UNIVERSITY OF VETERINARY MEDICINE BUDAPEST
DEPARTMENT OF CLINICAL PATHOLOGY AND ONCOLOGY

MINIREVIEW: BIOMARKERS IN VETERINARY ONCOLOGY – WITH THE FOCUS ON BLOOD BIOMARKERS
A LITERATURE REVIEW

MINIREVIEW: BIOMARKEREK AZ ÁLLATORVOSI ONKOLÓGIÁBAN – KÜLNÖS TEKINTETTEL A VÉRBEN MÉRHETŐ BIOMARKEREKRE
IRODALMI ÖSSZEFOGLALÓ

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ABSTRACT

There are several molecular markers which we determine to prove neoplastic processes in the human and animal body. Biomarkers have been used for clinical diagnostics in human medicine for many years, especially in the diagnostic and further workup of cancer. In the field of veterinary medicine, biomarkers are also used, however lagging a few steps behind its human counterparts. As life expectancy increases, the incidence of cancerous patients is also expected to remain high in the coming years. Consequently, this topic holds significant relevance for future veterinarians who may encounter cancer patients or those who could engage in disease screening and prevention efforts. The primary objective of this thesis is to provide a comprehensive overview of the key parameters that can effectively establish the presence of tumors or their metastasis within the body.

ÖSSZEFOGLALÓ

Molekuláris markerek számos neoplastikus folyamat kimutatására szolgálnak az emberi és az állati szervezetben. A biomarkereket már évek óta alkalmazzák az emberi orvoslás klinikai diagnosztikájában, különösen a rákos megbetegedések diagnózisánál és további vizsgálatánál. Az állatorvosi gyakorlatban is alkalmazzák a biomarkereket, bár az emberi orvoslással összehasonlítva néhány lépéssel lemaradnak. Ahogy az élettartam növekszik, várhatóan a rákos betegek előfordulása is magas marad a következő években. Ennek következtében ez a téma jelentős relevanciával bír a jövőbeni állatorvosok számára, akik rákbetegekkel találkozhatnak, vagy részt vehetnek a betegség szűrésében és megelőzésében. Ezen értekezés fő célja, hogy átfogó áttekintést nyújtson azokról a kulcsfontosságú paraméterekről, amelyek hatékonyan képesek meghatározni a daganatok jelenlétét vagy áttétüket a szervezetben.

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ABBREVIATIONS

ALP – Alkaline phosphatase
cNHL – Canine non-Hodgkin lymphoma
cLBT - Canine lymphoma blood test
CPSE - Canine prostate specific esterase
CEA – carcinoembryonic Antigen
CRP – C reactive protein
cTnI – Cardiac troponin l
ctDNA – Circulating tumor DNA
cfDNA – Circulating free DNA
EGRF - Epidermal growth factor receptor
ELISA – Enzyme linked immunosorbent assay
FNA – Fine needle aspiration
Hapt – Haptoglobin
HSA – Hemangiosarcoma
HPLC – High performance liquid chromatography
IHC – Immunohistochemistry
IL6 – Interleukin 6
IL1 – Interleukin 1
kDa – Kilodaltons
MCT – Mast cell tumor
MGT – Mammary gland tumor
NGS - next-generation sequencing
NHL – Non-Hodgkin Lymphoma
OSA – Osteosarcoma
PCNA - Proliferative cell nuclear antigen
PSA – Prostatic specific antigen
RIA – Radioimmunoassay
RT-qPCR - Reverse transcription-quantitative polymerase chain reaction
SAA – Serum Amyloyd A
TCC – Transitional cell carcinoma
TK1 – Thymidine Kinase 1

INTRODUCTION

Cancer encompasses a diverse range of diseases, each capable of originating in virtually any organ or tissue within the body. It emerges because of abnormal cell growth, where these cells not only proliferate uncontrollably but also breach their customary confines, infiltrating adjacent regions and potentially disseminating to distant organs. This complex process underlines the challenge posed by cancer in the field of medicine. Cancer arises as a result of a series of genetic and epigenetic changes, among them the inactivation of tumor suppressor genes and the promotion of activation of proto-oncogenes to oncogenes. [1]

Cancer is a prevalent health concern in the field of veterinary medicine, affecting a substantial number of animals across various species. Veterinary oncology encompasses a broad range of cancer types, with two of the most prevalent being lymphoma and mammary gland tumors. Treatment options in veterinary medicine include surgery, chemotherapy, radiation therapy and immunotherapy, to mention some. The primary goal of veterinary oncology is to improve the quality of life of the patients, even if a complete cure may not be achievable. In veterinary medicine, cancer stands as the leading cause of death in pets aged 10 and above, with 23% of all canine fatalities attributed to this disease. [2] Put differently, cancer is both pervasive and life-threatening, underscoring the critical significance of understanding how to treat and diagnose these patients. Hence, biomarkers serve as indispensable tools in both veterinary and human medicine, with the primary objectives of enabling early detection, precise diagnosis, personalized treatment, and continuous monitoring of disease progression.[1]

In this mini review, the discussion will focus on biomarkers used in different cancer types such as Lymphoma, Osteosarcoma, mammary gland tumor, hemangiosarcoma, canine prostate cancer and urinary bladder cancer. The discussion will encompass various cancer types, providing detailed insights into the clinical utilization of different biomarkers for the diagnosis, prediction, and treatment of patients, whereby influencing the disease´s progression. Ultimately, a summary will highlight the key parameters which are suitable to prove tumor existence or its spreading within the body.

DEFINITION

A biomarker, which is a shortened term for biological marker, is a measurable and objective indicator that defines a biological process. This measure is typically a molecule (e.g., a hormone, protein, metabolite, or gene) that is detectible in body fluids such as blood or saliva, and in general reflect normal biological processes or pathological changes within an organism. Put simply, a biomarker refers to any substance, structure or process that can be measured to anticipate the occurrence of a particular outcome of disease.[3]

The utilization of biomarkers is a growing trend in diagnostics both in human and veterinary medicine, which have been amplified especially over the last 10 years. Although biomarkers play a huge role in oncology, it can be used in a variety of fields for specific risk assessment, diagnosis of diseases, prognostication, monitoring of treatment response, and detection of relapse. Three types of biomarkers are accessible: prognostic markers, predictive markers, and diagnostic markers. The different markers can aid in cancer screening, inform treatment decisions, and guide the timing of intervention. [4] Nevertheless, at present, there are few tumor markers that are perfectly developed and understood in veterinary practice, mirroring the situation in human oncology. Anticipated in the near future is the development of marker panels and algorithms that hold the potential to significantly improve diagnostic accuracy. Furthermore, if tumor markers can predict treatment outcomes early in the planning stage, it can positively impact the quality of life and economic considerations of veterinary care. Additionally, detecting microscopic disease before clinical relapse can lead to earlier and potentially more successful rescue therapy, especially for rapidly growing tumors that may be more responsive to treatments like chemotherapy.[5]

The aforementioned tumor marker stands as a promising candidate for integration into veterinary oncology, albeit its full realization remains pending. Nevertheless, there exist a range of biomarkers that can contribute to the diagnosis and therapeutic strategies in veterinary practice. Additionally, it is plausible that in the forthcoming years, this marker may reach a state of full development and accessibility, aligning with its intended clinical application. [6]

BIOMARKERS USED IN LYMPHOMA

Lymphoma is one of the most common cancer types in dogs and cats, accounting for 15-20% of new cancer diagnoses in dogs. [7] It can be categorized into four different types, where multicentric (systemic) lymphoma is by far the most common. The disease usually presents as painless swellings in the peripheral lymph nodes, and diagnosis can often be complicated by different lymphoid proliferations in organs such as the alimentary tract and bone marrow. For a definite diagnosis, a fine needle aspiration (FNA) is taken from the affected tissue, or a biopsy and histological examination. T-cell lymphoma generally exhibit poorer prognosis and shows greater resistance to chemotherapy than B-cell lymphoma.[8] To determine whether the malignancy is from B- or T-cell origin, an immunophenotyping is recommended. Lymphoma is more commonly diagnosed in middle-aged to older dogs, and some breeds are more predisposed than others.[7]

Patients with lymphoma seems to response relatively well to chemotherapy. However, high-grade lymphoma consists of malignant cells with a high mitotic rate resulting in the disease developing rapidly. According to Purdue university College of veterinary Medicine and their canine lymphoma research, the second round of treatment is less effective, as the patient's immune system will start the development of resistance to the chemotherapy.[7][9]For this reason, close follow-up and monitoring of these patients is highly important. One of the gold standard examinations for close monitoring of a lymphoma diagnosed dog, includes the palpation of the lymph nodes. As this can be subjective and the veterinarian requires a good experience, it does not always detect the disease.[7] The other, and more precise way of detection are the biochemical methods, which looks for circulating biomarkers of the disease. Previously results have shown that C-reactive protein and Haptoglobin could be included in the differential diagnosis of dogs with severe acute lymphatic neoplasia, such as high-grade lymphoma. [10, 11]

Non-blood biomarkers of lymphoma

The non-blood biomarkers and diagnostic methods complement blood biomarkers in veterinary medicine and help provide a more comprehensive understanding of the presence, type, and extent of lymphoma in animals. The choice of diagnostic method often depends on the specific clinical presentation and the need for accurate diagnosis and staging.

Histopathology, immunohistopathology, fine needle aspiration, moreover, flow cytometry, PCR-techniques and western blot analysis of tissues are valuable diagnostic techniques in veterinary medicine, particularly in the context of lymphoma. Even though they are not categorized as markers themselves, they play a crucial role in the diagnosis and assessment of this type of cancer. Immunophenotyping is a non-blood biomarker used in lymphoma, which involved the analysis of specific cell surface markers or antigens on lymphoma cells to determine their origin and type. This technique can help differentiate between B-cell and T-cell lymphoma, which is crucial for treatment decisions. Furthermore, certain molecular biomarkers, such as gene mutations or overexpression or specific proteins, can provide insights into the aggressiveness and behavior of lymphoma. The typical tool for the analysis of such issues can be determined by the PCR for receptor rearrangement (PARR).[12] Moreover, the presence of certain mutations like P53 or Bcl-2 may have prognostic implications.[13]

Blood biomarkers of lymphoma

Blood biomarkers, such as haptoglobin (HAPT) and C-reactive protein (CRP), are emerging as valuable tools in the realm of small animal medicine, particularly in the context of lymphoma. [7] In this exploration, theoretical concept, scientific findings, clinical significance, limitations, availability, and methods, as well as future perspectives of these biomarkers are examined.

