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Serumtymidinkinas 1-aktivitet hos friska katter

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SUMMARY

Lymphoma is one of the most common neoplastic disorders in domestic cats. The disease is a challenge for clinicians to diagnose and prognosticate due to many different possible anatomic locations and various, unspecific clinical signs. The personal value of cats as pets is increasing for cat owners, demanding more advanced veterinary care for their cats. Lymphoma is a cancer which is increasing in cats, thus giving yet another reason for improving possibilities in handling feline lymphoma patients. A tumor biomarker to help detect early stages of lymphoma, to help differentiate lymphoma from other diseases with similar clinical signs, to monitor treatment and as an aid to prognosticate would be of great use in feline oncology.

Tumor biomarkers are widely used in human medicine for these purposes as well in some extent in canine oncology. Thymidine kinase is an enzyme involved in the DNA-synthesis, and levels of this enzyme in sera is correlated to cell proliferation, i.e. serum thymidine kinase (sTK) levels are higher in cancer patients due to the rapid cell division rate in cancer cells.

The aim of this study was to examine the serum thymidine kinase 1 (sTK1) activity levels in healthy cats to be able to calculate a reference value, and to see if there was an individual variation in sTK1 activity levels depending on age or gender. The study group consisted of 35 healthy cats with various age, gender and breed. To be included in the study the cats had to be evaluated as clinically healthy on clinical examination, and to be healthy on blood screening (hematology and serum biochemistry profile).

The study resulted in a reference value of <0.933 pmol/min/mL, and showed that there was no individual variation depending on age or gender. The results from this study is a good starting point for future studies as it is a consistent group of healthy cats to use as comparison for sick cats.

SAMMANFATTNING

Lymfom är en av de vanligaste neoplastiska sjukdomarna hos domesticerade katter. Sjukdomen är en utmaning för kliniker att diagnostisera och prognosticera på grund av många olika anatomiska variationer och ospecifika kliniska symptom. Det personliga värdet för katter som husdjur ökar hos kattägare som då kräver en mer avancerad veterinärvård, vilket bidrar till en önskan om att förbättra diagnostiken och möjligheterna i vårdandet av dessa katter. En biomarkör för att kunna upptäcka sjukdomen i ett tidigare stadie, för att lättare differentiera lymfom från andra sjukdomar med liknande kliniska symptom, för att kontrollera behandling och som en prognostisk faktor skulle vara till stor nytta inom veterinärmedicinen.

Biomarkörer används i hög utsträckning inom humanmedicinen för dessa syften och även till viss del inom veterinärmedicinen, då framförallt på hundar. Tymidinkinas är ett enzym som är involverat i DNA-syntesen, och nivåerna av enzymet i serum är korrelerade till cellproliferation, alltså är serumtymidinkinas (sTK)-nivåer högre hos cancerpatienter på grund av den snabba celldelningen hos cancerceller.

Syftet med den här studien var att undersöka sTK1-aktivitetsnivåer hos friska katter för att beräkna att referensvärde, samt undersöka om det förelåg en individvariation beroende på kön eller ålder. Studiegruppen bestod av 35 friska katter av varierande ålder, ras och kön. För att inkluderas i studien behövde katten klassas som frisk vid klinisk undersökning och på blodprover (hematologi och flera olika kemianalyser).

Resultaten av studien ledde till ett referensvärde på <0,933 pmol/min/mL och visade att det inte finns någon individvariation beroende på kön eller ålder. Resultaten utgör en bra utgångspunkt för framtida studier inom samma ämne.

CONTENT

INTRODUCTION

Lymphoma is one of the most common neoplastic disorders in the domestic cat, with an estimated incidence of 200 per 100,000 cats at risk, making it the most common malignancy in this species. It is a form of cancer that originates from lymphoreticular cells, thus affecting lymphoid tissues. Feline lymphoma is often disguised behind many different clinical signs and can be found almost anywhere in the body, making it a challenge for clinicians to diagnose and to obtain a prognosis for affected patients. Due to the great variation of feline lymphoma, it is difficult to generalize clinical signs, signalment and prognosis (Withrow *et al.*, 2013).

