD (2.0 mg per cat q48–72h PO), Cyclosporine; an immune-modulating drug inhibiting T-cell function effective in cats with refractory IBD (5 mg/ kg q12 to 24h PO) (Norsworthy *et al.*, 2011).. In case of severe IBD or alimentary lymphoma and when biopsy is not an option, it might be necessary with a multimodal approach. That is prednisolone, Chlorambucil, hydrolyzed diet, and antibiotics might be appropriate.

## IBD Prognosis

Lymphocytic/plasmacytic colitis can often be managed with an appropriate diet alone (Norsworthy *et al.*, 2011). Lymphocytic/plasmacytic IBD is often controllable with proper pharmaceutical therapy and diet. Pancreatic or hepatic involvement typically indicates less promising prognosis. Less common forms of IBD such as eosinophilic IBD are tougher to foretell.

## Inflammatory Bowel Diseases (IBD) versus High Grade Lymphoma

IBD is a diagnosis of exclusion that entails elimination of other diseases that may cause intestinal inflammation (Hall *et al.*, 2005). Infectious and systemic diseases, dietary allergy and intolerance, intestinal parasites, and alimentary lymphoma are the major differential diagnoses of IBD. Clinical symptoms must be related to histological gastroenteritis signs, and other chronic mucosal inflammation causes should be eliminated by proper diagnostic methods. Therapeutic treatments using anthelmintics or hypoallergenic diets may be appropriate in cases of parasitic or dietary causes of enterocolitis. Studies indicate that up to 30% of cats with idiopathic gastrointestinal diseases might suffer from food sensitivities.

Immunohistochemistry applications may also aid in the diagnosis of alimentary neoplasia (Hall *et al.*, 2005). Immunohistochemistry methods are typically used to distinguish between an early-stage alimentary lymphoma and lymphocytic FIBD, as it is particularly challenging at the histopathological level to distinguish between the two. In the case of lymphoma, the infiltrate is clonal, why almost all of the lymphocytes will be of one phenotype. In other words, B-lymphocytes or T-lymphocytes will be expected. In the case of an inflammatory process a mix of the phenotypes may be found.

Many gastrointestinal diseases are clinically similar to FIBD, as the gastrointestinal mucosa answers to many insults with an inflammation (Norsworthy *et al.*, 2011). Thus, histological indication of inflammatory infiltrate alone does not constitute an IBD diagnosis. Differential diagnoses to contemplate are allergies, intolerance, parasites, bacterial and other infectious agents, i.e. *Giardia*, nematodes, heartworms, *Tritrichomonas*, *Cryptosporidium*, *Helicobacter*, diabetes mellitus, hyperthyroidism, feline immunodeficiency virus, feline leukemia virus, salmonellosis, campylobacteriosis, feline infectious peritonitis, adenocarcinoma, alimentary lymphoma, chronic renal failure, chronic pancreatitis, exocrine pancreatic insufficiency or hepatic disease. Thus, a systematic diagnostic procedure along with a thorough anamnesis is critical in order to diagnose IBD with confidence.

## MATERIAL OCH METHODS

### Thymidine kinase assay

TK1 activity was assessed by radiochemical assay using DE-81 filter paper. The following was added to the mixture: 20 mM Tris-HCl pH 7.6, 2 mM dithiothreitol (DTT), 5 mM MgCl<sub>2</sub>, 5 mM ATP, 50 μM [<sup>3</sup>H]-dThd and 10 μL of serum making the volume 40 μL per sample (Sharif & von Euler *et al.*, 2012).

20 μL of the reaction mixer was pored in 1,5 mTubes. 10 μL of centrifuged serum and 10 μL distilled water was added to each sample.

