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C-reactive Protein and Albumin Based Predictive Indices in Canine Cancer

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Abstract

Inflammation is believed to be one of the hallmarks of cancer. Inflammatory mechanisms play a role in cancer development as well as progression and metastasis. Systemic inflammation has been demonstrated in multiple neoplastic processes in humans. A well standardised and readily available method to assess systemic inflammation is the measurement of acute phase proteins. CRP and albumin being a major positive and a negative acute phase protein respectively appear to be an attractive way to assess systemic inflammation in patients suffering from neoplastic diseases. Their ratio (CRP to albumin ratio, CAR) as well as prognostic scores, namely the Glasgow Prognostic Score (GPS) and the modified Glasgow Prognostic Score (mGPS) offers a combination to assess them together in a single parameter. Numerous human studies have evaluated these and demonstrated their usefulness in survival prognostication before treatment initiation as well as advanced inoperable disease, along with predictive value for response to therapy and side-effects. Some of these aspects have been demonstrated in multiple types of cancer and appear to be independent of other measures of disease activity, such as stage or performance status. However, veterinary literature on this topic is scarce.

Materials and methods

Retrospective analysis of dogs investigated in the Veterinary Haematology and Oncology Centre, Budapest, suffering from various neoplastic conditions in various stages of their disease is conducted. A cohort of healthy dogs and dogs suffering from other non-neoplastic disorders. Descriptive as well as statistical methods were used to assess CAR, GPS and mGPS in this group of patients.

Results

Statistically, significant differences were found in albumin concentration between the healthy and neoplastic as well as the other diseases and neoplastic cohorts but not between the healthy and other disease cohorts. In terms of CRP all cohorts differed from each other. CAR values were significantly different between healthy and neoplastic as well as other diseases and neoplastic cohorts but not between healthy and other cohorts. Higher GPS and mGPS scores were observed in the neoplastic group. Furthermore, it appears that dogs suffering from neoplastic diseases with active disease show significantly higher CRP and CAR values but not lower albumin then those in remission. Further studies are necessary to evaluate the prognostic value of these markers in dogs suffering from neoplastic disease.

Absztrakt

A tumorok egyik fémjele a gyulladás. A gyulladásos jelenségek szerepet játszanak a tumorok kialakulásában ugyanúgy, ahogy a progressziójukban és áttétképzésük folyamán. Emberekben számos daganatos folyamat során mutattak ki szisztámás gyulladást. Ennek kimutatására jól sztenderdizált és könnyen elérhető módszer az akut fázis fehérjék vizsgálata. A CRP egy major akut fázis fehérje, míg az albumin negatív akut fázis fehérje Ezek mérése a szisztémás gyulladás vizsgálatának vonzó lehetőségét rejti. Arányuk (CRP:albumin arány, CAR) illetve a használatukkal készített prognosztikai pontozásos módszerek (a Galsgow Prognosztikai Index – GPS - és a módosított Glasgow Prognosztikai Index – mGPS) összetett vizsgálati lehetőséget biztosít, mellyel egy eredményben bírálható el a két parameter. Számos human tanulmány vizsgálta ezek prognosztikai értékét különféle körülmények között: kezelés előtt ugyanúgy, ahogy előrehaladott áttétes, inoperábilis daganatos betegek körében, a kemoterápia hátékonyságának előrejelzésében valamint a súlyos nemkívánatos mellékhatások jelentkezésével kapcsolatban is. Az előbbiek közül néhány paraméter számos daganatos megbetegedésben egyéb változóktól (stadium, a betegek teljesítőképessége) független prediktív paraméternek bizonyult. Mindezek ellenére az állatorvosi irodalmi adatokból cask kevés áll rendelkezésre.

Anyag és módszer

Az Állatorvosi Hematológiai és Onkológiai Központ beteganyagából retrosepktív vizsgálatot végeztünk. Daganatos betegek mellett egészséges és egyéb, nem tumorous megbetegedésben szenvedő állatok vérmintáinak eredményét hasonlítottuk össze, statisztikai módszerek mellett leíró módszerek használatával.

Eredmények

Szignifikáns eltérések mutatkoztak albumin koncentráció tekintetében az egészséges és daganatos betegek valamint az egyéb betegségekben szenvedő és a daganatos betegek között is, ugyanakkor az egészséges és az egyéb betegségekkel küzdő betegek között nem volt különbség. Mindegyik csoport különbözött egymástól CRP koncentráció tekintetében. A CAR tekintetében az albuminhoz hasonló eredmények születtek. A többi csoporthoz képest magasabb GPS és mGPS érték mutatkozott a daganatos betegek körében. E mellett az aktív tumorous megbetegedésben szenvedő kutyák magasabb CRP koncentrácciót és CAR értéket mutattak, mint a remisszióban lévő társaik. Az előbbi két csoport mintáiból mért albumin koncentráció nem mutatott szignifikáns különbséget. További tanulmányok szükségesek

kutyákban a daganatos megbetegeédsek mellett jelentkező szisztémés gyulladás vizsgálatára alkalmas prognosztikai indexekkel kapcsolatban.

List of Abbreviations

AGP - α-1 acid glycoprotein

APP – Acute Phase Protein

BCG - Bacillus Calmette-Guerin

cL – canine lymphoma

CAR – C-reactive protein to albumin ratio

CRP – C-reactive protein

ECOG - Eastern Cooperative Oncology Group

GPS - Glasgow Prognostic Score

GM-CSF – Granulocyte-Macrophage Colony-Stimulating Factor

Hp – Haptoglobin

hsCRP - high sensitivity CRP

IBD – Irritable Bowel Disease

IL-1 – Interleukin-1

IL-6 – Interleukin-6

IMHA – Immune-mediated haemolytic anaemia

IPI - international prognostic index

LOB - limit of blank

LOD - limit of detection

LOQ - limit of quantification

LoS – Length of hospital stay

LPE - Lymphocytic Plasmacytic Enteritis

MAPK – Mitogen-Activated Protein Kinase

M-CSF – Macrophage Colony-Stimulating Factor

MCT – Mast Cell Tumours

mGPS – modified Glasgow Prognostic Score

mTOR – mammalian Target Of Rapamycin

NHL – Non-Hodgkin Lymphoma

NLR - Neutrophil to Lymphocyte Ratio

OHPSCC - Oropharyngeal and Hypopharyngeal Squamous Cell Carcinoma (OHPSCC)

OS - overall survival

PI3K – Phosphoinositide 3-kinase

PNR - Platelet to Neutrophil Ratio

PNI - prognostic nutritional index

ROC – Receiver Operating Characteristics

SAA – Serum Amyloid-A

Th1 – helper T-cell

TNF-α – Tumour Necrosis Factor α

TNM – Tumour Node Metastasis

VEGF – Vascular Endothelial Growth Factor

WBC – White Blood Cell

WHO – World Health Organisation

Introduction

Domestic dogs have a long history with humankind, from their domestication to man's best friend or invaluable work colleague, often being regarded as entire family members [46]. Dogs now live in great numbers in our households and us humans take care of them more and more especially with veterinary medicine evolving [4]. Therefore, dogs are generally living longer than they use to due to better general and veterinary care which predisposes them to cancer [71]. Ageing is the most significant risk factor for cancer demonstrated both in humans and animals [67] [71].