The etiology of feline lymphoma is of either non-viral or retrovirus-associated origin, with the non-viral origin being most common to date, unlike before the 1980's when the retroviral lymphomas were more common. The retroviruses that increase the risk of feline lymphoma are FeLV and FIV. These viruses still increase the risk of lymphoma in infected cats, but the number of infected cats have decreased due to improved diagnostics and introduction of the FeLV vaccine on the market (Louwerens *et al.*, 2005).

As earlier mentioned there is a great variation in clinical signs, mostly because of multiple anatomic locations, thus making diagnostics and prognostication challenging for the clinician. Diagnosis is generally achieved via cytology or histopathology and there are multiple chemotherapeutic controls used in the disease management. The factors used today for obtaining a prognosis are clinical response to treatment, location of the lymphoma and clinical staging (which is difficult to do in feline lymphoma patients). In canine patients with lymphoma there are more prognostic factors to use such as immunophenotyping, tumor markers and better possibilities for clinical staging. Unfortunately, these alternatives are not equally usable for feline lymphoma patients (Nelson & Couto, 2009).

Since feline lymphoma is such a common malignancy and that there is no optimal way to prognosticate it, a biomarker would be of great use, both as a prognostic indicator as well as for diagnostics. Thymidine kinase is an enzyme involved in DNA-replication and therefor exists in higher levels in rapidly dividing cells. This has been used in human and canine oncology where this enzyme is used as a serum biomarker. A few studies have been made to try to find a way to apply this in feline medicine as well. One study by Taylor *et al.* (2013) found a serum thymidine kinase 1 (sTK1) activity reference interval of <5.5 U/l and that cats with lymphoma had significantly higher sTK 1 activity levels than healthy cats.

Aim of the study

In this study, serum thymidine kinase 1 (sTK1) levels were measured in healthy cats by radiochemical assay using the DE-81 filter paper technique. The aim of the study was to:

- Obtain a reference interval/value for sTK1 activity in healthy cats.
- Examine if there is an individual variation of sTK1 activity in healthy cats, e.g. in gender or age.

LITERATURE REVIEW

Feline lymphoma

Lymphoma (i.e., malignant lymphoma, lymphosarcoma) is one of the most common neoplastic disorders and the most common neoplasm of the hematopoietic system seen in cats, with an estimated incidence of 200 per 100,000 cats at risk. It is a form of neoplasm that origin from lymphoreticular cells, which means that the affected organs are those that consist of lymphoid tissues, such as lymph nodes and spleen, i.e. the cancer begins in the cells of the lymph system. There is also a form of lymphoma that origins from the bone marrow; lymphoid leukemia. Due to the anatomic distribution of the lymphoid system, lymphoma can be found almost anywhere in the body (Withrow *et al.*, 2013).

The anatomic variation of feline lymphoma makes it difficult to point out specific symptoms of the disease since the clinical findings correlate to the anatomic site of the neoplasm. It is also hard to state a typical signalment for cats with lymphoma due to the frequent occurrence of unspecific clinical signs and the often no apparent enlargement of peripheral lymph-nodes in contrary to what is common in canine lymphoma patients. Four anatomic forms of presentations are described in table 1 below; multicentric, mediastinal, alimentary and extra nodal, where the alimentary form is most common in cats (Nelson & Couto, 2009).

Table 1. *Different forms of lymphomas seen in cats*

Form of lymphoma	**Anatomic site**	**Clinical presentation**
Multicentric	Generalized lymphadenopathy with either hepatic, splenic or bone marrow involvement and sometimes a combination of these	Often nonspecific symptoms such as weight loss, anorexia and lethargy. Owners may seek veterinary care only due to the discovery of subcutaneous masses, i.e., enlarged lymph-nodes
Mediastinal	Mediastinal lymphadenopathy, with or without bone marrow infiltration	Dyspnea, coughing, regurgitation due to compression of the respiratory and upper digestive tract from enlarged mediastinal lymph-nodes. Some cats present with uni- or bilateral Horner´s syndrome
Alimentary	Solitary, diffuse or multifocal gastrointestinal tract infiltration with or without intraabdominal lymphadenopathy	Vomiting, anorexia, diarrhea and weight loss
Extra nodal	May affect any organ or tissue, for example: renal, neural, nasopharyngeal, ocular	Variable, depends on the location of the lymphoma

Etiology of feline lymphoma

The etiology of feline lymphoma is often unknown, but it can be divided in to two basic types; those that are retrovirus-associated and those that are of non-viral origin. It is a recognized fact that retroviral infected cats have an increased risk of this cancer. FeLV (feline leukemia virus) increases the risk 62 fold, FIV (feline immunodeficiency virus) increases the risk 6 fold whereas a simultaneous infection of both FeLV and FIV infection increases the risk 77 fold (Louwerens *et al.,* 2005; Shelton *et al.,* 1990).

