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**The use of APTT and PT as predictive markers in canine and
feline oncology patients**

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Abstract

Coagulation abnormalities are often observed in canine and feline oncology patients and are thought to correlate with disease progression and neoplastic severity. This can be explained by the cancer's direct effect on the coagulation pathways or indirect effect through the inflammatory response of the body to the tumor.

Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) are widely used coagulation assays, both in human and veterinary medicine, which measure different pathways of the clotting cascade. In human oncology, these markers are used for monitoring cancer patients at risk of coagulopathies or thromboembolic events and for managing anticoagulation therapy. In contrast, their use in veterinary oncology is growing but has not been established yet. While PT and aPTT are used for monitoring coagulation in veterinary oncology patients, their predictive value for cancer-related coagulopathies is still under investigation. Studies have suggested that prolonged clotting times are associated with advanced-stage cancers and may predict worsened outcomes. Thus, this study aims to evaluate the potential of PT and aPTT as predictive markers for disease progression in canine and feline oncology patients.

We conducted a retrospective analysis on more than 100 canine cancer patients and more than 40 feline patients presented to Á.H.O.K. Állatorvosi Hematológiai és Onkológiai Központ, Állatpatika. We assessed PT and aPTT values in relation to tumor type, stage, metastasis and clinical outcomes. In addition, paraneoplastic findings, particularly the presence of inflammatory processes, were detected. The statistical analyses will be provided in the thesis.

According to our hypothesis, prolonged PT and aPTT are associated with a hypocoagulable state, often driven by disseminated intravascular coagulation (DIC), in advanced or aggressive cancers. Moreover, isolated prolongation of PT or aPTT suggests the involvement of the extrinsic or intrinsic pathway, respectively. Conversely, shortened PT and aPTT are linked to hypercoagulable states, likely associated with systemic inflammatory response and increased thrombin generation induced by the tumor. Additionally, mixed coagulopathies, where PT and aPTT fluctuate over time, may occur in patients with advanced or metastatic disease. These differences may be caused due to changes in the tumor burden, inflammatory responses or as side effects of the treatments.

These findings suggest that monitoring of PT and aPTT in canine and feline oncology patients could provide valuable predictive information. Future studies are needed to further establish the value of these tests as reliable prognostic markers for cancer-related coagulopathies in dogs and cats.

A véralvadási rendellenességek gyakran megfigyelhetők kutyák onkológiai betegeinél, és összefüggésbe hozhatók a betegség előrehaladásával és a daganat súlyosságával. Ez magyarázható a daganatok közvetlen hatásával a véralvadási útvonalakra vagy közvetett hatással a szervezetben a daganatok okozta gyulladáson folyó folyamatokra.

A Prothrombin Idő (PT) és az Aktivált Parciális Tromboplastin Idő (aPTT) széles körben alkalmazott véralvadási vizsgálatok, mind az emberi, mind az állatorvosi orvoslásban, amelyek a véralvadási kaskád különböző útvonalait mérik. Az emberi onkológiában ezeket a markereket a rákbetegek véralvadási zavarainak vagy tromboembóliás események kockázatának figyelemmel kísérésére, valamint az antikoaguláns terápia kezelésére használják. Ezzel szemben az állatorvosi onkológiában ezek használata egyre gyakoribb, de még nem teljesen elfogadott. Míg a PT és aPTT alkalmazása a véralvadás monitorozására az állatorvosi onkológiai betegeknél egyre gyakoribb, prognosztikai értéküket a daganattal összefüggő véralvadási zavarok tekintetében még vizsgálták széleskörűen. Tanulmányok azt sugallják, hogy a meghosszabbodott véralvadási idők összefüggésben állnak a rák előrehaladott stádiumával, és rosszabb kimenetelre utalhatnak, így jelen tanulmány célja a PT és aPTT potenciáljának értékelése, mint a betegség előrehaladásának prediktív markerei a kutyák onkológiai betegeinél.

Retrospektív elemzést végeztünk több mint 100 kutya rákos betegen, akiket az Á.H.O.K. Állatorvosi Hematológiai és Onkológiai Központban vizsgáltak meg. A PT és aPTT értékeket a daganat típusa, stádiuma, áttétek és klinikai eredmények függvényében értékeltük. Ezenkívül paraneoplastikus leletek, különösen gyulladáson folyó folyamatok jelenlétét is kimutattuk. A statisztikai elemzéseket a dolgozatban mutatjuk be.

Hipotézisünk szerint a meghosszabbodott PT és aPTT hypocoagulabilis állapothoz társul, amelyet gyakran a disszeminált intravaszkuláris koaguláció (DIC) okoz előrehaladott vagy agresszív rákos esetekben. Továbbá, a PT vagy aPTT izolált meghosszabbodása az extrinsic vagy intrinsic útvonal érintettségére utal. Ezzel szemben a rövidült PT és aPTT hypercoagulabilis állapotra utal, amelyet valószínűleg a tumor által kiváltott szisztémás gyulladáson válasz és fokozott trombingeneráció okoz. Emellett vegyes koagulopátiák, amelyekben a PT és aPTT időszakosan változik, előfordulhatnak előrehaladott vagy áttétes betegségben szenvedő betegeknél. Ezek a különbségek a tumor tömegének változásai, a gyulladáson válaszok vagy a kezelések mellékhatásai miatt következhetnek be.

Table of Contents

1	List of abbreviations.....	1
2	Introduction	2
3	Literature review	3
3.1	Hemostasis, physiological background.....	3
3.2	Hemostatic alternations in cancer patients.....	6
3.3	Biological markers.....	8
3.4	The use of biological markers in veterinary medicine.....	10
3.4.1	Activated partial thromboplastin time (aPTT)	10
3.4.2	Prothrombin time (PT).....	15
4	Goals	19
5	Materials and Methods.....	20
5.1	Population	20
5.2	Sample collection	22
5.3	Coagulation tests	23
5.4	Statistical analysis	23
6	Results	24
7	Discussion.....	35
8	Acknowledgements.....	39
9	References	40
10	Appendices	45

1 List of abbreviations

ALKP; Alkaline Phosphatase

ALT; Alanine Aminotransferase

ANOVA; Analysis of Variance

aPTT; Activated partial thromboplastin time

CRP; C-reactive protein

DIC; Disseminated Intravascular Coagulation

FDP; fibrin degradation product

HAS; Hemangiosarcoma

IL-6; Interleukin-6

MCT; Mast cell tumor

MGT; Mammary gland tumor

OAT; Oral anticoagulant therapy

PCV; Packed cell volume

PLT; platelets

POC; Point of care

PT; Prothrombin time

SD; Standard Deviation

TEG; Thromboelastography

TNF- α ; Tumor Necrosis Factor-alpha

TT; thrombin time

VTE; venous thromboembolism

2 Introduction

Hemostatic alternations in veterinary cancer patients have been described in the literature to a lesser extent compared to human medicine. However, due to physiological similarities between the species, recent studies suggest that canine and feline oncology patients show hemostatic abnormalities related to tumor type and progression, similar to those seen in humans.

Different biological markers have been developed to assess the hematological alterations associated with various neoplasms. While they are widely used in human patients to determine coagulation abnormalities, monitor therapy efficacy and guide treatment decisions, often these markers lack information regarding their effectiveness in oncology veterinary patients. Therefore, further research is needed regarding their usage in diagnosing and treating oncology veterinary patients.

This study examines the correlation between the biomarkers Activated Partial Thromboplastin Time (aPTT) and Prothrombin Time (PT) and their role in the evaluation of cancer types and progression, as well as the presence of inflammatory processes which may affect the severity of the disease.

3 Literature review

3.1 Hemostasis, physiological background

Hemostasis is a general term used to describe different processes initiated within the body, aiming to stop the bleeding in case of tissue or blood vessel injury. The term originates from the Greek words "haeme" and "stasis", which mean "blood" and "stop" respectively [1]. This complex process consists of three major steps [2]:

- Vasoconstriction – Narrowing of the blood vessel lumen due to constriction of muscular elements in the blood vessel wall.
- Platelet aggregation - Formation of primary platelet thrombus, also called primary hemostasis [3].
- Coagulation - Formation of polymerized fibrin network that stabilizes the platelet plug, initiated by the intrinsic/extrinsic/common pathway. This process is called secondary hemostasis [3].

The process of hemostasis may be initiated in two main ways. The first involves trauma to the tissues, which results in bleeding, while the second includes denaturation of skin proteins resulting from cell death or physical injury [4].

As described by Honrado and Murakami [4], vasoconstriction occurs due to activation of the sympathetic system. Penetration of the skin results in the release of vasoactive amines, which in turn stimulate the secretion of epinephrine and norepinephrine at the injured site. In addition, the injured cells release prostaglandins, such as thromboxane, further contributing to the vessels' vasoconstriction.

Moreover, the damage caused to the endothelial cells results in the exposure of collagen fibers, the release of tissue factors and the production of Von Willebrand Factor (VWF), which helps the aggregation and formation of the platelet plug [4]. Non-adhered platelets are activated by platelet-derived ADP and thromboxane [5]. The aggregation of the platelets causes further release of alpha granules, dense bodies, lysozymes and other factors (i.e. platelet-derived growth factor, fibroblast growth factor-2), which play a role in the healing process [4].

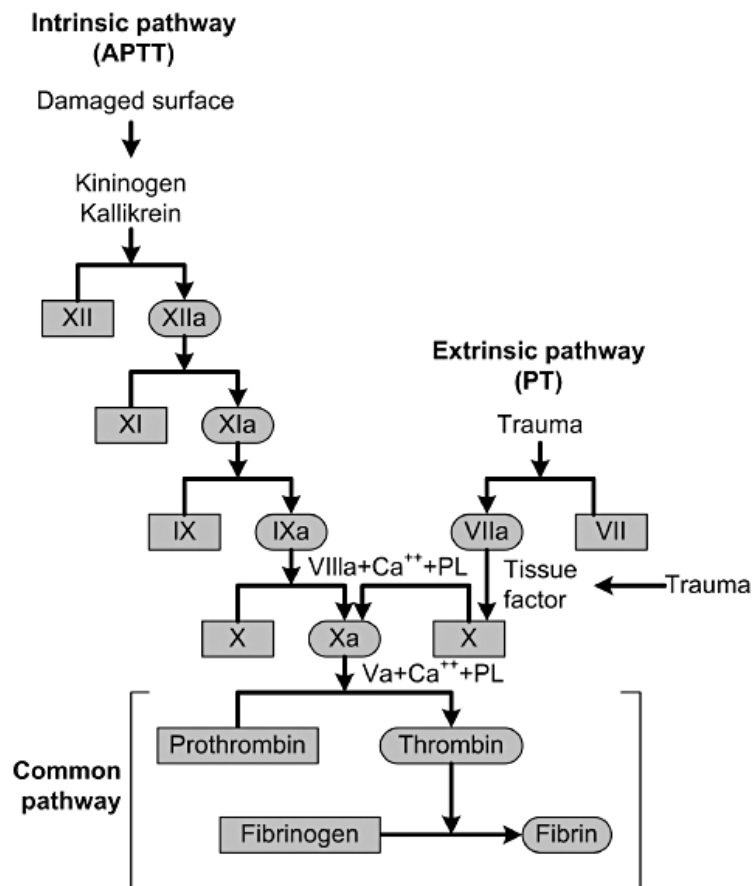
According to Mackavey and Hanks [6], the coagulation cascade involves a series proteolytic activation of proteins called clotting factors. Each clotting factor is a serine protease enzyme in an inactive form called zymogen, which is activated by its glycoprotein co-factor that

enables it to catalyze the following reaction. The activation of the proteins happens in multiple ways: the extrinsic, intrinsic, and common pathways. Interaction between all the paths leads to stable blood clot formation.

As shown in Figure 1, exposure to different substrates leads to the activation of either the extrinsic or intrinsic pathways. Both pathways lead to the common pathway by activating factor X to Xa [7]. The extrinsic pathway is initiated due to endothelial injury, exposing factor III (i.e., tissue factor) into the blood. If vitamin K is present, factor VII can be activated and bind to factor III together with calcium [6]. In comparison, the intrinsic pathway is initiated when factor XII (i.e. the Hageman factor) is activated by collagen and kallikrein, which is bound to high molecular weight kininogen (HMWK) [5]. This is followed by the activation of factor XI by activated factor XIIa, which in turn activates factor IX together with calcium ions [7]. Furthermore, factor VIII, found in the blood and often activated by thrombin (activated factor II), forms a complex with activated factor IXa, factor VIIIa and calcium. This results in the activation of factor X and initiation of the common pathway [2].