HAPTOGLOBIN

Hapt is an acute phase protein primarily synthesized in the liver. It is a glycoprotein, meaning it is a biological molecule consisting of a protein covalently bonded to one or more carbohydrate chains. Its molecular weight varies, but its typically around 90kDa. [14]

Synthesis of Haptoglobin

As hapt is synthesized primarily in the liver, it makes it one of the major acute phase proteins produced by hepatocytes. The synthesis and secretion of hapt are tightly regulated in response to various physiological and pathological stimuli, with inflammation and tissue injury being the primary triggers. Hepatocytes, which are the main parenchymal cells of the liver, are the primary site of hapt production. [15]The synthesis of hapt occurs within the liver cells in response to specific signaling pathways that are activated during inflammatory processes. The

production of hapt is significantly upregulated in response to a range of inflammatory mediators, including interleukin-6 (IL-6), interleukin-1 (IL-1), and tumor necrosis factor-alpha (TNF-a). These cytokines are released during inflammatory responses to various stimuli, such as infections, tissue injury, or neoplastic growth. The genes encoding hapt are subject to transcriptional regulation. When hepatocytes receive signals from inflammatory mediators, these genes become more active, resulting in increased synthesis of hapt mRNA. Once the mRNA of hapt is synthesized, the protein is translated in the endoplasmic reticulum and then modified through glycosylation in the Golgi apparatus. The post-translational modification results in the addition of carbohydrate chains to the hapt protein, enhancing its stability and function. After synthesis and glycolisation, hapt is secreted into the bloodstream. It circulates as an acute phase reactant in its soluble form and is readily available to bind to free hemoglobin, limiting the oxidative damage of hemoglobin in circulation. [16]

The tights regulation of hapt synthesis allows it to be an essential component of the hosts innate immune response. By sequestering free hemoglobin and minimizing oxidative stress, hapt plays a vital role in protecting tissues and organs from damage caused by hemolysis and inflammation. Its synthesis is part of the intricate web of host defense mechanisms aimed at maintaining homeostasis during various pathological conditions. Research into the synthesis of hapt and its regulation continues to provide insight into the broader field of host responses to inflammation and tissue injury. Understanding these processes at the molecular level can have implication for disease diagnosis and therapeutic interventions, including the monitoring of conditions such as lymphoma. [16]

Function

In its normal state, hapt plays a crucial role in binding free hemoglobin, which is released from damaged red blood cells, preventing the oxidative damage of hemoglobin in circulation. This protects tissues from oxidative stress. Pathologically, hapt levels rise significantly in response to inflammation or tissue injury, making it a valuable marker for various diseases, including cancer. Elevated levels are indicative of an acute phase response and ongoing inflammatory processes.[17]

History

The history of hapt dates back to the early 1930s when its existence was first recognized by Polonovski and Jayle. Researchers initially identified a substance in the serum that had the remarkable ability to bind free hemoglobin. This discovery was particularly intriguing because it prevented the loss of hemoglobin thorough the kidneys into the urine, preserving the body´s iron and minimizing oxidative damage. [18]

Analytical method

Hapt levels can be determined using various analytical methods, including spectrophotometry, ELISA, and HPLC. These methods offer quantitative measurements of hapt in biological samples.[14] The normal range of hapt varies across species, in serum samples from healthy cats the normal range is less than 3mg/ml, whereas in healthy dogs it´s generally < 2,7mg/ml. The Feline & Canine Haptoglobin ELISA test offers a sensitivity of 0,051mg/ml. Both serum, plasma and cell culture is accepted sample types.[19]

Diagnostic importance

Elevated hapt levels in the blood can serve as a valuable indicator of ongoing inflammation or tissue damage. In the context of lymphoma, hapt is used as a non-specific marker in the commercial canine lymphoma blood test cLBT for disease monitoring. Scientific findings have highlighted the utility of hapt in the monitoring remission and relapse during lymphoma treatment.[11] In one study, CRP and hapt levels were assessed in dogs with different types of blood malignancies, including high-grade lymphoma (16 dogs), acute lymphoblastic leukemia (11 dogs), chronic lymphocytic leukemia (7 dogs), and multiple myeloma (8 dogs). Additionally, 25 healthy dogs were used a control group. Hapt was measured based on its ability to bind hemoglobin. In comparison to the healthy control dogs (median concentration of 0.59g/L), dogs with acute lymphoblastic leukemia had the highest haptoglobin levels (6,8 g/L, significant with P <0.0001), followed by dogs with malignant lymphoma (3,8g/L, significant with P < 0.0001), and chronic lymphocytic leukemia (3,2g/L, significant with P = 0,0008). This finding suggests that dogs experiencing severe and acute lymphatic neoplasia exhibit an increase in hapt levels. This underscores the importance of considering these conditions as potential cause when interpreting elevated blood levels of hapt. [20]

Species differences

Hapt is not confined to a single species: instead, it is a widely conserved protein present in many mammals, including dogs, humans, cats, cattle, and various other animal species. This broad distribution underscores its evolutionary significance in host defense mechanisms against oxidative stress and inflammation. While the fundamental function of hapt remains consistent across species, there are notable differences in its structure and isoform. In some animals, such as in humans and dogs, hapt exists in different forms, known as isoforms, which are the result of genetic variations. [18]These isoforms can impact the way hapt is functioning and may have diagnostic implications in specific diseases.[16]

Present and future

Currently, hapt is a valuable adjunct marker for monitoring remission and relapse during lymphoma treatment. Future research may further refine its diagnostic and prognostic significance in lymphoma and other diseases. For instance, current research endeavors may investigate the potential of hapt to predict therapeutic responses and develop personalized treatment approaches for individuals diagnosed with lymphoma.

C-REACTIVE PROTEIN

Description

C-reactive protein (CRP) is another acute phase protein. It's a pentameric protein, meaning it consists of five subunits arranged in a circular pattern. Each subunit is a glycoprotein, which means it contains carbohydrate (sugar) molecules attached to the protein structure. CRP has a molecular weight of approximately 115kDa. Under typical, non-inflammatory conditions, CRP levels in the blood are very low, often undetectable or at trace levels. This is because CRP is produced in response to specific stimuli, such as inflammation. [21]

Synthesis

The liver is the primary site of CRP synthesis. Hepatocytes respond to pro-inflammatory signals, such as IL-6 and IL-1, which are released in response to various inflammatory triggers. Upon exposure to these pro-inflammatory cytokines, hepatocytes initiate the synthesis of CRP. [21]Acute phase proteins are a group of proteins whose levels in the blood increase rapidly in response to inflammation or infection. The production of CRP is part of the acute-phase response, which is a systemic reaction of the body to a variety of insults, including infection, trauma, surgery, and inflammatory conditions. CRP is rapidly synthesized by hepatocytes in

response to the inflammatory stimulus, and it is released into the bloodstream within hours. The increase in CRP levels in the blood is one of the earliest measurable indicators of the acute-phase response. CRP plays a role in the innate immune response. It can bind to various molecules associated with damaged cells or pathogens, such as phosphorylcholine on the surface of bacteria. By binding to these targets, CRP can activate the complement system, which is an integral part of the immune response that helps eliminate pathogens and damaged cells. The activation of complement can facilitate the removal of pathogens by phagocytic cells and contribute to the overall immune defense against infection. [22]

Function

CRP serves several distinct physiological roles in the body. It is part of the innate immune response, the body´s first line of defense against infections and other threats. In this capacity, CRP contributes to immune surveillance and early responses to potential dangers. It also recognizes pathogens and damaged cells, as CRP can bind to a variety of molecules present on the surface of damaged or infected cells, one example is the phosphorylcholine mentioned previously. Furthermore, CRP can activate the complement system, a complex cascade of proteins involved in the immune response. Activation of complement can have several effects, including the recruitment of immune cells to the site of infection or injury and the clearance of pathogens and damaged cells. When CRP levels is increased in the bloodstream, we talk about its pathological function. In other words, elevated CRP levels in the blood are indicative of pathological conditions, including infections, autoimmune diseases, tissue damage, and various inflammatory states. It is used in both human and veterinary medicine to assess the presence and severity of inflammation.[11]

History

CRP was initially identified by Tillett and Francis in 1930. The name “C-Reactive protein” originated from its discovery as a substance in the serum of individuals with acute inflammation, demonstrating reactivity with the “c” carbohydrate antigen found in the pneumococcus bacterial capsule.[23]

Analytical method

CRP levels can be measured using various analytical methods, including ELISA, nephelometry, and high-sensitivity CRP assays, which provide highly sensitive quantitative measures.[23] Normal ranges of CRP in dogs is usually below 10mg/L, however some healthy individuals

might have higher values, up to 25mg/L.[24]. Based on the analysis of the ROC curve, a CRP threshold of 54.1 mg/L serves as an indicator of advanced-stage canine lymphoma, offering the potential to be employed as a biomarker for forecasting cancer spread.[25]

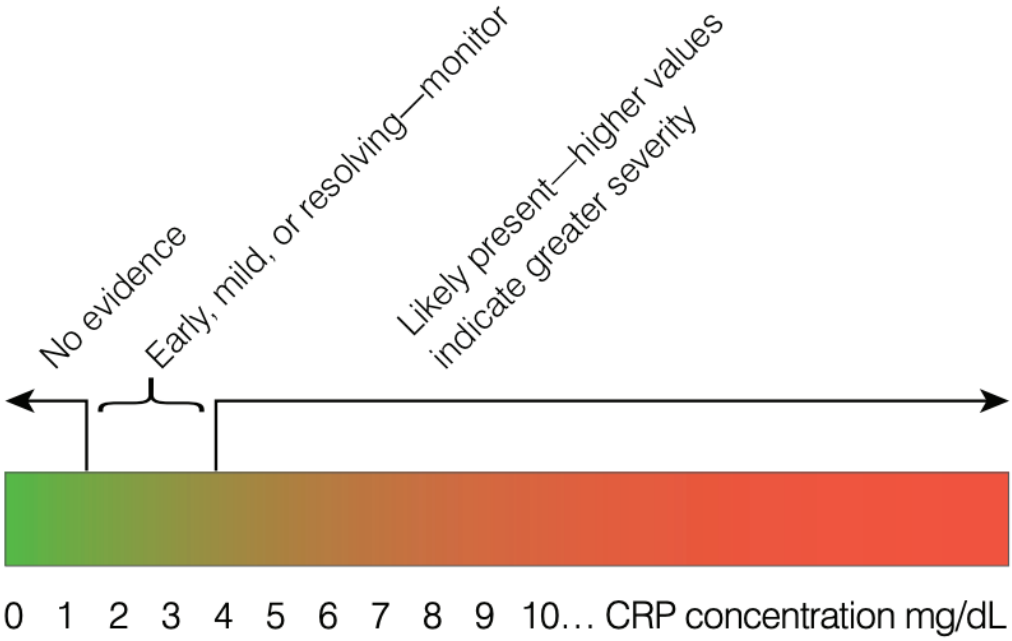

Figure 1: Interpretation of canine CRP. Please note that concentration here is given in mg/dL. With concentrations <1mg/dL, systemic inflammation is doubtful. Concentrations above 3mg/dL however, systemic inflammation is likely. Patients with values ranging from 1-3mg/dL should be monitored as it could be sign of an early or resolving inflammation.[26]. Advanced-stage canine lymphoma can be indicated at 5,41mg/dL, making it a biomarker to predict cancer dissemination. [25]

Diagnostic importance

CRP is a valuable marker for monitoring inflammation and response to treatment, including in lymphoma cases. Elevated CRP levels are often associated with lymphoma activity and other inflammatory processes. Scientific findings demonstrate that CRP can differentiate between dogs in remission and those with recurring lymphoma before clinical signs manifest, aiding in early detection and intervention.[11]

Species differences

CRP is present in various species, making it a valuable marker in veterinary and human medicine. The acute phase proteins vary among the different species, and while CRP is

commonly known in dogs and humans, horses and cats for instance have serum amyloid A as an acute phase protein.[27]

Species	Acute phase proteins
Bovine	SAA, CRP, hapt
Canine	SAA, CRP, hapt
Caprine	SAA, hapt
Equine	SAA hapt
Feline	SAA, hapt
Human	CRP, SAA, hapt
Rabbit	CRP, SAA, hapt
Rat	CRP, hapt

Table 1: acute phase proteins in different animal species, for a better understanding of the variations. [27]

Present and future

CRP is a valuable tool for monitoring remission and recurrence in lymphoma patients. Its clinical utility extends to other diseases marked by inflammation. Ongoing research aims to enhance the specificity and diagnostic accuracy of disease monitoring and treatment guidance. Further research may explore its potential in predicting therapeutic responses and optimizing individualized treatment strategies in lymphoma patients.

