

Prognostic biomarkers of cancer in animals: A systemic approach

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Received: 15.4.26; Accepted: 7.6.26

ABSTRACT

Cancer is a condition characterized by uncontrolled cell proliferation, tissue invasion and metastasis. Animal cancers, which are on rise in the recent past, resemble human cancers and serve as valuable models for comparative research in cancer biology and drug development. Biomarkers are defined as measurable molecules indicating biological processes, disease or treatment response to cancer. Historical evolution of biomarkers spans from simple protein markers like alpha-fetoprotein (AFP) to advanced microRNAs and circulating tumour cells (CTCs). Ideal biomarkers must be tumor-specific, detectable in minimally invasive samples, correlate with tumor burden and enable high-throughput testing. Important proliferation markers of cancer include Ki-67, which is higher in malignant tumors, PCNA (Proliferating Cell Nuclear Antigen) which correlates with tumor grade and invasion, AgNORs (Argyrophilic Nucleolar Organizer Regions) which distinguish tumors as benign/malignant and telomerase which is 95% tumor-specific. Tumor suppressors like p53 and adhesion molecules like E-cadherin predict aggressiveness of tumors. Serum markers like AFP, TK1 (Thymidine Kinase-1), VEGF (Vascular Endothelial Growth Factor) aid in diagnosis and prognosis of hemangiosarcoma, osteosarcoma and prostate cancers. Tumor biomarker detection includes immunological methods, cytogenetic analysis and genetic analysis. Tumour biomarkers are beneficial in early detection of cancer, assessing prognosis, therapy response and monitoring relapse across species.

Keywords: Biomarkers, Detection, Diagnostics, Tumours

INTRODUCTION

Cancer remains a major cause of death in both humans and animals, particularly companion animals, despite notable advances in diagnostic and therapeutic methods in recent years. It encompasses a group of disorders characterized by uncontrolled cell proliferation, tissue invasion, and sometimes metastasis. Cancer is the second leading cause of mortality in humans and the first leading cause in dogs and cats. Globally, about 13% of annual deaths are attributed to cancer, with 70% occurring in low and middle income countries. Spontaneous tumors in domestic animals—especially in dogs, whose cancers often resemble those found in humans—provide valuable models for comparative research and for gaining insights into cancer biology and drug development. The age-related cancer incidence per 100,000 individuals per year is estimated to be about 381 in dogs and 264 in cats, compared with approximately 105 in humans¹.

BIOMARKER

According to the Working Group of the U.S. National Institutes of Health (NIH) and the Biomarkers Consortium, a biomarker is any characteristic that can be objectively measured and used to indicate normal biological activity, disease-related processes, or the body's response to a therapeutic intervention. The National Cancer Institute (NCI) further defines biomarkers as biological molecules present in blood, other bodily fluids, or tissues that signal normal or abnormal processes or the presence of a disease. They can also help assess how effectively the body responds to treatment and are sometimes referred to as molecular markers or signature molecules. The World Health Organization (WHO) defines a biomarker as any measurable substance, structure, or process within the body or its products that influences or predicts the occurrence or outcome of a disease².

How to cite this article : Nithyashree S., Ravikumar P., Kavitha Rani B., Manjunatha S.S., Sunil Chandra U. and Bagalakote, P.S. 2026. Prognostic biomarkers of cancer in animals: A systemic approach. Indian J. Vet. Pathol., 50(3) : 193-203.

HISTORY

Early cancer biomarkers were mainly protein-based, such as alpha-fetoprotein (AFP) and prostate-specific antigen (PSA), which were among the first to be developed for clinical use. With technological progress, biomarker research expanded to include circulating tumor DNA (ctDNA) like mutated EGFR, various forms of RNA (especially miRNA), genes released during cell division, peptides, proteins, lipids, metabolites, circulating tumor cells (CTCs), and extracellular vesicles such as exosomes. Cell-free DNA (cfDNA) was first reported in

1948, and later studies showed that tumor-specific genetic changes—such as mutations, methylation abnormalities, and chromosomal variations—could be detected in cfDNA, enabling non-invasive cancer diagnosis. In the 1970s and 1980s, interest in non-invasive biomarkers grew. The first clinically useful biomarker discovered through antibody-based methods was carcino-embryonic antigen (CEA) in 1965. By the late 1970s and 1980s, several other markers, including PSA, AFP, and cancer antigens like CA19-9, CA72-4, CA125, and CA15-3, were introduced. The development of monoclonal antibodies and immunoassays significantly advanced biomarker detection. Genetic discoveries followed, including BRCA1 and BRCA2 in the 1990s, which were useful as key to assessing hereditary cancer risk. From the late 1990s onward, genomic technologies such as microarrays, high-throughput sequencing, and proteomics broadened biomarker research. Major initiatives like the Human Genome Project (HGP) and The Cancer Genome Atlas (TCGA) provided deeper insights into cancer-related genetic alterations. Liquid biopsy emerged as a promising non-invasive approach for detecting ctDNA and monitoring disease. Recent progress includes single-cell sequencing to study tumor heterogeneity, immune biomarkers like PD-L1 for immunotherapy, and the use of artificial intelligence (AI) and machine learning (ML) to analyze large datasets³.