The inflammation process is intrinsically linked to the neoplasia formation as it is part of the immune system defense mechanism against neoplasia. One of these responses is the acute phase response which is a component of a non-specific and complex host immune response to inflammation [68]. The acute phase response involves the increased or decreased production of acute phase proteins (APPs). APPs with increased production include C-reactive protein (CRP) and serum amyloid A (SAA), α-1 acid glycoprotein (AGP) and haptoglobin (Hp) whereas the most important APP with decreased production is albumin. CRP is a common inflammatory marker, although non-specific, it is highly sensitive [63]. Albumin being a "major" negative APP as it decreases in response to an inflammation, disease, or injury [67] [3]. Systemic inflammatory response correlates strongly with hypoalbuminaemia and in human cancer patients, weight loss and relates to a decreased complete remission and survival time in NHL [38]. Hypoalbuminaemia was revealed in canine lymphoma and also related to a reduced remission and survival time [38].

Traditionally, it was assumed that in humans APPs were solely hepatocyte derived, now there is stronger confirmation to reinforce extra-hepatic generation in neoplastic and other disease states such as primary renal tumours, survival in colorectal cancer and oesophageal squamous cell carcinoma [68]. In humans, CRP has been revealed to be valuable in recognising metastatic disease from primary renal tumours [17] as well as exhibiting promise for measuring rejection in renal transplant patients [7]. Survival in colorectal cancer correlates with serum CRP [37] and oesophageal squamous cell carcinoma [72] while cancer activity, stage, and prognosis in gastric tumours have been found to correlate with SAA concentrations [39] [16]. Tumour tissue may itself create APPs [37] with a poorer survival outcome according to immunohistochemical studies in people with oesophageal carcinoma.

A similar association has been found in colorectal tumours, ovarian carcinoma and AGP [66] [70].

Due to the non-specific nature of APPs, with noticeable variation in their response inflammation and tissue damage, simultaneously measuring several APPs is likely to be more advantageous than analysing a single value in isolation [68]. Clinical use may be optimised by using APP profiles that use at least one major, one moderate and one negative APP [67].

In human medicine, merged use of various biomarkers is preferred to a single isolated biomarker and in cancers. Scoring systems which integrate serum biomarkers of systemic inflammation; for example, the international prognostic index (IPI), prognostic nutritional index (PNI), neutrophil lymphocyte ratio (NLR) and the Glasgow Prognostic Score/modified Glasgow Prognostic Score (GPS/mGPS) are utilised to forecast prognosis, conduct treatment decisions, and ameliorate patient outcome [38] [36]. However, in canine lymphoma no veterinary prognostic scoring structures are currently in regular use [38].

The inflammation process is intrinsically linked to the neoplasia formation such as lymphoma as it is part of the immune system defense mechanism against neoplasia. One of these responses will be the aim of this study; the acute phase protein (APP) response which is a component of a non-specific and complex host immune response to inflammation [68]. APPs include C-reactive protein (CRP) and serum amyloid A (SAA), while moderate response APPs include α-1 acid glycoprotein (AGP) and haptoglobin (Hp). CRP is a common inflammatory marker, although non-specific, it is highly sensitive [61]. Albumin being a “major” negative APP as it decreases in response to an inflammation, disease, or injury [67] [3]. Hypoalbuminaemia correlates strongly with the systemic inflammatory response and weight loss in human cancer patients and relates to reduced complete remission and survival time in NHL [38]. Hypoalbuminaemia was revealed in canine lymphoma and also related to reduced remission and survival time [38].

Neoplastic diseases are diverse, and their therapies are varied, involving surgery, chemotherapy, and radiation therapy amongst others. Due to this diverse nature prognostication of the disease may require extensive diagnostic workup. It appears that the close link of inflammation to cancer may aid this prognostication and acute phase proteins and derived prognostic indices or scores offer a readily available method helping clinicians and patients alike.

Literature Review

Cancer and inflammation

Association of cancer and inflammation has long been described and studied. Initially in the 19th century Marjolin described cancer development at a posttraumatic site [29] and later Virchow observed inflammatory cells in tumours suggesting a role of inflammation in cancer [34]. It has also been observed that chronic inflammatory disorders such as obesity promotes cancer development and that the use of non-steroidal anti-inflammatory medication mitigates cancer development risk [44]. In fact, systemic inflammation is described as the seventh hallmark of cancer [2].

Inflammation itself has diverse roles and both antineoplastic and tumour promoting effects were observed. On one hand it was observed that an increased inflammatory response may lead to cancer regression, first described by Coley, who employed bacterial extract to induce remission in selected patients with cancer [34]. This phenomenon is currently used routinely in some cases in human medicine, notably bacillus Calmette-Guerin (BCG) is a therapeutic adjunct in bladder cancer patients improving outcomes via proinflammatory pathways [63]. Although, it is well known that inflammatory conditions may lead to tumorigenesis some exceptions exist, such as psoriasis in people, where the inflammation associated with the disease favours the selection of M1 macrophage through Th1 (helper T-cell) activation. The M1 macrophages appear to have anti-tumour activity which prevents the development of epithelial neoplasia in these patients [53].

However, systemic inflammation in most cases seems to play a different, pro-neoplastic role and contributes to cancer morbidity and mortality. Systemic inflammation promotes tumorigenesis and has been described in numerous studies. For example, obesity leads to a hypoxic tissue environment; in addition, overproduction of insulin, leptin and other hormones lead to the activation of pro-oncogenic molecular pathways (MAPK, mTOR, PI3K) [34]. Neoplastic diseases can induce systemic inflammation too, although the exact pathomechanism is not fully elucidated. Non-specific immune responses to tumour necrosis or hypoxia may be responsible for the initiation of a systemic response through pro-inflammatory cytokine production (IL-6, VEGF, M-CSF, GM-CSF). As a result, neutrophil granulocytes, myeloid derived suppressor cells and regulatory T-cells accumulate in tissues inhibiting lymphocyte function and inducing an immunosuppressive macrophage phenotype

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which leads to the amplification of inflammation. This cascade further promotes the recruitment of inflammatory cells to the cancer site where these cells contribute to the promotion of cancer, neovascularisation and later the formation of metastases [34]. It is hypothesised that systemic inflammation contributes to up to 15-20 % of cancer related death in people [53].

Inflammation based prognostic scores: CRP to albumin ratio (CAR), Glasgow Prognostic Score, modified Glasgow Prognostic Score

The acute phase response occurs directly after tissue injury and may be caused by a span of different sources, including infectious, inflammatory, neoplastic, traumatic, or immunological disease [68]. It is moderated by pro-inflammatory cytokines such as interleukin-1 (IL-1), tumour necrosis factor α (TNF-α) and interleukin-6 (IL-6) [31] [32] [69] and triggered by the disease process [47] [67]. The early response to insult or injury is an essential role of the acute phase response and plays an important part in the survival of the host during these initial stages [68].

