Serum TK1 activity and SAA concentration as biomarkers to differentiate lymphoma from gastrointestinal disease in cats

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TK1-aktivitet och SAA-koncentration i serum som biomarkörer för att särskilja lymfom från gastrointestinal sjukdom hos katt

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Abstract

Alimentary lymphoma is one of the most common malignant neoplasms in the gastrointestinal (GI) tract in cats. Since the clinical signs often are identical to those of inflammatory bowel disease (IBD), and the findings on diagnostic imaging and endoscopic mucosal biopsies overlap, invasive biopsy sampling from the GI tract is needed to be able to differentiate these two diseases from each other. Thymidine kinase 1 (TK1) is a cell cycle specific cytosolic enzyme involved in DNA synthesis and cell repair, and is commonly used as a tumor biomarker in human oncology since the TK1 activity increases with high cell turnover which is commonly seen in tumors. The aim of this study was to evaluate if sTK1 can be useful as a biomarker to differentiate cats with lymphoma from cats with acute to chronic GI disease. The relation between sTK1 activity and a marker for inflammation, the acute-phase protein serum amyloid A (SAA), was also examined, to evaluate if there is a correlation between sTK1 activity and SAA in cats with GI disease and lymphoma, and if a combination of the two assays strengthens the accuracy and predictive value.

Private-owned cats of varying breeds, ages and genders were recruited into three different groups; one group of 41 healthy control cats that were classified as healthy based on clinical examination and blood analysis; one group of 54 cats with GI disease that was presenting with vomiting, diarrhea, weight-loss and/or anorexia and of which a diagnosis related to the GI tract had been reached, and one group of 14 cats with confirmed lymphoma. Sera was collected from all cats and sTK1 activity was measured using the [^{3}H]-dThd phosphorylation assay while SAA was measured with a latex turbidimetric immunoassay. The results showed that cats with lymphoma had a significantly higher sTK1 activity compared to both healthy cats ($p<0.01$) and cats with GI disease ($p<0.05$), and the sTK1 activity assay for lymphoma had a higher accuracy, sensitivity and specificity compared to the SAA concentration assay for this group. In contrast, the SAA concentration in was significantly higher in cats with GI disease compared to healthy cats ($p<0.01$) and showed a higher accuracy, sensitivity and specificity compared to the sTK1 activity assay. The combination of sTK1 and SAA analyses showed a small, but statistically significant, added sensitivity and specificity in cats with GI disease, but not in the lymphoma group. There was also a weak correlation between TK1 and SAA in cats with lymphoma and cats with GI disease.

This study concludes that sTK1 is not useful as a biomarker for differentiating between lymphoma and GI disease in cats, as there is a high degree of overlap between the two groups. There are however indications that TK1 might be useful in malignancy assessment, monitoring of disease progression and prognosis estimation. Another conclusion is that there is a correlation between TK1 and SAA, although the correlation is weak and the combination of the two values do not strengthen the accuracy or predictive value for cats with lymphoma or GI disease, which means that SAA is not useful as an additional biomarker to sTK1 for this purpose. However, this study indicates that cats with inflammatory disease and pancreatitis may have an elevated sTK1 and SAA in the acute phase of disease that is later decreased if the tissue damage is reduced. More studies with a larger lymphoma group and with follow-up samples for the cats with GI disease and lymphoma are necessary to confirm these conclusions. Further studies on the potential use of both sTK1 and SAA in disease monitoring, malignancy assessment and evaluation of prognosis are also recommended.

Keywords: lymphoma, gastrointestinal lymphoma, feline lymphoma, inflammatory bowel disease, IBD, thymidine kinase 1, TK1, biomarker, cancer, cats.

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Abbreviations

AL	Alimentary lymphoma
DNA	Deoxyribonucleic acid
dThd	Deoxythymidine
GI	Gastrointestinal
HGAL	High-grade alimentary lymphoma
IBD	Inflammatory bowel disease
LGAL	Low-grade alimentary lymphoma
LGITL	Low-grade intestinal T-cell lymphoma
REA	Radioenzymatic assay
SAA	Serum amyloid A
sTK1	Serum thymidine kinase 1
TK1	Thymidine kinase 1
TK1a	Thymidine kinase 1 activity
TK1p	Thymidine kinase 1 protein
UDS	Universitetsdjursjukhuset (University Animal Hospital)

1. Introduction

Hematopoietic tumors represent one-third of all tumor cases in cats, and 90% of these are lymphomas (Richter 2003). In turn, lymphoma is the most common form of all neoplasms in this species, with an estimated annual incidence of 200 per 100 000 cats at risk (MacVean *et al.* 1978; Withrow *et al.* 2013), Alimentary lymphoma (AL) is recognized as the most common anatomical form in cats (Gabor *et al.* 1998; Vail *et al.* 1998; Louwerens *et al.* 2005; Milner *et al.* 2005; Vezzali *et al.* 2010; Weiss *et al.* 2010; Stützer *et al.* 2011). The clinical signs of AL are identical to those of inflammatory bowel disease (IBD), most commonly vomiting, diarrhoea, anorexia and weight-loss (Tarns 1993). Differentiating IBD from AL is a challenge due to their overlap in clinical picture, and invasive diagnostic methods such as histopathological examination of biopsies from the GI tract are required to confirm the diagnosis (Evans *et al.* 2006; Kleinschmidt *et al.* 2010).

Thymidine kinase 1 (TK1) is a cell cycle specific enzyme involved in DNA synthesis (Bello 1974) and cell repair (Chen *et al.* 2010). Cancer tissue is characterized by a high rate of cell proliferation, which causes an elevation of TK1 that remains throughout the cell cycle (Hengstschläger *et al.* 1994) and can be detected in serum using enzyme activity assays and immunoassays (Jagarlamudi & Shaw 2018). TK1 is an established tumor biomarker that is used clinically in human oncology and is valuable in malignancy assessment, monitoring of treatment results and early detection of recurrence (von Euler & Eriksson 2011). There have been many corresponding studies made on canine lymphoma and other malignancies (Nakamura *et al.* 1997; von Euler *et al.* 2004, 2006, 2008; Sharif *et al.* 2012) and currently, TK1 is available as a preliminary test method for early detection of tumor growth in dogs (Jagarlamudi *et al.* 2015).

Only a few studies have evaluated TK1 in cats. These studies indicate that TK1 may be a potentially useful biomarker for feline lymphoma, but further research is required to fully assess its utility (Taylor *et al.* 2013; Wang *et al.* 2021). Because an early diagnosis is important, and it is desirable to be able to select the patients who will benefit from undergoing more comprehensive diagnostic examinations, a good serum biomarker for GI lymphoma in cats would optimize both animal welfare and cat health.

1.1 Aim of the study

The aim of this study was to evaluate if sTK1 can be useful as a biomarker to differentiate cats with lymphoma from cats with acute to chronic GI disease. Furthermore, the relation between sTK1 activity and serum amyloid A (SAA) were examined, to evaluate if there is a correlation between sTK1 activity and SAA levels in cats with GI disease and lymphoma, and to assess if a combination of the two assays strengthens the accuracy and predictive value, compared to the two as single determinates.

2. Literature review

2.1 Structure of DNA

Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) are the carriers of genetic information in all living organisms. The building-blocks of RNA and DNA are ribonucleoside and deoxyribonucleoside phosphate respectively, also called nucleotides, which are composed of a nitrogenous base that is covalently linked to a five-carbon sugar and one, two or three phosphate groups. (Alberts *et al.* 2019). The nitrogen-containing base is a derivate from either purines or pyrimidines. The purine bases are adenine (A) and guanine (G), while the pyrimidine bases are cytosine (C) and, depending on DNA or RNA, thymine (T) or uracil (U) respectively. A ribonucleoside is produced when adding a ribose to a base, while a deoxyribonucleoside is produced when adding 2-deoxyribose. The addition of one or more phosphate groups to a nucleoside creates a nucleotide (Ferrier 2017).

Deoxynucleosides that are used for deoxynucleotide synthesis can be generated either *de novo* or through utilization of salvage pathways. When generated *de novo*, pyrimidine and purine ribonucleotides are reduced to deoxyribonucleotides by the enzyme ribonucleotide reductase. In the salvage pathway, the nucleosides are derived from nutrients, metabolic decomposition, or degraded DNA, and taken up from the extracellular space, transferred across the cell membrane and then phosphorylated to deoxyribonucleoside monophosphates by deoxyribonucleoside kinases. The charged monophosphates can then be further phosphorylated to DNA triphosphates. There are four types of deoxyribonucleoside kinases: deoxycytidine kinase (dCK), thymidine kinase 1 (TK1), thymidine kinase 2 (TK2) and deoxy-guanosine kinase (dGK). These are usually the rate-determining enzymes in the salvage pathway (Arnér & Eriksson 1995).

The nucleotides within a DNA strand are connected by phosphodiester bonds at the 3’-hydroxyl group of one sugar to the 5’ phosphate group of another, which creates a chemical polarity to each polynucleotide strand. In a DNA double helix, a purine base always pairs with a pyrimidine base. This is called a base pair, and the genetic information is encoded by the sequence of the nucleotides along each DNA strand.

The double stranded DNA molecules are in turn packed into chromosomes in the nucleus (Alberts *et al.* 2019). Domestic cats have 38 chromosomes, consisting of 18 autosomal pairs and the sex chromosome pair, X and Y (Lyons 2012). A cell contains two copies of every chromosome, except the gametes and highly specialised cells that lack DNA entirely (Alberts *et al.* 2019).

2.2 Cell cycle and normal cell division

The cell cycle is the process when a cell duplicates its contents and then divides itself into two. This process can be described in four phases. The G1 phase is when the cell increases in size, which is followed by the S phase where the cell replicates its DNA, and G2 phase where the cell prepares to divide itself. These three phases are together called the interphase. The M phase is when mitosis, i.e., cell division, occurs (Alberts *et al.* 2019). The G0 phase is a non-proliferative phase between the M and S phase. External factors such as growth factors and cell adhesion molecules stimulate the G0 cell to enter the G1 phase in the cell cycle (Withrow *et al.* 2013).

This entire process is managed by the cell-cycle control system, which ensures that the cell cycle progresses properly by the activation and inactivation of protein kinases and protein phosphatases. The protein kinases are also known as cyclin-dependant protein kinases (CKDs), since their activation in the cell cycle is controlled by cyclins. These ensures that the protein kinases are only activated during appropriate times in the cell cycle phase (Alberts *et al.* 2019). This activation is also controlled by phosphorylation by CKD activating kinases (CAK). The CKDs regulate a series of checkpoints that mediate mitogenic and inhibitory signals. The cell cycle can also be paused by inhibitory proteins called cyclin-dependant kinase inhibitors (CDKI) that blocks the G1/S progression (Withrow *et al.* 2013). These checkpoints ensures that all steps have been completed and that the environmental conditions are favourable before the cell enters the next phase in the cell cycle (Alberts *et al.* 2019).

2.2.1 Response to cell damage

The cells can respond in two ways when exposed to stress signals, DNA damage or oxygen depletion. This is to either undergo cell cycle arrest in G1, S and G2, or to enter programmed cell death (apoptosis). The protein p53 is a tumor suppressor protein that regulates checkpoints in response to DNA damage and plays an important role in maintaining genomic stability by initiating cell arrest or apoptosis to prevent genome mutation and cancer (Withrow *et al.* 2013).