SENSITEST

HAPT and CRP, both acute phase proteins, are integral to the commercial canine lymphoma blood test (cLBT) known as SENSITEST. These proteins are nonspecific markers, initially used for assessing various disease processes, including cancer. When combined into an algorithm, they offer diagnostic aid for lymphoma. The cLBT excels in detecting relapse even before physical signs emerge, making it a valuable tool for monitoring remission during and after treatment.[11]

In a study involving 57 dogs undergoing lymphoma chemotherapy, the cLBT effectively monitored remission and recurrence, demonstrating its utility in differencing dogs in remission

from those with recurring disease before lymphadenopathy appears (Lee J et al, 2017). It also showcased prognostic potential, with dogs achieving lower cLBT scores during treatment experiencing longer survival times. The cLBT grades remission status, aiding in clinical decision making. The cLBTs clinical significance lies in its ability to provide early relapse detection and prognosis assessment. However, it´s important to note that these biomarkers are not specific to lymphoma or cancer. Thus, their use should complement other diagnostic tools, and consultation with qualified oncologists is advisable when interpreting results. [11]

The cLBT offers a rapid turnaround time of 24 hours for results. Avacta provides updates graphical representations of a dog's disease status with each sequential sample, aiding in treatment monitoring. The grading system allows for clear categorization of disease status, aiding in clinical decision support. Future perspectives includes refinement, as ongoing research may refine these biomarkers and improve their specificity for lymphoma, enhancing diagnostic accuracy. [11]

In conclusion, HAPT and CRP biomarkers, as exemplified by the cLBT, offer promising insights into lymphoma diagnosis, monitoring, and prognosis in small animal medicine. While not standalone diagnostic tools, they provide valuable adjuncts in clinical decision-making. As our understanding of these biomarkers and disease processes evolves, their role in small animal oncology is likely to expand and become more refined. [11]

Dysregulation of cytokine response

The University of Lisbon performed a study of canine multicentric lymphoma (cNHL) and its potential as a model for studying lymphoma in humans. The research aims to better understand cytokine regulation in cNHL, which is a type of non-Hodgkin lymphoma (NHL) in dogs. NHL is a common cancer in both dogs and humans, and the similarities between cNHL and human NHL make it a valuable model for developing new immunotherapies. The study involved the creation of a multicentric cNHL biobank and the analysis of cytokine mRNA profiles in tumor tissue and circulating blood cells of affected dogs compared to healthy dogs. Cytokines are molecules that play a crucial role in the interaction between tumors and their microenvironment. In human NHL, cytokines are important biomarkers for treatment response and prognosis, but their role in cNHL is less understood. [28]

The result of the study reveals dysregulation of cytokine mRNA expression in both tumor tissue and the systemic response in cNHL. Specifically, there is a downregulation of certain cytokine responses within the tumor microenvironment, and immunosuppression is evident in the systemic response. Notably, the concentration of IL-10, a cytokine, is significantly increased in cNHL. The study also identifies correlations between cytokine levels and hematological parameters in dogs with cNHL. Additionally, baseline pretreatment levels of IFN-gamma mRNA in tissue are found to be predictive of a favorable response to chemotherapy. In conclusion, the research shows that cNHL exhibits dysregulation in both local and systemic cytokine responses. This information could have implications for diagnostic, prognostic and therapeutic purposes in veterinary oncology, adding value to comparative oncology efforts. [28]

In summary, while individual biomarkers and panels hold potential, they are not flawless tests and should not be solely relied upon in isolation. In veterinary medicine, there is ongoing research to determine the best ways to utilize these assays for cancer patients. The optimal applications of these tests are still being studied and refined as we gain a deeper understanding of complex biomarkers and disease processes, which cannot be adequately represented by a few markers alone. While most of the biomarkers are not lymphoma-specific, they offer early relapse detection and prognostic assessment. The cLBT test offers rapid results, graphical representation of disease status, and clear categorization, facilitating clinical decision support. The dysregulated cytokine response has diagnostic, prognostic and therapeutic implications in veterinary oncology, cont

BIOMARKERS USED IN OSTEOSARCOMA

Osteosarcoma is an aggressive neoplasm of the bone which is characterized by the production of Osteoid. Clinical symptoms often include lameness, swelling at the site and pain, with forelimbs more commonly affected then the hindlimbs. Diagnosing the patient include physical examination and radiography of the lesion. Standard treatments include limb amputation, chemotherapy and palliative therapy which in the last couple of years has shown an increase in survival rates, however the mortality rate and development of lung metastasis still remains high. Compared to humans, canines have a remarkable higher incidence per year (13,9/100,000 per year compared to 1,2/100,000).[29]

Canine osteosarcoma is a predominant malignant bone tumor mainly affecting large breeds. It exhibits significant heterogeneity in terms of location, metastasis, radiographic presentation, histopathological subtypes, and response to treatment. Dogs typically present with lameness or bone fractures, and the disease often occurs in weight-bearing long bones but can also affect axial and extra-skeletal sites. OSA is characterized by its highly aggressive nature, with local skeletal destruction and frequent metastases, primarily to the lungs. The current gold standard for diagnosis involves histopathological examination, which can vary in appearance and grading.[30]

Early diagnosis and accurate prognostication are paramount for effective treatment planning. Biomarkers have emerged as invaluable tools in this quest, shedding light on disease mechanisms and guiding clinical decisions. In this chapter I discuss biomarkers for osteosarcoma, exploring their theoretical foundations, scientific applications, clinical significance, limitations, availability, and methods. Biomarkers for osteosarcoma in dogs and cats have their origins in the molecular underpinnings of cancer.

Non-blood biomarkers in osteosarcoma

Non-blood biomarkers used in osteosarcoma in veterinary medicine help in diagnosing, monitoring, and understanding this aggressive cancer. Anaplastic lymphoma kinase (ALK) is a cell surface receptor protein, and its overexpression or rearrangement has been associated with certain canine osteosarcomas. Detectible ALK alterations though immunohistochemistry or molecular techniques can help identify specific subtypes and guide treatment decisions.

[31]Another non-blood biomarker is the Ki-67 which is a nuclear protein that plays a role in cell proliferation. It is often measured though immunohistochemistry to assess the rate of cell decision in tumor samples. High Ki-67 expression is associated with more aggressive osteosarcomas and may influence treatment choices. Furthermore, tumor protein 53 (P53) is a tumor suppressor that helps regulate cell growth. Mutations in the P53 gene are common in osteosarcoma and can be detected using immunohistochemistry or molecular techniques. P53 status can provide prognostic information and guide treatment planning.[29]

Blood-biomarker in osteosarcoma

While diagnostic imaging and tissue biopsies play an important role in confirming osteosarcoma, blood biomarkers have emerged as a valuable and non-invasive adjunct in the diagnostic workup. Different blood-biomarkers such as ALP, LDH, CPR, osteocalcin, beta2microglobulin, and TK1 provide valuable insights to guide treatment decisions and improve the management of osteosarcoma cases.[32]

ALKALINE PHOSPHATASE

Description

Alkaline phosphatase (ALP) comprises a group of enzymes that facilitate the hydrolysis of phosphate esters under alkaline conditions. It exists in several isoforms, with varying molecular weights, ranging from 70-150 kDa. In the context of osteosarcoma, the bone-specific isoform of ALP is often of particular interest.[33]

Synthesis

ALP is synthetized in different organs. The liver is one significant source of ALP production. Hepatocytes generate and release ALP into the bloodstream. Hepatic ALP is involved in various metabolic processes and plays a role in dephosphorylating compounds in the liver. In the bones, osteoblasts also synthesize and secret a specific form of ALP known as bone-specific ALP. This isoform is instrumental in bone mineralization and plays a pivotal role in the formation of healthy bone tissue. The small intestine is another site of ALP production. Intestinal ALP is essential for the absorption of nutrients, particularly phosphates and fatty acids, from the diet.

It contributes to the regulation of intestinal pH and is involved in various digestive processes. During pregnancy, the placenta produces ALP, which aids in the transport of nutrients between the mother and the developing fetus. Placental ALP is part of the alkaline phosphatase system in the body.[34]

In context of bone health and osteosarcoma, the bone-specific ALP is of particular interest. Osteoblasts release this isoform into the bloodstream during periods of increased bone turnover and activity, such as bone growth, repair, or pathological conditions like osteosarcoma. High levels of bone-specific ALP can be indicative of increased bone metabolism, including the growth of bone tumors like osteosarcoma.[35]

Function

ALP is an enzyme with several isoforms that play essential roles in normal physiological processes. Its primary normal functions include bone mineralization: one of the key functions of ALP is its involvement in bone mineralization. In this context, ALP, particularly the bone-specific isoform, is crucial for the formation of healthy bone tissue. Osteoblasts release ALP during bone growth and repair, and it helps in the deposition of minerals like calcium and phosphate, in the bone matrix. Intestinal ALP is essential for the absorption of nutrients, including phosphates and fatty acids, from the diet. It plays a role in maintaining the balance of pH in the intestines and contributes to digestive processes. Hepatic ALP produced by the liver, is involved in various metabolic processes, including the dephosphorylation of compounds. Elevated ALP levels can indicate underlying pathological conditions, and ALP is often used as a marker of disease or tissue damage. Pathological functions of ALP include bone disorders including osteosarcoma, liver disease, intestinal issues, placental complications or metabolic disorders. Understanding the balance between the normal and pathological functions of ALP is crucial in the clinical interpretation of ALP measurements. [35]

History

In 1923, Robert Robison, PhD, first identified alkaline phosphatase in London. His 1932 research conducted in New York expanded on his initial hypothesis, suggesting that ALP played a role in skeletal calcification by releasing inorganic phosphate (Pi) for the formation of hydroxyapatite crystals, potentially through the involvement of unidentified factor that influenced this process.[36]

Analytical method

ALP levels in blood samples are typically determined thorough enzymatic assays. These assays involve the use of specific substrates that react with ALP to produce a measurable product. Various methods can be employed, including spectrophotometric assays, ELISA and enzymatic tests. These techniques offer quantitative measurements of ALP activity in biological samples. [35] The references range of total alkaline phosphatase vary across age groups in dogs. For dogs aged 1-2 years, the normal range is 36-128U/L, for those aged 2-3 years, it is 16-106U/L, for the age group of 3-7years, it is 0-235U/L, and for dogs aged 7 years and above, the reference range is 0-251u/L. [37] In a study involving 61 dogs with appendicular osteosarcoma, serum samples were collected both before and after treatment (chemotherapy), to measure total ALP activity and its constituent bone, liver, and corticosteroid-induced isoenzymes. The relationship between ALP activities and survival was analyzed using cow proportional hazard regression and Kaplan-Meier log rank analysis. The results showed that mean total ALP, bone-ALP, and liver-ALP activities decreased significantly after treatment. Additionally, dogs with normal total ALP and liver ALP activities before treatment had significantly longer survival times compared to those with increased activities (P .001 and .003 respectively). Specifically, dogs with normal total ALP activity have a median survival of 12,5 months, while those with increased total ALP has a median survival of 5,5 months. Dogs with normal bone ALP activity has a median survival of 16,6 months, whereas those with increased bone ALP has a median survival of 9,5 months. These findings suggest that ALP activity can be a valuable factor for stratifying randomization in future clinical trials and tailoring chemotherapy based on individual patient needs. The study also found that the strength of the relationship between the pre-treatment ALP activities and survival can be gauged from the results of relative risk analysis. It reveals that with each 100U/L increase in total ALP or bone ALP activity, the risk of mortality during any specific time interval rises by approximately 25%. [37]