Acute phase protein (APP) response differs between species [73] and involve major, moderate, and minor response levels as well as negative acute phase proteins. A major APP will have low concentration in healthy animals but will appear in a considerable rise (100-1000-fold) on stimulation with a peak between 24 to 48 hours and then a rapid decrease [68]. A moderate response APP will expand 5 to 10-fold following incitement over 48 to 72 hours to peak and will decline gradually [68]. A minor response APP will expand only steadily and up to twice its resting level [68]. In dogs, major response APPs include C-reactive protein (CRP) and serum amyloid A (SAA), while moderate response APPs include α-1 acid glycoprotein (AGP) and haptoglobin (Hp). The positive APPs (Hp, CRP, caeruloplasmin, SAA, AGP, fibrinogen) expand in response to release of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α while negative APPs (albumin, transferrin) decline in response to inflammation [67]. The acute phase protein reaction can be misleading as it can accompany both acute and chronic states, including cancers [12].

Considering that systemic inflammation appears to play a key role in cancer progression it is judicious to hypothesise that markers of this phenomenon may serve as prognostic markers of the ongoing disease. Indeed, several of these have been described and analysed and a growing body of evidence is gathered. Acute phase proteins as well as inflammatory

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cells and platelets serving as non-specific inflammatory markers have been investigated in various neoplastic disorders under various circumstances. Their standardised and routine measurement along with usually high precision (low coefficient of variation) facilitates their use [41]. Recently in a thesis at UVMB Réka Bozsó demonstrated that thrombocytosis is a negative prognostic factor of survival in urothelial carcinoma in dogs [30].

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A combination of these markers may lead to an even better prognostication possibility. Hence, CRP to albumin ratio (CAR), neutrophil to lymphocyte ratio (NLR) and platelet to neutrophil ratio (PNR) came forward amongst others.

CRP and albumin are extensively investigated in human medicine. In addition to the aforementioned CRP to albumin ratio prognostic scores appeared: the Glasgow Prognostic Score (GPS) [41] and the modified Glasgow Prognostic Score (mGPS). These scoring systems allow for a simplified approach, assigning a value of 0 or 1 to CRP and albumin if their value is within reference range or above (in the case of CRP) or below (in the case of albumin) of their respective upper or lower reference limits. In the case of mGPS low albumin only matters if it is combined with a high CRP value. This way a score between 0-2 can be assigned to a patient in both scoring systems.

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mGPS appreciates that in most cases the GPS score of 1 (i.e. either elevated CRP or decreased albumin) is attributable to an elevated CRP [41]. In colorectal cancer patients it was observed that low albumin as a cause of a score of 1 happens in less than 10 % of the cases and it was concluded that low albumin alone does not correlate with decreased survival [59]. Hence, nowadays the mGPS is used more frequently than GPS.

The value of CAR, GPS and mGPS in neoplastic diseases in humans is extensively studied. A search in PubMed reveals that more than 2,100 articles were published on CAR, GPS and mGPS in the last 2 decades. However, only a handful of articles were published on canine diseases and mostly individual acute phase proteins were investigated.

CAR, GPS and mGPS in the human literature

CAR, GPS and the mGPS utilise the same biomarkers, CRP and albumin in order to determine the severity of systemic inflammation. CRP is a major positive acute phase protein whose concentration increases rapidly, sometimes as much as 10-100x of the baseline value on inflammation. Albumin on the other hand is a negative acute phase protein whose concentration in plasma decreases in inflammatory conditions [50]. This stereotypical response has been demonstrated across several cancer types [64].

Glasgow prognostic score was introduced in 2003 by Forrest *et al*., [41] investigating the outcomes of inoperable non-small-cell lung cancer patients receiving chemotherapy. They found that GPS is an independent prognostic indicator for survival in these patients and is comparable to other, conventional methods. Yet, they suggested that the ease of measurement and routine availability along with high reliability of the methods involved is the major advantage of GPS [41]. Indeed, conventional methods such as clinical staging or the assessment of performance status (such as the ECOG performance status) requires more investigation and – in the case of performance status - is less objective than GPS.

One proposed advantage of CAR, GPS and mGPS is that apart of systemic inflammation they are also reflecting the nutritional status of the patient. Albumin concentration is partially dependent on tissue cell mass and hence, decreased nutritional status leads to hypoalbuminaemia. It has long been established that weight loss (in particular sarcopenia) is associated with poor chemotherapy tolerance in cancer patients and therefore contributes to poor survival as well [64].

CAR, GPS and mGPS can be utilised in several scenarios. Their prognostic value has been demonstrated in pre-treatment scenarios. Additionally, their use was advocated in patients with advanced non-resectable neoplastic disease. Yet, another value is that in some cases they may predict treatment failure in neoplastic disease well before other diagnostic methods (imaging) are usually performed allowing for early treatment modification. The latter two scenarios suggest a possible longitudinal use of these markers. In some studies, severe side-effects of chemotherapy were associated with higher scores.

It was also found that these scores are prognosticators independent of the stage of the disease and treatment modality in most studies and also superior to white blood cell count or ECOG (Eastern Cooperative Oncology Group) performance status (a test assessing the patient's ability to perform ordinary tasks).

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CAR sometimes appear to be a better marker than GPS or mGPS. Being a continuous variable, more subtle changes can be appreciated, and clinical cutoff values proposed based on patient data. Being a continuous variable is better suited for longitudinal studies as well. Moreover, it was demonstrated in numerous cases that CAR was superior to other prognostic scores due to higher overall sensitivity/specificity reflected by the area under the curve in ROC analysis [5].

Selected studies on the use of CAR, GPS and mGPS in the human literature

A large cohort study by Proctor *et al*., [65] and Cui *et al*., [5] demonstrated that higher CRP and lower albumin and thus higher mGPS scores are seen in cancer patients compared to people with other diseases. Altogether more than 200,000 patients were included, of which more than 22,000 were diagnosed with cancer. Elevated CRP concentration was demonstrated in 47 % of cancer patients (vs 39 % of others) and a decreased albumin concentration in 19 % (vs 13 % of others). It was demonstrated that the mGPS scores of 1 and 2 are more frequently found in patients with cancer and this change was attributed to a decreased albumin concentration. Elevated mGPS scores were seen in breast, prostate, and pulmonary cancer cases (21, 46 and 68 % respectively). Additionally, a relation between mGPS scores and 5-year survival rates were hypothesised based on higher proportions of patients with an mGPS score of 0 in cancers with higher 5-year survival rates. It was therefore suggested that mGPS not only has a prognostic value within cancers but across various cancers too.

A follow-up publication by the same group of authors indicated that mGPS appears to predict survival independent of cancer site with breast, bladder, gynaecological, prostate, gastroesophageal, haematological, renal, colorectal, head and neck, hepatopancreatic biliary and pulmonary neoplasia investigated in more than 7,000 patients. Notably routine blood sampling was allowed in 2 years preceding cancer diagnosis which was based on the assumption that cancer was in development, yet not diagnosed within this period of time [65]. A mGPS of 2 indicated 160 % decreased survival times across all groups investigated.