The common pathway begins when a complex (i.e. prothrombinase complex) is formed from the activated factor Xa, Va, and calcium. This complex then activates prothrombin (i.e. factor II) into thrombin (IIa), which subsequently cleaves fibrinogen (factor I) into fibrin (Ia). In addition, thrombin cleaves and activates factor XIII (i.e. stabilizing factor), which binds with calcium and creates fibrin crosslinks (stabilizing the clot). The activated thrombin (IIa) has several functions, including activation of platelets and factors V, VIII, and IX [6].

Figure 1 The Coagulation cascade, a multi-levelled activation process [8]



As described by Mackavey and Hanks [6], Clot formation is regulated by three main antithrombotic control systems:

- The first is the antithrombin serpin enzyme, a serine protease inhibitor. The enzyme results in the inactivation of factors VIIa, IXa, Xa, XIa, as well as thrombin (IIa).
- The second is the protein C pathway, in which the thrombomodulin molecule induces thrombin conformational change. As a result, the conversion of fibrin to fibrinogen and activation of platelets are inhibited, while protein C is activated. The activation of protein C requires a cofactor named protein S. The activated protein C can inactivate factors Va and VIIIa.
- The third pathway, tissue factor pathway inhibitor, includes a single polypeptide chain that reversibly inhibits factor Xa and VIIa-TF complex.

Clots are generally removed by the body by the process of fibrinolysis. As described by Hattey et al. [9], whenever fibrin forms in the circulation, it will initiate activation of the fibrinolytic system. This is due to the effect of fibrin on tissue plasminogen activator (t-PA).

Consequently, cleavage of fibrin as well as other clotting factors by plasmin will result in the release of fibrin degradation products into the circulation. The generated plasmin will form a complex with α 2-antiplasmin named plasmin-plasmin inhibitor complex (PPIC).

According to Mackavey and Hanks [6], Fibrinolysis is also regulated by three main inhibitory pathways:

- Plasminogen activator inhibitor 1, which inactivates tissue plasminogen activator.
- Alpha 2 antiplasmin, which inactivates plasmin.
- Thrombin activatable fibrinolysis inhibitor, that acts on fibrin and makes it more resistant to degradation.

3.2 Hemostatic alternations in cancer patients

Generally, in cancer patients, different hemostatic alternations can be observed [10]. This is due to either the tumor malignancy or the body's response to the tumor or as a result of the cancer therapy. In human cancer patients with hematopoietic malignancies or disseminated solid tumors, these are more prevalent than in patients with localized solid tumors [11]. In comparison, in veterinary cancer patients, the literature lacks information regarding the occurrence in subpopulations with specific tumor types, although the majority of cases are linked to cancer therapy itself [11] [10].

For several reasons, understanding and recognizing hematologic abnormalities associated with specific tumor types is essential. Firstly, this may shorten diagnosis time, which is of extra value, especially for cancer patients. Secondly, they may indicate a response to tumor therapy or remission status. Thirdly, hematologic abnormalities may require additional treatment and, therefore, should be addressed. Lastly, some may serve as prognostic indicators in certain tumor types [12].

As described by Childress [11], these hematologic abnormalities can be classified into several groups:

- **Abnormal decrease in circulating blood cell concentrations**
 - **Anemia:** One of the most common conditions in human cancer patients, 30-50% of solid tumors and 70% of hematopoietic tumors [13], [14]. Although not reported in the literature, it is common in canine and feline cancer patients. Mainly in hematopoietic and vascular tumors like lymphoma and hemangiosarcoma. Often, a multifactorial problem, which several pathways

may cause, all lead to reduced ability of the blood to carry sufficient amount of oxygen to the different tissues [11].

- **Thrombocytopenia:** Resulting in decreased numbers of platelets in the blood. Frequent in veterinary cancer patients, especially in cats and dogs with hematopoietic and vascular tumors like acute leukemia and disseminated histiocytic sarcoma [11].
- **Leukopenia:** In cancer patients, it is mainly associated with neutropenia caused by myelosuppressive cancer therapy (i.e., chemotherapy). True cancer-associated neutropenia is less common and mostly linked to hematopoietic malignancies [11].
- **Bicytopenia and pancytopenia:** Bicytopenia is the reduction of two cell lines, while pancytopenia is the reduction of cells in all lineages in the blood. Both cases originate due to abnormalities in the bone marrow. Commonly in patients with hematologic cancers, in particular acute leukemia, lymphoma, multiple myeloma and disseminated histiocytic sarcoma [15], [16].
- **Abnormal increase in circulating blood cell concentrations**
 - **Erythrocytosis:** Uncommon in veterinary cancer patients. It may be relative or absolute, with the latter being primary or secondary. In relation to cancer patients, it originates mainly from either myeloproliferative disease or paraneoplastic syndrome, which is seen most often in renal neoplasia [11].
 - **Thrombocytosis:** In contrast to human cancer patients, where thrombocytosis occurs up to 60%, it is less described in veterinary cancer patients [11]. However, this could be due to underdiagnosis, as in one retrospective study, neoplasia was the most diagnosed disease concerning thrombocytosis [17]. Thrombocytosis can be further classified into physiologic, reactive, or neoplastic. However, all three forms can occur in cancer patients [11].
 - **Leukocytosis:** This is the absolute increase in the number of leukocytes, which can be attributed to either granulocytes, lymphocytes, or monocytes. Although not pathognomonic to cancer patients, neoplasia is the most common cause of persistent lymphocytosis, primarily in lymphoid malignancies [11].
- **Abnormal cells in circulation:** The appearance of abnormal blood cells in the hemogram of the veterinary cancer patient may occur, especially in hematopoietic neoplasms that affect the bone marrow like leukemia, lymphoma [11].
- **Coagulopathies**

- **Hypercoagulability:** A common condition both in human and veterinary cancer patients, leading to an increased risk of thrombosis. In human cancer patients, it is the second leading cause of death [18]. While in canine cancer patients, it was confirmed as the most common hemostatic abnormality found [12].
- **Hypocoagulability:** Less common condition, leading to an increased risk of bleeding. It may occur due to a decrease in platelets number or alternation in their function, alternations in clotting factors, production of neoplastic anticoagulant substances and changes in plasma viscosity [11].
- **Disseminated Intravascular Coagulation (DIC):** A complex condition including both thrombosis and bleeding. This is due to excessive activation of the coagulation cascade, leading to widespread microthrombosis and multiorgan failure [19]. It can manifest as per acute and acute forms and may be preceded by the chronic form, which is most common in advanced or metastatic tumors [11].
- **Platelet Function Disorders:** Platelet dysfunction may contribute to various pathological conditions such as bleeding and thrombosis as well as diseases affecting hemostasis like cancer [18]. Platelet function can be effected either by the tumor itself or anticancer treatments, leading to hyper- or hypo-coagulability. Moreover, studies have suggested that platelets may contribute to tumor growth via angiogenesis and provide physical and mechanical support to evade the immune system and spread to secondary organs [18].
- **Dysproteinemias:** Among these, hyperglobulinemia is the most diagnostically relevant. The serum protein profile should be further characterized by electrophoresis. If a broad-based peak characterizes the electrophoretogram, it is primarily due to acute or chronic inflammatory disease. If the peak is tall and narrow, it is consistent with monoclonal gammopathy, which in dogs and cats is mainly associated with neoplasms involving B-lymphocytes or non-neoplastic causes, including ehrlichiosis, leishmaniasis, heartworm disease, chronic pyoderma, and plasmacytic gastroenterocolitis [11].

3.3 Biological markers

The National Institute of Health Definitions Working Group (NIH DWG) defines a biological marker (or 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" [19]. They consist of biological features that have been shown to correlate with the physiologic or pathologic state in question [20], and can be used to assess the health status of the individual [21].

Based on their application, biomarkers can be subdivided into five major categories: 1) antecedent biomarkers, which are applied to identify the risk of disease development, 2) screening biomarkers for the screening of disease, 3) diagnostic biomarkers used to diagnose a disease, 4) staging biomarkers which assist in categorizing disease according to its severity, and 5) prognostic biomarkers to predict future course of disease, including recurrence, response to therapy, and monitoring efficacy of therapy [22].

Creating a useful biomarker is a complex process that consists of several steps. The first step includes identifying the target population and disease status. Understanding the pathogenesis of the disease, as well as the pharmacodynamics and pharmacokinetics of the treatment options, is crucial in finding a single biological measurement that will correlate with the disease in question. These are followed by data collection, application of suitable assays and analysis, validation and regulatory approval process. All are necessary for the production of new biomarkers [23].

Biomarkers require large populations of both healthy and diseased animals, which is financially and time-consuming. Thus, practical feasibility and cost-benefit ratio must also be considered when transitioning from experimental to clinical setup. There are multiple challenges in producing a reliable biomarker arising from the complex nature of biological systems. Unintentional biases can be challenging in establishing accurate cut-off values for biomarkers. They can occur due to biological factors such as species, size and age, or technical aspects, including variations in sample handling, environments, and the animal's stress response. Therefore, animal models and standardized protocols are crucial to minimize bias and regulate these variables. Furthermore, validating ensures the biomarker's suitability for clinical settings. Challenges in specificity and sensitivity often hinder the translation of a biomarker assay from the lab to clinical use, causing many studies to fail before reaching the large-scale validation phase [24], [25].

Moreover, establishing a reliable biomarker faces two significant challenges. The first is related to the ability to differentiate healthy from diseased individuals, considering that various conditions in healthy subjects can alter biomarker values. The second is related to

specific disease detection. The biomarker should help detect specific diseases, even though many abnormal conditions can cause similar alterations in biomarker levels.

The ideal biomarker should be safe and easy to measure, cost-efficient, consistent across individuals and adjustable according to the treatment [21]. Moreover, reliable biomarkers must not only be associated with the disease, but also predict or accurately correlate with the clinical outcomes [4].

3.4 The use of biological markers in veterinary medicine

The evaluation of the hemostatic status of a patient requires a comprehensive approach using a combination of laboratory tests in order to assess different aspects of the coagulation cascade and platelet function. These, in return, help the diagnosis of bleeding and thrombotic disorders, guide treatment decisions, and monitor patient response to therapy [20].

Hemostatic alternations, particularly subclinical ones, have been shown to be related to cancer type and disease progression as described by Andreasen et al. [12]. There have been multiple laboratory examinations which have been investigated to demonstrate the correlation between the two, each used to pinpoint a different hemostatic route that may be altered [12]. However, it must be taken into consideration that in cancer, any hemostatic biomarker can be slightly altered without necessarily leading to hemostatic clinical disorders.

3.4.1 Activated partial thromboplastin time (aPTT)

Theoretical background

The term 'activated partial thromboplastin time' can be broken down for a better understanding of the test's background. As described by Tripodi and Mannucci [26], 'Thromboplastin' is a term which is used to define the complex formed by different plasma clotting factors, leading to the formation of thrombin and, consequently, the development of a fibrin clot. The complex is activated due to the addition of phospholipid preparation, which leads to the acceleration of clotting but not to the correction of the prolonged clotting times of hemophilia patients [27]. The word 'partial' indicates the presence of phospholipid but not tissue factor [26].

aPTT test originates back to 1953, when Brinkhous et al. designed a test to purify factor VIII from plasma. In the beginning, only the phospholipid concentration was controlled. Thus, the coagulation time was measured after adding platelet substitutes, namely exogenous phospholipids (cephalin), and calcium chloride. Later, the test underwent another modification by Proctor and Rapaport, who added kaolin to optimize the activation of

coagulation. This change helped reduce the coagulation times and variability of the results [5], [26].

In 1960, commercial kits became available and the test became widely used for hemorrhagic diseases. Another development in the use of aPTT was related to the incidental finding of Conley and Hartmann [28]. The two discovered that there was a circulating anticoagulant in the blood of patients with lupus erythematosus, which resulted in prolonged aPTT times despite the fact normal plasma was used. This finding led to the search for aPTT reagent, which was less responsive to the circulating anticoagulant called "lupus anticoagulant". This viewpoint shifted when Bowie et al. [29] observed alternation in blood coagulation in patients with systemic lupus erythematosus who had circulating anticoagulants and prolonged aPTT. Following this discovery, aPTT has been widely used to detect lupus anticoagulants in patients with a history of thrombosis or fetal loss [30]. In the early 1970s, aPTT became the test of choice for dose adjustment of unfractionated heparin, which was the drug used in the treatment of acute venous thromboembolism (VTE) [31].