2.3 Cancer mechanism

There is a wide range of mechanisms involved in the development of tumors and tumors can be derived from a diverse spectrum of tissues, which makes it difficult to define a cancer cell (Withrow *et al.* 2013). According to the National Cancer Institute (NCI), cancer is "a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body" (NCI 2021). Tumors can be classified into three broad types. Benign tumors are local tumors that can appear in any tissue but do not metastasize. In situ tumors are often small tumors that arise in the epithelium and do not invade the basement membrane or the supporting mesenchyme. Lastly, cancer is a malignant tumor that can both locally invade other tissues and metastasize to other parts of the body (Withrow *et al.* 2013).

The six hallmarks of cancer were first defined in 2000 by Hanahan and Weinberg, as following: self-sufficient growth, insensitivity to antigrowth signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis (Hanahan & Weinberg 2000). Since then, four more emerging hallmarks have been incorporated: abnormal metabolic pathways, evasion of the immune system, unlocking phenotypic plasticity and senescent cells, along with four enabling characteristics: genome instability, inflammation, polymorphic microbiomes and non-mutational epigenetic reprogramming (Hanahan & Weinberg 2011; Hanahan 2022).

2.4 Biomarkers

The Biomarkers Definition Working Group (2001) defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Biomarkers Definitions Working Group 2001). Thus, it can be used to differentiate a patient with a disease from a healthy individual. Biomarkers can be found in excretions, secretions, body tissue or in the circulation and can include almost any activity or substance such as proteins, nucleic acids, antibodies, peptides or gene expression (Henry & Hayes 2012).

Diagnostic biomarkers are characteristics that are associated with a specific disease but not necessarily a clinical outcome or treatment response (Webster *et al.* 2011). Prognostic biomarkers are markers that are associated with a clinical outcome regardless of therapy and may be used to evaluate the possible disease progression and outcome. Predictive biomarkers are associated with a treatment outcome and can be an indicator of a therapeutic effect, which can help to make choices between different treatment options for a patient (McShane *et al.* 2005; Webster *et al.* 2011).

2.4.1 Thymidine kinase 1

Thymidine kinase (TK) is one of the four deoxyribonucleoside kinases involved in the salvage pathway of pyrimidine synthesis, and it catalyses the phosphorylation of deoxythymidine (dT) to deoxythymidine monophosphate (dTMP) using adenosine triphosphate (ATP) as the phosphate donor. dTMP is then further phosphorylated to deoxythymidine diphosphate (dTDP) and deoxythymidine triphosphate (dTTP) (Arnér & Eriksson 1995).

Thymidine kinase exists as two isoenzymes: TK1 and TK2. Thymidine kinase 1 (TK1) is a cytosolic enzyme and has the most restricted substrate specificity among the deoxyribonucleoside kinases as it only phosphorylates thymidine and deoxyuridine (Arnér & Eriksson 1995). TK1 has a cell cycle specific expression and is closely correlated to cell proliferation and cell synthesis. The enzyme activity is absent in resting cells, increases in the G1 phase and reaches its highest level at the S-phase and then decreases in the M phase (Bello 1974; Sherley & Kelly 1988). TK1 is also involved in cell repair, as the TK1 levels increase when DNA is damaged by radiation or chemotherapeutic agents, and depletion of TK1 in cells exposed to DNA damage can result in apoptosis (Chen *et al.* 2010).

Thymidine kinase 2 (TK2) is a mitochondrial enzyme and have a broader substrate specificity than TK1, however, the level of TK2 is low compared with TK1 in proliferating cells. TK2 is expressed in all cells irrespective of the cell cycle and it is the only pyrimidine deoxyribonucleoside phosphorylating enzyme found in resting cells (Arnér & Eriksson 1995).

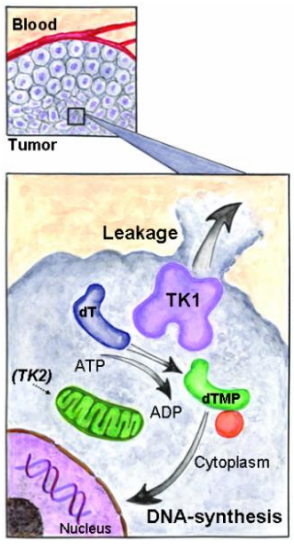

Figure 1. TK1 is a cytosolic enzyme that phosphorylates thymidine and is only expressed in proliferating cells. Cell apoptosis causes TK1 to leak out into the circulation. TK2 is a mitochondrial enzyme that is constitutively expressed in both proliferating and in resting cells. Illustration from von Euler et al. (2004) and published with permission from Wiley-Blackwell Publishing.

Thymidine kinase 1 as a tumor biomarker

Tumor biomarkers are biological molecules, activities or substances that are present in cancer tissues or body fluids, which can be used to provide information about current and future behaviour of cancer such as risk assessment, diagnosis, prognosis, and to predict the efficacy or toxicity of treatment and likelihood of recurrence (Henry & Hayes 2012).

TK1 as a biomarker for cancer have been studied since the 1960s (Weber & Lea 1966). Unlike normal tissue where TK1 activity is absent in mitotic and nonproliferating cells, tumor tissue causes the TK1 activity to increase and remain throughout the M and G2 phase (Hengstschläger *et al.* 1994) and the usual degradation of TK1 during mitosis is disrupted (Chang & Huang 1992). This is caused by an absence of a normal p53 response in cancer cells, which leads to a loss of the cell cycle specific control of TK1 activity (Schwartz *et al.* 2004). Malignant cells are characterized by a rapid and unregulated cell proliferation, which causes a higher rate of cell apoptosis. TK1 is released into the circulation when fast dividing cells die during mitosis (Jagarlamudi & Shaw 2018). The overall DNA synthesis and cell death in the replicative stage can therefore be reflected by an increase in extracellular TK1 activity (von Euler *et al.* 2004). The rate of cell proliferation and disruption in body tissue is usually measured with proliferation markers by immunohistology on biopsies, but the unique quality of TK1 is that it can also be measured in serum by using enzyme activity assays to detect the rate of TK1 enzyme activity (TK1a) and immunoassays to measure the level of TK1 protein (TK1p) (Jagarlamudi & Shaw 2018).

In human medicine, sTK1 has proven to be a useful biomarker regarding prognosis and treatment for various hematopoietic malignancies such as leukaemia, multiple myeloma, Hodgkin's lymphoma and non-Hodgkin lymphoma (von Euler *et al.* 2004), as well as solid tumors such as breast, colorectal, lung and gynaecologic cancers (von Euler & Eriksson 2011).

TK1 has also been evaluated as a possible biomarker in veterinary medicine, especially in canines. Nakamura *et al.* (1997) found that plasma TK1a was increased in dogs with malignant lymphoma and leukaemia. Later studies have also measured serum TK1a with radioenzymatic assays (TK-REA) (von Euler *et al.* 2004; von Euler *et al.* 2006) and non-radiometric assays, such as the TK-Liaison (von Euler *et al.* 2008) and [^{3}H]-deoxythymidine (dThd) phosphorylation assay (Sharif *et al.* 2012). These assays have shown to reflect the clinical stage and prognosis and to provide valuable information for treatment monitoring in canine lymphoma. In several studies, serum TK1a is significantly higher in dogs with hematopoietic tumors but there is no difference in serum TK1a in dogs with solid

tumors, compared to healthy individuals (von Euler *et al.* 2006; Sharif *et al.* 2012; Jagarlamudi *et al.* 2013). However, studies have found that the level of serum TK1p is much higher in both hematopoietic and solid tumors compared to healthy dogs, and that TK1p assays are more sensitive than TK1a when it comes to prognosis and treatment of solid tumors (Jagarlamudi *et al.* 2014, 2015)

Only a few studies have been made to evaluate TK1 in cats. Taylor *et al.* (2013) used a TK-REA to measure and compare sTK1 activity in cats with lymphoma, cats with inflammatory disease, cats with non-haematopoietic neoplasia and healthy cats. The results showed that cats with lymphoma had a significantly higher sTK1 activity compared to the other three groups (Taylor *et al.* 2013). This finding has also been confirmed in a more recent study using a [^{3}H]-dThd assay, where sTK1 activity in cats with lymphoma was higher compared to healthy cats and cats with inflammatory diseases or IBD (Wang *et al.* 2021).

2.5 Feline lymphoma

2.5.1 Classification

Lymphoma originates from lymphoreticular cells and can arise from almost any lymphoid tissue in the body, such as lymph nodes, spleen and bone marrow (Withrow *et al.* 2013). The tumor can be classified by anatomical location, histological grade and immunophenotype (Barrs & Beatty 2012). The four anatomical locations that are traditionally described are alimentary, mediastinal, multicentric and extra-nodal lymphoma (Hardy 1981; Barrs & Beatty 2012), with the alimentary tract being recognized as the most common location in many studies (Gabor *et al.* 1998; Vail *et al.* 1998; Louwerens *et al.* 2005; Milner *et al.* 2005; Vezzali *et al.* 2010; Weiss *et al.* 2010; Stützer *et al.* 2011).

Alimentary lymphoma (AL) is mostly seen in older cats, with a median age ranging between 10-13 years (Mahony *et al.* 1996; Gabor *et al.* 1998; Carreras *et al.* 2003; Waly *et al.* 2005; Kiselow *et al.* 2008; Lingard *et al.* 2009; Pohlman *et al.* 2009; Rissetto *et al.* 2011; Barrs & Beatty 2012a; Russell *et al.* 2012). The Domestic shorthair and Siamese breed seems to be overrepresented in several studies (Gabor *et al.* 1998; Vail *et al.* 1998; Kristal *et al.* 2001; Carreras *et al.* 2003; Waly *et al.* 2005; Rissetto *et al.* 2011) and some studies indicate that male cats are predisposed for AL (Gabor *et al.* 1998; Vail *et al.* 1998; Kristal *et al.* 2001; Carreras *et al.* 2003; Wilson 2008; Pohlman *et al.* 2009) while other studies do not support this finding (Waly *et al.* 2005; Lingard *et al.* 2009; Gianella *et al.* 2017).

AL is characterized by neoplastic lymphocyte infiltration of the GI tract, with or without involvement of mesenteric lymph nodes (Barrs & Beatty 2012a). The most common site of involvement is the jejunum, followed by the duodenum and ileum (Evans *et al.* 2006; Kleinschmidt *et al.* 2010; Briscoe *et al.* 2011; Moore *et al.* 2012). The original classification system was developed by the National Cancer Institute Working Formulation (NCIWF) to characterize human non-Hodgkin's lymphoma (The Non-Hodgkin's Lymphoma Pathologic Classification Project 1982) and it has since then been successfully used to characterize lymphoma in other species, including cats (Valli *et al.* 2000). The NCIWF classifies lymphoma into three histological grades based on progression and rate of mitosis: low-grade (LGAL), intermediate grade (IGAL), and high-grade (HGAL) (Valli *et al.* 2000).

The World Health Organization (WHO) classification system classifies lymphoma according to immunophenotype and it is based on the Revised European-American Classification of Lymphoid Neoplasms (REAL) classification developed by the International Lymphoma Study Group (ILSG) (Alaggio *et al.* 2022). Overall, feline lymphomas are usually of T-cell origin (Jackson *et al.* 1996; Mahony *et al.* 1996), although AL is reported to primarily be of B-cell origin in many studies (Jackson *et al.* 1996; Mahony *et al.* 1996; Gabor *et al.* 1999; Patterson-Kane *et al.* 2004; Waly *et al.* 2005; Pohlman *et al.* 2009). A strong association between anatomical location and immunophenotype has been identified, with a predomination of high-grade B-cell lymphoma in the stomach and large intestine, while low-grade T-cell lymphoma (LGITL) is almost exclusively localized to the small intestine (Gabor *et al.* 1999; Lingard *et al.* 2009; Pohlman *et al.* 2009; Moore *et al.* 2012).