An important limitation of the previous studies was that comorbidities (which might have contributed to an elevated CRP and therefore elevated mGPS) were not investigated.

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CRP to albumin ratio was investigated in a study by Miyamoto *et al*., [74]. Their approach was to investigate this parameter in various intervals before the death of patients. Neoplastic and non-neoplastic diseases were investigated concurrently. It was demonstrated that CRP increases whereas albumin decreases before death and higher changes (and therefore higher CRP to albumin ratio) indicate impending death. A cutoff of 2.9 for CAR carried a 2.1 odds ratio for death within 2 weeks. Similarly, a cutoff of 1.6 carried a 1.91 odds ratio for death within a month. mGPS was also investigated and a score of 2 in both cases was found to carry an increased risk of death, although the odds ratios were somewhat lower (1.34 and 1.71 for mortality within 2 weeks or a month respectively). This held true for all cases suffering from solid tumours, haematologic malignancies and non-cancer cases alike.

A larger metanalysis investigated publications on CAR in patients with solid tumours (head and neck, gynaecological, abdominal and others) [5]. High CAR was associated with poor overall survival as well as poor progression free survival and disease-free survival in all groups investigated.

CAR has been investigated in various haematological malignancies too. It has been demonstrated that high pre-treatment CAR was a predictor of worse overall survival in various diseases, including T-cell lymphoma/leukaemia [10] [26], diffuse large B-cell lymphoma (not only survival but response to induction therapy was also poorer,), acute myeloid leukaemia (with poor response to induction therapy as well [6], myelofibrosis (mGPS also investigated, associated with worse prognosis too [48]. A study on myelodysplastic syndromes demonstrated worse prognosis in patients with elevated CRP levels or decreased albumin levels in plasma – however, CAR in this study has not been evaluated [25]. It appears that low albumin contributes to lower CAR and worse outcomes [10]. One longitudinal study assessed CRP, albumin, and CAR in terminally ill cancer patients. A consistent increase of CRP, decrease of albumin and increase of CAR was demonstrated. CAR appeared to be an independent predictor of death, a noteworthy feature of this measurement [51]. A longitudinal study assessed CAR in elderly patients with various cancers. Two distinct groups were identified, one with relatively stable low CAR and another with an increasing CAR pattern. The latter was associated with worse overall survival [23]. Again, both studies analysed various types of tumours indicating that CAR is a useful parameter across various disease entities.

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Safety and efficacy of chemotherapy was investigated considering CRP to albumin ratio in various studies. In a cohort of patients suffering from pancreatic carcinoma it was observed that a decreased post-chemotherapeutic CAR is suggestive of better responses [9]. Increased CAR was associated with a decreased chemotherapy response rate in locally advanced breast cancer patients undergoing neoadjuvant chemotherapeutic treatment [21]. Worse response rates and worse prognosis are tightly associated, and this relationship hardly comes as a surprise.

One longitudinal study attempted to assess mGPS as a marker of disease progression in the setting of advanced metastatic renal cell carcinoma. mGPS appeared to correlate well with therapeutic response and served as an early indicator of disease progression (that is earlier than conventional imaging studies were routinely carried out), allowing for therapy modifications if necessary [33].

In some studies, adverse events were evaluated. In a study conducted by Tominaga *et al*., [11] it was observed that higher CAR was associated with more frequent occurrence of severe chemotherapy related adverse events (grade 3 and higher, including neutropenia, fatigue, anorexia, diarrhoea amongst others) and with chemotherapy discontinuation in patients with metastatic colorectal cancer. Not surprisingly survival was worse in the group with high grade adverse events. The authors suggest that high CAR indicates systemic inflammation, entailing higher levels of circulating cytokines which were previously described as predictors of adverse events in patients undergoing chemotherapy. Furthermore, poor nutritional status, reflected by albumin concentration, is also associated with worse chemotherapy related toxicity events. However, these findings were not confirmed in another study [24] investigating similar patients.

Another study on patients with unresectable mammary tumours response to therapy was worse in patients with higher mGPS values (clinical benefit observed in 6% of patients with mGPS 2 vs 16 and 45 % with mGPS 1 and 0 respectively). In the same study chemotherapy associated side effects (neutropenia, thrombocytopenia, gastrointestinal side effects and fatigue) were significantly more frequent in patients with higher mGPS scores, associated with treatment delays too [8].

It should be noted that CAR is used in non-neoplastic diseases as a prognosticator. Pancreatitis [20], myocardial infarction [22], COVID-19 disease [57], critically ill patients admitted to intensive care unit [58], sepsis [55] and rheumatoid arthritis [56].

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CRP and albumin in canine neoplastic disease

CRP is a common inflammatory marker and, although non-specific, it is highly sensitive [61]. The fast response and relatively short half-life are high clinical values which make C-reactive protein a good therapeutic outline. In many countries, the assessment is becoming part of the biochemistry blood work procedure in veterinary medicine with diverse tests available on the market. Despite its usefulness, particularly together with white blood cell count or other acute phase response proteins measurements, it does not permit an entire assessment as an individual parameter [61] [67]. Systemic inflammation activity and the efficacy of treatments can be detected and monitored by measuring canine CRP as it is more sensitive than shifts in leukocyte counts. In neoplasia patients such as dogs suffering from canine lymphoma, the CRP rise is a result of the secondary inflammatory or immune-mediated stimuli rather than the direct action of the tumour [61] [68]. Multiple studies suggest that a high CRP level leads to a poorer prognosis in canine lymphoma and classifies lymphomas into an advance stage in accordance with the WHO classification (World Health Organisation) [38] [68].

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According to Hart's *et al*., [62] study, a major problem in explaining the value of CRP measurements in any cancer state is that its relative blood level can change rapidly and in direct relationship to the stage and extent of progressive disease and/or associated complications (e.g. infections) that may conduct the disease development. A second restriction is that multiple studies did not completely distinguish the connection between CRP and the distinct variables that define cancer staging (e.g. tumour size in TNM staging of breast cancer in humans). However, the prognostic value of CRP in connection to neutrophil/lymphocyte ratio has also been put forward as well as the CAR [62]. Various research reports a maximum CRP level as a relevant diagnostic index, despite that, such values must be evaluated with reference to how prolonged cancer disease extends, the treatments used during disease, and the levels measured both before and after and surgical interventions [62].

Another complicating element in clarifying the diagnostic and prognostic value of CRP in cancer disease is the present focus on the value and significance of "high sensitivity CRP" levels (hsCRP) [38]. hsCRP is a novel essay used in humans to detect smaller changes in CRP levels than the conventional CRP essay. In healthy human individuals, baseline levels of CRP are normally described as being <1-2 µg/ml [62]. The literature described and

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distinctly interpreted cohort groups with hsCRP levels between 1-3 and >3µg/ml [62]. While the US Food and Drug Administration (FDA) suggests no advice on the diagnostic importance of such values in any disease, including cancer, it is important to note that baseline hsCRP levels are often normally associated with populations of individuals grouped by gender, age, ethnicity, degree of fitness and obesity [38] [62]. Sensitive assays for CRP being now readily available, many studies have manifested publishing that rather increased hsCRP levels are reflective of an elevation in developing and aggravating cancerous growth [62] [68].