The cocktail mixture was incubated for 60 minutes at 37°C, cooled on ice, spun and 10 μL applied on the marked DE-81 filter papers to dry. The filter papers were washed with 1 mM ammonium-formate for 5 min twice. Then the filter papers were put in tubes and eluted for 45

min in 0.1 M HCl and 0.2 M KCl. Scintillation liquid was added and the samples were incubated for 10 minutes. The radioactivity was measured by liquid scintillation counting and the activity was expressed as pmol/min/mL. The average per sample was based on 2-3 thymidine kinase assay results depending on the sample.

### **TK REA and assessing TK1 activity**

TK activity in serum was determined using the ProlifigenTK-REA (radio-enzyme assay). This assay is based on a radioenzymatic technique in which the substrate analogue <sup>125</sup>I-iododeoxyuridine is converted to <sup>125</sup>I-iododeoxyuridine monophosphate by TK (Von Euler *et al.*, 2004). The phosphorylated reaction product is absorbed to a separator tablet (aluminum hydroxide). The radioactivity then is measured after several washings. TK activity is reported as units per liter (U/L). The TK REA assay was conducted at The Swedish University of Agricultural Sciences Laboratories, Uppsala.

## **RESULTS**

### **Hypothesis**

The hypothesis was that TK1 activity in serum has the potential to become a valuable biomarker in feline clinical practice. Feline TK1 activity in serum from cats with known diagnosis was measured with the [3H]-dThd phosphorylation assay. The results were then compared with the proven and commercially available TK-REA assay. Several parameters in the [3H]-dThd phosphorylation assay were altered in order to optimize, if possible, the assay. A possible optimization of the assay allows for improved possibilities of using TK1 as a valuable biomarker in feline clinical practice.

### **Clinical cases**

Clinical cases were identified at SLU Small Animal Hospital in Uppsala and at The Animal Hospital in Jönköping following a search of clinical records for cats with the following diagnoses: lymphoma, cystitis, cutaneous lymphosarcoma and leukemia. Cases that were subject to TK-REA analysis were included in the study.

The samples consisted of 12 TK-REA serum samples from 8 cats with an average age of 8,7 years. The group included 1 intact male, 4 neutered males, and 3 spayed females. Four samples from four healthy non-pedigree cats were also included in the study, but not analyzed by TK-REA. Results for the healthy cats were 0,05, 0,16, (-0,17), and 0,19 pmol/min/mL.

Cases of lymphoma were included in the study. Those were diagnosed with lymphoma after histopathological or cytological examination of samples. Below table describes how the diagnoses by the clinicians were confirmed.

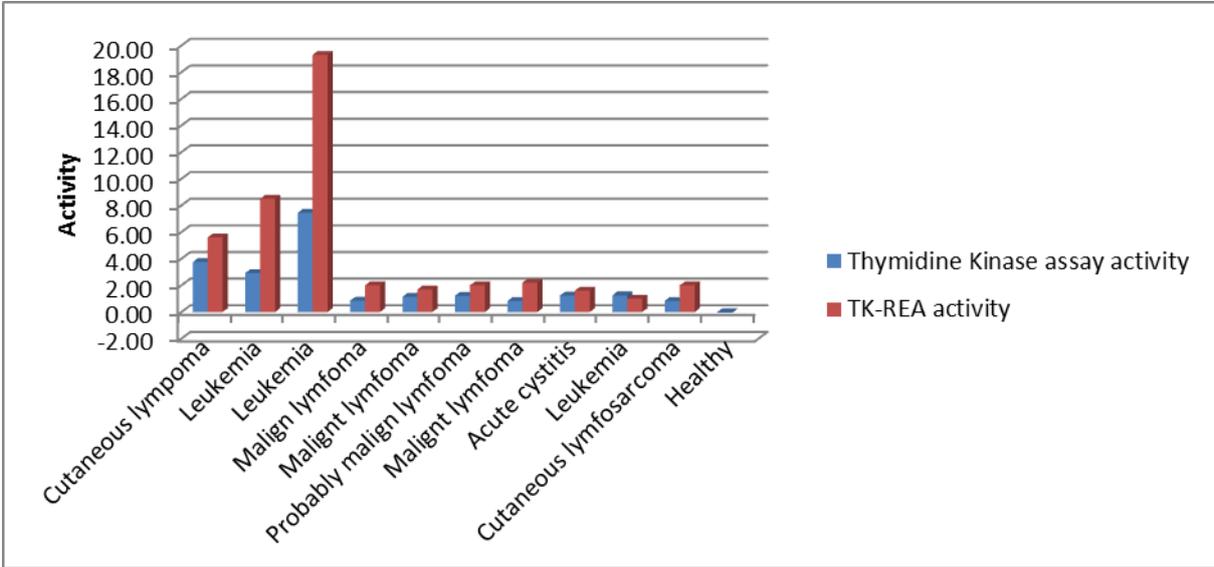
*Table 1. Diagnosis and assessment method for the samples used*