Non-retroviral-associated lymphoma

Currently, the largest group is however the non-retroviral-associated lymphomas, which are lymphomas of B- or T-cell lineage that tend to be more organ specific and solitary, whereas FeLV-associated lymphomas often are of T-cell lineage and a generalized form, and FIV-associated lymphoma are more likely to be of B-cell linage (Louwerens *et al.,* 2005). The underlying cause of the non-retroviral-associated lymphomas is not fully understood. Bertone *et al* (2002) suggests that chronic cigarette smoke exposure may increase the risk of lymphoma in cats. Multiple studies imply a correlation between feline lymphoma and chronic inflammation, such as the possible linkage between inflammatory bowel disease and alimentary lymphoma (Carreras *et al.,* 2003; Ragaini *et al.,* 2003).

Siamese cats appear to have a higher risk in developing lymphoma, which suggests a genetic predisposition as well (Dorn *et al.,* 1967). Louwerens *et al.* (2005) describe a group of twelve cases of FeLV-neagtive Oriental Short-hair cats with lymphoma, where all the cats in this group had an early onset (2 years old) and a similar clinical presentation (mediastinal lymphoma). This, combined with the absence of a viral etiology in these cats suggests a genetic, heritable form of lymphoma in this breed.

Lymphoma and FeLV

Lymphoma is as stated the most common malignancy of domestic cats, and FeLV, feline leukemia virus, is a recognized etiology of the disease. A FeLV-infected cat increases its risk 60 times of getting lymphoma compared to a non-infected cat (Shelton *et al.,* 1990). FeLV is a gammaretrovirus and is transmitted vertically and horizontally, infecting domestic cats around the world.

FeLV infection was more common in cats from the 1950s through the 1970s. In 1973, the first diagnostic test and elimination regimen made its appearance on the market which in combination with the first commercial vaccine in 1986 resulted in a rapid decline in FeLV-infected cats. Before the control by test and vaccination of FeLV started, more than 70% of cats with lymphoma were infected and hence the most important form of lymphoma was the retrovirus-associated form (Louwerens *et al.,* 2005).

Louwerens *et al.* (2005) found that even though the decrease in FeLV-associated lymphoma cases, lymphoma in cats is increasing. One explanation could be, according to the authors, that more clients may be considering treatment for their cats as well as the probable genetic lymphoma in young Siamese-cats, and the possible link between chronic inflammation and intestinal lymphoma. The authors suggest that the connection between chronic inflammation and intestinal lymphoma could be a result of dietary changes in cats.

Lymphoma and FIV

FIV, feline immunodeficiency virus, is a lentivirus and is described as an animal model for HIV infection and acquired immunodeficiency syndrome (AIDS) in humans. The virus itself does not cause severe clinical signs but increases the risk of opportunistic infections and tumors. In experimentally infected cats, the infection has several stages; acute phase, asymptomatic phase and terminal phase. The last terminal phase, is sometimes referred to as "feline acquired immunodeficiency syndrome" (FAIDS) and it is here the clinical signs, for example neoplasia, is seen (Hartmann, 2011).

As earlier mentioned, FIV infection increases the risk of lymphoma 6-fold and cats infected with FIV often have lymphoma. FIV is believed to play an indirect role in the development towards lymphoma, as it has immunosuppressive effects that dysregulates the immune system. The virus is only occasionally detected in tumor cells, also suggesting a direct role (Wang *et al.,* 2001). The virus may increase the risk of cancer in the following ways; by decreasing tumor immunosurveillance mechanisms, may promote tumor development through the immunostimulatory effects of replicating in lymphocytes and/or may impair immunological control of FeLV infection and accelerate the proliferation of transformed lymphoid cells (Hartmann, 2011).