Albumin is a "major" negative APP as it reduces in response to an inflammation, disease, or injury [3] [67]. Systemic inflammatory response and weight loss in human cancer patients correlates strongly with hypoalbuminaemia and relates to reduced complete remission and survival time in NHL [38]. Hypoalbuminaemia was revealed in canine lymphoma and also related to reduced remission and survival time [38].

Dog and cat patients suffering from lymphoma have been reported to have elevated CRP levels [27] [52] and complete remission status in dogs with multicentric lymphoma might be indicated by serum CRP [15].

Staging lymphoma with the basis of serum CRP concentrations was revealed to be an accurate method in Manachai's *et al*., study [36], however, the small sample size of dogs with lymphoma and the absence of survival analysis are limitations of this study. A higher stage of the disease was associated with a significantly higher CRP level (stage IV and V having higher CRP than I-III). The CRP cut-off level for the advanced lymphoma stage according to the Receiver Operating Characteristics (ROCs) is of 54.1 mg/l which can be used as a biomarker to predict cancer dissemination [36].

Fontaine's *et al*.'s study [38] hypothesised GPS and mGPS may be relevant to canine lymphoma patients. They investigated 77 dogs at the time of their diagnoses. Their study revealed that an increased mGPS was associated with stage and substage of the disease, weight loss, GI disturbances and lethargy. A mGPS score of 2 was associated with decreased overall survival and progression free survival. Hypoalbuminaemia was identified as the factor behind these findings as CRP did not seem to influence these outcome measures.

Mammary gland tumours are one of the most widespread neoplasms in female dogs and mostly seen in entire bitches [18]. Tecles *et al*., [18] aimed to investigate three positive APPs including CRP, SAA and haptoglobin and one negative APP, albumin in female dogs with

mammary neoplasia. An increase in the positive APPs was only detected in metastatic cases or in tumours greater than 5 cm in diameter with ulceration or in dogs with concomitant diseases [18]. Albumin was decreased with metastases and concomitant diseases. Different factors would influence acute phase proteins in female dogs with mammary gland tumours such as metastasis, large size of the primary mass and ulceration or secondary inflammation of the tumour [18].

Another study in mast cell tumours (MCTs) and sarcomas, identified that CRP and α-1 acid glycoprotein levels elevated with a simultaneous decrease in SAA levels in MCTs patients [68]. In sarcoma patients, CRP, α-1 acid glycoprotein and haptoglobin were increased [68]. These findings put forward that certain solid tumour types in dogs could be connected with particular changes in APP profiles [18] [68].

CRP and albumin in canine non-neoplastic disease

Plasma concentration of CRP was measured in 928 dogs with various diseases in Nakamura's *et al*., study [60] and compared to other inflammatory parameters. As already mentioned, elevation of CRP was mostly elevated in neoplastic diseases [60] but also in immune-mediated diseases. Diseases in which a CRP concentration was increased were ever case of pyometra, panniculitis, acute pancreatitis, polyarthritis, and hemangiosarcoma. However, in a handful of neurological diseases cases, such as epilepsy, meningoencephalitis, and hydrocephalus and endocrine diseases such as hypothyroidism, hyperadrenocorticism, and diabetes mellitus; CRP was only increased. The authors observed only a fragile correlation between CRP and white blood cell (WBC) counts but no correlation with band neutrophil counts [60]. Interestingly, the authors reported an absent correlation between CRP and albumin concentrations, but a weak negative correlation when eliminating chronic intestinal diseases and nephrotic syndromes which can cause protein loss [60]. This study concluded that CRP can be useful to detect inflammations that cannot be detected by WBC counts or band neutrophil counts, recommending that CRP concentration examination is essential as a standard diagnostic test.

Another study suggests that extremely high CRPs >100 mg/l occur in about 12% of patients in a referral veterinary hospital are characteristic of a severe systemic disease with guarded prognosis and are observed due to various aetiologies such as trauma, infection, immunopathy, and malignant neoplasia [75]. But such severe elevations in CRP do not allow

a resolution of the underlying aetiology or a distinction between bacterial and non-bacterial inflammation. The individual prognosis significantly depends on the specific underlying aetiology [75], further diagnostics are advised, and the patient should be closely surveyed.

CAR was measured in canine pancreatitis and its value was assessed as a prognostic marker for survival [42]. Patients with high CAR had a remarkably greater mortality than dogs with lower CAR: for every unit increased in CAR ratio, the probability of death over the examined period increased by 130% [42]. The optimal CAR cut-off point was 0.56 for predicting mortality, with a sensitivity and specificity of 88.9% and 68.2% respectively (AUC=0.82; $p<0.001$). As in humans, the CAR maybe promising, though not particularly specific, prognostic marker for elevated risk of death in dogs with acute pancreatitis.

A Brazilian study suggested that CAR was a more accurate marker than were CRP and albumin analysed separately [45]. The aim of this research was to create a reference interval for canine CAR and to analyse the prospect of CAR and their relationship, to serve as measures of disease severity, length of hospital stay (LoS) and mortality [45]. Plasma was collected from 190 dogs randomly selected at a veterinary hospital without distinction of gender, age or breed. It was analysed for evaluation of CRP and albumin. The reference range specified for CAR in dogs was 0.36-0.60, as determined by the confidence interval of mean resampling. The frequencies mean, and standard deviations of the variables, correlation analysis, and comparative analysis were calculated. Elevation (above reference) of CAR was established to be proportional to the severity of the underlying disease, and CRP means were reasonable. Besides, hypoalbuminemia was indicative of systemic disease, but not of severity [45].

Objectives

In the present retrospective descriptive study CRP to albumin ratio is assessed in a population of patients presented under various circumstances in the Veterinary Haematology and Oncology Centre (Állatorvosi Hematológiai és Onkológiai Központ, ÁHOK Kft), Budapest in a period of 2 years (2022-2023).

Materials and Methods

The LabsoftLIMS (LabsoftLIMS v 2.8., NetCare Kft, Kalocsa, Hungary) system used by the clinic was used for laboratory data extraction. Results from all patients in which CRP and albumin had been measured concomitantly were collected. CRP to albumin ratio as well as GPS and mGPS were then calculated from these data. Clinical data was then collected from the practice management software (Doki for Vets v10.00.1793, Alpha-Vet Kft, Székesfehérvér, Hungary) and included breed, sex, age, and diagnosis as well as comorbidities. Disease stage was also assessed where it was appropriate (neoplastic diseases).

Patient cohorts were then created as follows: healthy controls (samples from dogs arriving for vaccinations or routine health checks), neoplastic diseases and other diseases. Patients were considered in the other disease group if significant inflammatory comorbidities were present (i.e. lymphoma complicated by severe gastroenteritis).

Statistical analyses were performed in R (R v , R Foundation for Statistical Computing, Vienna, Austria). Mann-Whitney U-test was used for group comparison.