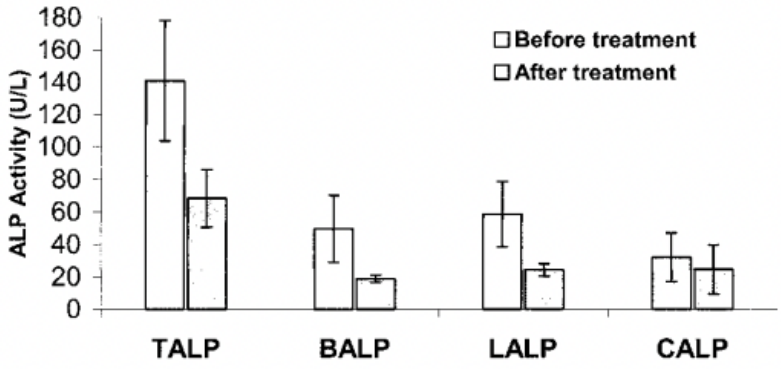

Figure 2 illustrates the average activities of total ALP (TALP) and its individual isoenzymes

both before and after treatment. The activities show a notable decrease following treatment. [37]

Diagnostic importance

One of the diagnostic importance of ALP is the tumor-associated elevations. Osteosarcoma can lead to release of ALP from tumor cells and the bone matrix into the bloodstream. As a result, elevated ALP levels in blood serum can be indicative of the presence of osteosarcoma. Another diagnostic importance is the differential diagnosis, as ALP is a valuable tool for distinguishing between benign and malignant conditions. Benign bone diseases, such as bone cysts or fractures, typically do not result in a significant increase in ALP levels, while OSA and other malignant bone tumors often lead to notably elevated concentrations. Furthermore, ALP levels can also serve as a marker to monitor the response to OSA treatment. Following surgical resection of the tumor or chemotherapy, a decrease in ALP levels can be indicative of a positive response to therapy. Conversely, rising or persistently high ALP levels may suggest treatment resistance or disease progression. In addition, ALP levels can carry prognostic significance in OSA cases. High baseline ALP levels before treatment may be associated with a poorer prognosis, while a decrease in ALP during treatment can be a positive prognostic indicator. Lastly, elevated ALP levels may also be associated with the presence of metastases in OSA, as the tumor has the potential to spread to the lungs and other sites. Serial monitoring of ALP can aid in the early detection of metastatic disease.[35]

Species differences

Species differences in alkaline phosphatase levels can occur and are primarily attributed to variations in normal reference ranges for different species. These differences are observed in the context of ALP measurements in blood tests, and they are important to consider when assessing ALP levels in osteosarcoma. Different species such as humans, dogs, and cats have distinct normal reference ranges for ALP in their blood. Therefore, ALP levels considered normal in one species may be higher or lower than the normal range in another. In addition to reference ranges, the source of ALP production may also vary. In humans, ALP is produced in various tissues, including the liver, bone and intestine. In dogs, liver and bone ALP isoenzymes are typically the most prominent sources, while in cats, ALP production primarily occurs in the liver. [38]These tissue-specific differences can impact ALP levels. Furthermore, age and growth can also influence ALP levels in animals. Young, growing animals may naturally have

higher ALP levels due to bone growth and development, which can sometimes complicate the interpretation of ALP levels. [31]

Present and future

ALP remains a valuable diagnostic and monitoring tool in the assessment of osteosarcoma. Currently, it is widely used to aid in the diagnosis of this primary bone tumor and to monitor treatment response. However, it´s important to note that while ALP is a valuable diagnostic and monitoring tool for osteosarcoma, it is not specific to this condition and can be elevated in other diseases affecting the bones and liver. Therefore, ALP results are typically interpreted in conjunction with clinical and imaging findings to arrive at a comprehensive diagnosis and treatment plan for OSA in both human and veterinary medicine. Overall, the present and future of ALP in osteosarcoma involve a continued role as a diagnostic, monitoring, and prognostic tool.[33]

Osteopontin (OPN) is an extracellular matrix protein originated from the bone matrix with a variety of different functions including cell adhesion and immunomodulation. It was found that the overexpression of this protein was linked to tumor progression. [39] Biomarkers have shown promising clinical applications in osteosarcoma management. ALP is used clinically for diagnosis and prognosis: higher levels correlate with advanced stage and poor prognosis. OPN is used mainly for prognosis and treatment response. Elevated levels predict disease progression and treatment resistance.

The advantages of the blood biomarkers are many, one of them is to enable the real-time assessment of disease processes within blood tests. They offer distinct advantages over traditional tissue biopsies, as they are minimally invasive and permit ongoing monitoring throughout a patient´s treatment journey. This non-invasive approach holds promise in OSA cases, especially since many patients undergo limb amputation, making tissue samples from secondary lung lesions challenging to obtain. Given OSA´s tendency to metastasize thorough the bloodstream, with blood acting as a carrier for both cells and cell products, numerous studies have focused on utilizing blood as a valuable source for analysis.

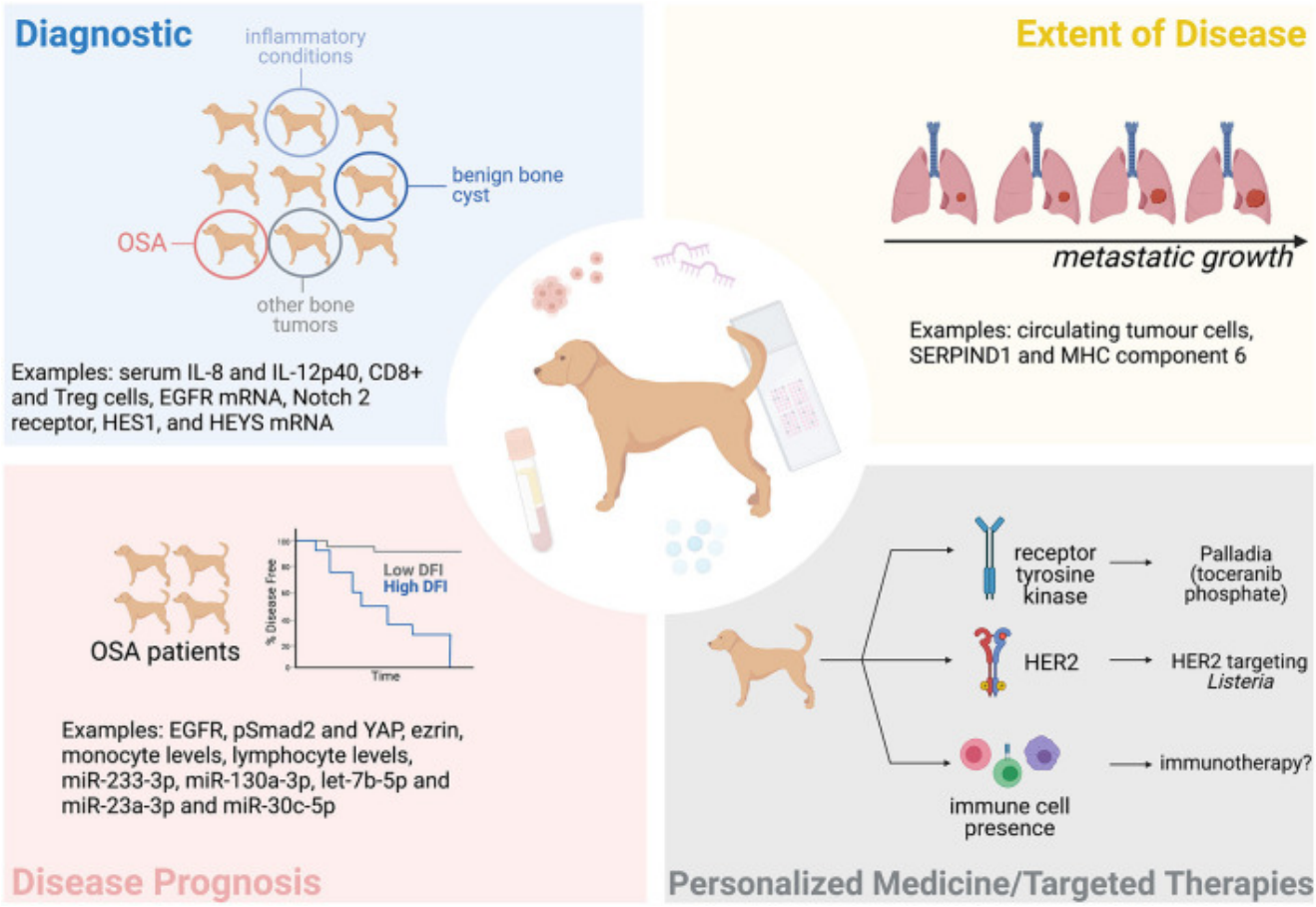

Figure 3: The potential biomarkers in the clinical management of canine osteosarcoma. [29]

Future perspectives of using the markers

The future holds promise for biomarkers in osteosarcoma management. Biomarkers continue to evolve as research advances. Future perspectives include the possibility of personalized medicine: tailoring treatment plans based on individual biomarker profiles. Furthermore, the use of multi-marker panels which means combining multiple biomarkers for enhanced accuracy and molecular profiling, which gives deeper insight into the genetic and molecular basis of osteosarcoma.

In conclusion, biomarkers for osteosarcoma in dogs and cats have come a long way in contributing to the diagnosis, prognosis, and treatment. While they hold immense potential, their limitations underscore the importance of ongoing research and clinical validation. As science progresses, the future of biomarkers in osteosarcoma management looks increasingly promising, offering hope for improved outcomes and better lives for out furry companions.

BIOMARKERS USED IN MAMMARY GLAND TUMORS

Mammary gland tumors refer to neoplastic growths originating from the mammary gland tissue, predominantly seen in canines and felines. These tumors can exhibit a variable histological profile, ranging from benign adenomas to malignant adenocarcinomas. Mammary gland tumors represent one of the most common neoplastic conditions in intact female dogs, with a reported prevalence of approximately 50% and a lower but still significant incidence in cats. Early detection and classification of these tumors are crucial for therapeutic decisions. Breeds like toy and miniature poodles, spaniels, and German Shepherds are more susceptible, with male dogs rarely affected. These tumors come in various sizes, shapes, and consistencies, and they may be movable or attached to underlying tissue. Some individuals may develop a single tumor in one gland, while others may develop multiple tumors in different glands or even within the same gland. Clinical signs of mammary tumors may vary; some individuals may not display any symptoms related to the tumor, and the mass might be discovered during petting or a routine exam. In advanced cases, tumors can become ulcerated, leading to bleeding. In case of metastasis, weight loss, poor appetite, vomiting, diarrhea and breathing difficulties might be present.[40]

Cancer researchers and veterinarians often rely on various biomarkers to understand and management breast cancer, both in humans and companion animals. These biomarkers provide insight into aspects such as cancer cell proliferation, apoptosis, metastatic potential, angiogenesis, and hormone receptor status. Understanding these biomarkers is essential for diagnosing, staging, and developing treatment strategies for breast cancer in all species.