FIV may also have a more direct role in the disease development since it is classified into the family retroviridae (same as FeLV) which is assumed to possess the potential for tumorigenesis (Endo *et al.,* 1997). Gabor *et al.* (2001) showed in a cohort study that the prevalence of FIV infection was 50% higher in cats with lymphoma compared to the FIV prevalence in the cat population without lymphomas, which also supports the theory of a more direct role in the pathogenesis of lymphoma.

Diagnosis

The diagnostic evaluation of a cat with suspected lymphoma should include a CBC with differential cell count, platelet count, a serum chemistry profile, urinalysis and FeLV/FIV screen. This will help to get an overall picture about the cat's general health status as well as, in some cases, reveal tumor involvement in certain organs (Withrow *et al.,* 2013). It can also help the clinician and the owner in the decision making whether to treat the patient or not. If the decision is to treat the patient, these parameters are a good baseline for further evaluation and also necessary for deciding what kind of treatment the patient will receive (Nelson & Couto, 2009).

A cytopathologic and/or histopathologic evaluation of lymph node or involved organ tissue is a must to establish a definitive diagnosis. This can be done via fine needle aspirate (FNA) or biopsy (surgical, endoscopic or needle-core). Nelson & Couto (2009) promotes a cytological evaluation by FNA rather than histopathological findings by biopsy due to the financial aspects of the method combined with the very low/non-existing morbidity compared to a biopsy which includes a higher cost for the owners as well as a greater risk for the patient. If needed, the test material can be further investigated by for example histochemical, immunohistochemical, flow cytometric analysis and molecular techniques (Withrow *et al.,* 2013).

It is also possible, and more and more common, to perform immunophentoyping of the lymphoma to determine B- versus T-cell origin of the tumor. This can be done by immunocytochemistry, immunohistochemistry, flow cytometry or PCR for clonality (Nelson & Couto, 2009). In dogs this can be of value for prognosis but unfortunately this is not the case in cats (Argyle *et al.,* 2008).

Clinical staging

There are staging systems for cats (more commonly used in canine patients with lymphoma), but due to the different anatomic forms and the variation in feline lymphoma they are generally less helpful than in dogs (Withrow *et al.,* 2013). One staging system is the one provided by the World Health Organization (WHO), a system based on TNM (tumor, node, metastasis) – a staging system used for neoplasms in humans, which is based on clinical and clinical pathologic information about the patient. All this is weighed together to obtain a prognosis, however this protocol cannot be used prognostically in cats according to the authors (Nelson & Couto, 2009). Nelson & Coutu (2009) thinks that there is no effective protocol for staging feline lymphoma to obtain a prognosis.

According to Argyle *et al.* (2008) the response to treatment is the most important prognostic factor in cats with lymphoma. FeLV is a negative prognostic factor in cats with lymphoma (Nelson & Couto, 2009). The prediction and prognosis of outcomes in cats with lymphoma is not possible to establish and generalize due to the great variation seen (Withrow *et al.,* 2013).

Treatment

Argyle *et al.* (2008) list 3 different therapeutic regimes; no treatment, single-agent therapy and combination chemotherapy. An example of single-agent therapy is prednisolone which is mostly seen as a palliative care and is often used in GI lymphoma. The best treatment of choice when assessing feline lymphoma is chemotherapy. There are two main chemotherapeutic approaches in feline lymphoma: induction chemotherapy followed by maintenance which is a less aggressive COP (cyclophosphamide, vincristine and prednisone)- based protocol, or a more aggressive chemotherapy for a set period of time and with no maintenance chemotherapy after that. In this more aggressive chemotherapy, a CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone)-based protocol is used (Nelson & Couto, 2009).

Many oncologists choose the COP protocol for cats since there is no strong evidence that doxorubicin provide any clinical benefit for cats and can also be nephrotoxic for some feline patients (Argyle *et al.,* 2008). The COP protocol has some of the highest rates of complete remission (CR, i.e, disappearance of all measurable tumors) and the longest survival times in the treatment of feline lymphoma with a minimal treatment-related toxicity (Teske *et al.,* 2002). The same study also showed that response to treatment with the COP protocol had a great prognostic potential. If the treated cat achieved CR it had a much longer survival time than those that did not.