Samples	Diagnosis	Assessment method
5399	Cutaneous lymphoma	Biopsy
4739	Leukemia	Fine Needle Aspiration
4471	Leukemia	Fine Needle Aspiration
6318	Malignant lymphoma	Biopsy
4773	Malignant lymphoma	Biopsy
4907	Probably malign lymphoma	Fine Needle Aspiration
6095	Malignant lymphoma	Biopsy
5206	Acute cystitis	Uricult + CLED
5841	Leukemia	Fine Needle Aspiration
8477	Cutaneous Lymphosarcoma	Biopsy

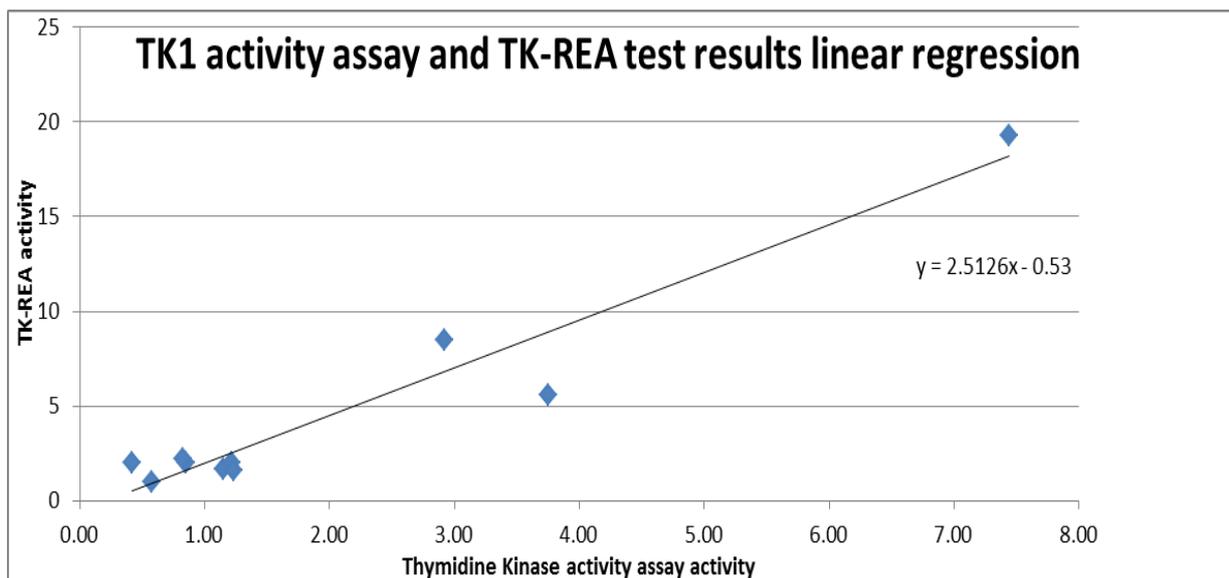
Some of the samples are from the same patient before, during and after therapy indicated in the table. Feline leukemia virus (FeLV) status was recorded in each case where available. The correlation between average activity using the thymidine kinase assay and the TK-REA test used by SLU Laboratory when running both test for eleven samples were 0.97 based on Microsoft Excel 2010 “KORREL” function. The [<sup>3</sup>H]-dThd phosphorylation assay activity had an average of 2.04 with a standard deviation of 2.07 as can be found in the table below. All of the samples were from sick cats with various diagnoses. The patient with the highest activity level in both tests were a Cornish Rex, four years of age, with the diagnosis leukemia, measuring 19.3 in the TK-REA test and 6.02 in the thymidine kinase assay test. The second highest level of activity was from a six year old forest cat with cutaneous lymphosarcoma, followed by three malignant lymphoma cats, another cat with cutaneous lymphosarcoma, and an acute cystitis. The cat with acute cystitis indicates that TK1 serum activity can be significantly elevated in not only malignancies but also non-malignancy diseases such as cystitis.