Clinical significance and usage

aPTT gives information about the intrinsic and common pathways, as it is triggered by surface activation (reproducing a similar effect to collagen, which is found on the inner surface of the blood vessels) of factor XII and activation of factor X by the addition of calcium [27].

Factors involved: XII, XI., IX., VIII., X., V., II., I., XIII [53].

As described by Raber [32], The activated partial thromboplastin time (aPTT) is an effective screening test for both inherited and acquired factor deficiencies. Prolongation of aPTT is often observed in inherited disorders such as hemophilia A (factor VIII deficiency) and hemophilia B (factor IX deficiency). Other congenital conditions involving factors associated with the intrinsic and common pathways may also be absent, although these conditions are rare. For example, abnormalities in factor XII are often associated with a prolonged aPTT without clinical signs of bleeding. Acquired factor deficiencies are more common and can occur due to liver dysfunction, vitamin K deficiency or anticoagulation therapy with warfarin. Other conditions that may result in a decrease in factor concentrations include disseminated intravascular coagulation (DIC), prolonged bleeding, and massive transfusion.

Another reason for prolonged aPTT is circulating inhibitors of coagulation. These conditions are mostly acquired and result in prolonged aPTT, even with the addition of normal plasma.

Circulating inhibitors consist of antithrombins (most often), such as heparin and fibrin degradation products (FDP). These substances are usually elevated in DIC and primary fibrinolysis. Another group of inhibitors involves antibodies, such as antibodies against factor VIII in patients with hemophilia A [32].

To differentiate between inhibitors and factor depletion, a mixing study is performed. In this test, normal plasma and patient plasma are mixed in a 1:1 ratio, followed by repeating the test on the mixed sample. Factor deficiency is indicated if the abnormal value is corrected completely. If the result does not change or is only partially corrected, it suggests the presence of an inhibitor [32].

Less often, the aPTT values are lower than normal. This finding may correlate with increased levels of activated coagulation factors and the patient is in a hypercoagulable state. This can be seen in the early stages of DIC but should not be considered diagnostic for this condition [32]. In addition, although previous studies have explored the significance of shortened aPTT (as reviewed in Ref. [33]), a case-control study directly examined this issue and identified an association between shortened aPTT and an increased risk of venous thromboembolism (VTE) [33].

Limitations

The reference range for aPTT clotting times lies between 27-35 seconds [27], with differences between species as shown in Table 1. However, values vary between laboratories depending on a few factors, such as the type of activator and reagent used, the incubation times applied, the method of endpoint detection (coagulometer) and whether automated or manual test was used [27], [34].

Table 1. aPTT values among different species (adapted from [26], [35], [36], [37])

	horse	cattle	swine	dog	cat
aPTT (sec')	35.8-48.6 35.0-46.1	30.0 – 58.0 23.90-31.90	17.8-34.2	11-17.5 10.4-12.9	15-21 11.2-16.0

Availability and methods

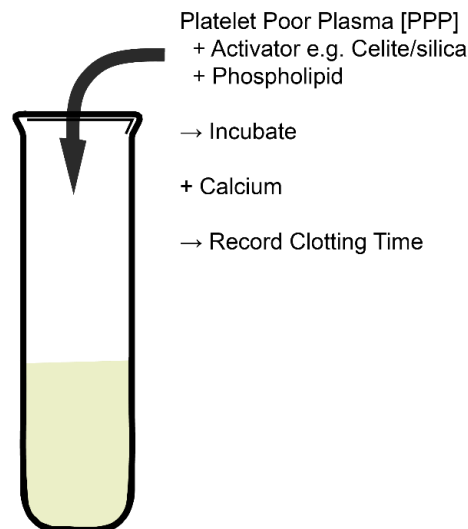
In the past, kaolin was used as a surface activator in aPTT tests. However, kaolin is rarely used nowadays in automated aPTT tests due to its opacity, which interferes with the optical detection of the fibrin clot endpoint. Instead, commonly used activators for automated analyzers include micronized silica and ellagic acid [27].

Cephalin, a phospholipid substitute, replaces platelet phospholipid in the test. Since the test uses platelet-poor plasma (PPP), an external source of phospholipid is necessary for coagulation to occur [27].

As shown in Figure 2, phospholipid (cephalin) and contact activator (Kaolin, micronized Silica or Ellagic acid) are added to preincubated PPP (at 37°C). This initiates the activation of factor XI to XIa. The addition of prewarmed calcium (at 37°C) activates the rest of the pathway, which results in clot formation. The time is measured from the addition of the calcium until the formation of the fibrin clot [27].

Figure 2 aPTT testing (adapted from [27])

Activated Partial Thromboplastin Time [APTT]



Mostly, automated analyzers are used to measure the time to clot formation, using the optical density of the mixture. The mixture becomes opaque when a clot is formed, and less light can pass through. However, false normal values may occur in case the patient's plasma is turbid (e.g. in case of hyperlipidemia, hyperbilirubinemia) [27].

Many different commercial kits have been developed, featuring a variety of reagents and phospholipids for use. Those kits which also use kaolin and human placenta are noted for their high sensitivity [38]. Each kit includes manufacturer-specific instructions, with reagents either requiring preparation or ready to use. An example is the Silimat reagent, which contains rabbit brain homogenate as PF3 (platelet factor 3) and micronized silica as a contact activator. The test involves preincubation of the citrated (decalcinated) plasma with the surface activator

(the reagent), to activate factors XII and XI [39]. CaCl₂ is then added to initiate a calcium-dependent cascade that results in a fibrin clot [38]. The time is measured simultaneously with this step, and depending on the technique used, the normal time values usually range between 25-35 seconds [32]. The coagulation activity (%) is calculated using reference curves specific to each reagent [38].

Future usage

Despite studies addressing the significance of shortened aPTT, most of the test results investigated correlate with aPTT prolongation as an indicator of loss of function (hypocoagulable state) [26]. Only in the last years, the correlation between shortened aPTT (hypercoagulable state) and venous thromboembolism has been investigated in humans [33].

Importance in cancer diagnosis

Although not described vastly in veterinary literature, Anjos et al. [10] describe the hemostatic alternations found in their study as part of a paraneoplastic syndrome associated with tumor non-invasive actions. Their results report on thrombocytosis, increased aPTT, PT and fibrinogen. Although no clinical alternations were seen (e.g., petechia), the elevated fibrinogen and aPTT were assumed to result from the body's systemic inflammation reaction to the tumor cells.

Andreasen et al. [12] found that the most prevalent hemostatic dysfunction in canine cancer patients was hypercoagulability. Non-mammary carcinomas had increased aPTT apart from increased clot strength (TEG) and fibrinogen. The increase in these parameters was thought to result from the pro-inflammatory response associated with the disease.

Another study [13] evaluated the hemostatic status of female dogs with mammary carcinomas free of distant metastases. The most common hemostatic dysfunction among the bitches was hyperfibrinogenemia, which appeared to correlate with disease progression. Additionally, an increase in platelet count, fibrinogen concentration, PT and aPTT were suggested to result from a proinflammatory response induced by the tumor, indicating a state of hyperfibrinogenemia. The abnormalities in coagulation parameters were influenced by the clinical stage of the disease and the histopathological grade.

In addition, 50% of dogs with multiple myeloma presented with bleeding diathesis and prolonged aPTT and/or PT. This can be explained by the M-component and thrombocytopenia, which may contribute to bleeding. The M component prevents platelet

aggregation and inhibits tissue factor and proteins S and C. Moreover, it results in abnormal fibrin polymerization and reduces available calcium [40].

3.4.2 Prothrombin time (PT)

Theoretical background

The first to recognize the serine protease thrombin was Alexander Schmidt in 1872, who hypothesized the existence of an enzyme that can convert fibrinogen to fibrin. Initially, it was named "fibrin ferment" until Rudolf Virchow introduced the term "thrombin." It was only a few years later that Pikelharing discovered the inactive precursor prothrombin [41].

The Prothrombin Time (PT) test was first introduced by Dr. Armand Quick in 1935 to identify coagulation disorders in chickens. This test later played a crucial role in the discovery of the anticoagulant warfarin during investigations into the cause of "sweet clover disease," which led to coagulopathy in cattle [42].

Clinical significance and usage

In comparison to aPTT, prothrombin time gives information about the function of the extrinsic and common pathways [32]. This is because the coagulation cascade is triggered by adding tissue factor (and calcium ions) to the dicalcinated plasma sample, for the activation of factor VII [32].

Factors involved: III, VII., X., V., II., I., XIII [43].

As described by Raber [32], prolonged PT may be associated with problems related to either factor deficiencies or circulating inhibitors of coagulation. Regarding factor deficiencies, the PT test is more sensitive than aPTT. Thus, even a small decrease in factor VII concentrations may prolong the PT time. Factor VII deficiency, whether inherited or acquired, is marked by a prolonged PT with a normal aPTT. While inherited deficiency is usually a rare bleeding disorder, acquired deficiencies are typically associated with conditions such as warfarin therapy, liver disease, or factor depletion due to consumptive coagulopathy. Circulating inhibitors, such as heparin and fibrinolysis products, most commonly target thrombin or factor X.

Similarly to the aPTT test, the distinction between the two is made by mixing the sample with normal plasma in a 1:1 ratio and repeating the test. In the case of circulating inhibitors, the prolonged PT cannot be corrected entirely [32].

PT test is a widespread coagulation test in routine laboratories. Some of its main indications include monitoring oral anticoagulant therapy (OAT) in humans [44], as well as preoperative assessment of bleeding tendencies. Moreover, it is used in the prevention and treatment of thrombotic states such as venous thromboembolism and atrial fibrillation [45].

Limitations

Despite the fact PT has been successfully standardized (compared to aPTT) [44], reference ranges may differ between laboratories. Each laboratory has its normal values, usually ranging between 10-15 seconds [32], [43].

Prothrombin Time (PT) has been standardized for domestic chickens, compared to non-domestic avian species, for which there is still a lack of validated coagulation tests. This influences the investigation of acquired hemostatic disorders in these species [20]. Table 2 shows the PT values among the different species.

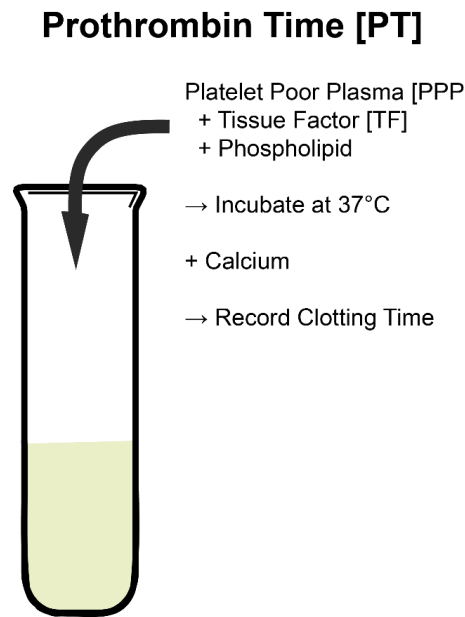
Table 2. PT values among different species (adapted from [35], [46], [36])

	horse	cattle	swine	dog	cat	chicken
PT (sec')	11.0-15.0	26.0-38.0	17.7-21.3	11.0 - 15.5	15.0-20.0	49-132
	10.7-12.0	23.8-27		7.0-9.3	10.0-15.3	
	23.8-32.4	18.50-37.50		5.4-11.9	5.4-11.9	

Availability and methods

As shown in Figure 3, the test involves an activating agent, usually thromboplastin (containing tissue factor and phospholipid), extracted from the animal brain, which is added to citrated plasma and incubated at 37°C. Calcium chloride is then added and the time is measured (manually or automatically) until the formation of the fibrin clot [32], [43].

Figure 3 Prothrombin time testing (adapted from [43])



There are two methods used for PT measurement: the Quick method and the Owren method [47]. The Quick prothrombin time was developed by Armand Quick and is the most widely used PT method worldwide. The method measures factors II, V, VII, X and fibrinogen [43]. The Owren prothrombin time was developed by Paul Owren and is used mainly in the Nordic countries, Benelux, Japan and to a lesser extent in Europe [48].