Promising biomarkers for canine mammary tumors include blood-biomarkers, miRNAs, cancer stem cells, circulating tumor cells and mutations in the BRCA1 and BRCA2 genes. Established biomarkers such as Ki-68, HER-2 estrogen receptor, progesterone receptor, COX-2, carcinoembryonic antigen and CA15-3 are reliable for detecting CMT in both serum and tissue samples. Additionally, other markers like p53, E-cadherin, vascular endothelial growth factor and micro RNA shows potential for diagnosis and prognosis. Despite progress, the widespread use of biomarkers in CMT cases remains limited, emphasizing the need for further research in this area.[41]

Non-blood biomarkers of mammary gland tumors

The diagnosis and management of MGT in veterinary medicine require a multifaceted approach to assess tumor characteristics, guide treatment decisions, and monitor disease progression. While blood biomarkers offer valuable insights into systemic changes associated with cancer, non-blood biomarkers emerge as a pivotal tool in regard to MGT. These non-blood biomarkers, encompassing a range of cellular and molecular factors such as Ki-67, PCNA, P53, E-cadherin, ER (estrogen receptor), PR (progesterone receptor), HER-1, EGRF (epidermal growth factor receptor), and HER-1, provide critical information at the tissue and cellular levels. Their assessment aids in understanding the biological behavior of tumors, predicts prognosis, and influences the selection of targeted therapies. This thorough investigation delves into the importance, diagnostic value, and evolving viewpoints regarding these non-blood biomarkers, within the realm of MGT. [41]

The protein p53 serves as a marker for neoplastic transformation and apoptosis control. Mutations in the p53 gene are common in both human breast cancer and canine mammary tumor and are linked to tumor progression. High p53 expression generally indicates poor survival in both species. Metastatic potential is a critical aspect of cancer, and biomarkers related to this are crucial. Cadherins, especially E-cadherin play a role in cell-cell adhesion and are associated with tumor metastasis. Downregulated E-cadherin expression is linked to poor prognosis in both humans and dogs.[13]

HER-2 is another important tumor marker expressed in both human and canine mammary tumors. In dogs, inhibitors like trastuzumab and cetuximab, which target EGRF show promise in treating HER-2 positive mammary tumors. Hormone receptors, particularly estrogen and progesterone receptor (ER and PR), are of interest in breast cancer. Er and PR influence tumor growth, but their role in prognosis varies among studies. Targeted therapies involving these hormone receptors are less common in canine mammary tumors due to potential side effects. Understanding these various biomarkers helps guide diagnosis and treatment strategies for breast cancer, but further research and standardization of evaluation methods are needed to improve their utility, particularly in veterinary medicine.

One widely studied biomarker for tumor proliferation and apoptosis is Ki-67. In human medicine, high Ki-67 is expression is correlated with a poor prognosis, but these tumors may

respond well to chemotherapy due to their high proliferative activity. In dogs, Ki-67 shows promise as a serum biomarker, with levels positively correlating with tumor grade. Another marker, PCNA (proliferative cell nuclear antigen), is frequently evaluated alongside Ki-67. High PCNA expression is associated with large tumor size, malignancy and poor prognosis in both humans and dogs.[41]

Blood-biomarkers used in mammary gland tumors

CARCINOEMBRYONIC ANTIGEN

CEA is a glycoprotein tumor marker that is used to assess and monitor the presence and activity of malignancies, including mammary gland tumors, both in veterinary and human medicine. It typically has a molecular weight of approximately 180-200kDa, although this may vary among different species and forms of the protein.[37]

Synthesis

CEA is primarily synthesized in the fetal gut and fetal liver during embryonic development. In adults, its production is significantly reduced, but it can be produced by various epithelial tissues. In pathological conditions, such as mammary gland tumors, CEA can be synthesized by the tumor cells themselves. This is an important aspect when evaluating its diagnostic significance. [42]

Function

In normal, healthy individuals, CEA has no established significant physiological function. Pathological function however, which means elevated levels of CEA are associated with various malignant tumors, including mammary gland tumors in animals. It is considered a marker of tumor activity and can indicate the presence of malignancy.[41]

History

CEA was first discovered as an antigen associated with colon cancer in humans. Its name “carcinoembryonic antigen” reflects its initial identification in embryonic tissue and its later association with carcinoma. The recognition of CEA as a tumor marker led to its application in various cancer types, including mammary gland tumors in veterinary medicine.[43]

Analytical methods

CEA levels are determined thorough immunoassays such as ELISA, radioimmunoassay, and chemiluminescent immunoassays. These tests rely on specific antibodies that bind to CEA and generate a measurable signal. [41] In radioimmunoassay, the reference values for serum CEA concentrations in healthy dogs were initially established, ranging between 0,12 to 0,23ng/ml.[32]

Diagnostic importance

Elevated CEA levels in the blood are suggestive of malignancy, including mammary gland tumors. It is a valuable marker for monitoring tumor activity, assessing response to treatment, and detecting recurrence. CEA it not specific to a particular type of cancer and may be elevated in various malignancies, emphasizing the need for further diagnostic investigations. In one study, by utilizing a Receiver operating characteristic (ROC) curve with a cutoff value of 1,08ng/ml, they determined that the sensitivity and specificity of serum CEA, measured via ELISA, for detecting the presence of mammary tumors were 82,14% and 95,24%, respectively. The sensitivity at this threshold was found to be 100% for tumors larger than 3 cm and metastasized tumors, but reduced to 70% for tumors smaller than 3 cm. These combined findings confirm that CEA serves as a relatively sensitive and specific diagnostic biomarker for canine mammary tumors, particularly in advances stages.[32]

Species differences

CEA is utilized in veterinary medicine, including the diagnosis and monitoring of mammary gland tumors in dogs and cats. Its application in veterinary oncology parallels its use in human medicine. [41]

Present and future

Currently, CEA is an established biomarker in veterinary oncology for evaluating mammary gland tumors. It aids in diagnosis and monitoring of treatment. The future of CEA may refine its diagnostic and prognostic value, potentially leading to advancements in the early detection and management of mammary gland tumors in animals.

VASCULAR ENDOTHELIAL GROWTH FACTOR

Description

VEGF is a glycoprotein that plays a pivotal role in angiogenesis, which is the process of forming new blood vessels from pre-existing ones. VEGF is a signaling protein that stimulated the growth and development of blood vessels. It is a crucial regulator of angiogenesis during embryonic development and in various physiological processes, including wound healing and tissue repair. VEGF has multiple isoforms with varying molecular weights, typically ranging from 20-45kDa. [44]

Synthesis

VEGF is produced by various tissues and cell types, with primary sources including endothelial cells, macrophages, and fibroblasts within the tumor microenvironment. Endothelial cells, which line the inner walls of blood vessels, are one of the primary sources of VEGF production. In response to certain stimuli, endothelial cells initiate the synthesis of VEGF. This process is part of the body's natural response to injury inflammation and hypoxia. In regard to MGT, an essential aspect of VEGF synthesis is the tumor microenvironment. Tumor cells themselves can produce VEGF, releasing it into the surrounding tissues. This localized VEGF secretion serves as a critical factor in promoting angiogenesis within the tumor. Tumor-associated macrophages, fibroblast, and other stromal cells in the microenvironment can also contribute VEGF production. Hypoxia, also known as oxygen deprivation, is a significant inducer of VEGF synthesis. When tumor cells experience oxygen shortage due to their rapid growth and limited blood supply, they increase VEGF production as a survival mechanism. VEGF then stimulates the formation of new blood vessels, enhancing oxygen and nutrient delivery to the tumor.[45]

Function

In healthy individuals, VEGF plays a crucial role in angiogenesis, the formation of new blood vessels. This is essential in various stages of life, including embryonic development wound healing, and the maintenance of existing blood vessels. In these scenarios, VEGF promotes the growth of blood vessels to supply oxygen and nutrient to developing tissues, aid in tissue repair, and maintain tissue health. It plays a crucial role both in normal physiological processes and in pathological conditions. However, in cancer such as mammary gland tumors, VEGF takes on a pathological role. Tumor cells often upregulate VEGF production to create a microenvironment conductive to their growth and progression. Elevated levels of VEGF promote the growth of new blood vessels within the tumor, a process known as tumor angiogenesis. This has several

important consequences such as enhanced nutrient and oxygen supply. This enhancement supplies oxygen and nutrient to the tumor, which is crucial for its uncontrolled growth and survival. Furthermore, the blood vessels created by VEGF can provide a pathway for tumor cells to enter the bloodstream and spread to other parts of the body, contributing to metastasis. [34,35]3

History

VEGF was initially discovered in the 1980s by researchers investigating the mechanisms of angiogenesis. Its role in promoting the growth of blood vessels became evident, leading to its recognition as a key player in cancer biology, including mammary gland tumor.[46]

Analytical methods

VEGF levels can be determined using various analytical methods, including ELISA, RIA, and multiplex immunoassays. These tests rely on specific antibodies to detect and quantify VEGF in biological samples.[46]

Diagnostic importance

Elevated VEGF levels in mammary gland tumors are indicative of increased angiogenesis, which is a hallmark of malignancy. This knowledge is valuable for assessing tumor aggressiveness and potential for metastasis. Furthermore, VEGF is a target for anti-angiogenetic therapies, and assessing its levels informs treatment decisions. Blocking VEGF can hinder the blood supply to tumors, potentially inhibiting their growth and spread.

Present and future

VEGF assessment is a valuable tool used in veterinary oncology for characterizing mammary gland tumors and guiding therapeutic intervention. Given its critical role in tumor angiogenesis, VEGF has become a target for antineoplastic therapies. Blocking VEGF can disrupt the blood supply to tumors, potentially inhibiting their growth and spread. Various anti-VEGF drugs and therapies have been developed to interfere with this process.[45]

Availability and methods of the different biomarkers

Biomarker		Availability	Methods
Blood-biomarker	Ca 15-3	Human and veterinary medicine, mainly human medicine	ELISA, tissue immunohistochemistry
Blood-biomarker	CEA	Human and veterinary medicine	ELISA, tissue immunohistochemistry
Blood-biomarker	VEGF	Human and veterinary medicine	IHC, serum ELISA
Tissue marker	P53	Human and veterinary medicine	IHC
Tissue marker	E-cadherin	Human and veterinary medicine	IHC
Tissue marker	Estrogen receptor	Human and veterinary medicine	IHC
Tissue marker	Progesterone receptor	Human and veterinary medicine	IHC

Table 2: *Please note that the availability of these biomarkers and the specific methods used for detection may vary between human and veterinary medicine, and their clinical utility in veterinary medicine is an active area of research. [13, 42, 47]*

Future perspectives of using the markers

The horizon of biomarkers in mammary gland tumors holds promise for enhanced diagnostics and tailored treatment approaches. For example, precision medicine: biomarker-driven treatment strategies for individualized patient care, or molecular profiling: unveiling the heterogenicity of mgt for improved prognostication. Furthermore, non-invasive monitoring is also a future perspective, exploring minimally invasive techniques for biomarker assessment.

The discussed MT biomarkers encompass a range of molecular indicators that provide valuable insights into the behavior and prognosis of mammary gland tumors in both human and veterinary medicine. Ki-67 are biomarkers of proliferation, while p53 reflets neoplastic transformation and apoptosis. E-cadherin is linked to adhesion, while CEA and CA15-3 are glycoproteins associated with intracellular adhesion. VEGF and EGFR indicate angiogenesis and metastasis potential, and HER-2 is an important tumor marker. Additionally, estrogen and

progesterone receptors offer insight into hormone responsiveness. These biomarkers are detected through various methods, including immunohistochemistry and serum ELISA, and play essential roles in diagnosing, prognosticating, and treating mammary tumors, particularly in human medicine, while research continues to refine their clinical use in veterinary medicine.

BIOMARKERS USED IN HEMANGIOSARCOMA

Hemangiosarcoma is a highly aggressive and malignant cancer originating from the endothelial cells lining blood vessels. This hematopoietic neoplasm exhibits a proclivity for manifesting as visceral tumors, most notably affecting the spleen, heart, and liver. It is characterized by its insidious nature, rapid progression, and a propensity to metastasize, making it a formidable challenge in veterinary medicine. Early diagnosis and intervention are critical for improving outcomes, but the disease´s aggressive nature often limits treatment options and prognosis.[48]

The canine population, predominantly large and giant breeds, assumes center stage in the epidemiological narrative, with hemangiosarcoma exhibiting a predilection for certain breeds, such as the German Shepherd and Golden Retriever. Although genetic predispositions have been postulated, the exact genetic determinants remain elusive, calling for meticulous genetic investigations to unravel the genomic underpinnings of this malignancy. Biomarkers, such as cardiac troponin I (cTnI), serum collagen XXVII peptide, and thymidine kinase TK1), have emerged as valuable indicators in the context of canine hemangiosarcoma diagnosis and monitoring. [11]

Non-blood biomarkers used in hemangiosarcoma

Non-blood biomarkers used in the context of hemangiosarcoma in veterinary oncology is essential for diagnosing, monitoring and for better understanding. Immunohistochemistry involved staining tumor tissue samples with specific antibodies to identify proteins or markers associated with HAS. Markers like Factor VIII-related antigen and CD31 are commonly used to confirm the vascular origin of the tumor. Not only detection of the tumor, but also its potential to resist chemotherapy can be measured. P-glycoprotein is a cell membrane protein associated with drug resistance. Detecting P-gp expression in HAS tissue can provide insight into the tumor´s potential resistance to chemotherapy. Furthermore, E-cadherin is another valuable non-blood biomarker. It is a cell adhesion molecule, and its expression in HAS may offer insight into the tumor´s invasiveness and behavior.[49]

Blood-biomarkers used in hemangiosarcoma

Blood biomarkers used in hemangiosarcoma include hematocrit levels, platelet count, fibrinogen, D-dimer, cardiac troponin 1, and thymidine kinase I to name a few.