Table 2. Mean values from the two Serum TK1 assays of samples from 8 cats with date of birth, gender, breed and diagnosis as indicated. Samples 4739, 4471 and 5841 are from the same Forest Cat, and samples 6318 and 6095 are from the same non-pedigree cat. The cats 6318 and 6095 were both tested FeLV negative

Samples	TK1 Activity Assay		TK-REA	Breed	DoB	Gender	Diagnosis
	Activity	Std.dev.	Activity				
5399	3.75	1.11	5.60	Forest Cat	21-5-2005	spayed	Cutaneous lymphoma
4739	2.92	1.55	8.50	Cornish rex	22-11-2008	spayed	Leukemia
4471	7.44	3.20	19.30	Cornish rex	22-11-2008	spayed	Leukemia
6318	0.85	1.17	2.00	Non-Pedigree	15-04-2001	neutered	Malign lymphoma
4773	1.15	0.29	1.70	Non-Pedigree	15-09-1999	neutered	Malign lymphoma
4907	1.22	0.41	2.00	Non-Pedigree	01-07-2007	spayed	Probably malign lymphoma
6095	0.83	1.10	2.20	Non-Pedigree	15-04-2001	neutered	Malign lymphoma
5206	1.23	1.14	1.60	Non-Pedigree	16-5-1998	neutered	Acute cystitis
5841	1.25	0.84	1.00	Cornish rex	22-11-2008	spayed	Leukemia
8477	0.84	0.49	2.00	Non-Pedigree	01-06-2010	male	Cutaneous lymphosarcoma



Graph 1. Mean TK1 activity values determined with the two assays in the samples described in table 2.



Graph 2. Graph of the linear regression analysis of the test results in table 2, using Microsoft Excel 2010 and the “KORREL” function . The correlation between the two sets of activity values was 0.97.

### Optimizing the <sup>3</sup>H phosphorylation assay

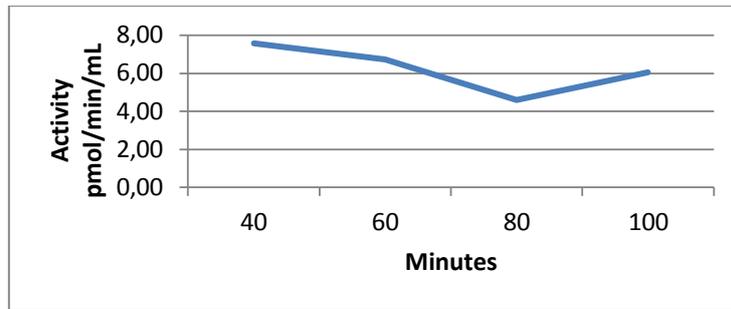
The parameters pH, reaction time, and temperature were altered in order to find out if the assay could be optimized. The activities presented are given as pmol/min/mL.

#### -pH

The highest activity measured using the thymidine kinase assay was from the Cornish Rex lymphoma cat at pH 6.5. The activity of the sample decreased as the pH decreased or increased from pH 7.3, suggesting an optimal pH of 6.5 for maximum enzyme activity. However, when measuring the activity from a healthy cat and from a cat with lymphoma, the maximum levels of activity were not identical. The assay does not seem to be sensible to pH variations between pH 6.5 - 8.1.