CHARACTERSTICS OF IDEAL BIOMARKERS

An optimal tumor marker should:

- 1. be produced specifically by premalignant or malignant cells early in disease development;
- 2. appear at detectable levels in all individuals with a particular cancer;
- 3. show organ-specific expression;
- 4. be measurable in easily obtained, minimally invasive samples;
- 5. correlate with tumor size, behavior, or disease progression;

- 6. have a short half-life so that changes in tumor burden or treatment response are quickly reflected; and
- 7. have a reliable, standardized, and validated quantitative test⁴.

CLASSIFICATION OF TUMOUR BIOMARKERS

Prognostic biomarkers are identified by characteristics that differentiate benign from malignant tumors. They may also be selected according to the degree of tumor differentiation, which can guide clinical decisions regarding treatment strategies. For instance, human papilloma virus (HPV) - associated oral tumors generally have a more favorable prognosis and longer survival outcomes, as they typically present in a relatively well-differentiated state⁵.

Predictive biomarkers, also known as response markers, are used specifically to evaluate the effectiveness of a particular therapeutic agent. These biomarkers enable clinicians to tailor chemotherapy by selecting drugs most likely to be effective for an individual patient. For example, Herceptin® is effective only in breast cancer cases that exhibit Her2/Neu over expression, while tamoxifen is more suitable for other types of breast cancer. Consequently, Her-2/Neu serves as a predictive biomarker for certain breast cancer treatments ⁶. Similarly, targeted therapies such as erlotinib and gefitinib are effective only in lung cancer patients harboring specific mutations in the epidermal growth factor receptor (EGFR) gene⁷.

Diagnostic biomarkers may be detected at any point during cancer development^{8,9}. Human papilloma virus (HPV) is a known diagnostic biomarker for cancers of the uterus and cervix since it is found in over 90% of tumour lesions. The identification of HPV as a diagnostic biomarker has been pivotal in advancing cervical cancer screening programs and in the development of preventive vaccines¹⁰ (Table 1).

CANINE CANCERS AND THEIR BIOMARKERS

Canine cancer patients serve as valuable models for

Table 1. Methods of detection of tumor markers¹¹

Cytogenetic analysis	• Spectral karyotyping • Comparative genomic hybridization • Fluorescent in-situ hybridization
Genetic analysis	• Sequencing (automated) • Reverse transcription • Gel electrophoresis • DNA micro-array analysis
Proteomics	• Surface-enhanced laser desorption / Ionization
Immunological	• Immunohistochemistry • Radio Immuno Assay • Enzyme-linked Immuno Sorbent Assay • Flow cytometry

human cancer research because many canine tumors closely resemble human cancers in histopathology, biological behavior, and treatment response. Apart from their significance in human cancer research, studying cancers in dogs is crucial for improving canine health and welfare. This emphasizes the importance of exploring the biological mechanisms underlying canine cancers and using comparative research approaches to identify therapeutic targets that can benefit dogs¹².

Canine mammary gland tumors (CMGTs) are the most frequently occurring neoplasms in intact female dogs and rank as the second leading cause of tumor-related death after skin cancers. Roughly half of all CMGTs are diagnosed as malignant based on histopathology, showing aggressive behavior with tendencies for metastasis and recurrence¹³. Spontaneously arising canine mammary tumors have been examined more extensively than any other canine neoplasm worldwide and are considered the most common, accounting for about 52% of tumors in female dogs¹⁴.

Ki-67

Ki-67 is one of the most extensively investigated biomarkers of cell proliferation and apoptosis in canine tumors and has been identified in a wide range of neoplastic types. In mammary tumors, it is the most commonly applied prognostic marker. Ki-67 is a non-histone nuclear protein present only in the nucleus during interphase and throughout mitosis. During mitosis, it relocates to the surface of the chromosomes. Its expression begins in the G1 phase, increases through the S and G2 phases, reaches its peak in the M phase, and then declines rapidly after cell division. It is absent in quiescent (G0) cells. Ki-67 can be measured both as a serum and tissue marker using various molecular techniques. Relying solely on the mitotic index from routine hematoxylin and eosin staining is less accurate than assessing Ki-67 via immunohistochemistry (IHC), because the mitotic index reflects only the cells in mitosis, whereas Ki-67 is expressed during multiple stages of the cell cycle. Moreover, Ki-67 can be assessed in cytology samples obtained by fine-needle aspiration, making it valuable for early detection of mammary tumors. Ki-67 can be detected by both serum ELISA testing and tissue immunohistochemistry (IHC). Increased Ki-67 expression is strongly associated with aggressive tumor behavior, including metastasis, larger tumor size, inflammation, ulceration, local tissue invasion, and lymph-node involvement. Dogs with high Ki-67 expression have shorter survival times and poorer overall prognosis. Older dogs generally exhibit lower Ki-67 expression than younger dogs, likely due to decreased cellular proliferative activity with aging¹⁵.