CARDIAC TROPONIN 1

Cardiac troponin I (cTn1) is a protein that is primarily found in cardiac muscle. It is one of the three subunits of the troponin complex, along with troponin C and troponin T, that plays a critical role in regulating muscle contraction in the heart. Specifically, cTn1 is involved in the regulation of cardiac muscle contraction by controlling the interaction between actin and myosin, two proteins that are essential for muscle contraction. cTn1 is an important biomarker for assessing cardiac muscle damage. When the heart muscle is injured, such as in the case of a heart attack (myocardial infarction), cTn1 is released into the bloodstream. Elevated levels of cTn1 in the blood are highly specific indicators of cardiac muscle damage and are commonly used in clinical practice to diagnose and monitor heart conditions. The protein has a molecular weight of approximately 24-28 kDa. While its primary role is in cardiac muscle, cTn1 is also found in small amounts in skeletal muscles.[50]

Synthesis

Cardiac troponin I is synthesized in cardiac muscle cells, also known as cardiomyocytes. The synthesis of cTnI is a complex process that involves the transcription and translation of the TNNI3 gene, which encodes the cTnI protein. Primarily, the synthesis of any protein, including cTnI, begins with the transcription of the corresponding gene. In the case of cTnI, the TNNI3 gene is transcribed into messenger RNA in the nucleus of the cardiomyocytes. Secondly, the mRNA molecule carries the genetic code for cTNI from the nucleus to the cytoplasm of the cell. In the cytoplasm, ribosomes read the mRNA sequence and facilitate the translation of this genetic code into an amino acid sequence, which forms the cTnI protein. Thirdly, after the initial translation, cTnI undergoes various post-translational modifications. These modifications can include phosphorylation, acetylation, and other changes that fine-tune the protein´s function within the cardiac muscle. Once synthesized and modified, cTnI becomes part of the sarcomere, which is the basic contractile unit of the muscle. The troponin complex, which includes cTnI, cTnT and cTnC regulates muscle contraction by interacting with other muscle proteins, such as actin and myosin.[50, 51]

Function

Cardiac troponin I´s primary function it to regulate muscle contraction by inhibiting the binding of myosin to actin in the absence of calcium ions. When calcium ions are released during muscle contraction, they bind to cTnC, causing a conformational change in the troponin complex that allows myosin to interact with actin, leading to contraction. To summarize, in its normal state, cTNI plays a pivotal role in regulating muscle contraction in the heart. Pathologically however, elevated cTnI levels in the blood are indicative of cardiac muscle damage, making it a valuable marker for heart conditions and HSA.[11, 50]

History

The recognition of cTnI as a biomarker is a relatively recent development. Researchers identified it's a highly specific and sensitive marker for cardiac muscle damage, particularly in case of myocardial infarction. [52]

Analytical method

The determination of cTnI levels in the blood is typically accomplished using immunoassays, such as ELISA or chemiluminescent immunoassays. These methods provide accurate quantification of cTnI concentration. The normal reference range of cTnI in dogs is <0,15ng/ml, and respectively <0,06ng/ml in cats.[53]

Diagnostic importance

In the context of hemangiosarcoma, elevated cTnI levels may indicate cardiac involvement or secondary cardiac complications due to tumor infiltration. Monitoring cTnI can aid in the early detection of cardiac issues associated with hemangiosarcoma, providing valuable information for clinical decision-making.

Species differences

Cardiac Troponin I is a relevant biomarker in various species, including dogs, cats, and humans. It has been extensively studied in veterinary medicine for its diagnostic significance in heart conditions.

Present and future

The current use of cTnI in veterinary oncology, particularly in case of hemangiosarcoma, highlights its role as a critical indicator of cardiac involvement. Future perspectives may

involve refining its application in diagnosing and managing cardiac complications associated with hemangiosarcoma, contributing to improved patient care and outcomes.

To conclude, Cardiac Troponin I (cTnI) is a highly specific marker for heart muscle damage, cTnI has demonstrated its utility in identifying cardiac HSA in dogs with pericardial effusion. It also aids in recognizing cardiac involvement in HSA originating from other sites in the body. This biomarkers boats high sensitivity and specificity, making it a reliable tool.

THYMIDINE KINASE 1

Description

Thymidine Kinase 1 is an enzyme that plays a crucial role in the synthesis of DNA by catalyzing the phosphorylation of thymidine, a deoxynucleoside. This phosphorylation converts thymidine into thymidine monophosphate, which is an essential precursor for DNA synthesis. In the context of hemangiosarcoma and other cancers, TK1 becomes a valuable biomarker. TK1 is a cytosolic enzyme, and its molecular weight typically ranges from 25 to 30 kDa.[54]

Synthesis

The synthesis of TK1 primarily occurs in the proliferating cells, particularly during the DNA synthesis phase of the cell cycle (S phase). Various tissues and organs can produce TK1, but its expression significantly increases in rapidly dividing cells, including cancer cells- In the case of hemangiosarcoma, tumor cells themselves contribute to elevated TK1 production. The synthesis of TK1 is tightly linked to DNA replication, and its levels in the bloodstream rise as cell actively divide. Once synthesized and modified, TK1 becomes part of the cellular machinery involved in DNA replication. It plays a role in phosphorylating thymidine, which is essential for DNA synthesis.[54, 55]

Function

In normal physiological conditions, TK1 severs as an3 essential role in cellular DNA replication. It phosphorylates thymidine, facilitating its incorporation into the growing DNA strand, thereby enabling cell division and tissue growth. However, in pathological conditions like cancer, TK1 levels significantly increase. Elevated TK1 in the bloodstream indicated increased cell proliferation, making it a valuable biomarker for cancer diagnosis and monitoring. [56]

History

The recognition of TK1 as a potential biomarker for cancer diagnosis and monitoring is a relatively recent development. Researchers identified its elevated levels in the bloodstream of cancer patients, indicating a link between increased TK1 activity and cancer cell proliferation. In the context of hemangiosarcoma, the specific association of TK1 with the disease´s pathogenesis and progression has contributed to its relevance as a diagnostic tool. [54, 57]

Analytical method

Various analytical methods are employed to determine TK1 levels in blood samples. These methods include ELISA, spectrophotometry, RIA, HPLC, and mass spectrometry. ELISA is one of the most commonly used methods for its ease of use and precision. These techniques rely on specific antibodies that bind to TK1, enabling quantification of its concentration in biological samples. [55]

Diagnostic importance

The measurement of TK1 in the bloodstream is of paramount diagnostic importance in veterinary oncology, particularly in the context of HSA. Elevated TK1 levels indicate increased cell proliferation, making it a sensitive biomarker for early cancer detection, assessment of tumor burden, and monitoring the effectiveness of cancer treatment. In HAS, it aids in distinguishing malignant masses from benign ones, which can be challenging through other methods. TK1 is utilized in specialized panels such as the “TK canine cancer panel” which helps in confirming the presence of cancer, monitoring treatment effectiveness, and verifying remission status.[58] In one study, the use of TK1 was investigated as a biomarker for canine malignant lymphoma. The study found that in the control group, serum TK activities ranged from 1-6U/L, with a reference interval of normal 0-7 U/L. In the canine malignant lymphoma group, serum TK activities ranged from 8,9 to 470U/L. All dogs with malignant lymphoma showed a 1,3-67 times higher serum TK activity compared to normal dogs. These results suggests that TK1 activity is significantly elevated in dogs with malignant lymphoma compared to healthy dogs and can serve as a potential biomarker.[59]

Species differences

TK1 has been determined and extensively studies as a biomarker in various species, including dogs and cats, in the context of cancer diagnostics. Its relevance extends to human medicine as well. The conserved role of TK1 in DNA synthesis makes it a valuable marker for assessing cell proliferation in diverse species.

Present and future

Currently, TK1 stands as a valuable tool in veterinary oncology, particularly in the diagnosis and management of hemangiosarcoma. Its sensitivity and specificity contribute to its utility in detecting cardiac involvement in hemangiosarcoma and assessing metastatic spread. The future of TK1 as a biomarker in veterinary medicine holds exciting possibilities.

Serum collagen XXVII Peptide is another biomarker used in the context of HSA. It is a protein breakdown product, associated with invasive and angiogenic processes like HSA, exhibits significantly elevated levels in dogs with large metastatic HSA burdens compared to healthy dogs. Monitoring changes in collagen XXVII peptide levels post-surgery helps assess tumor recurrence, indicating its potential as a useful HSA biomarker. Notably, dogs with other neoplasia or inflammatory diseases showed increased levels, albeit consistently lower than those for HSA.

Elevated serum TK1 activity correlated with DNA synthesis in proliferating cells, showing significantly higher levels in dogs with HSA compared to healthy counterparts. However, distinguishing benign splenic masses from HSA using TK1 alone is not possible. TK1 has also found application in detecting various cancers, including lymphoma, in both dogs and cats. Commercially available panels such as the ”TK canine cancer panel” and “TK Feline cancer panel” from veterinary diagnostic institute, utilize TK1 and CRP to generate a “neoplasia index” that aids in confirming cancer presence, monitoring treatment effectiveness, and verifying remission status. While these panels provide valuable insights, official diagnosis still requires cytology or biopsy confirmation.[11]

In summary, biomarkers like cTnI, collagen XXVII peptide and TK1 offer valuable diagnostic and monitoring tools in the realm of hemangiosarcoma. Their sensitivity and specificity make them promising aids for early detection and disease management, while specialized panels enhance cancer diagnosis and treatment assessment in a clinical setting.

Future perspectives of using the markers

While the use of TK1, serum collagen XXVII peptide and cTnI as biomarkers in the context of HSA has shown promise in diagnosis and monitoring, their future perspectives hold several exciting possibilities in veterinary medicine. Refinement of early detection is one perspective, while predictive markers another one. The possibility of combination biomarker panels: combining multiple biomarkers, including TK1, serum collagen XXVII peptide and cTnI, into a comprehensive panel could enhance diagnostic accuracy and prognostication. Such panels might provide a broader view of the disease status and response to treatment. The integration with imaging: combining biomarker information with advanced imaging techniques such as US or MRI, could offer a more holistic understanding for the disease. This integrated approach may enable better surgical planning and treatment decisions. Furthermore, the opportunity for a non-invasive monitoring through development of non-invasive methods for biomarker assessment, such as liquid biopsies, may become more feasible. This would minimize patient discomfort and allow for more frequent monitoring during treatment. In addition, a clinical decision support could be possible, with biomarker-based algorithms and decision support tools which could assist veterinarians in making more informed decisions regarding treatment strategies, including the choice of surgery, chemotherapy or targeted therapies.