As stated by Berlin et al. [47], while both methods are appropriate for controlling anticoagulant treatment, they have a few differences. The coagulation factors tested in the Quick method include fibrinogen, factor II, factor V, factor VII and factor X. In comparison, the reagent in the Owren method includes fibrinogen and factor V, making the test sensitive to deficiencies in factors II, factor VII, and factor X only. Another significant difference between the tests is the sample volume in the mixture. Compared to the Quick method, in which 50 microliters of plasma is added to 100 microliters of reagent, the Owren method uses 10 microliters of plasma and 50 microliters of diluent, which are added to 140 microliters of reagent [45]. This difference makes the Quick method more sensitive to preanalytical variables [47], while it increases the sensitivity of the test in the Owren method and decreases the effect heparin may have on the test [43]. In addition, the coagulation time differs between the two tests. While it is equal to about 10 seconds in the Quick method, in the Owren method it is doubled to about 20 seconds.

Despite these differences, both tests use reagents consisting of tissue factors extracted from natural sources. In the Quick method, these include natural sources such as bovine/human/rabbit brain, human placenta and rabbit lung, compared with the Owren method which uses bovine/monkey/rabbit brain [45].

Point of care testing (POCT) is a new sampling methodology for monitoring patients on oral anticoagulant therapy (OAT). The sample used is non-anticoagulant capillary whole blood and the testing can be done at home. Thromboplastin activation is necessary and coagulation time is measured [45]. In dogs, using a human point-of-care (POC) test for prothrombin time (PT) is a reliable and cost-effective method for assessing hemostasis. However, when a prolonged PT is observed, it is recommended to confirm the findings using a standard method [20]. For horses, preliminary studies suggest that this POC device is also specific and sensitive to abnormal PT values. However, its reliability decreases in horses with a low packed cell volume (PCV) of less than 0.25, requiring further confirmatory studies [47].

Future usage

PT has been shown to be effective in monitoring oral anticoagulant therapy (OAT) in humans, specifically rivaroxaban. Although generally laboratory testing for dose adjustment is not needed, it might be useful before initiation of treatment, before invasive procedures such as surgery, in case of thrombotic events and if reversal of the anticoagulant therapy is needed [49].

Importance in cancer diagnosis

As mentioned above, PT was shown to be prolonged in cancer patients with or without clinical alternations, as described by Anjos et al. [10]. This is supported by a study [13] that was done in female dogs with mammary carcinomas free of distant metastases. As part of the coagulation alternations induced by the tumor, prolongation of PT was observed, which was influenced by the clinical stage of the disease and histopathological grade. Moreover, another study [40] was done in dogs with multiple myeloma which presented with bleeding, found that PT was prolonged as well.

4 Goals

We aimed to find changes in PT and aPTT in canine and feline patients with tumors and non-neoplastic disease and predictive information about the state of the animals.

This study's main objective was to assess the differences in PT and aPTT values between healthy dog and cat populations and those diagnosed with cancer, in order to identify the potential changes in the coagulation profiles. Moreover, the analysis included whether certain types of cancer correlate with more pronounced alterations in PT and aPTT levels in canine and feline patients, with the purpose of evaluating whether these parameters can be used as potential predictive markers for disease progression.

Another objective of this study included the evaluation of PT and aPTT as markers for inflammatory state. This could be explained as the body's response to the tumor and may affect the severity of the disease. Thus, the correlation of PT and aPTT with inflammatory processes may also be valuable when constructing a prognosis for cancer patients.

The identification of cancer-specific alterations in PT and aPTT will improve the understanding of cancer-associated coagulopathies in canine and feline oncology patients and will contribute to the development of potential guidelines for the use of PT and aPTT as diagnostic or prognostic markers in managing those patients. This will aid veterinarians in the early detection of coagulation abnormalities and improve the management of canine and feline cancer patients.

5 Materials and Methods

5.1 Population

This was a retrospective cohort study carried out at the Á.H.O.K. Állatorvosi Hematológiai és Onkológiai Központ, Állatpatika, in which we retrospectively and prospectively analyzed 192 dogs and 45 cats within 6 months period. The number of male and female dogs was 115 and 77, respectively, whereas there were 13 male and 32 female cats. The number of neutered dogs and cats was 102 and 23, respectively. Their average age was 9.22 (± 3.9) years for dogs and 9.66 (± 4.57) years for cats.

The following groups were established, which can be seen on Table 3 and in Figure 4 and Figure 5.

Table 3. Grouping of animals based on disease type

Dogs		Cats	
Disease type	Num'		Num'
Healthy	11	Healthy	7
Inflammatory	28	Inflammatory	7
Benign tumor	20	Abdominal tumor	10
Intestinal tumor	7	Sarcoma	8
Sarcoma	15	Lymphoma	8
Hemangiosarcoma (HSA)	6	Benign or non-neoplastic chronic disease	5
Hepatic tumor	8		
Adrenal tumor	3		
Lymphoma	13		
Leukemia	3		
Mast cell tumor (MCT)	29		
Melanoma	3		
Mammary gland tumor (MGT)	14		
Oral cavity tumor	7		
Lower urinary tract and genital tumor	8		
Other tumor types	9		
Non-neoplastic chronic disease	8		

Figure 4 Disease groups in dogs

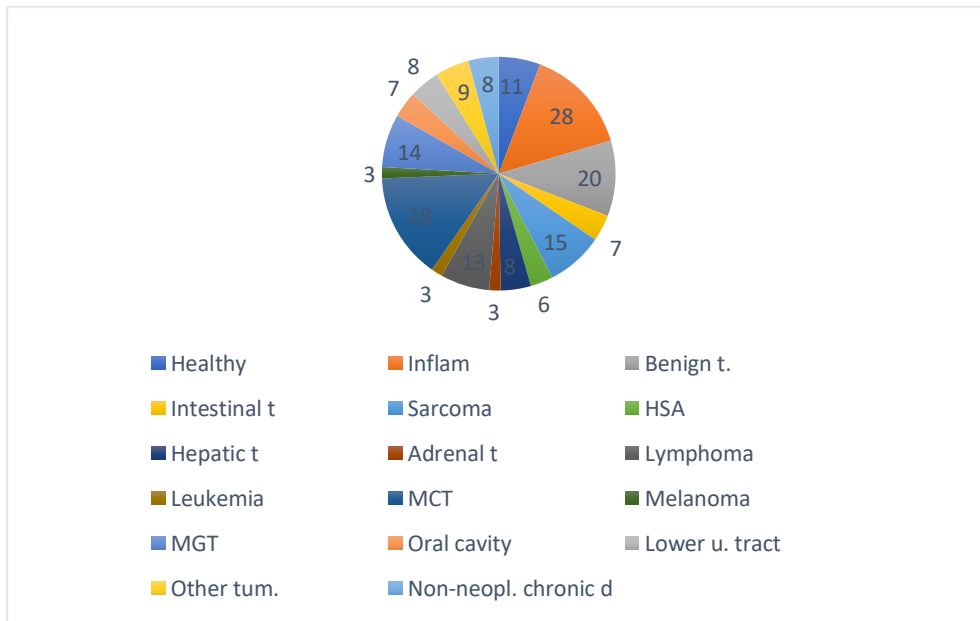
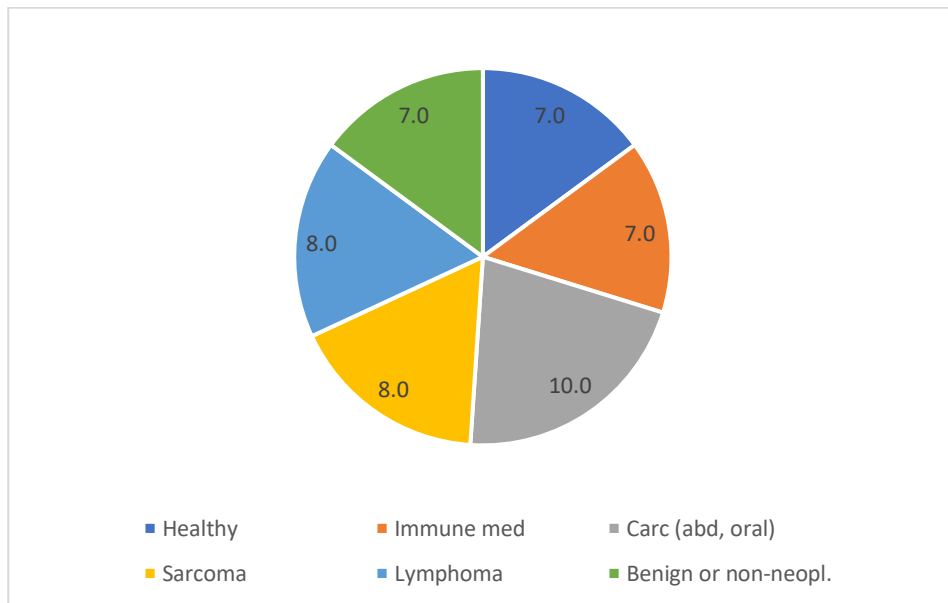


Figure 5 Disease groups in cats



Within the dog's Inflammatory group, there were immune-mediated anemia cases (No: 8), immune-mediated thrombocytopenia cases (No:6), patients with fever (No:12), osteomyelitis (No:1) and enteritis (No:1).

Other tumor types group in dogs included cerebral neoplasms (No:2), thyroid carcinoma (No:1), osteosarcoma (No:2), myeloma (No:2), renal neoplasm (No:1) and polycythemia vera (No:1).

In cats within the Healthy group (No: 7) were healthy cats that came for routine control (No: 3), squamous cell carcinoma lasted for more than 1 year with a diameter of <2 mm, without complaint (No: 3), and one with basal cell tumor on the chin (diameter is 0,3 mm). The inflammatory group consisted of 7 cases with immune-mediated hemolytic anemia, but within this group, there was one with thrombocytopenia, another one with endometritis, and another with concomitant rectal tumor. The abdominal Tumor (No: 10) group consisted of cats with liver neoplasm (biliary carcinoma, No: 4), mammary carcinoma (No: 4), and intestinal carcinoma (No: 2). Sarcoma group (No: 8) consisted of cats with feline injection sarcoma (No: 4), limb fibrosarcoma (No 3), and osteosarcoma (No: 1) type. The Lymphoma group (No: 8) consisted of cats with 4 alimentary (No: 4) multicentric lymphoma type (No: 4). Benign or Non-neoplastic Chronic Disease group (No: 5) consisted of cats with toxemia (No:1), hyperthyreosis (No:1), progressive histiocytosis (No:1), basal cell tumor (No:1) and kidney failure (No:1).

The dogs and cats included in this study were examined due to their complaints at our clinic. During the 1st examinations, or before surgical interventions, or when the animal was already examined but there was a relapse occurring and that state of the animal necessitated blood sampling. The animals were routinely examined by routine clinical, ultrasonographic and, in oncological cases, radiographic examinations. The endoscopy was done on 20 dogs and 3 cats. The final diagnosis of the diseases was made by morphological examinations of the blood smears and/or the fine needle aspiration samples. Histopathology was performed and provided a reliable diagnosis.

5.2 Sample collection

During the first diagnostic examinations, we took blood samples from the patients into the following test tubes:

For hematology analysis we used sample tube, K3 EDTA, 4 ml, Cap violet, (LxØ): 75 x 12 mm (Sarstedt, SARSTEDT AG & Co. KG, Sarstedtstraße 1, D-51588 Nümbrecht).

For clinical chemistry analysis we used serum tubes (Greiner Bio-One Tubes de sérum: Activateur de caillot avec séparateur (Fisher Scientific SAS - Boulevard Sébastien Brant - F67403 Illkirch Cedex – France).

For hemostasis examinations we used the citrated tubes (Micro sample tube Citrate 3.2%, 1.3 ml, screw cap, ISO | Prepared blood, SARSTEDT AG & Co. KG, Sarstedtstraße 1, D-51588 Nümbrecht).

The evaluations of the blood samples were done within 12 hours, except for aPTT and PT measurements, which were done within 30 minutes.

5.3 Coagulation tests

Fresh samples were processed via KC4, Amelung analyzer. The analyzer provides measurement of the clotting time of the plasma samples via detection of the changes in the optical density of the sample during the coagulation process. Thus, the formation of fibrin clot is detected by monitoring the changes in the light passage through the plasma sample. The formation of the clot causes a change in the optical density, which is measured by the analyzer.

For the measurement of PT, the plasma samples were mixed with thromboplastin reagent, which consists of tissue factor and calcium chloride (CaCl_2).