Proliferating Cell Nuclear Antigen (PCNA)

PCNA is an indicator used to assess the proliferation

index (PI). It functions as an accessory protein for DNA polymerase δ and is present in the nuclei of cells during the DNA synthesis phase of the cell cycle. PCNA participates in DNA repair, regulation of the cell cycle, chromatin organization, and RNA transcription. Its levels rise in late G1, peak during the S phase, and remain elevated through G2 and M phases because of its long half-life. It plays a key role in ensuring continuous cell-cycle progression and acts on both the leading and lagging DNA strands. In human oncology, PCNA is considered a useful proliferation marker, but only when interpreted alongside other breast cancer biomarkers such as ER, PR, Ki-67, or HER-2. One drawback is that PCNA is not exclusively linked to proliferation, as it is also involved in DNA repair. In veterinary medicine, PCNA is commonly measured in mammary tumors. Positive nuclear immunostaining for PCNA is associated with tumor size, histological type, level of differentiation, nuclear grade, mitotic activity, overall malignancy grade, and involvement of lymph nodes. Much like Ki-67, PCNA expression is higher in more aggressive tumors - those that are larger, ulcerated, of higher histologic grade, or associated with lymph-node metastasis. Elevated PCNA expression is linked with poorer prognosis and reduced survival. However, cytokines such as TGF, EGF, and PDGF may stimulate PCNA expression without initiating DNA synthesis, and its involvement in DNA repair also complicates interpretation. For this reason, PCNA is best evaluated in combination with additional biomarkers—especially Ki-67, whose expression is more strictly tied to cell-cycle activity¹⁵.

A retrospective analysis examined the expression of proliferating cell nuclear antigen (PCNA) in canine round cell tumors. Forty cases – involving transmissible venereal tumors (TVT), canine cutaneous histiocytomas (CCH), and mast cell tumors (MCT) - were assessed using immunohistochemistry. Most samples showed moderate to strong PCNA staining. The proportions of PCNA-positive cells were 42.1% in TVT, 57.1% in CCH, and 57.1% in MCT. A statistically significant correlation ($P < 0.05$) was found between mitotic counts and PCNA expression in TVT and CCH. PCNA levels in TVT varied from 17.4% to 56.3%, indicating differing growth rates within this tumor type. Overall, the findings suggest that PCNA immunohistochemistry can serve as a useful supplementary method for evaluating the biological behavior of canine round cell tumors¹⁶.

Argyrophilic Nucleolar Organizer Regions (AgNORs)

AgNOR is a molecular marker widely used to evaluate the rate of cellular proliferation in various types of tumors¹⁷. Nucleolar organizer regions (NORs) are DNA loops located within the nucleoli that contain genes encoding ribosomal RNA (rRNA), which are transcribed by RNA polymerase I¹⁸. These regions are

associated with specific argyrophilic proteins known as NOR-associated proteins (NORAPs). In rapidly proliferating tumor cells, these proteins accumulate as a result of active transcription and can be visualized as distinct black dots using silver staining on routine histopathological sections; these structures are referred to as argyrophilic nucleolar organizer regions (AgNORs)¹⁹. The number of AgNORs observed during interphase in continuously dividing cells correlates closely with the rate of cell proliferation²⁰. Counting mitotic figures in hematoxylin and eosin-stained sections, along with AgNOR enumeration, represents a simple, cost-effective, and practical approach for assessing cellular proliferation. Both the mitotic index and AgNOR count reflect tumor proliferative activity²¹.

Telomerase Activity

Recent research has focused on telomerase and its role in canine oncology, exploring whether telomerase is universally expressed in canine tumors and could serve as a promising target for therapy. Canine telomeres are composed of large, heterogeneous repeats ranging from 3 to 23 kbp. Telomere shortening has been observed *in vitro* in aging canine fibroblast cultures, and telomeric instability has also been documented *in vivo*. Telomerase activity is primarily restricted to tumor tissues, with little or no activity detected in normal somatic tissues such as lung, brain, liver, muscle, and heart. Exceptions include lens epithelial cells (both normal and cataractous) and testicular tissues, consistent with the high proliferative capacity of germ cells. Overall, the pattern of telomerase expression in canine somatic tissues closely resembles that in humans, underscoring that telomerase is not only one of the most widely expressed tumor markers in humans but also the most extensively recognized tumor marker in canines¹².

p53 Tumour Suppressor Gene

p53, often referred to as the “guardian of the genome,” plays a critical role in sensing DNA damage and inducing apoptosis in cells that are irreparably damaged. In humans, its involvement in carcinogenesis is well documented: p53 is mutated or lost in roughly half of all tumors, and in cases where it remains intact, its activity is frequently suppressed. In breast cancer specifically, p53 mutations occur in about 30% of cases and are typically linked to more aggressive disease, including triple-negative subtypes. Moreover, elevated p53 expression is associated with poorer prognosis and reduced overall survival²². p53 is a key biomarker involved in neoplastic transformation, regulation of cell proliferation, and apoptotic pathways. This protein plays a central role in cell-cycle control and programmed cell death and functions as a tumor suppressor. Alterations in the p53 gene are considered among the most frequent genetic changes observed in human breast cancer (HBC) and canine mammary

tumors (CMT), and these mutations are linked to tumor progression. Mutant p53 can also promote the expression of p21, a cyclin-dependent kinase inhibitor; however, p21 expression may also be induced through p53-independent mechanisms. Increased p53 expression is generally linked to unfavourable overall survival¹⁵.