#### -Reaction Time

Sample (4471) from cat with the diagnosis leukemia was tested, using the thymidine kinase assay for different time periods; 40, 60, 80, and 100 minutes. The results show that the reaction continues in a linear fashion throughout this timespan, suggesting that 60 – 100 minutes are optimal reaction times for this assay (Graph 3).

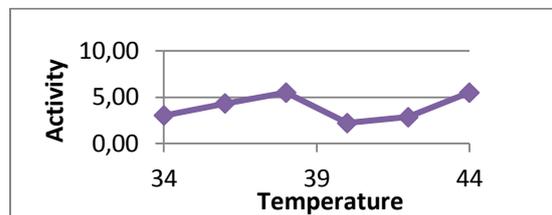


Graph 3. The graph shows enzyme activity over time in a sample from a cat with leukemia (sample 4471). Activity is measured in pmol/min/mL.

### -Temperature

Different temperatures, i.e. 34, 36, 38, 40, 42, and 44 degrees Celsius were used with a sample with the diagnosis leukemia (4471). Below the activities are shown, indicating that the feline TK1 enzyme activity increased to 38° C (Graph 4). Thus, an optimal temperature is most likely around 38° C.

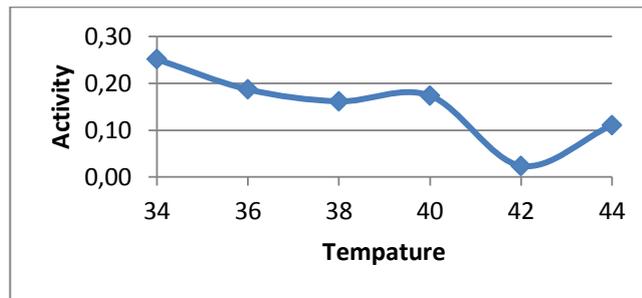
Temp	Activity
34	3,04
36	4,33
38	5,49
40	2,21
42	2,85
44	5,49



Graph 4. Enzyme activity measured at different temperatures in serum from a cat with leukemia.

Below are the results from the same test but using samples from a healthy cat. The enzyme activity in samples from a healthy individual are shown in graph 5, and they appeared to be more constant between 36° C and 40° C compared to the cat with leukemia. However, these enzymes levels are low, i.e. below the detection limit and are included here mainly to show the variation of the background activities. They cannot be used to draw any conclusions regarding the stability of serum TK1.

Temp	Activity
34	0,41
36	0,19
38	0,16
40	0,17
42	0,02
44	0,11



Graph 5. Enzyme activity measured in serum from a healthy cat.

The parameters used in the assay were optimized for canine TK1. The alteration of parameters indicates that the temperature may be raised from 37° C to 38° C for optimal feline TK1 activity. Besides the temperature, it seem like the optimal parameters for canine TK1 are similar to those of feline TK1.

## DISCUSSION

The aim of the pilot study was to provide a starting point for future feline TK research. The results demonstrate a correlation between the thymidine kinase assay and TK-REA. Both assays show a significant increase in TK activity in cats with lymphoma, leukemia and cystitis versus clinically healthy cats with a significant correlation, indicating that TK may serve as a diagnostic tool in clinical practice. However, the discrepancy in activity level between a cat with lymphoma and a healthy cat is not as clear cut as the difference in activity level between a dog with lymphoma and a healthy dog.

TK1 is used as a biomarker in clinical oncology to assist with early diagnosis of malignancy, prognosis of tumors, response to therapy evaluation and relapse detection (Von Euler & Eriksson, 2011).

Previous results revealed that the dThd phosphorylation assay is suitable for measurement of TK1 in hematologic malignancy (lymphoma and leukemia) in dogs and provided valuable information regarding the disease recurrence. Significant correlations were observed between the [<sup>3</sup>H]-dThd assay and the two commercial TK1 activity assays (Sharif & von Euler *et al*, 2012).