The commonly used COP protocol is most often administrated intravenously (IV). This can however be a problem for some cats who are unwilling to be restrained and injected IV, causing stress and discomfort for the cat. Sedation is a possible solution but adds costs to an already expensive treatment. This led Teske *et al.* (2014) to investigate intraperitoneal (IP) administration of the COP protocol. The authors implied that this route of administration reduced stress for the patient and improved safety for the administrator. The results were that IP administration is a safe and effective route for the administration of COP protocol, thus adding another option for treatment administration (Teske *et al.*, 2014).

Combination protocols tend to be more effective than single-agent ones. According to Nelson & Couto (2009) cats who are treated with multiple-agent chemotherapy protocols are expected to live 6 to 9 months while the approximate survival time for untreated cats is 4 to 8 weeks. The same authors opinion is that cats tolerate chemotherapy for lymphoma quite well and that most owners are happy with the choice to treat their cat, and most cats get an improvement in quality of life after therapy.

Biomarkers

In human medicine the use of tumor biomarkers is considered standard practice to provide the clinician with diagnostic and prognostic information. This is also examined in veterinary medicine, most of all on canine patients where thymidine kinase is one biomarker that is used in the clinic (Taylor *et al.*, 2013).

Diagnostic markers

There are often specific characteristics of a patient or a disease, such as clinical, molecular or pathologic features that can be associated with a specific disease. This is used as a clinical tool to help the clinician to obtain the diagnosis of a patient. These markers are not necessarily associated with a clinical outcome or response to treatment (Webster *et al.*, 2011).

Prognostic and predicitive markers

These are markers that are associated with a clinical outcome or a treatment outcome, and are used to identify the likely progression of a patient´s disease and to determinate modalities that are the most appropriate and efficacious for that specific patient (Webster *et al.*, 2011).

Tumor biomarkers

Tumor biomarkers are substances that are produced by cancer cells, or by other cells in the body as a response to cancer. Some tumor markers are made by normal cells as well as cancer cells, but are produced much faster in cancer cells due to their rapid cell division (Holland *et al.*, 2003).

In human medicine, different tumor markers are used to evaluate tumor progression, response to therapy and also as diagnostic tools. An elevated level of a tumor biomarker is however not enough to diagnose cancer, but is combined with other tests, such as biopsies of affected tissues. The markers can be found in blood, urine, stool, tumor tissue, or other tissues or bodily fluids in some cases. PSA, CD20 and AFP are examples of different tumor markers used in human medicine (Holland *et al.*, 2003; Sharif *et al.*, 2012).

An important aspect when developing a tumor marker test is to find the true positive cases. This is partly achieved by setting a high bar for the reference value. This will however increase the risk of more cancer cases being missed, thus stating the fact that a biomarker should be used as a tool for the clinician, and not as the final decision maker. It may also be used as a monitoring control by following the values in a patient with diagnosed and treated cancer (Taylor *et al.*, 2013).

Thymidine kinase

Thymidine kinase (TK) is a cellular enzyme that convert deoxythymidine to deoxythymidine monophosphate as part of the one-step salvage pathway of pyrimidine synthesis, thus have a function in DNA synthesis, leading to higher levels of the enzyme in rapidly proliferating cells. This is used as a tumor marker in humans and dogs and especially as a prognostic indicator for lymphoma (Taylor *et al.*, 2013). Figure 1 illustrates the role of TK in the salvage pathway.

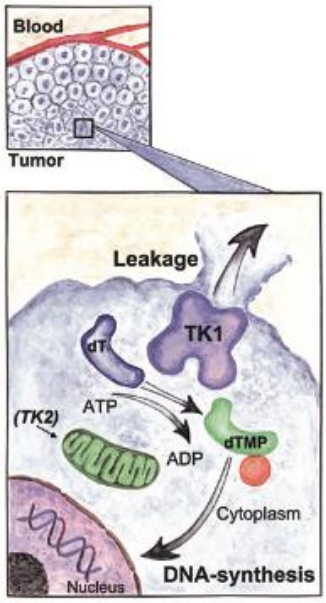

Figure 1. *The role of TK in the salvage pathway of the pyrimidine synthesis. From von Euler et al. (2004) and published with permission from the authors.*