A study aimed to examine the involvement of the p53 tumor suppressor gene in canine mammary tumors. Mutations in exons 5 and 7 were assessed in 15 mammary tumor tissue samples using PCR–single strand conformational polymorphism (PCR–SSCP) analysis followed by direct DNA sequencing. PCR–SSCP analysis detected abnormally migrating bands indicative of mutations in the amplified exon 5 (codons 117–175) of the p53 tumor suppressor gene in 3 (20%) cases of spontaneous canine mammary tumors. PCR–SSCP screening of the p53 gene region spanning amplified exon 5 (from codon 165) through exon 7 (up to codon 241) showed identical banding patterns across all 15 samples, indicating no detectable mutations in this segment²³.

BRCA-1 and BRCA-2

Among the various genetic alterations implicated in mammary tumorigenesis, mutations in the BRCA1 and BRCA2 genes play a particularly significant role in both human and canine mammary gland tumors. The BRCA1 gene, located on human chromosome 17q21, is widely expressed in mammalian tissues and encodes a nuclear phosphoprotein involved in regulating the cell cycle of mammary epithelial cells. Loss of BRCA1 expression leads to impaired DNA repair, disruption of normal cell-cycle control, increased apoptosis, and enhanced tumor development, confirming its function as a tumor suppressor gene. In human medicine, numerous genetic mutations have been identified in breast cancer cases. Germline mutations in BRCA1 and BRCA2 are inherited and confer a markedly elevated lifetime risk of developing human breast cancer, estimated at 56–84%²⁴. Approximately 5–10% of all breast cancers in women are attributed to mutations in these two genes. In veterinary oncology, the hereditary basis of canine mammary tumors (CMT) remains under investigation, although certain dog breeds appear to be predisposed to the disease¹⁵. In one study analyzing mutations across ten breast cancer-related genes in CMT using iPLEX genotyping of 63 single-nucleotide polymorphisms, only BRCA1 and BRCA2 showed a significant association with tumor development²⁵. Reduced nuclear expression of BRCA1 was particularly linked to malignant tumors, a finding consistent with earlier reports²⁶. No significant difference in BRCA2 expression was observed between benign and malignant tumors in that study²⁵.

Cadherins

Cadherins are calcium-dependent transmembrane glycoproteins that mediate cell–cell adhesion and play

a crucial role in preserving normal tissue architecture. The cadherin family includes E-, P-, and N-cadherins. P-cadherin, also known as placental cadherin, is typically expressed in myoepithelial cells, and its overexpression in high-grade tumors is associated with increased tumor aggressiveness and an unfavorable prognosis. However, E-cadherin (epithelial cadherin) is the most extensively studied member of this family due to its central role in epithelial cell adhesion. Loss of E-cadherin is a hallmark of epithelial-mesenchymal transition (EMT), a process that facilitates tumor cell dissemination by enhancing migration and invasiveness. In veterinary oncology, since most canine mammary tumors originate from epithelial cells, decreased E-cadherin expression is associated with increased tumor progression, higher malignancy, metastatic aggressiveness, and shorter survival times. E-cadherin functions in conjunction with catenins, particularly β -catenin, which binds to its cytoplasmic domain and promotes the formation of adherens junctions. Disruption of this complex leads to weakened cell adhesion. During neoplastic transformation, β -catenin degradation is impaired, resulting in its accumulation within the cytoplasm and nucleus of tumor cells. Elevated β -catenin expression is associated with tumor progression, metastasis, and poor prognosis. Under normal conditions, circulating β -catenin levels are typically assessed alongside E-cadherin. In both human and veterinary patients, low E-cadherin expression—most commonly evaluated by immunohistochemistry in tumor tissues—is correlated with an unfavorable prognosis. However, its prognostic value is enhanced when interpreted in combination with other biomarkers, such as Ki-67. Despite its significance, E-cadherin is not among the most routinely assessed biomarkers in canine mammary tumors¹⁵. E-cadherin, in particular, is known to influence tumor progression and metastasis, as its loss is frequently linked to a more invasive and poorly differentiated phenotype in various carcinomas, including pancreatic cancer. On the other hand, N-cadherin (neural cadherin) is associated with increased cancer invasiveness. Recent research has shown that elevated N-cadherin expression in breast cancer is connected to greater invasiveness, driven by interactions between cancer cells and stromal cells mediated by N-cadherin²⁷.