Results

110 concomitant measurements were identified. 10 of these were excluded due to neoplastic disease combined with an apparent inflammatory disorder. Altogether, 100 samples from 86 patients were kept for analysis. Mean age was 8.9 years at testing. 15 females, 25 neutered females, 22 males and 20 neutered males were sampled. Mixed breed dogs were over-represented. Population characteristics for all these and the cohorts are presented in table 1.

Table 1.
Population characteristics and cohorts. F – female, FN – neutered female, M – male, MN – neutered male

Cohort	N Patients/measurements	Age Mean (range)	Sex (F/FN/M/MN)
Neoplastic	47/53	10.4 (4-16)	9/12/13/12
Other	29/35	7.5 (1-15)	3/9/9/8
Healthy control	10/13	6.6 (1-14)	3/4/1/2
All	86/100	9.0 (1-16)	15/25/23/22

In the control group, the following diseases were identified: cardiovascular disorders (degenerative mitral valve disease, n=1), gastrointestinal disorders (chronic gastroenteritis not otherwise specified, IBD, LPE (Lymphocytic Plasmacytic Enteritis), giardiasis, n=1, 3, 1 and 1 respectively), haematologic disorders (immune mediated haemolytic anaemia, immune thrombocytopenia and non-regenerative anaemia not otherwise specified n=1-1 each), hepatobiliary disorders (hepatopathy not otherwise specified and hepatic cirrhosis n=2 and 1 respectively), integumentary/dermatologic disorders (allergodermatitis, autoimmune dermatopathy not otherwise specified, pemphigus, otitis externa and panniculitis, n=7,1,1,1) musculoskeletal disorders (immune-mediated polyarthritis, arthrosis and disco spondylitis n=2, 1 and 1 respectively), neurologic disorders (epilepsy, n=2), other disorders (cachexia, other non-neoplastic disorders n=1 and 4 respectively).

In the neoplastic group, the following disorders were identified: cardiovascular tumours (chemodectomas, n=3), endocrine tumours (thyroid carcinoma, n=1), gastrointestinal tumours (oral malignant melanoma and intestinal adenocarcinoma, n=3 and 1 respectively), haematologic tumours (peripheral T-cell lymphoma not otherwise specified, chronic

lymphocytic leukaemia, diffuse large B-cell lymphoma, cutaneous epitheliotropic T-cell lymphoma, hepatosplenic lymphoma, multicentric lymphoma not otherwise specified, histiocytic sarcoma, n=7, 2, 2, 1, 1, 1 and 5 respectively), hepatobiliary tumours (cholangiocarcinoma and hepatic carcinoid, N=2 and 1 respectively), integumentary tumours (mast cell tumours, melanocytic tumours, anal sac apocrine gland adenocarcinoma, perianal adenoma, n=3, 2, 1, 1 respectively), connective tissue and musculoskeletal tumours (fibrosarcoma and osteosarcoma, N=2 and 1 respectively), mammary gland tumours (n=1), respiratory tumours (pulmonary carcinoma, n=2), splenic tumours (hemangiosarcoma, N=5), urogenital tumours (urothelial carcinoma, n=2).

CRP, albumin, and CAR values were obtained from these aforementioned groups and are presented in table 2.

Table 2.

Albumin, CRP and CAR values across neoplastic and other diseases groups according to anatomical classification.

Group (n: number of measurements, neoplastic/other disease groups)	Albumin (g/L) mean (SD)		CRP (mg/L) mean (SD)		CAR mean (SD)	
	neoplastic	other diseases	neoplastic	other disease	neoplastic	other disease
cardiac (n=3/1)	24 (3)	34.4 (NA)	25.2 (32.1)	6.0 (NA)	1.11 (1.46)	0.17 (NA)
endocrine (n=1/0)	22.5 (NA)		25.7 (NA)		1.14 (NA)	
gastrointestinal (n=6/6)	32.3 (3.4)	33.2 (3.5)	21.0 (36.1)	21.0 (39.2)	0.74 (1.41)	0.62 (1.65)
haematologic (n=24/3)	27.8 (6.5)	35.4 (2.7)	53.8 (49.8)	14.8 (17.0)	2.13 (1.94)	0.40 (0.56)
hepatobiliary (n=3/3)	27 (6.3)	36.3 (7.6)	51.4 (42.2)	21.0 (20.1)	2.23 (2.1)	0.68 (0.76)
integumentary (n=2/11)	34 (4.1)	32.7 (3.6)	20.9 (22.5)	17.3 (20.4)	0.65 (0.74)	0.58 (0.73)
mammary (n=1/0)	38.3 (NA)		5.0 (NA)		0.13 (NA)	
mesenchymal (n=3/0)	31.7 (1.9)		33.1 (42.1)		1.09 (1.42)	
musculoskeletal (n=0/4)		32.8 (2.6)		85.9 (84.6)		2.78 (2.86)
neurologic (n=0/2)		31.2 (0.77)		12.5 (4.3)		0.40 (0.15)
respiratory (n=2/0)	34.6 (2.0)		5.0 (NA)		0.07 (0.08)	
splenic (n=5/0)	27.8 (7.9)		58.5 (63.6)		3.03 (4.27)	
urogenital (n=2/0)	31.9 (1.2)		42.0 (52.3)		1.28 (1.58)	
other (n=0/5)		29.4 (5.7)		19.7 (21.6)		0.85 (1.17)

CAR, GPS and mGPS scores were observed in the investigated groups. The results are presented in table 3.

Table 3.

GPS, mGPS and CAR in the 3 cohorts.

	GPS 0/1/2 n (%)	mGPS 0/1/2 n(%)	CAR mean (SD)
Healthy control	0 (100 %)	0 (100 %)	0.17 (0.06)
Other	21 (60) / 13 (37.1) / 1 (2.9)	21 (60)/13 (37.1)/1 (2.9)	0.85 (1.35)
Neoplastic	22 (42.3) / 18 (34.6) / 12 (23.1)	24 (46.1) / 16 (30.8) / 12 (23.1)	1.72 (2.09)

Statistical analyses for CRP, Albumin and CRP to Albumin ratio were performed between groups. p values for the comparison of CRP and Albumin are presented in tables 4 and 5 as well as images 1 and 2. CRP to albumin ratio is presented separately in table 6 and image 3.

Table 4.

CRP and albumin concentrations in various groups.

Cohort	CRP mean (SD) mg/L	Albumin mean (SD) g/L
Healthy control	5.46 (1.7)	32.5 (3.08)
Other	25.5 (39.7)	32.8 (4.1)
Neoplastic	42.3 (45.6)	29.0 (6.1)

Table 5.

CRP and Albumin concentration compared amongst the patient cohorts investigated. p values associated with significant differences are typesetted in bold.