There are two forms/isoenzymes of TK; TK1 (cystolic form) and TK2 (mitochondrial form). TK2 is expressed in all cells independent of stage in the cell cycle. TK1 is however cell cycle-regulated and is highly associated with cellular proliferation. In normal cells TK1 activity is present only for a short period of time in the early S-phase of cell division, but is often much higher in abnormal cells. An extracellularly increase of TK1 activity is correlated to the rate of cell division which is particularly high in hematopoietic malignancies such as lymphoma (Taylor *et al.*, 2013). As excessive and uncontrolled, rapid cell proliferation is one of the hallmarks of cancer, and the fact that TK1 expression is correlated to the overall level of proliferation being a S phase-specific protein, it has come to be of great value, most of all in human oncology (Bello, 1974).

A study performed by Taylor *et al.* (2013) showed that cats with lymphoma had a significantly higher sTK1 activity than clinically healthy cats, thus suggesting that sTK1 activity is a potential biomarker for feline lymphoma. The use of sTK1 activity as a tool in diagnosing feline lymphoma would be of great use due to the various clinical signs in cats with lymphoma as stated before.

MATERIAL AND METHODS

Healthy cats

Blood samples (sera and EDTA) from 35 healthy cats were collected at the University Animal Hospital, Swedish University of Agricultural Sciences, Uppsala. The selection of cats was not limited by age, gender or breed. All cats included in this study were classified as healthy after being clinically examined by a veterinary student and after blood tests were run at The Clinical Pathology Laboratory, at the University Animal Hospital, Swedish University of Agricultural Sciences, Uppsala. The blood tests included hematological profile and serum biochemistry profile, more specifically CBC, ALT, albumin, ALP, SAA, bile acids, glucose, calcium, potassium, creatinine and total protein. To be included in the study the cat had to be classified as healthy clinically and on the blood tests.

TK1 assay

TK1 activity was measured by radiochemical assay using the DE-81 filter paper technique. The reaction mixture used contained Tris-HCl pH 7.6, 10 mM; DTT, 2mM; $MgCl_2$, 5 mM; NaF, 5mM; ATP, 5mM; μM [^{3}H]-dThd and 10 μL serum in a final volume of 40 μL. The reaction mixture was incubated for 1 h at 37° C. Two aliquots of the reaction mixture were applied to the DE-81 filter paper discs and dried. The filters were then washed twice with 1 mM ammonium formate for 5 minutes and the products were

eluted for 45 minutes in 0.1 M HCl and 0.2 M KCl. Finally, the radioactivity was measured by β scintillation liquid counting and the activity was expressed as pmol/min/mL (Sharif *et al.*, 2012).

Statistical analysis

To calculate a reference interval, the formula Mean±SD was used. To examine if there was an individual variation in sTK1 activity levels (for example, does it matter if the cat is male or female), a scatter plot including age, gender and sTK1 activity was made. To compare sTK1 activity in males vs females and cats ≤1years old vs cats ≥2 years old, the Mann-Whitney U test were used.

RESULTS

sTK1 activity

Sera from 35 clinically healthy cats was analyzed by radiochemical assay as described in Material and methods. The mean and median age of the study group were 4.7 and 3.5 years respectively. The youngest cat was 6 months old and the oldest was 16 years old. The group consisted of 16 females (11 spayed, 5 entire) and 19 males (16 neutered, 3 entire), 8 pedigree cats and 27 non-pedigree cats. Figure 2 shows the sTK1 activity distribution.

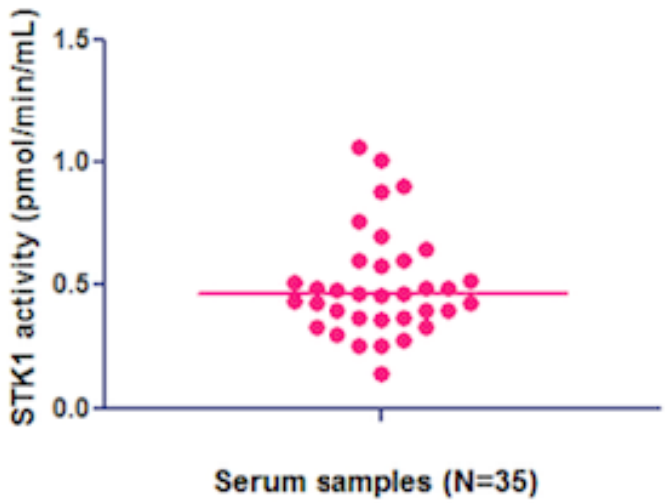

Figure 2. *Serum TK1 activity distribution in healthy cat sera. N=35, Mean=0.503, Median=0.470, Cut-off value=Mean±2SD=0.503±0.211=(0.073-)0.93 pmol/min/mL.*