Vimentin

Intermediate filaments (IFs), such as vimentin, perform roles that differ from those of microfilaments and microtubules. Early studies demonstrated that vimentin contributes to cellular integrity by providing structural stability and enhancing resistance to mechanical stress²⁸. The structural and physiological roles of IFs are closely linked²⁹. IFs participate in wound healing processes by regulating fibroblast proliferation, TGF β 1–

Slug signaling, collagen deposition, and epithelial-mesenchymal transition (EMT). In addition, they influence cell proliferation³⁰, adhesion³¹, migration and invasion^{28,32} and act as positive regulators of cellular stemness³³. Numerous studies have demonstrated that vimentin expression patterns differ significantly between normal and malignant epithelial tissues in the prostate, gastrointestinal tract, breast, central nervous system, and lung. Although the transcriptional and translational mechanisms regulating vimentin during cancer progression are varied^{34,35}, its overexpression is consistently linked to a more invasive and metastatic phenotype across these malignancies²⁸.

EGFR (Epidermal growth factor receptor)

Epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase that belongs to the human epidermal growth factor receptor (HER) family. This receptor family includes EGFR (HER-1/ ErbB-1), HER-2/ErbB-2, HER-3/ErbB-3, and HER-4/ErbB-4, all of which are typically localized to the cell membrane. EGFR signaling enhances angiogenesis and metastatic potential and has been associated with unfavourable clinical outcomes in patients with breast cancer. Elevated EGFR expression, most often assessed by immunohistochemistry in tumor tissues, correlates with larger tumor size, presence of tumor necrosis, increased mitotic activity, higher histological grade, and more advanced clinical stage. Additionally, a relationship between EGFR expression and the age of affected dogs has been reported. Positive correlations have also been identified between EGFR and CD31 expression, as well as between EGFR and cyclooxygenase-2 (COX-2) levels. EGFR contributes to angiogenesis by regulating the production and release of pro-angiogenic factors such as vascular endothelial growth factor (VEGF)¹⁵.

Androgen Receptors

The androgen receptor (AR) is a key steroid hormone receptor essential for male sexual differentiation, development, and the maintenance of secondary male characteristics, as well as the initiation and regulation of spermatogenesis³⁶. Functioning as a ligand-dependent transcription factor, AR is activated upon binding to the androgenic hormone's testosterone or dihydrotestosterone³⁷. Both normal prostate tissue and prostate cancer (PCa) rely on androgens for growth, and proper prostate development requires a functional androgen receptor (AR). Testosterone, the main circulating androgen in males, is primarily synthesized by Leydig cells in the testes³⁸. Within the prostate, testosterone is converted by the enzyme 5 α -reductase into 5 α -dihydrotestosterone (DHT), which binds to AR and stimulates the growth of male urogenital tissues. AR serves as the principal mediator of androgen signaling in the prostate. Activation of AR by DHT is essential for full

prostate development, as individuals lacking functional 5 α -reductase exhibit only rudimentary prostate tissue or complete absence of the prostate³⁹. Prostate cancer is likewise initially androgen-dependent and requires functional AR signaling; tumors typically undergo temporary regression following castration. AR expression persists in both androgen-dependent (AD) and androgen-independent (AI) PCa and is maintained throughout disease progression to hormone-refractory stages^{40,41}. Consequently, PCa treatment strategies primarily aim to inhibit androgen signaling, with androgen ablation leading to atrophy of the prostate epithelium. First-line therapy commonly involves androgen deprivation therapy (ADT), achieved by suppressing androgen production through surgical or chemical castration and/or administration of anti-androgens such as bicalutamide or enzalutamide (MDV3100). Despite initial responsiveness, many cases progress to an advanced androgen-independent state that is resistant to existing treatments, termed castration-resistant prostate cancer (CRPC), which arises due to reactivation of AR signalling⁴².

Cyclooxygenase - 2

Cyclooxygenase (COX) is an enzyme of the myeloperoxidase family that catalyzes the conversion of arachidonic acid into prostanoids. These products—including prostaglandins, prostacyclin, and thromboxane—are biologically active molecules that regulate a wide range of physiological functions in both humans and animals⁴³. Over expression of COX-2 induces a phenotypic shift from benign to malignant cells, a process associated with dysregulated growth and proliferation, enhanced resistance to apoptosis and immune surveillance, stimulation of angiogenesis, and increased invasive capacity⁴⁴. Research on COX-2 expression in animal populations across different countries has shown that the enzyme is frequently overexpressed in a variety of canine epithelial tumors. These include mammary gland adenocarcinomas and carcinomas, ovarian and prostate tumors, transitional cell (urothelial) carcinoma (TCC), colorectal and small intestinal tumors, squamous cell carcinoma (SCC) of the skin and oral cavity, osteosarcoma, and melanoma^{45,46,47,48}. In contrast, COX-2 is either minimally expressed or absent in canine lymphoma, sarcoma, fibrosarcoma, glioma, and mastocytoma⁴⁹. Studies on small groups of feline patients indicate that, in contrast to dogs, COX-2 is expressed less frequently and at lower levels in cats⁵⁰.