BIOMARKERS USED IN CANINE PROSTATE CANCER

Disease conditions involving the canine prostate gland are a common occurrence in small animal practice. The conditions most commonly encountered are benign prostatic hyperplasia, prostatitis, prostatic cysts, and prostatic neoplasia. [60] In recent years, there has been a growing demand for accurate and precise identification of prostatic diseases in dogs. [61] Prostate cancer in dogs, like in humans, present unique challenges. Especially older male dogs are affected by prostate cancer, and the tumors are typically diagnosed at an advanced stage, often when they have already metastasized, making treatment decisions difficult.[62] Unlike humans, where prostate-specific antigen (PSA) is a well-established biomarker, there isn´t yet an equivalent single marker for canine prostate cancer. However, canine prostate specific esterase has shown similar properties, and the study of this biomarker is still ongoing.

Among various diagnostic tools, seric canine prostate specific esterase (CPSE) has emerged as a reliable and specific biomarker for prostatic disorders in dogs. It has shown significantly higher levels in dogs affected by various prostatic abnormalities, including benign prostatic hyperplasia, bacterial prostatitis, and prostatic carcinoma. Consequently, measuring CPSE levels in serum has introduced a novel diagnostic and screening method. The utilization of CPSE in everyday clinical practice serves three primary purposes: diagnosing BPH, proactively screening for prostatic disorders in healthy dogs, and lastly monitoring individuals with prostatic disorders throughout and after medical treatment. CPSE levels remain unaffected by circadian rhythms or transrectal palpation performed during andrological examinations, however, to ensure accurate results, it’s advisable to observe a sexual rest period of at least 24 hours before CPSE measurement, since ejaculation can impact its levels.[61]

In a study conducted by Pinheiro et al, noteworthy variations in median CPSE levels were observed between the control dog group and the hyperplastic prostatic dog group (at 29.1 ng/ml and 160.7 ng/ml, respectively). Moreover, CPSE exhibits diagnostic characteristics similar to those of human prostate specific antigen (PSA), suggesting its potential in indicating prostate disorders in humans. However, please note that the detection of CPSE alone is not indicative of CPC. Other biomarkers used in canine prostate cancer include microRNAs and circulating tumor cells.

BIOMARKERS USED IN URINARY BLADDER CANCER

Urinary bladder cancer is a significant concern in veterinary medicine due to its high occurrence in several domestic animal species and its life-threatening nature. Bladder cancer in companion animals exhibits a complex and not yet fully understood biopathology, limiting therapeutic advancements. Nevertheless, recent progress has been made in identifying tumor markers with clinical applications for diagnosis, prognosis, and therapy, including the recognition of pathological BRAF mutations, flagging the way for targeted treatments. Urinary bladder cancer has a prevalence of approximately 2 percent of all reported malignancies in dogs, in contrast to cats which are less commonly affected.[63, 64] Most canine bladder tumors are malignant and of epithelial origin, with transitional cell carcinoma (TCC), also known as urothelial carcinoma (UC), being the most prevalent type. The etiology of this canine disease is believed to be multifactorial, involving factors such as exposure to older topical insecticides, obesity, female gender, herbicides, and breed predisposition.[65, 66]

Bladder tumors are also frequently observed in cattle grazing on pastures infested by toxic ferns, mainly Pteridium species. The etiology of bladder cancer in ruminants is better understood than in companion animals and is closely linked to the geographical distribution of toxic ferns. This condition is part of a syndrome called bovine enzootic hematuria. Bladder lesions in cattle have been experimentally induced using the fern of its toxin ptaquloside, an agent that damages DNA, leading to mutations and chromosomal abnormalities.[64]

In dogs with transitional cell carcinoma, clinical signs are often nonspecific and can include symptoms commonly associated with urinary tract disease, such as dysuria, hematuria, and pollakiuria. Many TCC cases co-occur with urinary tract infractions. Tumor growth can lead to the obstruction of the ureters or urethra, affecting normal urethral sphincter function. Physical examination may reveal thickening of the urethrae, the trigone region of the bladder, and enlarged iliac lymph nodes. In some cases, a bladder mass or distension may be palpable. Urinary tract obstruction can occur before lethal metastasis develops, and it is a common cause of death in dogs with TCC. However, it’s important to note that a normal physical examination does not rule out TCC. The differential diagnosis of canine TCC includes other neoplasms, chronic cystitis, polypoid cystitis, fibroepithelial polyps, granulomatous cystitis, calculi, and other conditions. In cattle, bladder cancer typically presents with symptoms like hematuria and weight loss, often as part of bovine enzootic hematuria syndrome. [64, 65]

Diagnostic procedures and staging diagnosing TCC involve various diagnostic procedures, including a complete blood cell count, serum biochemistry profile, urinalysis, urine culture to rule out lower UTIs, and cancer staging. A definite diagnosis of TCC usually requires histopathological examination of tumor tissue or cytology of a representative sample. Biopsies can be obtained through invasive methods such as cystotomy or traumatic catheterization, often performed under general anesthesia. These procedures carry a risk of tumor implantation or dissemination, especially after surgical manipulation. A less invasive diagnostic technique is urine sediment cytology. It can provide a diagnosis if tumor cells are present, but negative results do not exclude TCC. Clinical staging for canine TCC include radiography, ultrasonography, and specific imaging for the urinary tract. CT scans are increasingly used for diagnostic and staging purposes, helping to evaluate the urethra and detect metastases.[65]

Recent advances in diagnostic techniques for diagnosing canine TCC is challenging due to its similarity to other urinary tract disorders. This often leads to delayed diagnosis, allowing the tumor to progress. Early identification methods are essential to improve treatment outcomes and survival rates. Urine, being a potential source for TCC-specific molecules and biomarkers, can aid in non-invasive detection. Several biomarkers have been explored for diagnostic and prognostic purposes, some of which are commercially available. These biomarkers offer promise for early detection and improved clinical management. [65]

The BRAFV595E mutation in dogs is a genetic change in canine chromosome 16, causing a specific amino acid substitution. Studies have shown that this mutation is present in a significant percentage of invasive TCC bladder tumors in dogs, with high prevalence in urothelial carcinoma and prostatic carcinoma. A droplet digital PCR assay has been developed to detect this mutation in urogenital tumors and urine samples from affected dogs. Research indicated that terrier breeds might have a higher predisposition to this mutation. However, the mutation doesn´t seem to correlate with the histological grade of the tumor or impact the survival of affected dogs. Detecting this mutation, especially through non-invasive methods, has potential clinical utility for diagnosing TCC, monitoring disease progression, and assessing treatment response. Commercially available tests, based on ddPCR, offer a means of screening for this mutation.[65, 67]

The bladder tumor antigen test, originally designed for humans, has been adapted for use in veterinary medicine known as the V-BTA test. It employs a rapid latex agglutination dipstick

colorimetric approach to qualitatively detect specific tumor-related substances in urine. This method utilizes antibodies to identify a glycoprotein complex associated with urinary bladder tumors, containing proteins from the basement membrane. These proteins are released into the urine when the urinary tract is affected or damaged, potentially including immunoglobulins. Test results yield either positive or negative outcomes. Several studies in canine patients, encompassing various conditions, have assessed the V-BTA test. The results generally showed that the test has high sensitivity but lower specificity when it comes to detecting canine TCC. Therefore, it's not recommended as a definitive diagnostic tool for urinary tract TCC in dogs, especially in those presenting with urinary tract disease symptoms. However, it can be valuable as a preliminary screening test, particularly for dogs at high risk of developing the disease. For example, in a group of elderly dogs, if the test is positive, only a small fraction (less than 3%) of these dogs would be expected to have TCC, whereas a negative result would be highly reliable (99,9%) in ruling out the disease.[65, 68]

Other biomarkers used in bladder tumors include basic fibroblast growth factor (bFGF), microRNAs, HER-2, P63, Ki67 and epidermal growth factor receptor (EGFR) to mention some. Ongoing research in blood-based liquid biopsies is anticipated in the coming years, aiming to parallel advancements made in human medicine. Minimally invasive techniques have proven to be more effective biomarkers that tissue-based approaches. [65]

Biomarker	Sample	Availability, diagnostic utility
BRAF-mutation	Tissue, urine, blood	Commercially available for dogs - CADET® *BRAF* mutation detection assay.
BTA	Urine	Commercially available – V-BTA test
bFGF	Urine	Commercially available, for research only. Quantikine® HS ELISA, Human FGF basic Immunoassay, Canine BFGF ELISA Kit, Nori® Canine FGF Basic ELISA Kit.
microRNAs	Tissue, urine, blood	Not commercially available
HER-2	Tissue	Not available
P63, Ki67, β-catenin	Tissue	P63 could potentially be used as a clinical marker for diagnosing canine TCC.
EGFR	Tissue	EGFR expression could potentially be used as a marker to aid canine TCC diagnosis

Table 3: summary of different biomarkers in bladder tumors (Rasteiro et al, 2022). [65]

MICRO-RNA AS A TUMOR SPECIFIC BIOMARKER

MicroRNAs (miRNAs), are small non-coding RNA molecules found within cells that play an important role in regulating gene expression by inhibiting mRNA translation. These molecular components have been implicated in a wide range of diseases, including cancer, due to their involvement in critical cellular processes. In regards to cancer, miRNAs can act as either tumor suppressors or oncogenes, making them attractive candidates for diagnostic and therapeutic applications.[69]

Function

Micro RNAs are essential for the regulation of gene expression and maintaining cellular homeostasis. However, in pathological conditions such as cancer, miRNAs can be dysregulated, leading to significant disruptions in cellular processes such as proliferation, apoptosis, and angiogenesis. [69]

Analytical methods

There are several analytical methods that can be employed to determine miRNA levels in biological samples. These include spectrophotometry for quantification of miRNA concentration, reverse transcription-quantitative polymerase chain reaction (RT-qPCR) for specific miRNA profiling, and microarrays for high throughput analysis. Additionally, ELISA can be used to detect specific miRNAs in serum or plasma.[69]

Diagnostic importance

In veterinary oncology, determining the levels of miRNAs in tumor tissues, blood or other biological fluids can offer critical diagnostic information. MiRNAs can serve as tumor-specific biomarkers, aiding in cancer detection, subtyping, and prognosis.

A study with a focus on the potential of circulatory exosomal miRNAs as diagnostic biomarker for cancer explored their utility in identifying cancer patients at an early stage, which could significantly improve the effectiveness of cancer treatment and staging (Agarwal et al, 2021). Exosomal miRNAs was isolated from a variety of canine cancer cell lines, including those associated with lymphoma, mast cell tumors, histiocytic cells, osteosarcoma, melanoma, and breast tumors. Canine models were chosen for their relevance, as dogs share similar environmental exposures and genetic similarities with humans, making them valuable subjects

for cancer research. While previous research predominantly has focused on miRNAs associated with specific cancers, this study aimed to find miRNAs with broader applications. Such miRNAs could form the basis for a non-invasive diagnostic test, often referred to as a liquid biopsy. The study employed next-generation sequencing (NGS) to comprehensively profile these circulatory exosomal miRNAs and identify those consistently elevated across multiple cancer types. Subsequently, quantitative real-time PCR (Q-RT-PCR) was used to validate the findings. The study identified six miRNAs (cfa-miR-9, -1841, -1306, -345, -132, and -26b) that were consistently elevated across all cancer types. While NGS results indicated significant elevation of these miRNAs, the Q-RT-PCR results showed lower fold differences. Notably, cfa-miR-9 was consistently elevated in both NGS and Q-RT-PCR, making it a promising candidate for diagnostic purposes. The goal of this study was to identify circulatory miRNAs that could serve as diagnostic biomarkers for cancer, offering a potential breakthrough in cancer diagnosis. This research is a crucial step toward a non-invasive and reliable approach for early cancer detection, which could significantly improve cancer patient care and outcomes. Further validation of these findings in in vivo human patient samples is required to fully assess their diagnostic utility in the clinical setting. [69]

Species differences

The relevance of miRNAs as tumor-specific biomarkers is not limited to humans, but extends to various animal species, particularly those susceptible to cancer. Dogs, for example, are commonly used as models for studying cancer due to their genetic diversity and spontaneous tumor development, making them an ideal candidate for miRNA research. [69] Cats and other domestic animals have also been investigated.[70]

Present and future

Tumors identified by miRNAs analysis for diagnostic biomarkers includes lymphoma, mast cell tumor, histiocytic cell line, osteosarcoma, melanoma and mammary gland tumors.[30, 69]In the future, miRNAs are likely to play a central role in personalized medicine for pets, guiding treatment decisions and improving outcome in veterinary oncology. Additionally, collaborative research between human and veterinary medicine may reveal shared miRNA biomarkers, advancing our understanding of cancer across species and ultimately benefiting both animal and human patients.