The current system that has been developed by Moore *et al.* (2012) to classify feline AL contains the two following definitions: "mucosal lymphoma", which are usually low-grade alimentary lymphomas (LGAL) of small T-cell type, and "transmural lymphomas", which are more likely to be high-grade alimentary lymphomas (HGAL) of either small or large cell size and of either B- or T-cell type. Large granular lymphocyte lymphoma (LGLL) is recognised in the REAL/WHO scheme as a separate histological subclassification of AL, and it can be of any histological grade (Pohlman *et al.* 2009; Barrs & Beatty 2012a; Moore *et al.* 2012).

The majority of feline lymphomas have been classified as intermediate or high-grade in several studies (Vail *et al.* 1998; Gabor *et al.* 1999; Valli *et al.* 2000), although LGAL is considered to be the most common subtype of alimentary lymphoma according to most studies (Valli *et al.* 2000; Pohlman *et al.* 2009; Barrs & Beatty 2012a; Moore *et al.* 2012; Paulin *et al.* 2018).

2.5.2 Ethiopatogenesis

Non-retroviral-associated lymphoma

The non-retroviral associated lymphomas make up the largest group of lymphocytic cancers in felines, but the origin is still not fully understood (Louwerens *et al.* 2005). Bertone *et al.* (2002) showed that cats exposed to passive smoking have a higher risk of developing lymphoma.

A linkage between alimentary lymphoma and chronic inflammatory bowel disease (IBD) has also been suggested, and IBD has been proposed as a risk factor for the development of AL, although there are no direct evidence (Mahony *et al.* 1996; Krecic & Black 2000; Carreras *et al.* 2003; Ragaini *et al.* 2003; Louwerens *et al.* 2005; Waly *et al.* 2005; Roccabianca *et al.* 2006). It has also been suggested that diet may have an impact on the development of AL, but no definite proof exists (Ragaini *et al.* 2003; Louwerens *et al.* 2005).

Retroviral-associated lymphoma

A significant correlation between lymphoma and infection with feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) have been shown (Shelton *et al.* 1990; Liem *et al.* 2013). Infection with FIV, FeLV, or both, increases the relative risk of developing lymphoma 5.6, 62.1 and 77.3 times, respectively, compared to uninfected cats (Shelton *et al.* 1990).

Before 1980, FeLV accounted for approximately 70% of feline lymphoma cases (Shelton *et al.* 1990). Since the development of the first diagnostic test for FeLV in the 1970s and the first vaccine in the 1980s, infection with FeLV have declined in the total cat population. However, the incidence of feline lymphoma have increased, and the disease has changed from one that mostly used to affect younger, FeLV-positive cats to one that now has an increasing prevalence for older, FeLV-negative individuals (Hardy 1993; Louwerens *et al.* 2005). The median age of lymphoma occurrence in FeLV-positive cats is 4-6 years, whereas it is 11 years for the FeLV-negatives (Vail *et al.* 1998; Withrow *et al.* 2013).

Studies have also reported an association between retroviral infection and anatomic localisation and immunophenotype. FeLV-associated lymphomas are more likely to be of T-cell origin while FIV-associated lymphomas tend to be of B-cell lineage (Louwerens *et al.* 2005). Overall, multicentric T-cell lymphoma is the most common form in cats with retroviral infection, while cats that are negative usually have alimentary or thymic B-cell lymphoma (Shelton *et al.* 1990; Hardy 1993; Vail *et al.* 1998; Patterson-Kane *et al.* 2004; Louwerens *et al.* 2005).

2.5.3 Treatment and prognosis

Chemotherapy is the treatment of choice for most forms of feline lymphoma. The COP protocol was originally described by Cotter in 1983 and is considered the standard chemotherapy combination treatment in Europe. It includes vincristine, cyclophosphamide and prednisone (Moore *et al.* 1996; Vail *et al.* 1998; Kristal *et al.* 2001; Teske *et al.* 2002), and the recommended treatment duration is at least one year (Moore *et al.* 1996; Withrow *et al.* 2013). This protocol is well tolerated and has minimal treatment-related toxicity (Teske *et al.* 2002). However, cats with low-grade lymphoma (LGAL), which is the most common form, usually have a better prognosis and do not require as aggressive chemotherapy protocols compared to other histological grades and immunophenotypes. Treatment with chlorambucil and prednisone have resulted in a response rate of more than 90% and a median survival time of two years or longer for these patients. A less durable therapy response and a shorter survival time is seen in cats with high-grade B-cell or T-cell lymphoma, with a median survival of 45-100 days, regardless of treatment (Withrow *et al.* 2013). The approximate survival time in untreated cats with lymphoma is 4-8 weeks (Nelson & Couto 2014). Response to therapy is a positive prognostic factor, and achieving complete remission (CR) is necessary for longer survival (Ettinger 2003). A poor clinical condition when diagnosed is associated with a worse response to chemotherapy and shorter survival time (Mahony *et al.* 1996).

The CHOP protocol, i.e., the addition of doxorubicin, has been demonstrated to increase duration of remission in some studies. Cats that achieved CR with COP therapy and then received doxorubicin as maintenance therapy had longer remission times compared to cats that continued to receive COP chemotherapy (Moore *et al.* 1996; Vail *et al.* 1998). However, doxorubicin is not effective as a single treatment agent (Peaston & Maddison 1999; Kristal *et al.* 2001) and toxic side-effects, such as bone marrow suppression, gastrointestinal signs and renal toxicity, have also been reported (Kristal *et al.* 2001).

According to a study by Vail *et al.* (1998), anatomic site and FeLV-status is also a prognostic for remission duration and survival time. In their study, nasal lymphoma had the longest remission and survival times, followed by alimentary lymphoma. They also showed that FeLV-negative cats that achieved CR after COP treatment had longer remission and survival times compared to FeLV-positive cats, especially with the inclusion of doxorubicin in the treatment protocol (Vail *et al.* 1998). Another study by Moore *et al.* (1996) also showed that the longest remissions for cats treated with COP were in cats with alimentary lymphoma. However, this contrasts with a study by Mahony *et al.* (1995), where anatomical location and FeLV-status had no correlation with survival time.

2.6 Inflammatory bowel disease (IBD)

2.6.1 Etiopathogenesis

Inflammatory bowel disease (IBD) is a collective name of a group of chronic intestinal disorders in which no specific cause can be determined, although it is thought to occur in human as a combination of environmental factors, such as the enteric microbiota, and genetical factors, such as the host susceptibility and mucosal immunity. If the mucosal immune response to enteric bacteria is reduced in a susceptible host, it can lead to IBD (Jergens 2002; Jergens & Simpson 2012).

IBD usually affects middle-age to older cats, with a median age of approximately 8 years (Evans *et al.* 2006; Jergens *et al.* 2010; Kleinschmidt *et al.* 2010; Marsilio *et al.* 2019b; a; Freiche *et al.* 2021), and it is one of the most common causes of persisting or intermittent chronic GI signs in cats, such as vomiting, diarrhea, anorexia and weight-loss (Tarns 1993; Waly *et al.* 2005). Other important differential diagnoses to these clinical signs in cats are food responsive GI disease, chronic endoparasitism, neoplasia or hyperthyroidism (Tarns 1993; Jergens 2002, 2012; Kleinschmidt *et al.* 2010; Barrs & Beatty 2012b). Pancreatitis in cats is often seen with IBD and/or cholangitis in cats, a condition called “triaditis” (Simpson *et al.* 2001; Armstrong & Williams 2012; Jergens 2012; Černá *et al.* 2020).

IBD is characterized by a diffuse inflammatory cell infiltration within the lamina propria. The cell types include lymphocytes, plasma cells, eosinophils, neutrophils, and macrophages. The most common form in cats is lymphocytic-plasmocytic enteritis (LPE) (Tarns 1993; Gianella *et al.* 2017), followed by benign lymphocytic enteritis and lymphocytic-plasmocytic colitis (Tarns 1993).

2.6.2 Treatment and prognosis

Corticosteroids, such as prednisone, is considered the standard treatment for IBD. In addition, dietary therapy may also be helpful such as hypoallergenic diets (Tarns 1993; Jergens 2002; Jergens *et al.* 2010) that contains a new or hydrolysed protein source and highly digestible carbohydrates. It should also be gluten-free, low in lactose and fibres, nutritionally balanced and palatable (Jergens 2012). Prebiotic and probiotic therapy can reduce the intestinal inflammation by modifying the intestinal bacterial populations (Jergens 2002; Jergens & Simpson 2012).

There are few studies regarding the prognosis for cats with IBD. Many cats respond well to medical and dietary therapy, while some fail to have adequate treatment responses. The reason could be low compliance, severe intestinal inflammation, concurrent disease or an occult lymphoma diagnosis (Jergens *et al.* 2003).

2.7 Differentiating LGITL from IBD

To diagnose a chronic GI disease, it is important to first rule out other differential diagnoses. Recommended baseline tests include a biochemical profile, complete blood count (CBC), urine analysis, fecal examinations, serum thyroxine test and tests for FeLV and FIV (Tarns 1993; Jergens & Simpson 2012). After excluding non-gastrointestinal diseases, feline chronic enteropathy (FCE) usually remains. It is an umbrella term that includes food-responsive enteropathy (FRE), inflammatory bowel disease (IBD) and low-grade intestinal T-cell lymphoma (LGITL) (Jergens *et al.* 2010; Barrs & Beatty 2012a; b; Jergens 2012; Moore *et al.* 2012; Marsilio 2021b), and it is defined as GI signs that have been present for more than 3 weeks without any extraintestinal, infectious, obstructive or localized neoplastic explanations (Marsilio 2021b).

LGITL is the most common subtype of AL in cats, as it represents 60–75% of all cases. It is characterized by a mucosal infiltration of neoplastic T-cells in the small intestine (Paulin *et al.* 2018). Differentiating LGITL and IBD is difficult due to overlapping clinical signs, laboratory results, diagnostic imaging, and histological morphology (Waly *et al.* 2005; Briscoe *et al.* 2011; Kiupel *et al.* 2011; Barrs & Beatty 2012a; b; Norsworthy *et al.* 2015; Sabattini *et al.* 2016; Paulin *et al.* 2018). Other challenging aspects is that both diseases can be concurrent in the same patient (Lingard *et al.* 2009; Briscoe *et al.* 2011; Kiupel *et al.* 2011), and the possibility of IBD to predispose for lymphoma (Mahony *et al.* 1996; Krecic & Black 2000; Carreras *et al.* 2003; Ragaini *et al.* 2003; Louwerens *et al.* 2005; Waly *et al.* 2005; Roccabianca *et al.* 2006).

The Feline Enteropathy Activity Index (FCEAI) is a clinical scoring system designed to assess inflammatory activity in cats with chronic enteropathy and includes five independent variables that correlates to histopathologic inflammation: GI signs, endoscopic lesions, serum total protein, serum ALAT/ALP, and serum phosphorus concentrations. This index has shown to be suitable for monitoring the effect of treatment and can be used to assess disease activity in cats with either IBD or FRE (Jergens *et al.* 2010; Mitze *et al.* 2017). However, lymphoma and non-lymphoma groups cannot be differentiated only by this method (Mitze *et al.* 2017).