CRP and Albumin	Healthy control	Other	Neoplastic
Healthy control	-	**0.037**/0.68	**0.001**/**0.033**
Other	-	-	**0.05**/**0.001**

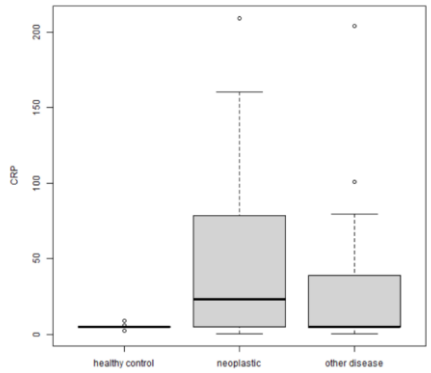

Image 1.

CRP concentrations in the 3 cohorts.

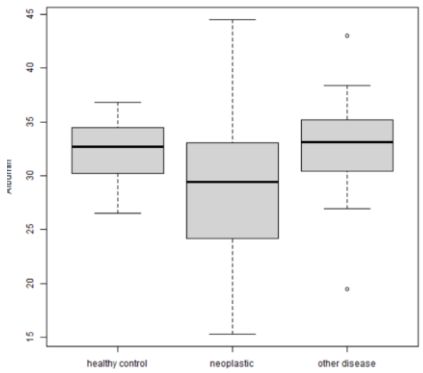

Image 2.

Albumin concentration in the 3 cohorts.

Table 6.

CAR comparison in the patient cohorts investigated. P-values associated with significant differences are typesetted in bold.

CAR	Healthy control	Other	Neoplastic
Healthy control	-	0.1	**0.002**
Other	-	-	**0.019**

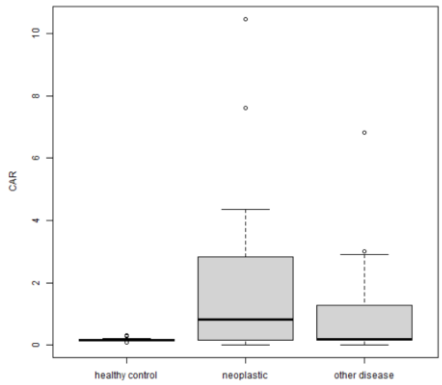

Image 3.

CAR values in the 3 cohorts.

The neoplastic disease cohort was investigated for differences amongst active disease and remission groups. Results are presented in table 7 and 8 and images 4 to 6.

Table 7.

CRP, Albumin and CAR comparison amongst active disease/remission groups. Significant differences are typesetted in bold.

	CRP mean (SD) mg/L **p=0.03**	Albumin mean (SD) g/L p=0.06	CAR mean (SD) **p=0.02**
Active disease (n=41)	46.7 (44.1)	28.5 (6.1)	1.83 (1.75)
Remission (n=12)	26.0 (49.6)	31.0 (5.8)	1.30 (3.12)

Table 8.

GPS and mGPS comparison in neoplastic disease groups.

	GPS 0/1/2 n(%)	mGPS 0/1/2 n(%)
active disease	13 (31.7) / 17 (41.5) / 11 (26.8)	15 (36.6) / 15 (36.6) / 11 (26.8)
remission	9 (81.8) / 1 (9.1) / 1 (9.1)	9 (81.8) / 1 (9.1) / 1 (9.1)

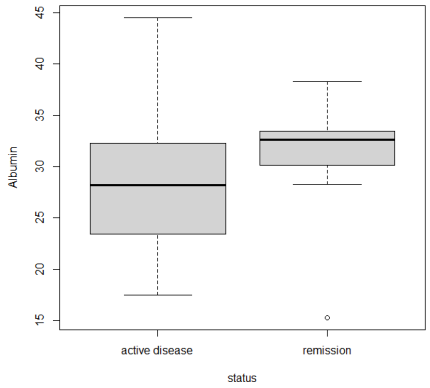

Image 4.

Albumin concentrations in the active disease and remission groups.

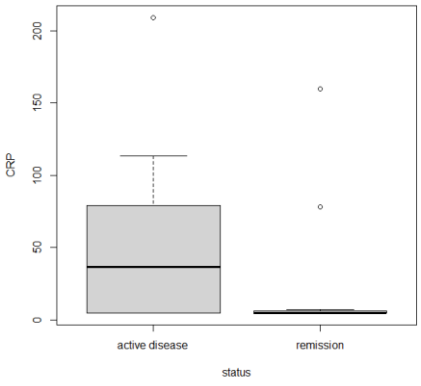

Image 5.

CRP concentrations in the active disease and remission groups.

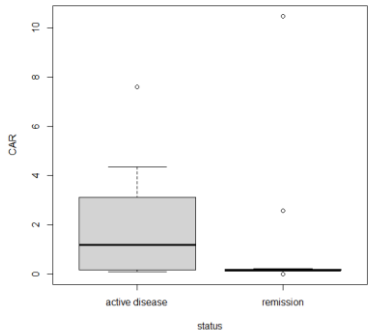

Image 6.

CAR values in the active disease and remission groups.

Discussion

An over-representation of neutered female and male dogs was present in the sample size. A wide range of diseases were identified both in the control and neoplastic groups.

CRP and albumin appear to be negatively correlated (higher CRP concentrations are accompanied by lower albumin concentrations), although the correlation appears to be weak (R^2=0,24 when all cohorts are considered). When analysed separately it appears that CRP is the highest and albumin is the lowest in the neoplastic disease group (image 7), which was previously described in table 4 and 5.

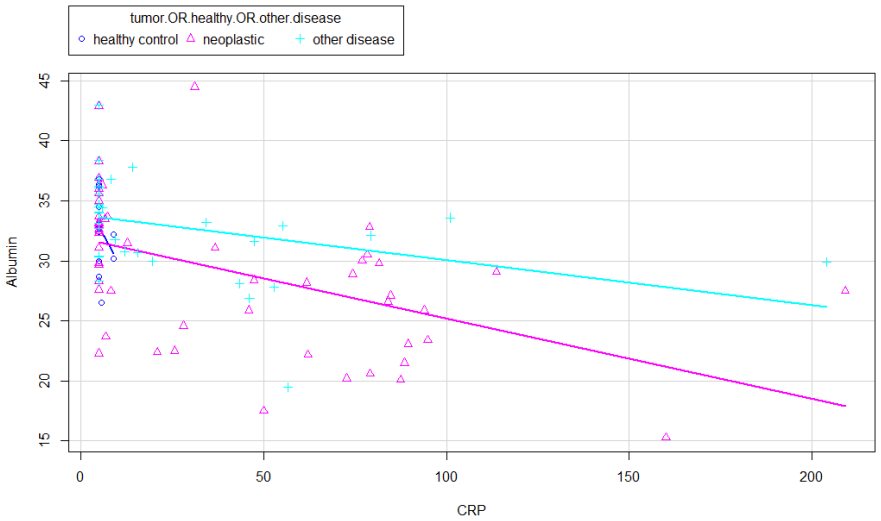

Image 7.

CRP plotted against albumin. Trend lines are added for the 3 cohorts.