For the measurement of aPTT, the plasma samples were pre-incubated with an activator reagent such as kaolin or ellagic acid. Then phospholipids and CaCl_2 were added to the mixture to trigger the clot formation, which is measured by the analyzer.

5.4 Statistical analysis

The mean and standard deviation (SD), were calculated for both aPTT and PT values among different tumor types in dogs and cats, as well as healthy controls. The data calculated for aPTT and PT in dogs can be seen in Figure 6 and Figure 7, and for cats in Figure 8 and Figure 9, respectively.

Statistical analysis was conducted using Two-way randomized block ANOVA, which was used to compare aPTT and PT values between the different groups of dogs and cats, using the healthy control groups as the reference. The significance level (α) was set at 0.05. In addition, Bonferoni and Kruskal-Wallis tests were used to calculate the significant differences.

Differences between groups were analyzed using Nonparametric linear regression analysis, Pearson's correlation, and unpaired Student's t-test. P-values below 0.05 were considered significant. Statistical analysis was carried out using Microsoft Excel 2010, R version 3.0.20

(Foundation for Statistical Computing, Vienna, Austria) and Stats Direct Statistical Software version 3.0.194 (StatsDirect Ltd, Altrincham, UK).

6 Results

Significant differences for dogs can be seen in the report by the Bonferoni comparison for aPTT, which provided almost the same results as Kruskal Wallis. However, for PT Kruskal Wallis paired analysis helped to identify significant differences between the healthy and the other groups of cats.

In dogs, using the Bonferoni comparison analysis, statistically significant differences in aPTT values were found for inflammatory diseases ($p=0.045$), lymphoma ($p=0.012$), leukemia ($p<0.0001$), melanoma ($p=0.001$), and non-neoplastic chronic diseases ($p=0.001$), when compared to the healthy controls. Using the Kruskal-Wallis test, a significant difference in PT values was found only for the inflammatory diseases group ($p=0.001$).

In cats, using the Kruskal Wallis paired analysis, statistically significant differences in aPTT and PT values were found for cases with abdominal neoplasia compared to the healthy controls ($p=0.0094$, $p=0.0093$).

The following results for aPTT and PT in dogs can be seen in Table 4 and Table 5, respectively.

Table 4 aPTT results in dogs

Variables	Mean	SD (±)	ANOVA comparison with the healthy values (p-values)
			Bonferoni
Healthy	15.89	1.36	
Inflammatory	15.78	4.98	0.045
Benign tumor	15.60	4.58	0.471
Intestinal tumor	17.76	1.70	0.250
Sarcoma	14.73	4.95	0.490
HSA	16.23	0.92	0.962
Hepatic tumor	14.08	5.79	0.936
Adrenal tumor	17.50	2.42	0.405
Lymphoma	16.84	5.01	0.012
Leukemia	24.73	4.69	< 0.0001
MCT	16.33	3.67	0.596
Melanoma	17.27	4.18	0.001
MGT	15.94	4.24	0.630
Oral cavity tumor	20.03	8.57	0.773
Lower u. tract tumor	18.91	3.05	0.237
Other tumors	18.00	3.13	0.511
Non-neoplastic chronic disease	16.91	7.74	0.001

Figure 6 aPTT mean and SD values calculated for dogs

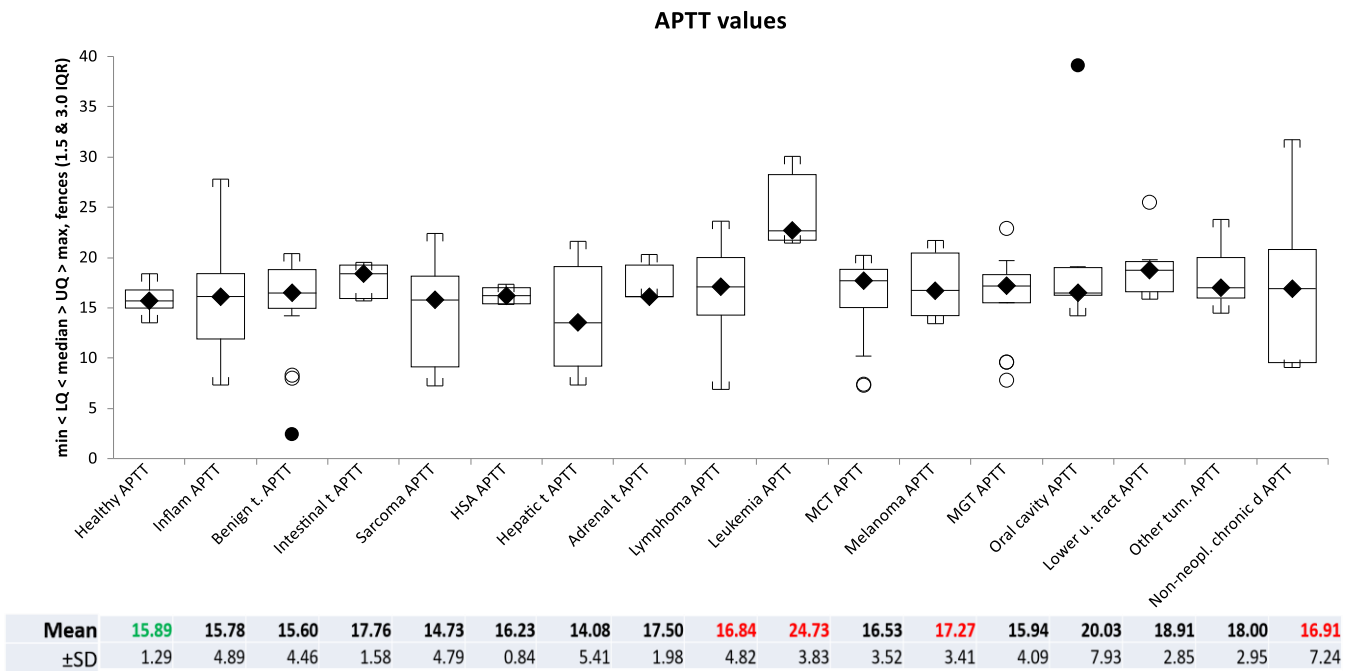
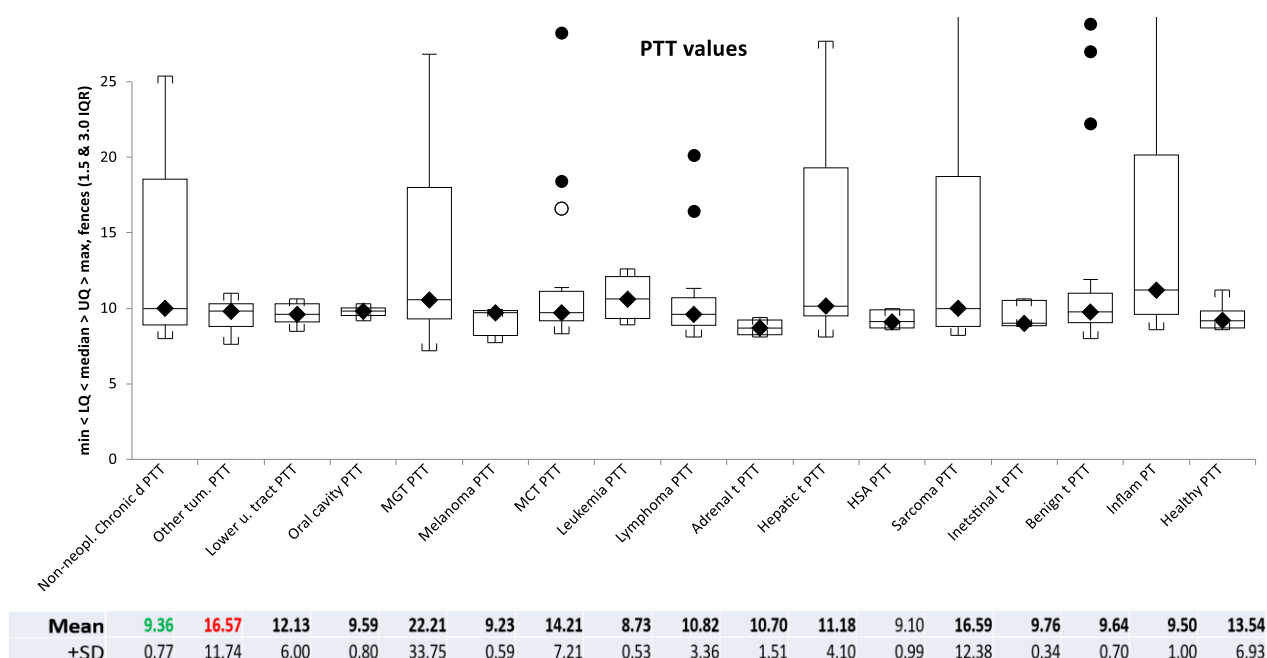


Table 5 PT results in dogs

Variables	Mean	SD (±)	ANOVA comparison with the healthy values (p-values)	
			Bonferoni	Kruskal-Wallis
Healthy	9.36	0.81		
Inflammatory	16.57	11.95	0.401	0.001
Benign tumor	12.13	6.16	0.833	0.103
Intestinal tumor	9.59	0.86	0.860	0.497
Sarcoma	22.21	34.93	0.280	0.169
HSA	9.23	0.64	0.673	0.763
Hepatic tumor	14.21	7.71	0.725	0.069
Adrenal tumor	8.73	0.65	0.295	0.350
Lymphoma	10.82	3.49	0.528	0.297
Leukemia	10.70	1.85	0.311	0.186
MCT	11.88	5.58	0.462	0.076
Melanoma	9.10	1.22	0.505	0.938
MGT	16.59	12.85	0.805	0.022
Oral cavity tumor	9.76	0.37	0.752	0.103
Lower u. tract	9.64	0.74	0.623	0.433
Other tumors	9.50	1.07	0.295	0.543
Non-neoplastic Chronic disease	13.54	7.41	0.441	0.283

Figure 7 PT mean and SD values calculated for dogs

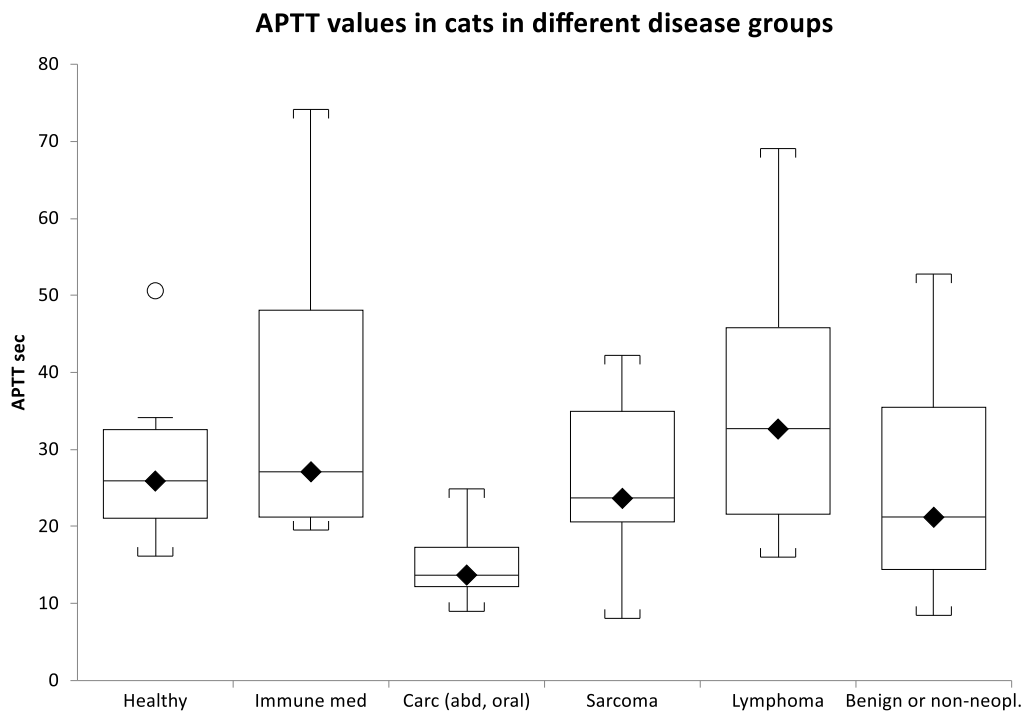


The following results in cats can be seen in Table 6 and Table 7, for aPTT and PT values, respectively.