Laboratory tests

Cats with IBD usually have normal baseline test results, but some deviations have been identified. Hyperproteinemia and a mild elevation in ALP and ALAT is often reported (Tarns 1993; Jergens *et al.* 2010; Jergens 2012), as well as decreased serum cobalamin and folate concentrations (Simpson *et al.* 2001; Reed *et al.* 2007). Hypoalbuminaemia is a common finding in both IBD and AL (Marsilio 2021b) and inorganic phosphorus may also be decreased (Reed *et al.* 2007).

Other anomalies associated with IBD are mild non-regenerative anaemia, neutrophilia without a left shift and/or eosinophilia (Tarns 1993; Jergens & Simpson 2012). Some cats may also have increased serum fPLI concentrations, which could suggest a concurrent pancreatitis (Jergens *et al.* 2010; Marsilio 2021a).

Imaging diagnostics

Ultrasonographic distinction between LGITL and IBD is usually not possible due to their great similarities. The most common findings include a diffuse thickening of the muscularis layer in the small intestines, disrupted wall layering and abdominal lymphadenopathy (Richter 2003; Evans *et al.* 2006; Lingard *et al.* 2009; Zwingenberger *et al.* 2010; Daniaux *et al.* 2014; Gianella *et al.* 2017).

Histopathology

A definite diagnosis of LGITL and IBD can only be made by histopathological evaluation of biopsies from the stomach and intestines. A challenge with alimentary tract biopsy is that it is necessary to obtain tissue at the correct location and of sufficient depth to be able to make the correct diagnosis. Biopsies can be obtained by endoscopy (EB), which is a non-invasive and quick method, although it generally only provides limited information since only samples from the mucosa of the gastrointestinal tract (GIT) can be obtained and it can rarely reach the jejunum or ileum in most cats. This may lead to an incorrect diagnosis and inappropriate treatment, which in turn could affect the prognosis. Another diagnostic method is exploratory laparotomy or laparoscopy, which is invasive, expensive and time-consuming but allows full inspection of the intestines and for full-thickness (transmural) biopsies to be taken from the GIT and other abdominal organs, including the mucosal, submucosal and muscularis layer (Evans *et al.* 2006; Kleinschmidt *et al.* 2010).

The histological features of LGITL and IBD (particularly LPE) can be similar, which makes them difficult to distinguish (Briscoe *et al.* 2011; Gianella *et al.* 2017). Microscopic findings in IBD include an infiltration of inflammatory cells in the lamina propria, along with mucosal structure disruption (Briscoe *et al.* 2011; Jergens 2012; Jergens & Simpson 2012). LGITL is histologically characterised by a mucosal infiltration of neoplastic lymphocytes, involving both the epithelium and lamina propria (Gabor *et al.* 1999; Carreras *et al.* 2003; Richter 2003; Briscoe *et al.* 2011; Gieger 2011; Kiupel *et al.* 2011; Moore *et al.* 2012). However, one defining criterium to differentiate LGITL from IBD is epitheliotropism, i.e., the ability for neoplastic T-cells to progress to the submucosal and transmural layer (Carreras *et al.* 2003; Richter 2003; Briscoe *et al.* 2011).

To differentiate IBD and LGITL and to ultimately confirm the diagnosis, histologic evaluation needs to be combined with immunohistochemical (IHC) and clonality analyses on biopsies (Moore *et al.* 2005; Waly *et al.* 2005; Evans *et al.* 2006; Kiupel *et al.* 2011; Mitze *et al.* 2017; Paulin *et al.* 2018). IHC can inform about the phenotype of the infiltrative lymphocytes (Kiupel *et al.* 2011), while clonality analysis by PCR for antigen receptor rearrangement (PARR) can differentiate neoplastic monoclonal lymphoid proliferations from inflammatory polyclonal lymphoid proliferations (Moore *et al.* 2005; Sabattini *et al.* 2016).

Other biomarkers

In a study by Tamamoto *et al.* (2013), serum amyloid A (SAA) was evaluated as a prognostic marker in cats with neoplastic, inflammatory, and other diseases. This study showed that SAA concentration can be a useful prognostic indicator in cats with various diseases, and that cats with elevated SAA had a significantly shorter median survival time compared to cats with non-elevated SAA. In this study, 46% of cats with neoplastic disease also had elevated SAA, compared to 28% in the inflammatory disease group and 37% in cats with other diseases. In turn, 60% of the cats with both neoplastic disease and elevated SAA had lymphoma (Tamamoto *et al.* 2013). Other studies have also shown a correlation between elevated levels of SAA and neoplasms in humans and animals (Cho *et al.* 2010; Wang *et al.* 2012; Zhang *et al.* 2012; Tamamoto *et al.* 2014).

Love *et al.* (2012) evaluated serum haptoglobin as a biomarker for IBD and concluded that it can be useful for distinguishing between healthy cats and those with IBD, but cannot differentiate IBD from lymphoma (Love *et al.* 2021).

Lactate dehydrogenase has been assessed as a possible biomarker for lymphoma and is used in human medicine as a predictive and prognostic marker for cancer, as its activity in serum increases significantly due to necrosis or tissue damage, especially in malignant tumor cells. However, less studies have been made on cats (Terragni *et al.* 2016). One study showed a correlation between an increase of serum LDH and a worse prognosis in feline lymphoma (Hadden *et al.* 2008). Furthermore, a study by Terragni *et al.* (2016) found no diagnostic accuracy in differentiating AL and IBD in the overall cat population.

3. Material and methods

3.1 Cats in the study

This prospective study was conducted at the University Animal Hospital (UDS) at the Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden. The study started in April 2022 and finished in November 2022. In the study, cats were recruited into three separate groups based on current disease status. The study was approved by the Swedish Animal Protection Ethical Committee (reference number 5.2.18-13750/19, diary number 5.8.18-04682/2020) and the samples were collected from patients with signed informed consent provided by their owner.

3.1.1 Healthy control group

The healthy control group consisted of privately owned cats of varying breeds, ages, and gender. These cats were, according to the owner's knowledge, clinically healthy and had no current or chronic medical disease. Blood samples (sera and EDTA) were collected from the cats and a clinical examination was performed by a final year veterinary student at UDS in September 2022.

In order to be included in the study, the cat also had to be classified as healthy based on clinical examination and blood parameters. The blood samples were run at the Clinical Pathology Laboratory at UDS and included a complete blood count (CBC) and a serum biochemistry profile, more specifically the following values: triglycerides, CK, chloride, sodium, potassium, cholesterol, urea, phosphate, calcium, SAA, GGT, fructosamine, bile acids, glucose, ALP, ALAT, creatinine, protein, albumin, amylase, ASAT, total bilirubin, iron, lipase, magnesium, zinc, globulin, unsaturated iron-binding capacity (UIBC), total iron-binding capacity (TIBC), transferrin saturation and GLDH. The sera samples were centrifuged within 60 minutes after collection, with a speed of 3 000 revolutions per minute (RPM) for 10 minutes. The serum was then separated and pipetted into empty cryotubes. After running the biochemistry profile, the sera samples were stored in -80 °C until TK1 analysis. All left over blood samples were then reposited in the Feline Genome Biobank at SLU.

Cats were considered healthy if they had blood parameters within the reference range, or if any deviations from the reference range could be interpreted as normal either relatively to the other blood parameters or due to physiological explanations, for example hyperglycaemia without any other deviations could be explained as a stress reaction. Cats with blood parameters that strongly differed from the other individuals, or where a combination of the deviant parameters were indicative of a medical disease, were considered subclinical unhealthy, for example an elevation of both glucose and fructosamine, or a more than ten-fold elevation of SAA.

3.1.2 Cats with gastrointestinal disease

The group of cats with GI disease consisted of cats of varying breeds, ages, and gender that had visited UDS during the time period between April 2022 and October 2022, and for which a stored excess serum sample was available. The cases were initially included if they presented one or more of the following symptoms: vomiting, diarrhea, anorexia, and/or weight-loss. Cases were further selected if they had a confirmed or presumptive inflammatory diagnosis related to the GI tract based on a combination of clinical signs, blood tests, diagnostic imaging, cytology and/or histopathology. Stored sera samples were collected from the Clinical Pathology Laboratory at UDS and were stored in -20 °C before analysis.

Cats with a confirmed primary diagnosis unrelated to the digestive system, such as primary renal disease, hyperthyroidism, or diabetes mellitus, as well as neoplasms, foreign bodies, infectious diseases, or toxic causes, were excluded. Cats that had received systemic corticosteroids or other immunosuppressant treatment in the last three months before sample collection were also excluded.

3.1.3 Cats with lymphoma

This group consisted partly of cats with a confirmed diagnosis of lymphoma in any form, which had participated in another study published in 2021. In the present study, previous information about the cats and data from serum samples that had been collected at the Oncology clinic at UDS between 2017 and 2020 were used (Wang *et al.* 2021). Additional sera samples from cats visiting UDS in 2022 were also collected. The samples were stored in -20 °C before analysis.

3.2 TK1 activity analysis

TK1 activity was measured with the [^{3}H]-dThd phosphorylation assay using the DE-81 filter paper technique. The reaction mixture contained 10 mM Tris-HCl pH 7.6, 2 mM DTT, 5 mM $MgCl_2$, 5 mM NaF, 5 mM ATP, 5 µM[^{3}H]-dThd and 10 µL serum in a final volume of 40 µL that was incubated for 60 minutes at 37 °C. Three

aliquots of the reaction mixture were applied to the DE-81 filter paper discs and dried for 30 minutes. The filters were washed two times with 1 mM ammonium formate for 5 minutes and were then sorted into vials. The products were eluted for 45 minutes in 0.1 M HCl and 0.2 M KCl.

Lastly, 2 ml of scintillation liquid was added, and the products were incubated for 10 minutes on agitation. The radioactivity was then measured by β-scintillation liquid counting and the activity was expressed as pmol/min/mL (Sharif *et al.* 2012).

3.3 SAA concentration analysis

SAA concentration was analysed at the Clinical Pathology Laboratory at UDS using an automated DxC 700 AU Chemistry Analyser from Beckman Coulter, Life Sciences Division Headquarters, IN, USA. The analysis method was a latex turbidimetric immunoassay (LZ test) from Eiken Chemicals Co, Ldt, Tokyo, Japan.

3.4 Statistical analysis

Descriptive statistics were performed on age, gender, breed, diagnosis, TK1 activity and SAA concentration using Microsoft® Excel. Distributions of sTK1 activity and SAA levels in healthy, diseased and lymphoma cat sera were evaluated for normality and skewness using the D'Agostino and Pearson omnibus normality test. Because the sTK1 activity and SAA concentration data was non-Gaussian distributed, the non-parametric Kruskal Wallis test, followed by the Dunn's Multiple Comparison post-test, was used to compare sTK1 activity and SAA across multiple groups. Spearman's correlation coefficient (rs) was used to determine correlations between different parameters. All statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA) and the level of significance was set at $P<0.05$.

Receiver operating characteristic (ROC) curves were constructed to evaluate the performance of the [^{3}H]-dThd phosphorylation assay and the SAA concentration assay in different groups. Combined ROC curves were constructed by using MedCalc 17.6.