Alpha Fetoprotein

Alpha-fetoprotein (AFP) is produced in large amounts primarily by the liver during embryonic development and represents one of the predominant proteins in fetal circulation. After birth, AFP expression is transcriptionally suppressed, resulting in very low serum

concentrations in adult animals. AFP has been reported to exert immunosuppressive effects and to promote angiogenesis, thereby supporting neovascularization in both fetal and neoplastic tissues. Because AFP is predominantly synthesized by the liver, veterinary research has mainly investigated its potential role as a biomarker for canine hepatic tumors. In dogs, serum AFP concentrations are markedly elevated at birth (approximately $14,080 \pm 5,944$ $\mu\text{g/ml}$) and progressively decline during growth, reaching values between 0.014 and 0.069 $\mu\text{g/ml}$ in adulthood. Increased AFP concentrations are observed in dogs with lymphomas and mast cell tumors. Although elevated serum AFP levels may indicate an increased likelihood of neoplastic disease, no definitive serum cut-off value has been established in dogs to determine diagnostic sensitivity and specificity. A significant reduction in serum AFP levels has been observed following surgical resection of affected liver lobes⁵¹. A study evaluated Serum alpha-fetoprotein (AFP) levels before and after surgical excision of tumor masses in four dogs diagnosed with hepatocellular carcinoma (HCC), and assessed AFP expression in tumor tissues by immunohistochemistry. In three dogs, preoperative serum AFP concentrations were 10–20 times higher than those observed in healthy dogs and returned to normal levels within one to two months following surgery. Correspondingly, AFP immunoreactivity was detected in the tumor tissues of these cases. In contrast, one dog exhibited low serum AFP levels and showed no AFP expression within the tumor tissue⁵².

CEA (Carcino Embryonic Antigen)

Carcinoembryonic antigen (CEA) is a membrane-associated glycoprotein primarily produced in specific regions of the gastrointestinal tract, where it functions in cell adhesion. Under normal physiological conditions, circulating CEA levels are very low. However, certain tumor cells can overproduce CEA and express it across their entire cell surface as a result of loss of cellular polarity. Because these cells no longer adhere to the basal lamina, CEA is released into the blood stream, leading to elevated serum concentrations in patients with particular malignancies. CEA tumor marker is used to detect and monitor malignancies, including mammary gland tumors, in both humans and animals. Its molecular weight is typically around 180–200 kDa, though this can vary across species and protein isoforms⁵¹. CEA is mainly produced in the fetal gut and liver during embryonic development. In adults, its expression is minimal under normal conditions but can be produced by epithelial tissues. In pathological states, such as mammary gland tumors, tumor cells themselves can synthesize CEA, which is important for its diagnostic relevance⁵³. CEA has no significant physiological role in healthy individuals. Elevated levels are associated with malignancies,

serving as a marker of tumor activity and indicating the presence of cancer, including mammary gland tumors in animals⁵⁴. CEA was initially identified in human colon cancer and named for its detection in embryonic tissues. Its recognition as a tumor marker led to its use across multiple cancer types, including veterinary mammary tumors⁵⁵. In dogs, reference serum CEA levels in healthy individuals are 0.12–0.23 ng/ml for radioimmunoassay⁵⁶.

Thymidine kinase (TK-1)

TK1 is an enzyme primarily synthesized in proliferating cells during the S phase of the cell cycle. While various tissues can produce TK1, its expression is markedly elevated in fast-dividing cells, including tumor cells. In hemangiosarcoma, cancer cells contribute significantly to circulating TK1 levels. The enzyme's synthesis is closely linked to DNA replication, and higher blood levels reflect increased cellular proliferation⁵⁷. Under normal conditions, TK1 plays a vital role in DNA replication by phosphorylating thymidine, which is then incorporated into the growing DNA strand, supporting cell division and tissue growth. In cancer, TK1 levels rise substantially, reflecting abnormal cell proliferation, making it a sensitive marker for cancer detection and monitoring⁵⁸. TK1's role as a cancer biomarker is a relatively recent discovery. Elevated TK1 activity in the blood was linked to cancer cell proliferation, establishing its potential for diagnosing and monitoring malignancies, including hemangiosarcoma⁵⁹. TK1 levels can be measured using techniques such as ELISA, spectrophotometry, radioimmunoassay (RIA), HPLC, and mass spectrometry. ELISA is commonly used due to its accuracy and practicality. These assays utilize antibodies specific to TK1, enabling precise quantification in biological samples⁵⁷. Measuring TK1 in the bloodstream is crucial in veterinary oncology, particularly for hemangiosarcoma. Elevated TK1 reflects increased cell proliferation, aiding in early cancer detection, evaluating tumor burden, and monitoring treatment response. In hemangiosarcoma, TK1 helps to differentiate malignant from benign masses, which may be challenging by other diagnostic methods. TK1 is also included in specialized panels, such as the "TK canine cancer panel," to confirm cancer presence, assess therapy effectiveness, and verify remission⁶⁰. For example, a study on canine malignant lymphoma showed that normal dogs had serum TK activity of 0–7 U/L, whereas dogs with lymphoma had levels ranging from 8.9 to 470 U/L—1.3 to 67 times higher than healthy dogs. These findings demonstrate that TK1 activity is significantly increased in dogs with malignancies, reinforcing its utility as a diagnostic biomarker⁶¹.