CIRCULATING CELL-FREE DNA AS A BIOMARKER

In both human and veterinary medicine, circulating DNA, which includes circulating tumor DNA (ctDNA) in cancer patients, can provide valuable insights into various diseases. In humans, advanced techniques like sequencing and digital PCR have enabled applications such as early cancer diagnosis, precise tumor characterization and prognosis for treatment decisions, and treatment monitoring based on ctDNA levels. In canine oncology, the CNRS "Dog Genetics" team from Institute Genetics & Development of Rennes has been developing ctDNA analysis tests for histiocytic sarcoma and lymphoma (Prouteau et al, 2021). They have successfully identified tumor-specific mutations share between canine and human histiocytic sarcomas, detectable in the plasma of affected dogs, providing a non-invasive method for diagnosing this cancer. As one of their findings, the team has demonstrated that tcDNA is detectable in 92,3% of lymphoma cases as diagnosis and can be used to monitor treatment response and predict relapse during chemotherapy in dogs with diffuse large cell B lymphoma. [71] These tests offer huge valuable tools for further clinical diagnostic workup in the field of oncology.[71, 72]

Description

Circulating cfDNA is a molecule found in the bloodstream and various other biological fluids. It consists of short fragments of double-stranded DNA. The molecular weight of cfDNA varies, but is typically around 180-200 base pairs.[73]

Synthesis

cfDNA is produced and released by cells from various organs and tissues throughout the body. These fragments are released into circulation primarily as a result of cell death, including apoptosis, necrosis and active secretion. Levels of cfDNA is low in healthy individuals, however increased in various pathological conditions. [71]

Function

In a normal physiological state, cfDNA levels are relatively low and primarily serve as genetic material maintenance in the body. Pathologically on the other hand, in conditions like cancer, cfDNA can carry valuable genetic and epigenic information specific to the tumor. This circulating tumor DNA is therefore characterized by genetic alterations that promote cancer development, progression, and resistance to treatment. [71]

History

Recent advances in sequencing and digital PCR techniques have enabled researchers to identify and analyze ctDNA in various pathological conditions, leading to its recognition as a valuable biomarker.

Analytical methods

There are various methods available to determine the presence and characteristics of cfDNA, including spectrophotometry, ELISA, HPLC, mass spectrometry RIA, and more. These techniques allow for the quantification and genetic profiling of ctDNA.

Diagnostic importance

cfDNA, particularly ctDNA, holds significant diagnostic value in veterinary oncology. The detection of ctDNA allows for early cancer diagnosis in animals, improving the chances of successful treatment. Furthermore, ctDNA analysis provides insights into tumor genetics, aiding in prognosis determination and the guidance of treatment choices. Another important diagnostic value is the monitoring of ctDNA levels during treatment to access treatment effectiveness. Decreased levels of ctDNA would indicate tumor regression.

Species differences

Research on cfDNA and ctDNA has been conducted in various animal species, particularly in dogs. The diagnostic and monitoring applications of cfDNA are relevant to both human and veterinary medicine.[72]

One study (Favaro et al, 2022) involved a feasibility analysis of ctDNA in dogs, specifically in dogs with either benign splenic lesions or malignant splenic tumors, notably hemangiosarcoma. They used shallow whole-genome sequencing (sWGS) of cell-free DNA for analysis. The sWGS approach allowed for cost effective ctDNA analysis. Dogs have numerous advantages as a model for ctDNA research. These include a high incidence of spontaneous cancers, molecular heterogeneity, the ability to collect serial blood samples, use of clinical annotation methods similar to human oncology, and comparable tumor sizes. Canine cancers replicate human cancer heterogeneity, allowing the study of clonal evolution and therapeutic resistance. This provides a unique opportunity to explore ctDNA in a naturally occurring cancer context.

Present and future

The use of cfDNA as a biomarker in veterinary oncology is a growing field. Non-invasive plasma tests for specific cancers, like histiocytic sarcoma and lymphoma, are becoming more widely available. As technology and research advance, cfDNA analysis is expected to play an increasingly role in early cancer diagnosis, treatment optimization, and the overall management of cancer in animals. This perspective holds promise for improving the well-being of both veterinary and human patients with cancer.

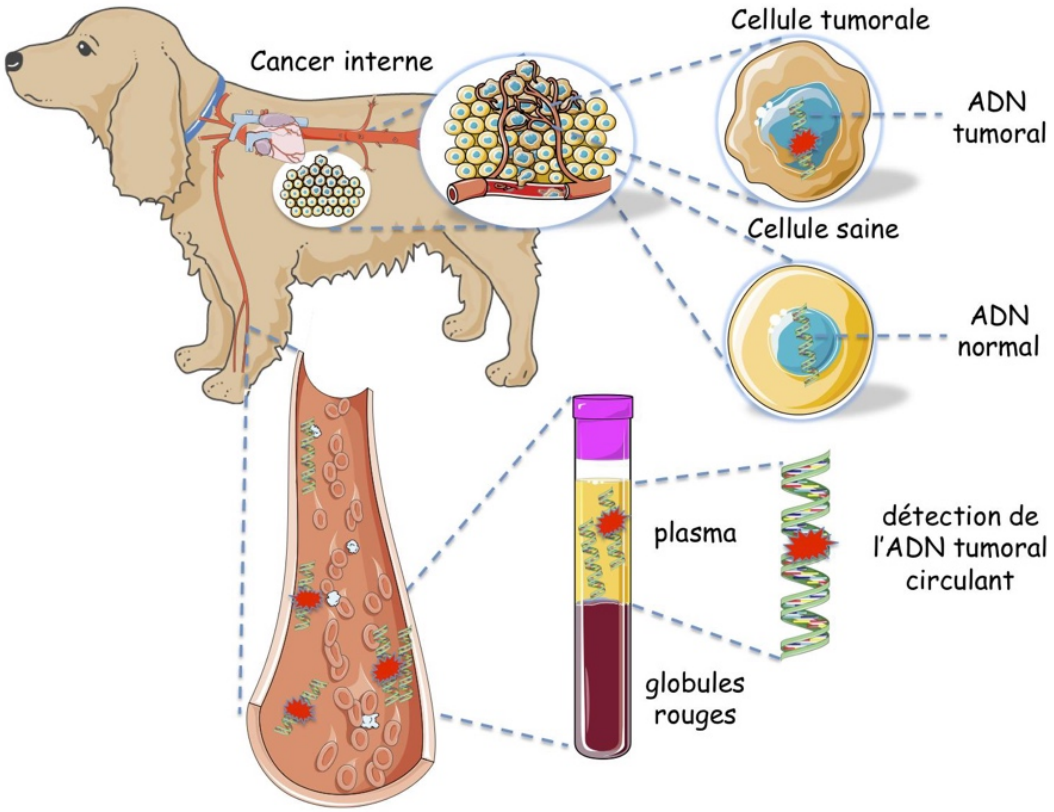

Figure 3: An illustration of ctDNA and its diagnostics [71]

SUMMARY

Lymphoma, a common cancer in dogs and cats, poses diagnostic challenges due to its varied forms and presentations. Fine needle aspiration and histological examination are vital for definite diagnosis. However, the importance of biomarkers in clinical workup is not only pivotal, but also non-invasive, cheaper and a good diagnostic tool. CRP and hapt serve as important biomarkers, both being commercially available as Sensitest. Aiding in disease differentiation and treatment response assessment, these acute phase proteins have proven valuable in the case of lymphoma.

In osteosarcoma, a challenging bone tumor, early diagnosis is essential for treatment success. Blood-biomarkers, including ALP, LDH and osteocalcin complement imaging and biopsies. ALP, specifically the bone-specific isoform, is of interest. Elevated ALP levels indicate increased bone metabolism, crucial form growth, repair, and pathological conditions like osteosarcoma. It serves a a diagnostic and differential marker, monitoring treatment response, and carrying prognostic value. Despite species differences and potential elevation in other diseases, ALP remains a valuable diagnostic tool.

Mammary gland tumors are common both in dogs and cats, and early detection and classification are essential for effective treatment. A variety of non-blood biomarkers, including Ki-67, P53, E-cadherin, and hormone receptors provide valuable insights, into tumor behavior, prognosis, and treatment strategies. Blood biomarkers on the other hand, like CEA and CA15-3 are essential for monitoring tumor activity and response to treatment. While the application of these biomarkers in veterinary medicine is still evolving, they hold promise for improving both diagnosis and management of mammary gland tumors in animals.

In the case of hemangiosarcoma, biomarkers like cardiac troponin I, serum collagen XXVII peptide, and thymidine kinase 1 have emerged as valuable tools for diagnosis and monitoring. These biomarkers offer sensitivity and specificity, aiding in the early detection of cardiac involvement in hemangiosarcoma and assessing the extent of metastasis. Their application is essential for understanding and managing the disease, which is notorious for its rapid progression.

While there is no single equivalent to human PSA in Canine prostate cancer, CPSE has emerged as a valuable biomarker for prostatic disorders. CPSE levels in serum provide a reliable method for monitoring and diagnosing individuals with prostatic conditions. In the realm or urinary bladder cancer, the search for effective biomarkers is ongoing, with the BRAFV595E mutation showing promise in diagnosis and monitoring. The VBTA test, although not definitive can serve as a preliminary screening tool. Several other biomarkers, such as bFGF, microRNAs, HER-2, P63 and Ki67 to mention some, are also under investigation, offering potential for early detection and improved clinical management.

MicroRNAs have emerged as tumor-specific biomarkers with diagnostic and prognostic significance in both human and veterinary medicine. Their dysregulation in pathologicsl conditions, such as cancer, makes them attractive candidates for diagnostic applications. Analytical methods are employed to determine miRNA levels, offering insights into cancer detection, subtyping, and prognosis. In the future, miRNAs are expected to play a central role in personalized medicine for pets and enhance out understanding of cancer across species.

Circulating cell-free DNA, particularly circulating tumor DNA is gaining prominence in veterinary oncology. CtDNA analysis allows for early cancer diagnosis, prognosis determination, and treatment monitoring in animals. The use of cfDNA as a biomarker is expanding, with non-invasive tests becoming more widely available for specific cancers. This advancement holds promise for early cancer diagnosis and treatment optimization, furthermore improved cancer management in veterinary and human patients alike.

In conclusion, biomarkers have made significant contributions to lymphoma, osteosarcoma, mammary gland tumors and hemangiosarcoma management in veterinary medicine. Their crucial advancement in diagnosing, monitoring, and understanding helps better understanding these challenging conditions. While their limitations are acknowledged, ongoing research offers hope for improved diagnostic accuracy and enhanced treatment strategies, ultimately benefiting out beloved animal companions.

Looking to the future, the perspectives for these biomarkers in veterinary medicine are exciting. Refinement of early detection methods, predictive markers, combination biomarker panels, integration with advanced imaging techniques, non-invasive monitoring, and clinical decision support tools all hold promise for enhancing the diagnosis and treatment of these conditions.

As research continues to advance in this field, the role of biomarkers in veterinary oncology is likely to expand, improving the care and outcomes for our patients.

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