4. Results

4.1 Cats in the study

4.1.1 Healthy control group

A total of 46 healthy cats were recruited to the control group, of which 41 cats were ultimately included in the statistical analyses. Five cats were excluded due to blood results that indicated an occult medical disease, more specifically two cats with elevated SAA, one cat with elevated urea and creatinine, one cat with hyperglobulinemia and hyperproteinemia, and one cat with elevated glucose, fructosamine, cholesterol and triglycerides.

The study group consisted of 18 females (15 spayed, three intact) and 15 males (14 neutered, one intact), and included 17 Domestic shorthair, four Domestic longhair, four Ragdoll, three European shorthair, one Norwegian Forest Cat, one Russian Blue, one Maine Coon, one Bengal and one La Perm. The median age was 62 months (range 3–164 months). The median weight was 4.5 kg (range 1.3–6.9 kg).

4.1.2 Cats with gastrointestinal disease

A group of 54 cats with GI disease were recruited to the study, which consisted of 20 females (17 spayed, three intact) and 34 males (24 neutered, ten intact), and included 30 Domestic shorthair, six Siberian, four Domestic longhair, three Maine coon, three Birman, two Abyssinian, two Ragdoll, one Cornish Rex, one Burma, one Norwegian Forest Cat and one Sphynx. The median age was 85 months (range 3–208 months). The median weight was 4.5 kg (range 1.9–7.1 kg).

Diagnoses that were reached for this group was acute gastroenteritis (n=17), chronic enteritis (n=16, one with lymphocytic-plasmocytic enteritis and one with food-responsive enteropathy), pancreatitis (n=8, four with additional cholangitis and hepatitis), triaditis (n=4), hepatopathy (n=3), colitis (n=3), acute gastritis (n=2) and typhlitis (n=1).

The diagnoses were reached based on a combination of clinical signs (n=54), blood results showing hypoalbuminemia (n=11), hypoproteinemia (n=10), elevated feline pancreas-specific lipase (n=9), hypoglobulinemia (n=6), elevated liver enzymes (n=8), elevated bile acids (n=4), low vitamin B12 (n=4), low folic acid (n=3), low phosphate (n=3) and hyperbilirubinemia (n=2), ultrasonography showing lymph-adenopathy (n=17), a thickened muscular layer in the small intestines (n=16), a hyperechoic liver (n=11), a heterogenous and hyperechoic pancreas (n=7), hepatomegaly (n=5), a dilated pancreatic duct (n=5), a dilated common bile duct (n=4), a thickened colonic wall (n=4), a torturous bile duct (n=3), hyperechoic content in the gallbladder (n=3), an enlarged duodenal papilla (n=2), a distended gallbladder (n=2) and a thickened gastric wall (n=2), cytology showing vacuolar hepatopathy (n=5) and reactive lymph nodes (n=2), histopathology of full-thickness biopsies (n=1) and post-mortem examination (n=1).

4.1.3 Cats with lymphoma

A total of 14 cats with lymphoma were used in this study, which included data from 11 cats that were used a previous study, and sera from three additional cats. This group consisted of five females (four spayed, one intact) and nine males (all neutered), and included eight Domestic shorthair, two Domestic longhair, two Siamese and two Norwegian Forest Cats. The median age was 152 months (range 65–213 months). One cat did not have an available SAA sample and could therefore not be included in the SAA analyses.

The lymphoma diagnoses that were included in this group was GI lymphoma (n=6), generalised lymphoma (n=5), cutaneous lymphoma (n=1), renal and GI lymphoma (n=1) and chronic lymphoid leukaemia (n=1).

4.2 sTK1 activity analysis

The median, mean and range sTK1 activity values and a ROC analysis for all three groups are presented in Table 1 and illustrated in Figure 2.

Table 1. Measurement of serum thymidine kinase 1 (sTK1) activity (pmol/min/ml) in healthy cats, cats with gastrointestinal (GI) disease and cats with lymphoma.

	Median	**Mean**	**Range**
Healthy cats (n=41)	0.75	1.04	0.36–5.14
GI disease (n=54)	0.95	1.68	0.32–22.81
Lymphoma (n=14)	1.45	4.94	0.47–35.33

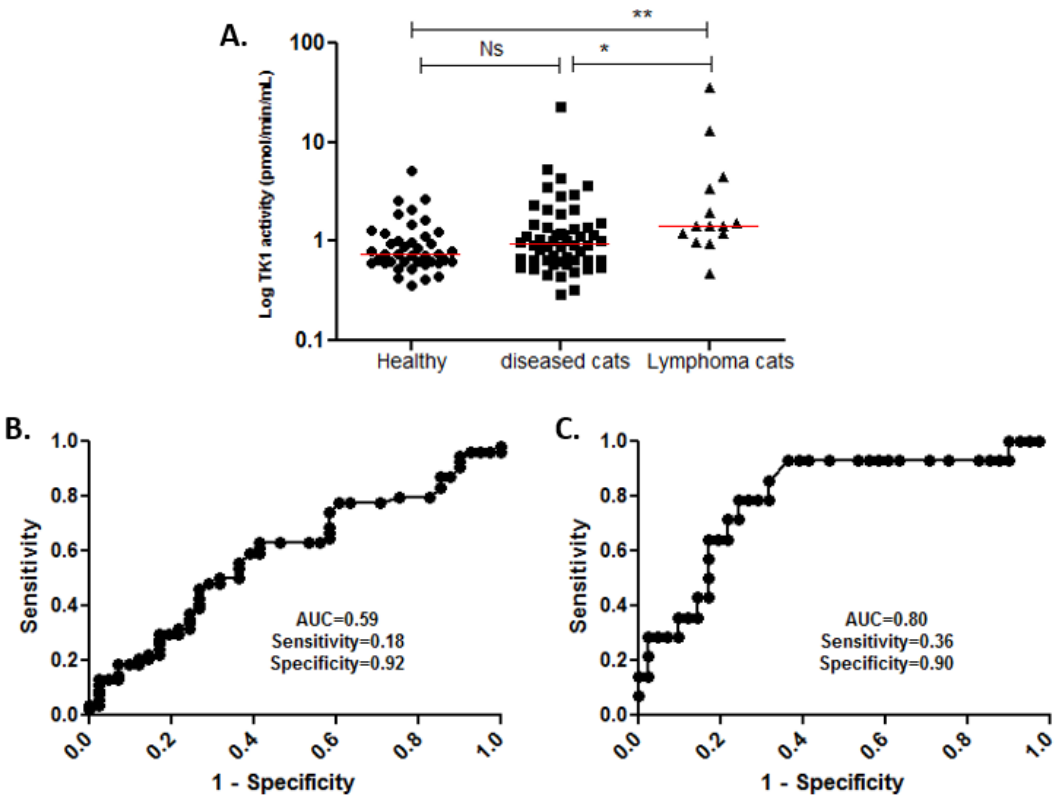

Figure 2. Analysis of serum thymidine kinase 1 (sTK1) activity in healthy cats, cats with gastrointestinal (GI) disease and cats with lymphoma. The red lines indicate the median value of respective group. A. Comparison of sTK1 activity in healthy cats (n=41), cats with GI diseases (n=54) and cats with lymphoma (n=14) using the Dunn's Multiple Comparison post-test. The level of significance was set to p<0.05; B. ROC analysis of sTK1 activity in the group with GI diseases; C. ROC analysis of sTK1 activity in the lymphoma group. The cut-off value for both ROC curves was set to 2.10 pmol/min/ml.

4.2.1 Healthy control group

The mean sTK1 activity in the healthy control group was 1.04 pmol/min/ml (range 0.36–5.14 pmol/min/ml) and the standard deviation 0.85 pmol/min/ml. The cut-off value was set to 2.10 pmol/min/ml based on a specificity of 0.90. Four cats (9.8%) had an sTK1 activity above the cut-off value at 2.09, 2.56, 2.66 and 5.14 pmol/min/ml.

4.2.2 Cats with gastrointestinal disease

The mean sTK1 activity was 1.68 pmol/min/ml (range 0.32–22.81 pmol/min/ml) in this group, with a standard deviation of 3.11 pmol/min/ml. Seven cats (18.5%) had an sTK1 activity above the cut-off value at 2.10, 2.10, 2.33, 2.89, 2.93, 3.55, 3.59, 4.35, 5.33 and 22.81 pmol/min/ml.

There was no significant difference between the sTK1 activity for the cats with GI diseases and healthy cats. The TK1 activity assay had an AUC of 0.59 and a sensitivity of 18% at a specificity of 92%.

Subgrouping of the diseased cats into acute gastroenteritis, chronic enteritis, pancreatitis, and other diseases did not provide additional information (Figure 3).

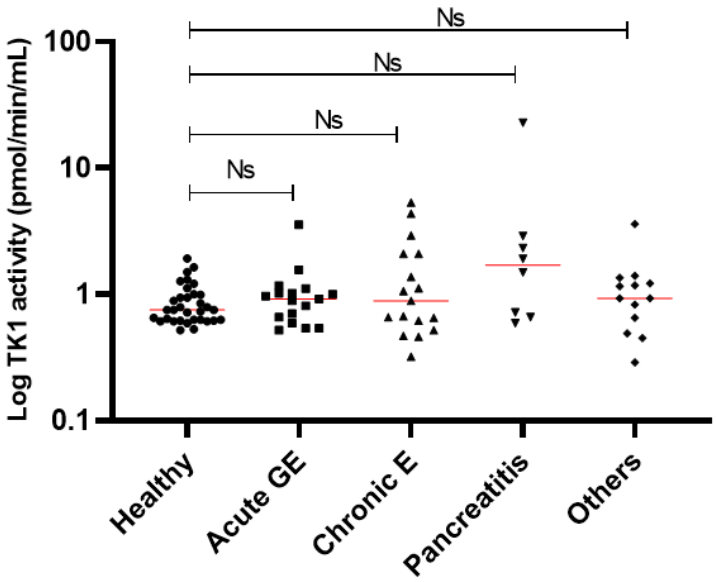

Figure 3. Comparison of serum thymidine kinase 1 (sTK1) activity in healthy cats (n=41) and cats with acute gastroenteritis (n=17), chronic enteritis (n=16), pancreatitis (n=8) and other diseases (n=13) using the Dunn's Multiple Comparison post-test. The red lines indicate the median value of respective group. The level of significance was set to p<0.05.

4.2.3 Cats with lymphoma

In the lymphoma group, the mean sTK1 activity was 4.94 pmol/min/ml (range 0.47–35.33 pmol/min/ml) and the standard deviation was 9.33 pmol/min/ml. Four cats (28.6%) had an sTK1 activity above the cut-off value at 3.38, 4.56, 13.29 and 35.33 pmol/min/ml.

Cats in the lymphoma group had significantly higher sTK1 activity compared to the healthy cats ($p<0.01$). The TK1 activity assay had an AUC of 0.80 and a sensitivity of 36% at a specificity of 90%. There was also a significant difference between cats with GI disease and cats with lymphoma ($p<0.05$).

4.3 SAA concentration analysis

The median, mean and range SAA concentration values and a ROC analysis for all three groups are presented in Table 2 and illustrated in Figure 4.

Table 2. Measurements of serum amyloid A (SAA) concentration (mg/ml) in healthy cats, cats with gastrointestinal (GI) disease and cats with lymphoma.