VEGF (Vascular endothelial growth factor)

Vascular endothelial growth factor (VEGF) is one of the most widely used biomarkers in human medicine

due to its central role in angiogenesis. VEGFs are encoded by four genes: VEGF-A, VEGF-B, VEGF-C, and VEGF-D. While VEGF-A and VEGF-B primarily regulate angiogenesis, VEGF-C and VEGF-D are involved in lymphangiogenesis. In normal tissues, VEGF regulates the formation, structure, and function of blood vessels. It promotes endothelial cell proliferation and migration, increases microvascular permeability—facilitating tumor cell escape and metastasis—prevents regression of newly formed vessels, stimulates endothelial invasion for new vessel formation, and inhibits apoptosis. Elevated VEGF levels are associated with tumor progression and metastasis, while reduced VEGF expression suppresses tumor growth. In canine mammary tumours (CMT), higher serum VEGF levels correlate with advanced clinical stage, poorer prognosis, and decreased survival, particularly in aggressive, infiltrative tumors or those with histological necrosis. VEGF is therefore considered a marker of tumor progression and metastatic potential. Both in human and veterinary medicine, VEGF serves as a reliable biomarker in tissue and serum and is particularly valuable for early detection of human and canine breast cancers. Its diagnostic sensitivity is further enhanced when combined with CA 15-3 measurements¹⁵.

Canine Prostate-Specific Esterase (CPSE)

Canine prostate cancer, similar to human cases, primarily affects older male dogs and is often diagnosed at an advanced stage, frequently after metastasis, which complicates treatment. Unlike humans, where prostate-specific antigen (PSA) is a standard biomarker, dogs lack a single well-established marker, though canine prostate-specific esterase (CPSE) has shown promise and remains under investigation. Serum CPSE has emerged as a reliable and specific biomarker for various prostatic disorders in dogs, including benign prostatic hyperplasia (BPH), bacterial prostatitis, and prostatic carcinoma. Measuring CPSE in serum provides a new diagnostic and screening tool, useful for diagnosing BPH, proactively screening healthy dogs, and monitoring dogs during and after treatment. CPSE levels are stable regardless of circadian rhythms or trans-rectal palpation; however, sexual rest for at least 24 hours is recommended before measurement, as recent ejaculation can affect CPSE levels. CPSE shows diagnostic characteristics similar to human PSA, highlighting its potential for indicating prostate disorders⁶².

Micro RNA

MicroRNAs (miRNAs) are small, non-coding RNA molecules present in cells that regulate gene expression by inhibiting mRNA translation. They are involved in many diseases, including cancer, due to their critical roles in cellular processes. In oncology, miRNAs can function as either tumor suppressors or oncogenes, making them promising targets for both diagnostic and

therapeutic applications. MicroRNAs are crucial for regulating gene expression and maintaining cellular homeostasis. In pathological conditions such as cancer, dysregulation of miRNAs can disrupt key processes like cell proliferation, apoptosis, and angiogenesis. Various techniques exist to measure miRNA levels in biological samples. Spectrophotometry can quantify miRNA concentrations, while reverse transcription-quantitative polymerase chain reaction (RT-qPCR) allows specific profiling. High-throughput analysis can be performed using microarrays, and ELISA can detect specific miRNAs in serum or plasma. In veterinary oncology, measuring miRNA levels in tumors, blood, or other biological fluids provides valuable diagnostic information. MicroRNAs can act as tumor-specific biomarkers, assisting in cancer detection, subtyping, and prognosis⁶².

CTCs (Circulating tumor cells)

Circulating Tumor Cells (CTCs) play a key role in the formation of metastases. These cells originate from solid tumors and gain the ability to detach and enter the bloodstream. Because CTCs are extremely rare—approximately 1 CTC per 10^6 – 10^7 leukocytes—detection requires highly sensitive methods, often involving enrichment and isolation techniques. These approaches rely on identifying cell surface markers, exploiting physical or biological properties (such as size, density, or invasiveness), or using molecular methods like RT-PCR to detect CTC-specific RNA. Currently, the only FDA-approved method for identifying, isolating, and counting CTCs (approved in 2004) combines an enrichment step with specific antibody-based detection and high-resolution imaging. CTCs identified by this method are characterized by the phenotype EpCAM⁺/CK⁺/DAPI⁺/CD45⁻. Due to this approval, most human oncology studies use this technology⁶³. In veterinary medicine, however, RT-PCR has been applied to detect CTC-specific mRNAs in canine blood⁶⁴.