Even though this study showed that samples from a leukemia-patient and a cystitis-patient had distinguishable activity levels, Pelle (8477) a cat with cutaneous lymphosarcoma, had an activity level, which was difficult to distinguish from a healthy cat.

Taylor's study shows there is a considerably higher serum TK activity in cats with lymphoma than in, cats with inflammatory diseases, or cats with non-hematopoietic neoplasia, or healthy cats (Taylor *et al.*, 2012). These findings suggest that serum TK activity is a biomarker for feline lymphoma. However, the ROC analysis in Taylor's study showed a relatively low sensitivity for the Prolifigen TK-REA; DiaSorin technique, i.e. with several lymphoma cases

having values within the reference interval. However, a high TK serum activity level was greatly indicative of a lymphoma diagnosis, while there is a very low predictability for cats with low TK serum activity level.

The preliminary study presented here show similar results. Thus, further research is needed in order to use feline TK1 activity measurements in serum as a reliable clinical biomarker. Furthermore, TK1 protein concentrations in serum should be analyzed in order to better understand the role of feline TK1 as a diagnostic biomarker. In many disorders the amount of protein can be more indicative than the protein activity. An ELISA based assay using antibodies measuring protein concentration versus the activity would most likely be beneficial.

Serum TK1 is most likely present in the blood of healthy cats. However, in the healthy cats the TK1 serum activity could not be quantified using the [<sup>3</sup>H]-dThd phosphorylation assay. As samples were scarce, a larger study with more samples should be done and will be more conclusive.

The complexity of the TK1 protein in serum is most likely a major contributing factor for the difficulty in developing effective determination methods (Sharif & Kiran *et al.*, 2012). Sharif et al found that serum TK1 exists in a mixture of different molecular sizes, and that only a fraction of the TK1 protein in serum is associated with TK1 activity. Reducing agents had a negative effects on serum TK1 activity but may be important in order to determine the TK1 protein levels with immunochemical methods.

This study shows that TK1 as a biomarker can be used to distinguish a cat with leukemia or a cat with cystitis from a healthy cat.

Feline inflammatory bowel diseases, allergies and intestinal lymphoma are commonly encountered maladies in clinical practice and difficult to tell apart. TK1 determinations in serum may serve as a biomarker provided and adequately sensitive TK1 assays may be able to aid with the differential diagnosis.