A reference interval of 0.073-0.933 pmol/min/mL was calculated based on the mean sTK1 activity of 0.503 pmol/min/mL and a standard deviation of 0.211 pmol/min/mL. Below the mean and median values from different groups are shown in table 2.

Table 2. *Mean and median sTK1 activity for different groups*

	Mean sTK1 activity (pmol/min/mL)	**Median sTK1 activity (pmol/min/mL)**
Complete study group (n=35)	0.503	0.470
Males (n=19)	0.486	0.470
Females (n=16)	0.504	0.435
Cats ≤1 years old (n=9)	0.596	0.490
Cats ≥2 years old (n=26)	0.459	0.430

Individual variation in sTK1 activity

Below, figure 3 shows the results in a scatter plot with sTK1 activity as a function of age. The plot implies that there seems to be no pattern, i.e. the sTK1 activity levels seems to not be affected by age.

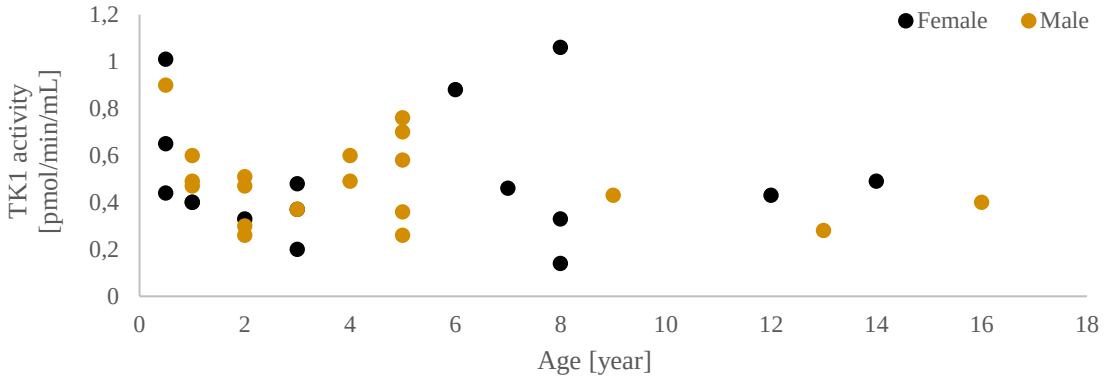

Figure 3. *A scatter chart of the results. Y=TK1 activity, x=age. The blue series represent the females and the yellow series the males.*

sTK1 activity and gender

The median sTK1 activity was 0.435 pmol/min/mL for females and 0.470 pmol/min/mL for males, and the mean sTK1 activity 0.504 pmol/min/mL and 0.486 pmol/min/mL respectively. By using the Mann-Whitney U test to compare if there was a difference in sTK1 activity between genders, a p-value of 0.920 was calculated, i.e. there was no significant difference in sTK1 activity between males and females.

sTK1 activity and age

To compare if there was a difference in sTK1 activity between ages, the study group was divided into two groups; ≤1 years old and ≥2 years old. The mean and median sTK1 activity for the young group was 0.596 pmol/min/mL and 0.490 pmol/min/mL. For the older cats the mean and median sTK1 activity was 0.459 pmol/min/mL and 0.430 pmol/min/mL. The Mann-Whitney U test was performed to calculate the difference between the groups. Due to a calculated p-value of 0.865, the result showed no significant difference in sTK1 activity between younger and older cats.

DISCUSSION

Tumor markers are widely used in human medicine and also in some extent in canine patients. These markers are used as a tool for clinicians to help diagnose, prognosticate and monitor their patients. Such a tool would be of great use for the clinicians facing feline lymphoma patients due to the challenge of diagnosing and prognosticate the disease in this species. Diagnosing different types of feline lymphoma, for example alimentary lymphoma, can be painful for the patient (biopsies from the GI-tract is often necessary) and costly for the cat owner. If there was a tumor marker to use, it could save time, pain and unnecessary suffering for the cat as well as money for the cat owner (von Euler & Eriksson, 2011).