Table 6 aPTT results in cats

Variables	Mean	SD (\pm)	ANOVA comparison with the healthy values (p-values)	
			Bonferoni	Kruskal-Wallis
Healthy (No: 7)	28,21	11,40		
Inflammatory (No: 8)	35,14	21,08	0.333	0.753
Abdominal tumor (No: 10)	15,51	5,31	0.0704	0.0094
Sarcoma (No: 8)	26,09	11,11	0.749	0.6211
Lymphoma (No: 8)	35,65	18,25	0.4873	0.6604
Benign or non-neoplastic tumor (No: 5)	25,72	16,98	0.5479	0.4601

Figure 8 aPTT mean and SD values calculated for cats

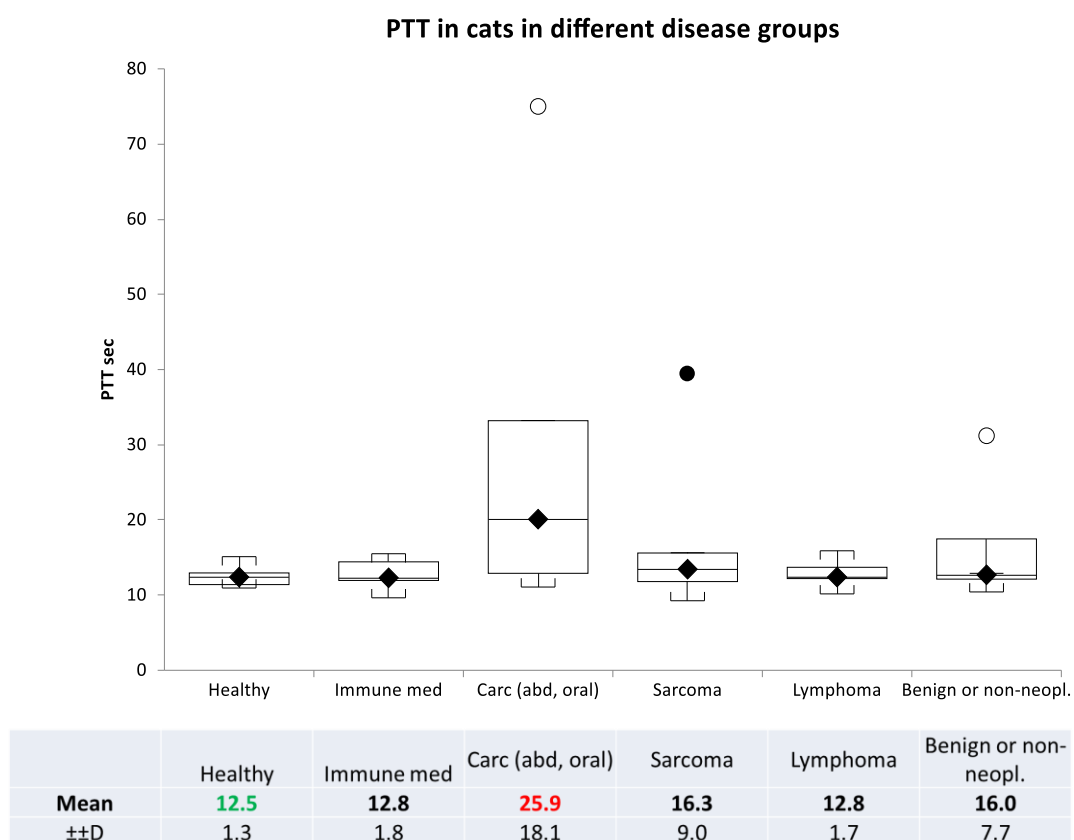


	Healthy	Immune med	Carc (abd, oral)	Sarcoma	Lymphoma	Benign or non-neopl.
Mean	28.2	35.1	15.5	26.1	35.7	25.7
\pm D	10.6	19.5	5.0	10.4	17.1	15.2

Table 7 PT results in cats

Variables	Mean	SD (\pm)	ANOVA comparison with the healthy values (p-values)	
			Bonferoni	Kruskal-Wallis
Healthy (No: 7)	12,47	1,38		
Inflammatory (No: 8)	12,77	1,95	0.996	0.8623
Abdominal tumor (No: 10)	25,85	19,06	0.0232	0.0093
Sarcoma (No: 8)	16,3	9,62	0.4278	0.2523
Lymphoma (No: 8)	12,83	1,79	0.966	0.8689
Benign or non-neoplastic tumor (No: 5)	15,98	8,57	0.6654	0.4703

Figure 9 PT mean and SD values calculated for cats



Correlation analyses between different variables were performed. Detailed descriptions and explanations are found in the Discussion session.

Table 8. Results of the correlation analysis of dogs included in hemostasis study (significant correlations – Pearson, $p < 0.05$)

Variable 1	Variable 2	r-values
Neutered	Gender	-0,3213
CRP	aPTT	0,242046
Ly.abs	CRP	-0,22458
Monocyte abs	aPTT	0,25467
Monocyte abs	CRP	0,35432
Monocyte abs	Ne.abs	0,685467
Monocyte abs	Ly.abs	0,263321
P	Krea	0,743808
ALKP	CRP	0,540611
ALKP	Ne.abs	0,303565
ALKP	ALT	0,277814
MCT Grade	CRP	0,843532
MCT Grade	Ly.abs	-0,56464
MCT Grade	Mon.abs	-0,44783
MCT Grade	ALKP	0,241041
Small (1) Large (2) tumor	CRP	0,364981
Small (1) Large (2) tumor	Ne.abs	0,265335
Small (1) Large (2) tumor	P	0,379108
Small (1) Large (2) tumor	MCT Grade	0,258199
Substage a:1, substage b: 0	aPTT	-0,26179
Substage a:1, substage b: 0	CRP	-0,6625
Substage a:1, substage b: 0	Mon.abs	-0,33777
Substage a:1, substage b: 0	ALKP	-0,23256
Substage a:1, substage b: 0	Small (1) Large (2) tumor	-0,36141

Figure 10 Results of the correlation analysis of dogs included in hemostasis study (Linear regression - CRP vs aPTT $p=0.167$, $r=0.24$)

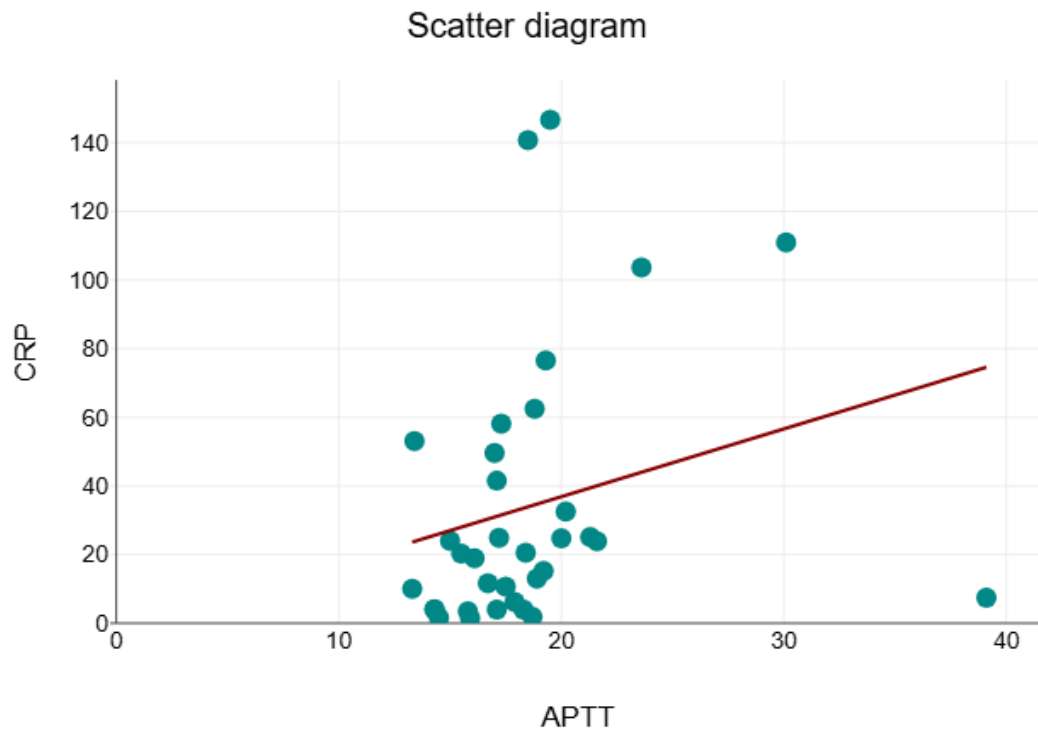


Figure 11 Results of the correlation analysis of dogs included in hemostasis study (Linear regression - Dogs Mon vs aPTT; $p=0.002$, $r=0.25$)

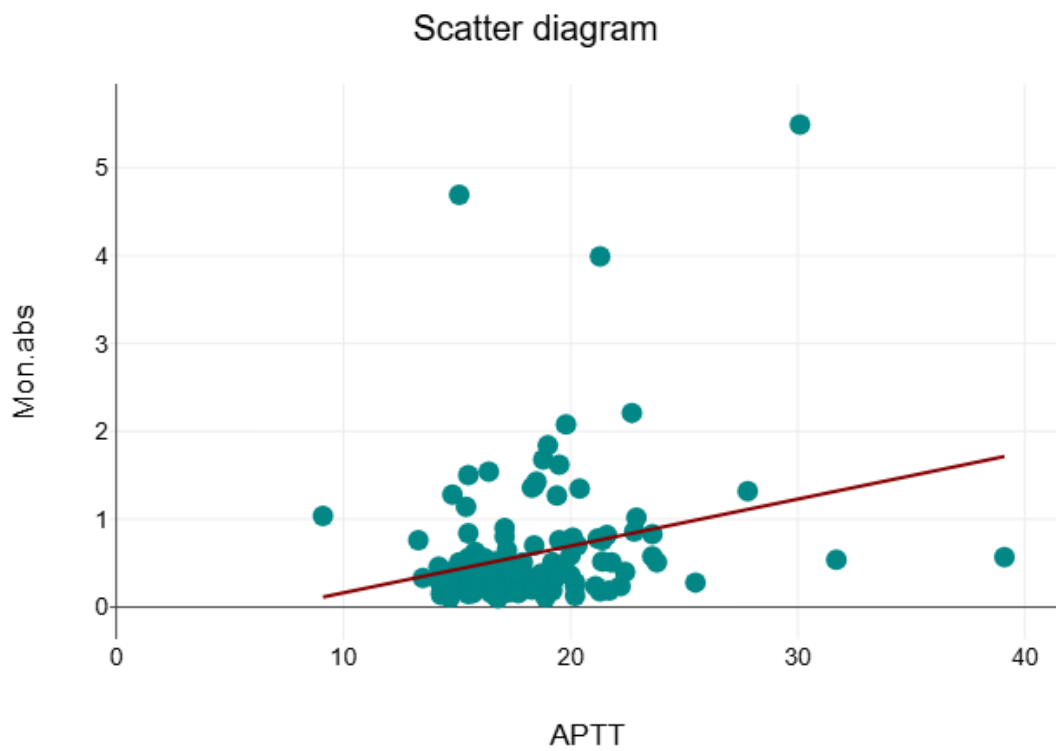


Figure 12 Results of the correlation analysis of dogs included in hemostasis study (Student's t-test for independent samples - Dog Substage a vs aPTT; $p=0.037$)