	Median	Mean	Range
Healthy cats (n=41)	5.00	5.63	5.00–23.00
GI disease (n=54)	5.00	32.24	5.00–400.00
Lymphoma (n=13)	5.00	33.31	5.00–125.00

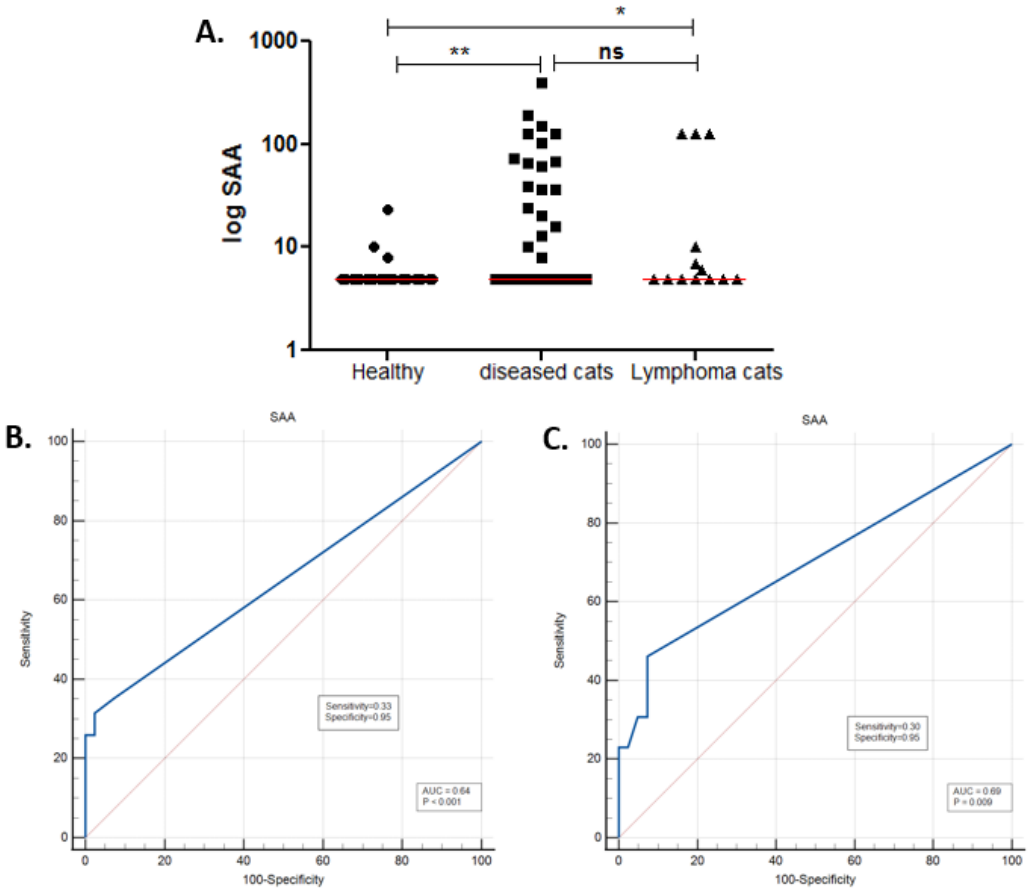

Figure 4. Analysis of serum amyloid A (SAA) concentration in healthy cats, cats with gastrointestinal (GI) disease, and cats with lymphoma. The red lines indicate the median value of respective group. A. Comparison of SAA concentration in healthy cats (n=41), cats with GI disease (n=54) and cats with lymphoma (n=13) using the Dunn's Multiple Comparison post-test. The level of significance was set to p<0.05; B. ROC analysis of SAA concentration in the group with GI diseases; C. ROC analysis of SAA concentration in the lymphoma group. The cut-off value for both ROC curves was set to 8 mg/ml.

4.3.1 Healthy control group

The mean SAA concentration in the healthy control group was 5.63 mg/ml (range 5.00–23.00 mg/ml) with a standard deviation of 2.92 mg/ml. The cut-off value was set to 8 mg/ml. Three cats (5.6%) had an SAA concentration above the reference interval at 8, 10 and 23 mg/ml.

4.3.2 Cats with gastrointestinal disease

In this group, the mean SAA concentration was 32.24 mg/ml (range 5.00–400.00 mg/ml), and the standard deviation was 65.86 mg/ml. Nineteen cats (35.2%) had an SAA concentration above the reference interval at 8, 10, 13, 16, 20, 24, 36, 36, 39, 62, 66, 68, 73, 103, 125, 125, 149, 193 and 400 mg/ml.

There was a significant difference in SAA concentration of diseased cats compared to healthy cats ($p<0.01$). The SAA concentration assay had an AUC of 0.64 and a sensitivity of 33% at a specificity of 95%.

Subgrouping of cats with GI disease showed a significant difference ($p<0.05$) in cats with pancreatitis compared to other groups (Figure 5).

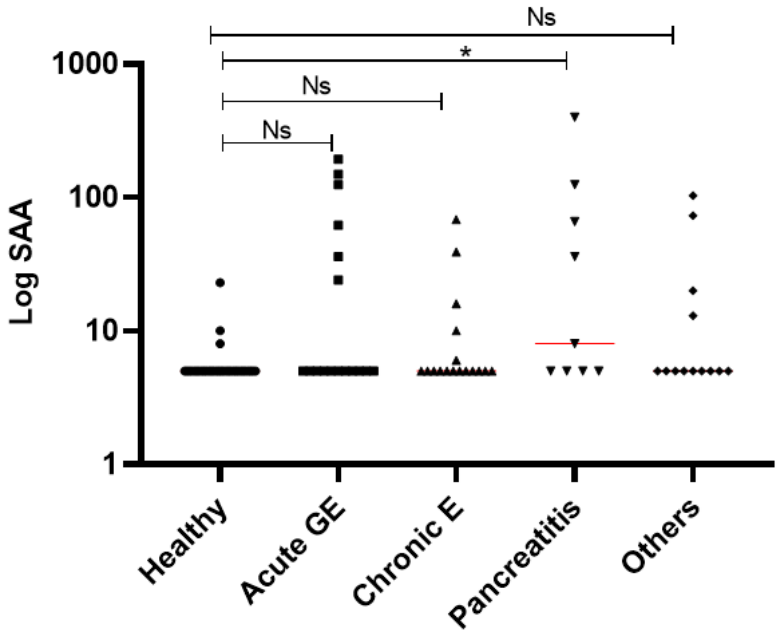

Figure 5. Comparison of serum amyloid A (SAA) concentration in healthy cats (n=41) and cats with acute gastroenteritis (n=17), chronic enteritis (n=16), pancreatitis (n=8) and other diseases (n=13) using the Dunn's Multiple Comparison post-test. The red lines indicate the median value of respective group. The level of significance was set to p<0.05.

4.3.3 Cats with lymphoma

The mean SAA concentration was 33.31 mg/ml (range 5.00–125.00 mg/ml) with a standard deviation of 52.29 mg/ml. Four cats (30.8%) had an SAA concentration above the reference interval at 10, 125, 125 and 125 mg/ml.

There was a statistical significance in SAA between cats with lymphoma and healthy cats ($p<0.05$), but no significant difference compared to cats with GI disease. The SAA concentration assay had an AUC of 0.69 and a sensitivity of 30% at a specificity of 95%.

4.4 Combination of TK1 and SAA

4.4.1 Cats with gastrointestinal disease

The combination of sTK1 activity and SAA concentration in cats with GI disease showed a significant difference ($p<0.012$), a sensitivity of 35% and a specificity of 95%, as well as an AUC of 0.65, which is illustrated in Figure 6 and Figure 7.

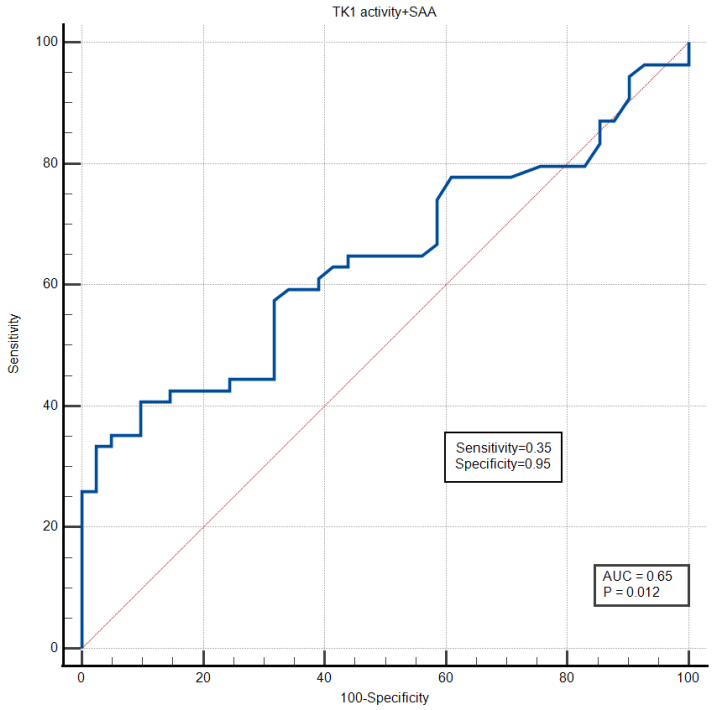

Figure 6. ROC analysis of a combination of serum thymidine kinase 1 (sTK1) activity and serum amyloid A (SAA) concentration in cats with gastrointestinal (GI) disease (n=54). The level of significance was set to $p<0.05$.

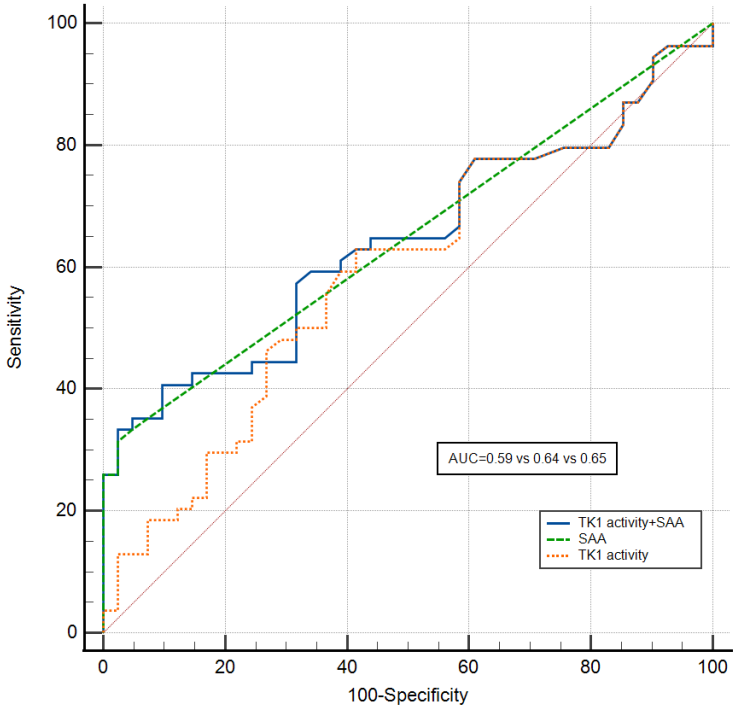

Figure 7. Three ROC analyses of serum thymidine kinase 1 (sTK1) activity, serum amyloid A (SAA) concentration and a combination of sTK1 activity and SAA concentration in cats with gastrointestinal (GI) disease (n=54). The level of significance was set to p<0.05.