## REFERENCES

- Argyle, D. J., Brearley, M.J. & Turek, M.M. (2008). *Decision making in small animal oncology*. Blackwell Publishing.
- Aufderklamm, S., Todenhöfer, T., Gakis, G., Kruck, S., Hennenlotter, J., Stenzl, A. & Schwentner, C. (2011). Thymidine kinase and cancer monitoring. *Cancer Letters* (Oct).
- August, J. R. (2006). *Consultations in feline internal medicine* 5<sup>th</sup> edition. Saunders Elsevier.
- Calladine, C. R., Drew, H. R., Luisi, B. F. & Travers, A. A. (2004). *Understanding DNA. The molecule and how it works*. 3<sup>rd</sup> Edition. San Diego: Elsevier Ltd.
- Henry, C.L. & Higginbotham, M.L. (2010). *Cancer management in small animal practice*. Saunders Elsevier.
- Chen, Z.H., Huang, S.Q., Wang, Y., Yang, A.Z., Wen, J., Xu, X.H., Chen, Y., Chen, Q.B., Wang, Y.H., He, E., Zhou, J. & Skog S. (2011). Serological thymidine kinase 1 is a biomarker for early detection of tumours. *Sensors (Basel)* 11(12), doi: 10.3390/s111211064. Epub 2011 Nov 28
- Edward J. Hall, James W. Simpson & David A. Williams (2005). *BSAVA Manual of canine and feline gastroenterology*, 2<sup>nd</sup> Edition. British Small Animal Veterinary Association.
- Eldredge, D. M., Carlson, D. G. & Giffin, J. M. (2008). *Cat owner's home veterinary handbook*. Wiley Publishing.
- Von Euler, H., Einarsson, R., Olsson, U., Lagerstedt, A.-S. & Eriksson, S. (2004). Serum thymidine kinase activity in dogs with malignant lymphoma: a potent marker for prognosis and monitoring the disease. *Journal of Veterinary Internal Medicine / American College of Veterinary Internal Medicine* 18(5), 696–702.
- Von Euler, H. & Eriksson, S. (2011). Comparative aspects of the proliferation marker TK1 in human and canine tumour diseases. *Veterinary and Comparative Oncology*. Mar;9(1), 1–15.
- Goulart, B. H. L., Clark, J. W. & Pien, H. H. (2007). Trends in the use and role of biomarkers in phase I oncology trials. *Clinical Cancer Research* (13), 6719–6726.
- Hallek, M., Wanders, L., Strohmeyer, S. & Emmerich, B. (1992). Thymidine kinase a tumor marker with prognostic value. *Clinical Cancer Research* (13) 6719-6726.
- He, E. Xu, X.H., Guan, H., Chen, Y., Chen, Z.H., Pan, Z.L., Tang, L.L., Hu, G.Z., Li, Y., Zhang, M., Zhou, J., Eriksson, S., Fornander, T. & Skog, S. (2010). Thymidine kinase 1 is a potential marker for prognosis and monitoring the response to treatment of patients with breast, lung, and esophageal cancer and non-Hodgkin's lymphoma. *Nucleosides Nucleotides Nucleic Acids*, 29(4-6), 352–8.
- Jergens, A. E. (2012). Feline idiopathic inflammatory bowel disease - What we know and what remains to be unraveled. *Journal of Feline Medicine and Surgery* 14(7), 445–458.
- Norsworthy, G. D., Fooshee Grace, S., Crystal, M. A. & Tilley, L. P. (2011). *The feline patient*. Wiley-Blackwell.
- Puntmann, V. (2009). How-to guide on biomarkers: biomarker definitions, validation and applications with examples from cardiovascular disease. *Postgraduate Medical Journal* 85(1008), 538–45.
- Russell, K. J., Beatty, J. A., Dhand, N., Gunew, M. & Lingard, A. E. (2012). Feline low-grade alimentary lymphoma - how common is it? *Postgraduate Medical Journal* 85 (1008) 538-45.
- Sharif, H., von Euler, H., Westberg, S., He, E., Wang, L. & Erikson, S. (2012). A sensitive and kinetically defined radiochemical assay for canine and human *Veterinary Journal* 194(1) 1-8.

- Sharif, H., Kiran K.J., Wang, L., He, E. & Eriksson, S. (2012). Quaternary structures of recombinant, cellular and serum forms of thymidine kinase 1 from dogs and humans. *BMC Biochemistry Journal* 13 (12) 1-10.
- Sharif, H. M. *Molecular properties of serum thymidine kinase 1 and use of the canine enzyme in disease monitoring*. Diss: Uppsala. Swedish University of Agricultural Sciences [online] (2012-09-27). Available from: [http://pub.epsilon.slu.se/8999/1/sharif\\_h\\_120827.pdf](http://pub.epsilon.slu.se/8999/1/sharif_h_120827.pdf). [Accessed 2012-09-27].
- Taylor, S., Dodkin, S., Papasouliotis, K., Evans, H., Graham, P., Belshaw, Z., Westberg, S. & von Euler, HP. (2012). Serum thymidine kinase activity in clinically healthy and diseased cats: a potential biomarker for lymphoma. *Journal of Feline Medicine and Surgery* Oct 17. [Epub ahead of print]
- Uzman, A., Johnson, J., Eichberg, J., Widger, W., Voet, D., Voet, J. G. & Pratt, C. W. (2012). *Fundamentals of Biochemistry 4th Edition - Solutions*. 4th. ed. Hoboken: John Wiley & Sons, Inc.