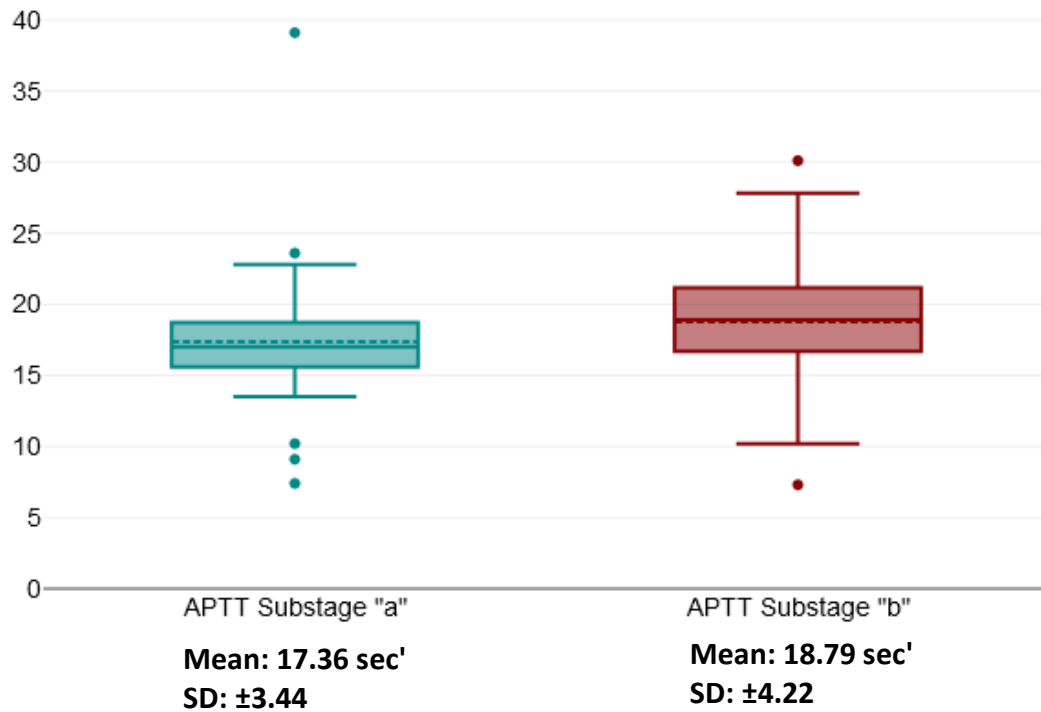


Figure 13 Results of the correlation analysis of dogs included in hemostasis study (Mann-Whitney U-Test - Dog Substage vs CRP; $p<0.001$)

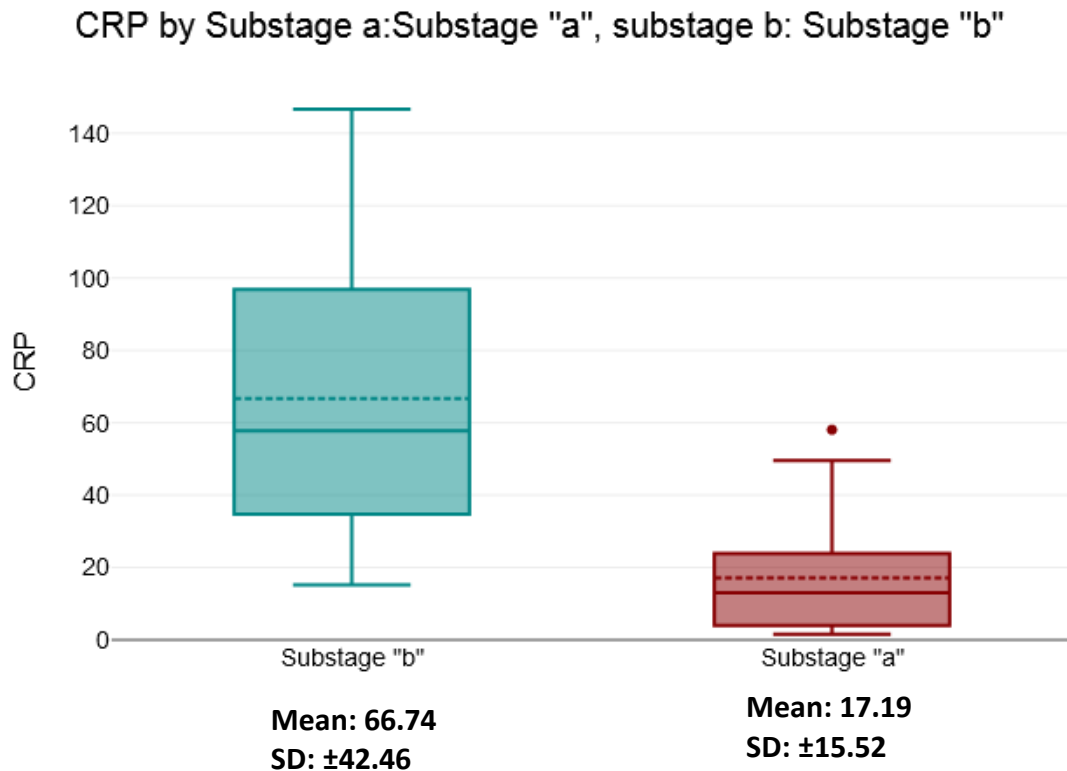


Table 9. Results of the correlation analysis of cats included in hemostasis study (significant correlations – Pearson, $p < 0.05$)

Variable 1	Variable 2	r-values
PTT	aPTT	-0,36731
Gender	aPTT	0,460746
Neutered	Years	0,300565
Lymphocyte abs	PT	0,298625
Creatinine	Gender	0,336853
Creatinine	Neutered	0,324969
P	aPTT	0,337766
P	Gender	-0,6486
P	Neutered	-0,42844
Substage a:1, substage b: 0	PT	-0,42511
Substage a:1, substage b: 0	Neutered	-0,32593
Substage a:1, substage b: 0	Small (1) Large (2) tumor	-0,48795

Figure 14 Results of the correlation analysis of cats included in hemostasis study (Linear regression - Cat PTT vs Lmyph abs; $p = 0.108$, $r = 0.3$)

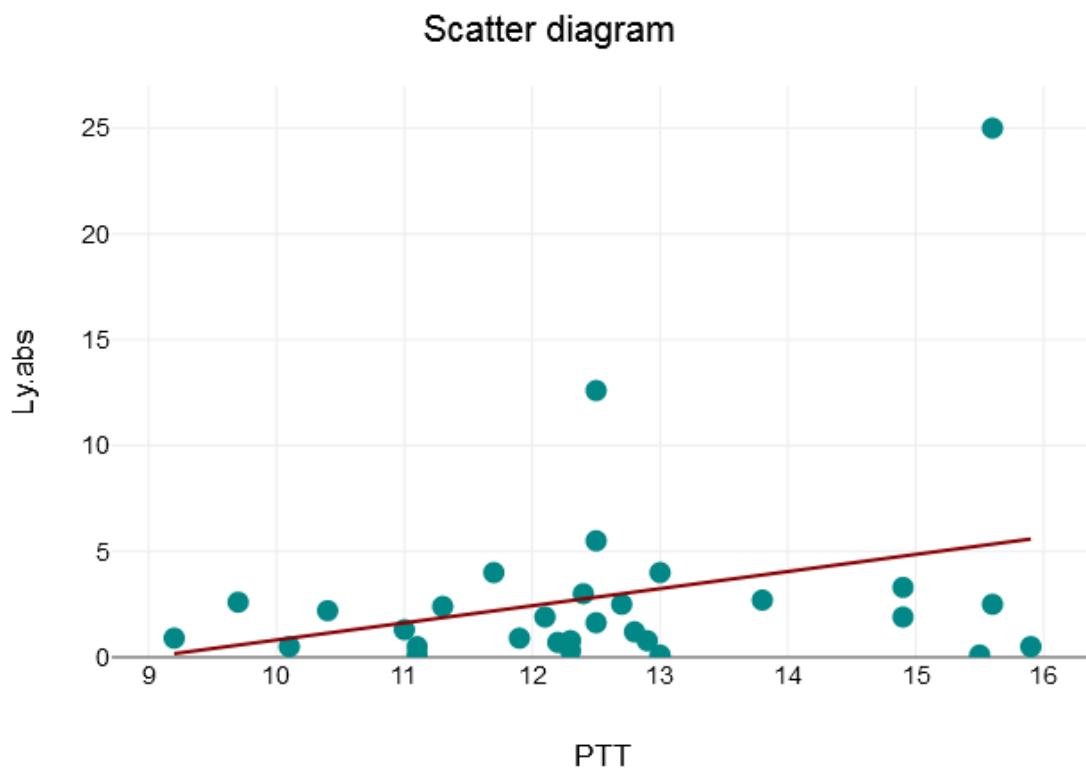
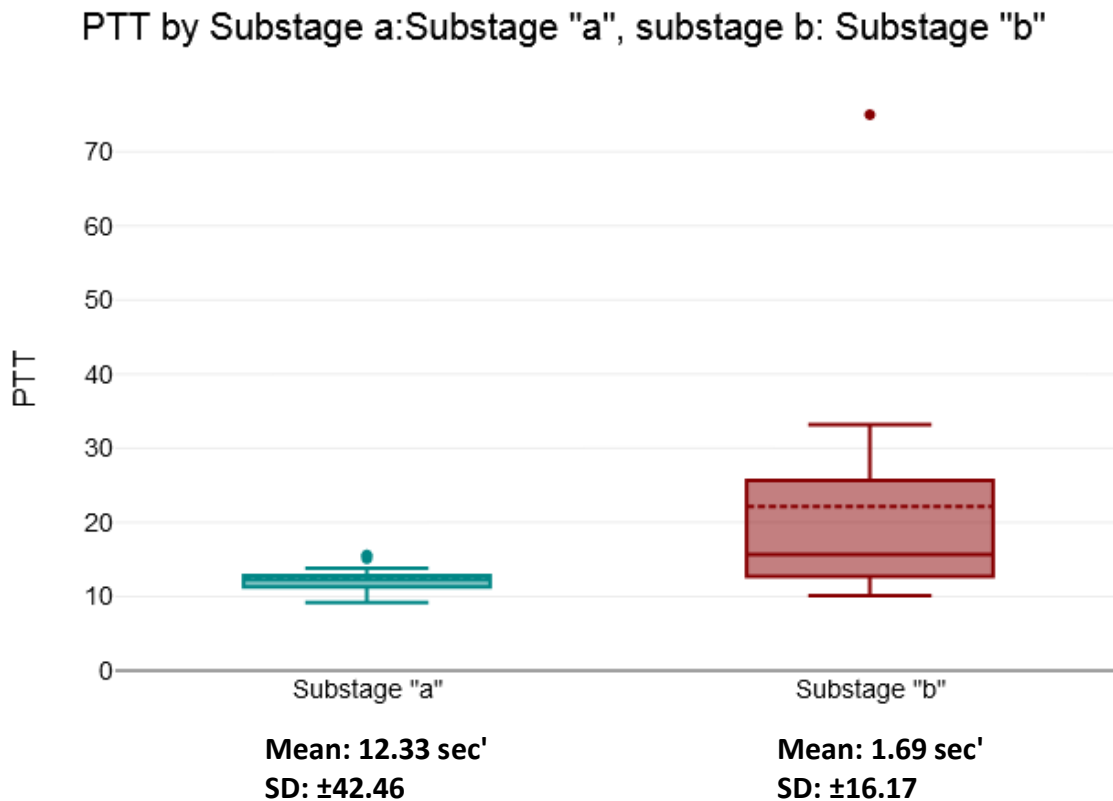


Figure 15 Results of the correlation analysis of cats included in hemostasis study (Mann-Whitney U-Test - Cat Substage vs PTT; $p=0.03$)

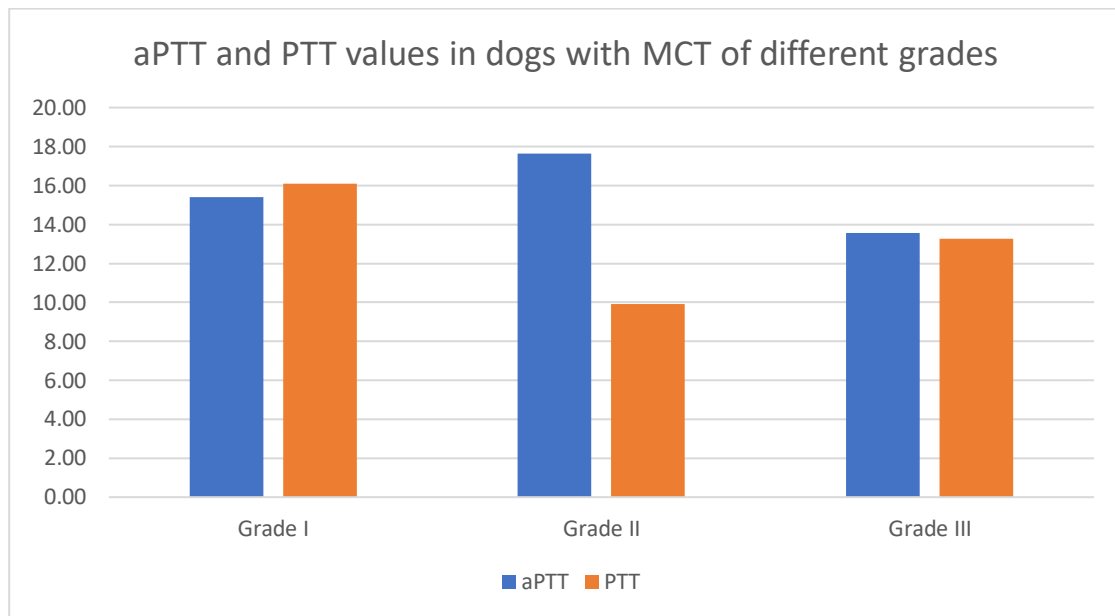


In addition, as shown in Table 10, we analyzed aPTT and PT in different MCT grades and there were no significant differences, but an increase in aPTT in grade 2. Results can be seen in Figure 16.