For three cats in the GI diseased group, a follow-up sera sample was available. The diagnosis, sample collection dates, sTK1 activity and SAA concentration is presented for each cat in Table 3.

Table 3. Diagnosis, sample collection dates from the first visit and follow-up visit, serum thymidine kinase 1 (sTK1) activity and serum amyloid A (SAA) concentration for three cats with different gastrointestinal (GI) diseases.

	Diagnosis	**Sample collection date**	**sTK1 activity (pmol/min/ml)**	**SAA (mg/ml)**
Cat 1	Chronic pancreatitis and cholangiohepatitis	2022-04-20	2.89	125
		2022-05-26	1.47	45
Cat 2	Typhlitis	2022-08-26	0.93	73
		2022-10-27	0.47	6
Cat 3	Acute pancreatitis and cholangiohepatitis	2022-10-04	22.81	400
		2022-10-20	1.60	5

4.4.2 Cats with lymphoma

Combination of SAA concentration to sTK1 activity did not show any added sensitivity or specificity in cats with lymphoma (Figure 8).

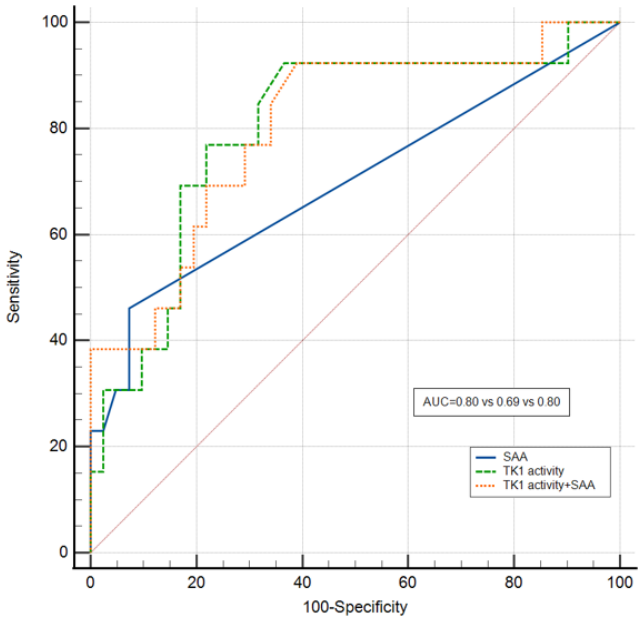

Figure 8. Three ROC analyses of serum thymidine kinase 1 (sTK1) activity, serum amyloid A (SAA) concentration and a combination of sTK1 activity and SAA concentration in cats with lymphoma (n=13). The level of significance was set to p<0.05.

4.5 Correlation between sTK1 and SAA

The correlation between sTK1 activity and SAA concentration in cats with lymphoma and cats with GI disease is presented in Figure 9 below. There was a significant (p=0.0002) weak positive (rs=0.435) correlation.

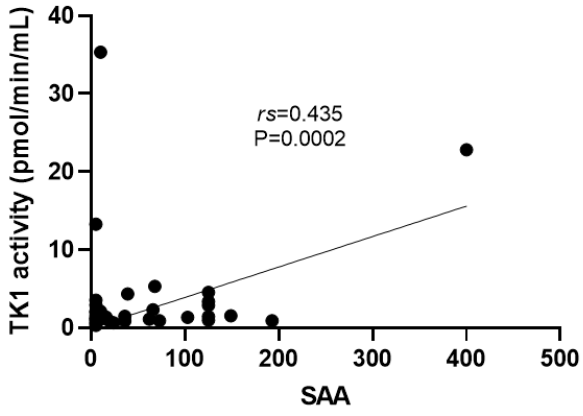

Figure 9. The correlation between serum thymidine kinase 1 (sTK1) activity and serum amyloid A (SAA) concentration in cats with gastrointestinal (GI) disease and cats with lymphoma (n=67) using Spearman's correlation coefficient (rs). The level of significance was set to p<0.05.

5. Discussion

5.1 sTK1 activity

In this study, sTK1 activity was evaluated in cats as a possible biomarker to distinguish lymphoma from GI disease. Cats with lymphoma had a significantly higher sTK1 activity compared to both healthy cats and cats with GI disease, which is a finding that is supported in previous studies (Taylor *et al.* 2013; Wang *et al.* 2021). At a cut-off value of 2.10 pmol/min/ml, the ROC analysis revealed an AUC value of 0.80 and a sensitivity of 36% at a specificity of 90% for the lymphoma group, whereas for the group of cats with GI disease the AUC value was 0.59 and the sensitivity 18% at a specificity of 92%. These results show a high degree of overlap of sTK1 activity between cats with lymphoma and those with GI disease, and even though there was a significant difference between the groups, the test sensitivity was low and only 28.6% of lymphoma cases generated an sTK1 activity above the cut-off value compared to 18.5% in the GI disease group. A similar result was presented in the study by Taylor *et al.* (2013), who used a TK-REA to measure sTK1 activity and showed that 39.4% of cats with lymphoma and 14.5% of cats with inflammatory disease had an sTK1 activity above their reference interval, as well as a ROC analysis that revealed a low sensitivity for the test.

This implies that sTK1 as a biomarker should not be used as a tool to confirm a lymphoma diagnosis, but should rather assist in selecting cases with a high sTK1 activity who are relevant for further diagnostics, as a high TK1 could indicate the existence of a process associated with a high cell turnover such as severe inflammation or a high-grade tumor. LGITL is the most common form of AL in cats and since it has a lower rate of mitosis and cell turnover (Valli *et al.* 2000) and since low-grade tumors of T-cell origin also have shown to generate a lower sTK1 value in previous studies (Selting et al. 2016; Boyé et al. 2019; Saellström et al. 2022), this could explain why there is an overlap in sTK1 activity between the lymphoma group and the GI disease group, as well as why many of the cats with lymphoma had an sTK1 activity within reference. As mentioned earlier, the only possible method to distinguish between LGITL and IBD is with histopathological evaluation of biopsies (Evans *et al.* 2006; Kleinschmidt *et al.* 2010). Based on this

information, combined with the results from this study, it could be assumed that neither sTK1 is very useful to differentiate LGITL from IBD, but could rather be more useful to flag for diseases with a high cell turnover, i.e. HGAL, and differentiate these from diseases with a lower cell turnover, i.e. LGAL or IBD. This is especially important in an early lymphoma stage, as the treatment choice and treatment aggression differs more between HGAL and LGAL compared to IBD and LGAL, and the overall prognosis is also worse for HGAL (Withrow *et al.* 2013). In a patient diagnosed with lymphoma through histopathologic examination, TK1 could potentially also be useful to help in deciding the right treatment, monitor treatment progression and estimate the prognosis, as well as providing an early indication of tumor growth before any other clinical signs are present, or recurrence after a time of remission.

Four cats with lymphoma generated an sTK1 activity above the cut-off value, and all of these cats were diagnosed with GI lymphoma. However, it was not possible to do any statistical analyses within the group since the group of lymphoma cases was so small, and since neither the tumor grades nor immunophenotypes was known for the lymphoma patients in this study, the extent of conclusions that can be made from this study is limited. Suggestions for further research is to evaluate if there is a difference in sTK1 activity between different types of lymphoma and if there is a higher tumor aggressivity in the patients with a higher sTK1 activity. It would also be interesting to follow how the sTK1 activity varies between these individuals during treatment, to see if it correlates with remission and/or relapse.

The low sensitivity in this study could be due to the broad range of distribution of TK1 activity in healthy sera from 0.36 to 5.14 pmol/min/ml, as well as many lymphoma cases generating values within the reference interval. The sTK1 cut-off value for healthy cats in this study was higher (2.10 pmol/min/ml) compared to previous studies (Pettersen 2018; Wang *et al.* 2021) and one healthy cat in this study had an sTK1 activity value above this. In the studies by Pettersen (2018) and Wang *et al.* (2021), the cut-off value was 0.93 and 0.97 pmol/min/ml, and 15 respectively 13 healthy cats in this study had a sTK1 activity above these values. The reason for the difference in this study is not clear, and follow-up samples would be necessary to confirm if the sTK1 remained high or decreased in time. If the former, it could be an indication of either an occult malignancy or due to individual variations, which would be an important observation regarding outliers for apparently normal cats and should be evaluated in future studies. If the latter, it could be an indication of subclinical disease, as inflammatory diseases have shown to cause temporary sTK1 activity elevations in humans and dogs (Gronowitz *et al.* 1984; Nakamura *et al.* 1997). Due to the limitations of this study, repeated testing was not possible.

5.2 SAA concentration

SAA is a major acute phase protein that is used in both human and veterinary medicine as a prognostic marker for inflammatory diseases and neoplasia, as well as non-inflammatory diseases such as diabetes and hyperthyroidism (Tamamoto *et al.* 2013). Previous studies have shown that lymphoma in particular can cause an elevation of SAA in humans and animals (Cho *et al.* 2010; Wang *et al.* 2012; Zhang *et al.* 2012; Tamamoto *et al.* 2013, 2014), and SAA have been demonstrated to be a useful prognostic indicator in cats with various diseases (Tamamoto *et al.* 2013).

In this study, SAA was examined to assess its utility in diagnosing lymphoma in cats, both in combination with sTK1 and by itself, and showed a significant difference between healthy cats and cats with GI disease ($p<0.01$) and cats with lymphoma ($p<0.05$). There was no significant difference between cats with GI disease and lymphoma. At a cut-off value at 8 mg/ml, the ROC analysis revealed an AUC value of 0.64 and a sensitivity of 33% at a specificity of 95% for the GI group, while the lymphoma group showed an AUC value of 0.69 and a sensitivity of 30% at a specificity of 95%. This means that a high SAA concentration could indicate that the cat has either GI disease or lymphoma, but the assay is not very accurate or sensitive and there may also be a risk that the SAA concentration is normal in these individuals. This finding is supported by the study by Tamamoto *et al.* (2013), where only 56% of the cats with lymphoma and 39% of the cats with inflammatory GI disease had elevated SAA.

One explanation for the low sensitivity in this study could be because the reference interval of normal SAA value was <5, which is difficult to use in ROC curves and the software therefore used it as 5 in both healthy and diseased cats. Many of the cats with lymphoma and GI disease also had an SAA concentration within the reference interval, which can lead to a lower sensitivity.

5.3 sTK1 and SAA in combination

The correlation between TK1 and the inflammatory biomarker C-reactive protein (CRP), as well as the added value in combining the two biomarkers, have been studied on dogs, and Selting *et al.* (2015) reported that the combined measurement of sTK1 and CRP provided a more effective method in screening dogs with occult malignancies, compared to using them separately (Selting *et al.* 2015). In a recent study by Saellström *et al.* (2022), it was also reported that there is a higher sensitivity when combining TK1p or TK1a with CRP, compared to either biomarker alone (Saellström *et al.* 2022). This indicates that the combination of TK1 and inflammatory biomarkers could be a useful tool for aiding the prognosis

and therapy monitoring in dogs, and the combination of TK1 and SAA, as well as the correlation between the two values, was therefore evaluated in this study to investigate whether a corresponding result can also be found in cats.