It can be hard to differentiate feline lymphoma (alimentary lymphoma in particular) from chronic enteropathies due to similar clinical signs. In 2017 a retrospective study on chronic enteropathies in cats was made. The study included 147 cats. 12% of the cats had lymphoma, and to be able to differentiate those, histopathological reviews of the intestines had to be performed. This means that 18 cats had to go

through, for them, an unnecessary surgical procedure (Mitze *et al.,* 2017). If there was a tumor marker for aiding the diagnostic process of feline lymphoma, these cats may have eluded unnecessary suffering.

Thymidine kinase is one tumor marker that is frequently used in human oncology and provides accurate information about prognosis, for monitoring outcome of tumor therapy and for early discovery of cancer (He *et al.,* 2010). In canine lymphoma patients serum thymidine kinase has been evaluated with good results and is in some extent used in the clinic (Jagarlamudi *et al.,* 2014). Hopefully, this is a future in feline oncology as well. Taylor *et al.* (2013) performed a study where they analyzed sTK1 activity in cats to set a starting point for this cause. Their study implicated that there is a significant difference between lymphoma cats and healthy cats, i.e. cats with lymphoma have a higher sTK1 activity.

The main object of this study was to examine the sTK1 activity in healthy cats, and to calculate a reference interval for the study group from the collected data. One aim was also to study if there was an individual variation in sTK1 activity levels depending on age or gender. The reference interval calculated was 0.073-0.933 pmol/min/mL, with the higher value of 0.933 pmol/min/mL being the cut off value. This implies that a healthy cat should have <0.933 pmol/min/mL sTK1 activity and higher values would give a suspicion of disease. However, no lymphoma cases or other clinical cases were included in this study for comparison. The results also showed that there were no significant difference in sTK1 activity depending on gender or age.

A master thesis from 2013 (Mountari, 2013) included 8 clinical cases diagnosed with lymphoma (cutaneous, malignant, leukemia), where sTK1 activity was measured by the same assay as in this study. Three of these cases had sTK1 activity values <0.933 pmol/min/mL (0.83, 0.84 and 0.85), but the other five cats had values >0.933 pmol/min/mL (1.15, 1.22, 1.25, 3.75 and 7.44). This implies that the sTK1 activity is not suitable to be the single tool in the diagnosis making, as three of these lymphoma cats would have been missed based on only sTK1 activity using the suggested reference value. However, 5 of the 8 cats were above the calculated reference value. Moreover all of the 8 lymphoma cats were above the reference group's median and mean values. This is of course not a statistical proposition but it might be seen as a positive trend that suggests further studies on this topic. It would also be interesting to study diagnosed lymphoma cats that were to be followed during treatment and to see whether the sTK1 activity could function as a monitoring test to evaluate the treatment.

A strength of this study was that the cats included had to be both clinically healthy and healthy on blood screening, thus using a subjective as well as an objective factor in the inclusion criteria, i.e. the cats included in this study were well examined and it can be assumed that the cats were truly healthy. Another strength is that sTK1 stability is known to be maintained in various temperatures etc. thus assuming that storage of the samples did not affect the results (Taylor *et al.,* 2013).

In this master thesis, the sTK1 activity was measured. A study that was performed in 2013 analyzed both sTK1 activity and sTK1 protein levels in healthy dogs and in dogs with lymphoma, concluded that the actual sTK1 protein levels were of greater clinical value, especially for solid tumors (Kiran Kumar *et al.,* 2013). This would be interesting to study in feline patients as well.

This study serves as a baseline for future studies. For example, it would be of great use to compare sTK1 activity levels in cats diagnosed with lymphoma. As shown here, there seems to be no specific individual variation in the sTK1 activity, meaning that the whole study group and the results can be used to make comparisons and statistical analysis based on results from cats diagnosed with lymphoma.

CONCLUSION

The reference value for sTK1 activity in healthy cats is, according to this study, <0.933 pmol/min/mL. The results also showed that there is no individual variation in sTK1 activity, i.e. there is no significant difference between males and females or between cats ≤1 years old and cats ≥2 years old.

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