Ct-DNA (Circulating tumour DNA)

In both human and veterinary medicine, circulating DNA, including circulating tumor DNA (ctDNA), offers valuable insights into disease processes. In humans, advanced technologies such as sequencing and digital PCR enable early cancer detection, detailed tumor characterization, prognostic evaluation, and treatment monitoring based on ctDNA levels. In canine oncology, the CNRS (French National Centre For Scientific Research). “Dog Genetics” team at the Institute of Genetics and Development in Rennes has developed ctDNA assays for histiocytic sarcoma and lymphoma. Their research identified tumor-specific mutations shared between canine and human histiocytic sarcomas, detectable in the plasma of affected dogs, providing a non-invasive diagnostic method. Notably, ctDNA was detectable in 92.3% of canine lymphoma cases at diagnosis and has

been used to monitor treatment response and predict relapse in dogs with diffuse large B-cell lymphoma. These tests represent important tools for clinical oncology research and diagnostics. Circulating cell-free DNA (cfDNA) consists of short, double-stranded DNA fragments, usually 180–200 base pairs in length, present in blood and other biological fluids. cfDNA is released by cells from various organs through processes such as apoptosis, necrosis, and active secretion. While levels are low in healthy individuals, they rise in pathological conditions, including cancer. Under normal conditions, cfDNA is present at low levels and contributes to genetic material maintenance. In cancer, however, cfDNA can include ctDNA carrying tumor-specific genetic and epigenetic alterations that reflect tumor progression, treatment resistance, and malignancy. Techniques for cfDNA and ctDNA analysis include spectrophotometry, ELISA, HPLC, mass spectrometry, and RIA. These approaches allow for quantification and genetic profiling of ctDNA⁶².

BOVINE CANCER BIOMARKER

Anti-Müllerian hormone (AMH) is a glycoprotein produced by sertoli cells in males and by granulosa cells of early-stage ovarian follicles in females. After puberty, AMH secretion decreases in several species, including men, bulls, and stallions. Consequently, AMH has mainly been investigated as a biomarker for granulosa cell tumors (GCT) and sertoli cell tumors (SCT) in humans and animals. In cows and mares, serum AMH concentrations accurately indicates the presence of GCT, with cut-off values of 0.36 ng/ml in cows and 4 ng/ml in mares, and sensitivities of 100% and 98%, respectively⁵¹. A study evaluated anti-Müllerian hormone (AMH) as a non-invasive biomarker for diagnosing granulosa-theca cell tumors (GTCTs) in cattle. It showed that AMH had the highest diagnostic value (AUC = 0.99). At a cutoff value of ≥ 0.36 ng/mL, AMH achieved 100% sensitivity, 99.1% specificity, and 99.2% diagnostic accuracy. These results indicated that plasma AMH concentration is a highly sensitive and reliable biomarker for the diagnosis of bovine GTCTs⁶⁵.

EQUINE CANCER BIOMARKER

A study evaluated whole-blood microRNAs (miRNAs) as long-term diagnostic and prognostic biomarkers for equine sarcoids. Based on clinical examination at 3 years of age and follow-up after 5–12 years, 77 horses were classified into regression, progression, newly affected, and tumor-free control groups. Expression of eight miRNAs was measured by RT-qPCR, and the effects of sex, breed, diagnosis, and prognosis were analyzed. Sex and breed significantly influenced miRNA expression. Eca-miR-127 distinguished sarcoid-affected horses from tumor-free controls, whereas no miRNA differentiated between sarcoid regression and progression. Expression

of eca-miR-125a-5p and eca-miR-432 differed in male horses that later developed sarcoids compared with male controls. Overall, the studied miRNAs were not predictive of sarcoid outcome but showed potential for identifying young, tumor-free male horses at risk of developing sarcoids. These findings emphasize the importance of sex- and breed-specific effects in equine miRNA biomarker studies⁶⁶.

POULTRY CANCER BIOMARKER

A study investigated the role of chicken telomerase reverse transcriptase (chTERT) in regulating the Wnt/ β -catenin signaling pathway and its contribution to the tumorigenicity of avian leukosis virus subgroup J (ALV-J) in vivo. The reported plasma levels of β -catenin and c-Myc were markedly higher in chTERT-overexpressing, ALV-J-infected chickens compared with controls. ALV-J tumors exhibited enhanced Wnt/ β -catenin pathway activity, increased c-Myc expression, reduced apoptosis, and higher viral replication relative to adjacent non-tumorous tissues, as confirmed by immunohistochemistry, western blotting, and RT-qPCR. These findings demonstrated that chTERT promotes ALV-J-induced tumorigenesis by activating the Wnt/ β -catenin signaling pathway, providing insight into the molecular mechanisms underlying ALV-J-associated tumors⁶⁷.

CONCLUSION

Biomarkers play critical tools in modern veterinary oncology, providing valuable insights beyond what imaging and histopathology can offer. They aid in early and accurate diagnosis, helping into differentiate benign and malignant lesions and even identify the tumor origin. Real time monitoring using serum or liquid biomarkers allows better assessment of therapeutic effectiveness and early detection of relapse. While biomarkers enhance diagnostic precision, their high cost and limited availability can restrict routine use in general veterinary practice.

ACKNOWLEDGEMENT

We acknowledge the Dean, Veterinary College Shivamogga for the technical facilities.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)- Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI- assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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