Even though this study did not show any added sensitivity or specificity in lymphomas when combining TK1 and SAA, there was a statistically significant weak correlation between TK1 activity and SAA concentration in cats with GI disease and cats with lymphoma, which indicates that there is an association between the two variables and that both variables tend to go up in response to one another, although the relationship is not very strong which means that they provide different type of information. There is a possibility that both these values could aid in evaluation of prognosis as well as monitoring of disease progression and treatment result in cats, similarly to the study made on dogs by Saellström *et al.* (2021), as both TK1 and SAA tend to be elevated in patients with a high cell turnover such as high-grade tumors and severe inflammation (Selting *et al.* 2015), but further studies are necessary to evaluate the potential use of sTK1 activity and acute-phase reactants in disease monitoring and prognosis.

Pancreatitis is an inflammatory disease characterised by a premature activation of trypsin in the exocrine pancreas, which leads to autodigestion and tissue damage. It can be either acute or chronic, and can differ a lot in severity (Nelson & Guillermo Couto 2014). Based on this, and the fact that TK1 is a biomarker for high cell turnover, it is possible to believe that more acute pancreatitis with a higher degree of cell destruction will cause a greater elevation of TK1 activity compared to more chronic and low-grade forms of pancreatitis. In the study by Taylor *et al.* (2014), all cats with pancreatitis also had an increased sTK1 activity. This is not the case in this study, and there was also no significant difference in sTK1 activity between the different subgroups of diseased cats. However, the group size of pancreatitis cases was small, which makes it difficult to draw any safe conclusions from these results.

An interesting finding in this study is that the cat with the highest sTK1 activity (22.81 pmol/min/ml) in the GI diseased group was a cat with pancreatitis, and the sTK1 activity for this cat was even higher compared to many of the lymphoma cases. One initial sample during the acute phase and one follow-up sample was available for this cat, as well as from two other cats that had been diagnosed with and treated for GI disease. The sTK1 activity and SAA concentration was above the reference interval in two respectively all cats in the first sample. In the follow-up sample, both sTK1 and SAA had decreased in all cats. All three cats had an sTK1 within the reference range and two of them had a normalised SAA value. Since it was only three cases, a statistical analysis was not possible, but these results can provide an indication of the behaviour and connection between sTK1 and SAA.

This also corresponds with previous studies made on dogs and humans (Gronowitz *et al.* 1984; Nakamura *et al.* 1997) and supports the theory that some inflammatory diseases, such as pancreatitis, can cause a temporary rise in sTK1 activity and SAA concentration that is later normalised when the acute phase has passed. In opposite to lymphoma, which is progressive rather than regressive, the sTK1 activity and SAA concentration in inflammatory diseases will decrease if the tissue damage is reduced. This means that cats with an elevated sTK1 should be recommended repeated resampling within a few days after treatment to be able to follow the sTK1 activity and SAA concentration, as these values could assist in evaluating whether the cat has a progressive or regressive process. Examples of further research could be to examine the severity of GI disease based on sTK1 activity and SAA concentration, for example if the cats with initially higher sTK1 and/or SAA have more severe clinical signs or a longer stay in the hospital ward. The correlation of sTK1 and SAA and how the values change over time should also be evaluated in future studies, since it might be a helpful indicator of disease progression, grade of malignancy and prognosis in these patients. It would also be interesting to study this further in cats with lymphoma, and compare it with cats with GI disease.

There have been several studies made on canine patients to evaluate sTK1 protein as a complement to sTK1 activity in diagnosing cancer (Jagarlamudi *et al.* 2013, 2014, 2015; Saellström *et al.* 2022), and a commercial test for dogs have been developed based on an ELISA assay (Jagarlamudi *et al.* 2015). As of today, there is no corresponding test for cats, but a suggestion for further research is to assess if the combination of TK1p to TK1a and SAA concentration will provide an added diagnostic and/or prognostic value.

5.4 Limitations

An important limitation in this study is the rather small group of lymphoma cases, which makes it difficult to draw any safe conclusions from the results. More studies with a larger group are necessary to confirm these data, and the results from this study should be seen as an indication for further research.

Many of the GI diseased cats did not have a confirmed diagnosis, which can affect the results. It would have been desirable to follow these patients during a longer time to be able to confirm the diagnoses, but due to the limitations of this study, this was not possible. This means that many of the cats who had not been definitively diagnosed or were assumed to have inflammatory bowel disease may in fact have a different diagnosis, even lymphoma, which makes the results less reliable. Patients with a complete and confirmed diagnosis which had been reached through a combination of diagnostic methods was prioritized when selecting the

cases, but since many owners do not want to let their cat go through too many diagnostic steps and because of the time limit of the study, the case selection was limited to mostly presumptive diagnoses that had been made primarily through clinical signs, blood tests and ultrasonography. Suggestions for further studies could be to follow up the patients and evaluate how the sTK1 activity and SAA concentration changes over time in both cats with lymphoma and cats with GI disease, as well as in cats that receive treatment and cats that do not.

In this study, the sTK1 cut-off value was higher compared to other studies, and one supposedly healthy cat had a sTK1 activity above this value. There is a chance that the cats with a higher sTK1 activity are just examples of individual variations, but there is also a risk that these cats might have an underlying subclinical disease. Since further testing was not possible within the limits of this thesis, these cats were included with the conscious risk that they might be true positives.

5.5 Conclusions

In summary, this study concludes that sTK1 activity is not a useful biomarker to differentiate lymphoma from GI disease in cats, and even though the sTK1 activity in cats with lymphoma was significantly higher compared to healthy cats and cats with GI disease, there was a high degree of overlap between the cats with lymphoma and the cats with GI disease. However, as TK1 is a biomarker for high cell turnover, the result from this study implies that TK1 can be useful in finding cases that are likely to have an aggressive process, and it can therefore be helpful in selecting cases that are more relevant for further diagnostics. It could potentially also be useful in a later diagnostic stage after a lymphoma diagnosis has been made through histopathological examination, by aiding in malignancy assessment, monitoring disease progression, decision of treatment, and estimation of prognosis.

Another conclusion is that there is a correlation between TK1 and SAA, although the correlation is weak and the combination of the two values do not strengthen the accuracy or predictive value for cats with lymphoma or GI disease, which means that SAA is not useful as an additional biomarker to sTK1 for this purpose. However, this study indicates that cats with inflammatory disease and pancreatitis may have an elevated sTK1 and SAA in the acute phase of disease that is later decreased if the tissue damage is reduced.

More studies with a larger lymphoma group and with follow-up samples for the cats with GI disease and lymphoma are necessary to confirm these conclusions. Further studies on the potential use of both sTK1 and SAA in disease monitoring, malignancy assessment and evaluation of prognosis are also recommended.

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Populärvetenskaplig sammanfattning

Lymfom är den vanligaste tumörsjukdomen hos katt och utgör cirka 30 % av fallen. Lymfom kan i sin tur delas upp i olika former baserat på anatomisk lokalisation, där den vanligaste formen är i mag-tarmkanalen, så kallat gastrointestinalt lymfom. Denna sjukdom ger upphov till symptom såsom kräkningar, diarré, avmagring och nedsatt aptit, vilket är symptom som även kan ses vid inflammatorisk tarmsjukdom, på engelska kallat ”inflammatory bowel disease” (IBD). Att skilja på IBD och gastrointestinalt lymfom går inte att göra enbart baserat på kliniska symptom eller bilddiagnostik, utan för att bekräfta diagnosen krävs invasiva metoder såsom histopatologisk undersökning av biopsier från mag-tarmkanalen, vilket oftast är förenat med en del risk och lidande för patienten.

Ett sätt att diagnosticera, utvärdera prognosen samt förutse effekten av behandling för tumörsjukdomar är användningen av så kallade tumörbiomarkörer. En typ av tumörbiomarkör är enzymet serum tymidinkinas 1 (sTK1), som finns inuti celler och är involverat i celldelning. Flera studier inom både human- och veterinärmedicin har visat att aktiviteten och nivån av TK1 stiger signifikant i blodet vid vissa tumörsjukdomar, framför allt hematopoetiska tumörer såsom lymfom. Detta innebär att sTK1 kan utnyttjas i diagnostiken av tumörsjukdomar genom ett enkelt blodprov. Det har gjort många studier på hund, och för närvarande finns sTK1 tillgängligt som en preliminär testmetod för tidig detektion av tumörtillväxt hos hund, men det finns ingen motsvarande metod hos katt och det har enbart gjorts ett fåtal studier för att utvärdera sTK1 hos katt.

Denna studie avsåg att utvärdera om sTK1 kan vara användbar som biomarkör för att särskilja katter med lymfom från katter med akut till kronisk mag-tarmsjukdom. Då det även har visat sig att akutfasproteinet serumamyloid A (SAA) kan stiga vid inflammatoriska såväl som neoplastiska sjukdomar, utvärderades också relationen mellan sTK1 och SAA för att se om det finns ett samband mellan dessa och ifall en kombination av sTK1-aktivitet och SAA-koncentration kan öka träffsäkerheten och det prediktiva värdet hos katter med lymfom, jämfört med de båda som enskilda determinanter.

I studien rekryterades katter av varierande ålder, kön och ras och delades in i tre olika grupper baserat på sjukdomsstatus: en frisk kontrollgrupp, en grupp med mag-tarmsjukdom (GI-sjukdom), samt en lymfomgrupp. För att klassas som en frisk kontroll behövde katten bedömas som frisk både baserat på klinisk undersökning och på blodprover, och i denna grupp ingick slutligen totalt 41 katter. I gruppen med GI-sjukdom inkluderades 54 katter som uppvisat symptom relaterade till IBD, såsom kräkning, diarré, avmagring och minskad aptit, samt katter som fått en primär diagnos relaterat till mag-tarmkanalen. Lymfomgruppen bestod av 14 katter där en lymfomdiagnos hade kunnat ställas. För samtliga katter analyserades TK1-aktivitet och SAA-koncentration i serum.

Studien visade att det finns en skillnad i sTK1-aktivitet hos katter med lymfom jämfört med friska katter och katter med GI-sjukdom. Det är dock en stor överlappning mellan katter med lymfom och katter med GI-sjukdom, vilket innebär att TK1-aktivitet inte är användbart för att skilja dessa två tillstånd åt. Däremot kan resultaten indikera att TK1 kan vara användbart för att hitta fall som sannolikt har en aggressiv process, och kan därmed vara hjälpsam i att välja ut patienter som är mer relevanta för vidare diagnostik. TK1 skulle potentiellt även kunna vara användbart i ett senare diagnostiskt skede efter att en lymfomdiagnos ställts genom histopatologisk undersökning, genom att hjälpa till med malignitetsbedömning, övervakning av sjukdomsprogression, beslut om rätt behandling och uppskattning av prognosen.

En annan slutsats är att det finns ett samband mellan TK1 och SAA, vilket indikerar att det finns en association mellan dessa två och att de följer varandra. Dock var sambandet svagt, och en kombination av de båda värdena gav inte heller någon ökad tillförlitlighet till testet hos katter med lymfom. Detta innebär att SAA inte är användbar som kompletterande biomarkör till sTK1 vid diagnostik av lymfom. Däremot visar denna studie på indikationer att katter med inflammatorisk sjukdom kan ha en ökad sTK1 och SAA i den akuta sjukdomsfasen, som sedan sänks ifall vävnadsskadan minskar.

Fler studier med en större lymfomgrupp och med uppföljande prover på katterna med GI-sjukdom och lymfom är nödvändiga för att bekräfta dessa slutsatser. Fler studier om den potentiella användbarheten för både sTK1 och SAA vid sjukdoms-övervakning, malignitetsbedömning och utvärdering av prognos rekommenderas också.

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