Table 10 aPTT and PT values in dogs with different grades of MCT (Student's t-test, $p<0.05$)

			aPTT	PTT
Grade I	No: 6	Mean	15,42	16,08
		SD	4,58	9,80
Grade II	No: 17	Mean	17,64	9,91
		SD	1,74	0,96
Grade III	No: 6	Mean	13,55	13,27
		SD	4,45	3,98
Student's t-test p-values	Grade I vs	Grade II	0,33	0,22
Student's t-test p-values	Grade I vs	Grade III	0,53	0,57
Student's t-test p-values	Grade II vs	Grade III	0,10	0,12

Figure 16 aPTT and PT values in dogs with MCT of different grade



7 Discussion

In accordance with our hypothesis, different types of cancers may lead to various coagulation abnormalities, which are associated with different aPTT and PT presentations. While aggressive or advanced cancers may lead to a hypocoagulable state in which prolonged aPTT and PT can be seen, shortened aPTT and PT correlate with a hypercoagulable state potentially linked to systemic inflammatory responses. Moreover, prolongation of either aPTT or PT may indicate the involvement of the intrinsic or extrinsic pathways, respectively. Furthermore, advanced or metastatic diseases may be associated with mixed coagulopathies, presenting fluctuation of aPTT and PT over time. These changes may originate from the tumor itself, the inflammatory response of the body, or the treatment's side effects.

Canine oncology patients were found to be more effected by changes in aPTT, associated with disturbances in the intrinsic and the common coagulation pathways. These changes can be attributed to different reasons, divided into three major groups: consumption of clotting factors, impaired production of clotting factors, or systemic inflammatory response that alters the normal coagulation process. Each condition may influence the balance needed for the coagulation system to work appropriately, leading to changes in aPTT. In regards to inflammatory diseases in dogs, both aPTT and PT values were shown to be altered. This can be explained by the widespread effect of inflammatory diseases on the different coagulation pathways.

According to our study, significant prolongation of aPTT in dogs was caused by inflammatory tumors, lymphoma, leukemia, melanoma and chronic diseases (Table 4). Inflammatory tumors may lead to a systemic inflammatory response involving the activation of cytokines (e.g., IL-6, TNF- α). These, in turn, can activate the coagulation cascade, resulting in the consumption of clotting factors. Inflammatory conditions may also lead to disseminated intravascular coagulation (DIC), which also may lead to clotting factors consumption and prolongation of aPTT. Lymphomas may be associated with paraneoplastic syndromes, which may produce substances that can promote or impair the coagulation process. Moreover, lymphomas can lead to the development of hematological abnormalities such as DIC or venous thromboembolism, resulting in the consumption of clotting factors. Melanomas, similarly to other solid tumors, may lead to prothrombotic states via the release of procoagulant factors. In addition, advanced melanomas may induce an inflammatory response, which can trigger DIC, leading to consumptive coagulopathy. Conditions such as

leukemias or chronic liver diseases may lead to impaired production of clotting factors or systemic inflammatory responses that alter normal coagulation processes. Although aPTT is increased in lymphoma, leukemia, melanoma, and chronic diseases compared to healthy ones, PT was prolonged in other tumor types like hepatic tumors, mast cell tumors, and mammary gland tumors.

Authors found that dogs with lymphoma showed prolonged PT and TT, decreased fibrinogen, increased FDP, and decreased platelet compared with the control group. The effect of disease stage was evaluated separately for dogs with stage II to IV lymphoma and dogs with stage V lymphoma; patients with stage II–IV lymphoma showed no significant differences, while in dogs with stage V lymphoma, a prolongation of PT and TT, a decrease in fibrinogen, an increase in FDPs and a decrease in PLT were found compared with the control group [50]. We could not detect increased PT in lymphoma-bearing dogs, but with other tumor types like mammary gland, mast cell, and hepatic tumors. Authors found according to their study, that there was increased aPTT and PT in 89% (49/55) and 50.90% (28/55), respectively, in the tumor group compared to normal [10], [12]. Other authors found that carcinomas showed increased hypercoagulability, except for mammary gland tumors [12]. While others found hemostatic dysfunction in dogs with mammary gland carcinomas [51].

The increase in aPTT in grade II of MCT (Table 10) can be relevant, although the small number of cases was insufficient for this comparison. However, other authors found that there is a significant alteration of aPTT between the control and mast cell tumor groups ($P = 0.012$) [10]. With respect to prothrombin time (PT) in dogs with different tumors, like MCT, there was no significant difference between tumor-bearing and control dogs ($P > 0.05$). According to our observations, aPTT was also increased in MCT cases compared to normal cases. Grade-influenced hemostatic differences have not been detected yet.

Inflammatory disease caused an increase in both aPTT and PT, too (Table 4, Table 5). It is, however, important that the inflammatory process has several stimulations to the intrinsic and extrinsic pathways [52]. In immune-mediated diseases, such as thrombocytopenia or immune-mediated hemolytic anemia, the body's immune system attacks its own cells. The damage caused to cells like red blood cells, platelets, or clotting factors can lead to various coagulation abnormalities. Moreover, the immune system activation may lead to systemic inflammation, which, as mentioned above, can result in states such as DIC and lead to the consumption of coagulation factors. In addition, immune-mediated diseases may also affect the liver,

indirectly impairing the production of clotting factors. As mentioned above, lymphomas have a widespread effect on the coagulation cascade, both through the extrinsic and intrinsic coagulation pathways, leading to abnormalities in PT and aPTT, respectively. Bening or non-neoplastic tumors are usually not aggressive or invasive. However, they may still lead to chronic systemic inflammation, causing the activation of the coagulation system and the consumption of clotting factors [53], [54].

In cats, significant differences were found in aPTT and PT in abdominal tumors only (Table 6, Table 7). These alternations are likely due to the combination of inflammation, clotting factor consumption, and potential liver involvement. The most common underlying disorders in cats causing hemostatic abnormalities and DIC were lymphoma, other forms of neoplasia, pancreatitis, and sepsis. These conditions suggest the involvement of the extrinsic and common coagulation pathways in cats, leading to prolonged aPTT and PT values [55].

Correlation analyses of variables in dogs (Table 8) revealed that inflammatory processes, marked by CRP values (Figure 10) and monocyte count (Figure 11), are associated with prolonged aPTT values. All other observed variables, like monocyte count, alkaline phosphatase activity, grade of mast cell tumors, and tumor size, are either correlating with the inflammatory marker CRP or with neutrophil and monocyte counts. All indicate the association of the mentioned variables with inflammation. Moreover, tumor sizes correlate with similar inflammatory markers. Inorganic phosphate (P) concentration correlates with tumor sizes due to the great phosphate content of the tumor mass. Mast cell tumor grade correlates with size, too. Although, we observed these findings in our earlier studies. Good general state (substage “a”) correlates with lower aPTT values (Figure 12), lower CRP (Figure 13), monocyte count, alkaline phosphatase activity, and smaller tumor size. These are those descriptive observations that have not been described earlier.

Correlation analysis in cats (Table 9) showed that males have higher aPTT and creatinine. Neutered cats were older. Although insignificant, the higher lymphocytic count was associated with increased PT (Figure 14). Males and neutered cats have higher creatinine values. Inorganic phosphate concentration correlates with the aPTT. This latter can be explained by tissue necrosis leading to phosphate release from the tumor mass, and due to the necrotic process, increased coagulation can be initiated. This can lead to increased coagulability marked by either increased or decreased aPTT. The necrosis rate can influence whether there is an increase in thrombin activation with an early decreased coagulation time

or increased coagulation time at a later stage, which can be phrased as pre-DIC. Male and neutered cats might be bigger and the males are more muscular. That is why the inorganic phosphate value is correlating with the male state. Good general state (substage “a”) correlates with lower PT (Figure 15). It is an important observation that the stage of the disease is associated with increased coagulability, as mentioned by authors examining lymphoma [50]. Similarly to dogs, we could prove in cats that the larger size of tumors is associated with a worse general state (substage “b”).

Our study suggests that aPTT and PT have the potential to assist in predicting, screening, and staging cancer in canine and feline oncology patients, when used in conjunction with other markers. The correlation of these markers with tumor types, stages, and systemic inflammation emphasizes their prognostic significance, particularly in identifying complications such as thrombosis or bleeding. Furthermore, our study reports on stage-related hemostatic alternations in MCT stage II. This tool could be valuable for veterinary oncologists, but it requires further investigation. Nevertheless, the sensitivity and specificity of aPTT and PT might be limited when used alone. Therefore, they should be accompanied by other biomarkers and clinical data for more accurate disease screening and evaluation. Although aPTT and PT are promising tools for monitoring disease progression and systemic effects, further research is essential to refine their application to specific cancers and patient populations.

In conclusion, apart from our important observations about detectable hypercoagulable states with different dog and cat tumors, further studies are required to establish the reliability of these tests as predictive markers for cancer-related coagulopathies in dogs and cats. Moreover, more research is needed regarding additional coagulation markers that may improve the diagnosis and management of coagulopathies in canine and feline oncology patients.

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10 Appendices

UNIVERSITY OF VETERINARY MEDICINE, BUDAPEST
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INTERNATIONAL STUDY PROGRAMS

Student name: Levy Narkis

ANNOUNCEMENT OF THE CHOSEN TOPIC OF THE THESIS

I would like to ask for the permission of the Head of the Department of Clinical Pathology and Oncology, to write my thesis in the following topic advertised and supervised by the Department.

Budapest, 28.08.2023

Levy Narkis

Topic of the thesis:

The study aims to evaluate the correlation between the clotting assays APTT (Activated partial thromboplastin time) and PT (Prothrombin time), in respect to the progression of cancer in canine and feline oncology patients

Title of the thesis:

Evaluation of APTT and PT as prognostic markers in canine and feline oncology patients

Name of the internal supervisor: Péter Vajdovich Vajdovich Péter

Name of the external supervisor:

Péter Vajdovich

I approve:

Signature of the Head of the
Department

Department of Clinical Pathology and Oncology



I hereby confirm that I am familiar with the content of the thesis entitled

Evaluation of APTT and PTT as predictive markers in canine and feline oncology patients



.....

.....

..... written by Narkis Levy.....

(student name) which I deem suitable for submission and defence.

Date: Budapest, 20 day 06 month 2023 year

.....
Vajdovich Péter
..... Supervisor name and signature

Department of Clinical Pathology and Oncology

.....

.....

..... Department




DECLARATION

I hereby declare that the thesis entitled *The use of aPTT and PT as*
predictive markers in canine and feline oncology
patients

is identical in terms of content and formal requirements to the TDK research paper submitted in
..... *2024* (year).

Date: Budapest, *28* day *11* month *2024* year

Narcis Levy 

Student name and signature



Thesis progress report for veterinary students

Name of student: Nancis Levy

Neptun code of the student: EU3VF2

Name and title of the supervisor: Dr. Vajdovich Peter

Department: Department of oncology

Thesis title: Evaluation of APTT & PT as predictive markers
in canine and feline oncology patients

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2024	3	1	preparation for study work	Vajdovich P
2.	2024	3	7	sample procedure, lab work	Vajdovich P
3.	2024	3	21	data collection, methodology	Vajdovich P
4.	2024	3	28	data collection, methodology	Vajdovich P
5.	2024	4	18	data collection, methodology	Vajdovich P

Grade achieved at the end of the first semester: 5 (sds)

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2024	6	25	preparation for writing	Vajdovich P
2.	2024	7	19	literature review	Vajdovich P
3.	2024	9	28	abstract	Vajdovich P
4.	2024	10	12	statistical analysis	Vajdovich P



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5.	2024	10	14	Final work	

Grade achieved at the end of the second semester: **5 (jeles)**

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

signature of the supervisor

Signature of the student:

Signature of the secretary of the department:

Date of handing the thesis in.... **15/10/2024**