However, large numbers of points with CRP 5 mg/L (in all cohorts) were due to the limit of detection of the reagent. A better correlation may be established by analysing more samples and with the use of high sensitivity CRP (hsCRP) [38]. hsCRP is a novel essay used in humans to detect smaller changes in CRP levels than the conventional CRP essay. Human standard levels of CRP in healthy individuals are generally described as being <1-2 µg/ml [62]. The literature described and differently interpreted cohort groups with hsCRP levels

between 1-3 and >3µg/ml [62]. While the US Food and Drug Administration (FDA) suggests no advice on the diagnostic significance of such values in any disease, including cancer, it is important to note that standard hsCRP levels are often mostly associated with populations of individuals grouped by gender, age, ethnicity, degree of fitness and obesity [38] [62]. Sensitive assays for CRP being now readily available, many studies have emerged publishing that rather increased hsCRP levels are reflective of increased developing and aggravating cancerous growth [62] [68].

A study measured automated hsCRP assay with the aim of validating it to be canine specific [1]. It was started by altering a commonly used canine-specific immunoturbidimetric CRP test (cCRP). Imprecision, linearity under dilution, limit of blank (LOB), limit of detection (LOD), and limit of quantification (LOQ) were regulated for the hsCRP test, as well as the presence of prozone effect and interferences. The imprecision, measured as intra-assay variation, was ≤2.7%. The assay was appropriately linear under dilution. An empirically relevant prozone effect was present for samples with CRP concentration >150 mg/L, and there were mild interferences from haemolysis and lipaemia. The LOB, LOD, and LOQ were 0.10 mg/L, 0.22 mg/L, and 0.50 mg/L, respectively. A method comparison study with a canine-specific enzyme-linked immunosorbent assay (ELISA) was conducted, showing poor consensus between the hsCRP test and the ELISA [1], rendering this novel method less useful.

In the neoplastic group, CRP results were quite variable. Cardiac and endocrine neoplastic disorders were close with 25.2 and 25.7 mg/l respectively, whereas only 6 mg/l were found in non-neoplastic cardiac and endocrine disorders. Gastrointestinal neoplastic and non-neoplastic groups had the same CRP of 21 mg/l. Haematologic neoplastic disorders had 53.8 mg/l CRP compared to 14.8 mg/L CRP in the non-neoplastic cohort. Hepatobiliary neoplastic disorders had 51.4 mg/l CRP compared to 21 mg/l CRP in the non-neoplastic diseases. The integumentary cohort had 20.9 mg/l CRP compared to 17.3 mg/l in the non-neoplastic group. Mammary diseases were only found if they were neoplastic with 5 mg/l CRP and so did mesenchymal diseases with 33.1 mg/l. Musculoskeletal diseases were only found as non-neoplastic with 85.9 mg/l CRP. Neurologic diseases were also only found in the non-neoplastic group with 12.5 mg/l. Respiratory, splenic, and urogenital diseases were only found to be neoplastic with 5 mg/l, 58.5 mg/l and 42 mg/l CRP respectively. Other non-neoplastic diseases had 19.7 mg/l CRP. In general, it appears that neoplastic groups had

higher CRP concentrations apart from gastrointestinal and integumentary disorders and the differences in mean CRP levels were roughly twofold if not higher.

Systemic inflammation or at least elevated CRP have been shown to be present in dogs with cardiac disease [13] [35]. However, those elevations may be mild in case of mitral valve disease for instance (median values <5 mg/L) and larger elevations (up to 70 mg/L) may only be seen in congestive heart failure. From the present study it appears that cardiac neoplasia may lead to higher elevation in CRP concentration then other cardiac disease.

Cardiac disease can lead to hypoalbuminaemia in dogs but possibly due to volume expansion and not decreased albumin synthesis[49]. Mild hypoalbuminaemia was observed in dogs suffering from cardiac tumours in this study. These patients were not suffering from congestion; therefore, hypoalbuminaemia may not be attributed to volume expansion and potentially it is related to systemic inflammation.

Only one patient with endocrine neoplasia was evaluated, a dog suffering from thyroid carcinoma. It appears that in this one patient CRP was moderately elevated whereas albumin was mildly decreased.

CRP in gastrointestinal disease in dogs was studied but neoplasia separately has not been assessed to the author's knowledge. It appears that CRP itself has a prognostic value for survival in inflammatory bowel disease, with high CRP concentrations associated with shortened survival. Therapeutic decisions can also be based on CRP concentration [28]. Considering the low number of analysed cases in each disease group these comparisons await confirmation.

Hypoalbuminaemia itself is a frequent finding in canine gastrointestinal disease, especially in chronic enteropathies. Interestingly in this investigation mean albumin concentrations were within reference range in neoplastic disorders and other diseases too. This is surely an incidental finding which influences the CAR values as well as the GPS and mGPS scores.

In haematologic disease it was found that neoplasia carried a higher CRP concentration than other diseases. One of the non-neoplastic diseases was immune mediated haemolytic anaemia (IMHA). In dogs it was demonstrated that IMHA leads to highly elevated CRP concentration [54], up to or above 200 mg/L. It was also demonstrated that albumin concentrations decrease [19]. Neither of these were observed in this study, moreover CRP concentration was 5 mg/L. This might be attributed to ongoing treatment or remission of the patient.

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CRP, albumin, and derived indices were investigated by Fontaine et al. [38] in canine lymphoma. The findings of this study are similar to the previously published results, although median CRP concentration in the present study appears to be higher (34.7 mg/L vs 51.4 mg/L). This may be in part due to several CRP measurements obtained from a patient suffering from histiocytic sarcoma, where CRP concentrations were in all measurements well above this mean concentration.

This latter patient was evaluated repeatedly. Initial CRP concentration was 209 mg/L whereas albumin was 27.5 g/L. CAR was 7.6 (a very high value in this study) but the mGPS score was only 1. With treatment initiation interestingly CRP concentration halved and remained relatively stable over time (6 months follow-up) but albumin also decreased, leading to a decreased, but still high (above 3) CAR score and an elevated GPS/mGPS score. Apparently, treatment initiation leads to a decrease in CAR but an increase in the GPS and mGPS scores. Hypoalbuminaemia may be due to hepatic involvement in this specific patient.

Hepatobiliary neoplasia cases were presented with higher CRP and lower albumin concentrations than other hepatobiliary diseases. This finding is contrary to the previously described changes. One study investigated dogs undergoing hepatic biopsy for various diseases and did not find significant differences in CRP concentration amongst neoplastic and non-neoplastic groups [14]. Low albumin concentration is also somewhat unexpected as hypoalbuminaemia is more characteristic of a diffuse hepatopathy rather than a nodular neoplastic disease. Nevertheless, low albumin concentration may be associated with systemic inflammation too.

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Integumentary disorders did not differ much from each other in terms of CRP or albumin concentrations. CAR was roughly similar too. Most cases from the non-neoplastic group were allergodermatitis. CRP in this cohort is described as non-elevated or only mildly elevated in the literature [40]. The non-neoplastic disease group contained several cases of mast cell tumours. For these it was described that CRP levels were much higher than the findings of this study; however, the cases here were in remission or were diagnosed early in the disease which may explain these differences.

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Mammary and mesenchymal (soft tissue sarcomas mostly) as well as respiratory, splenic, and urogenital groups were only seen in the neoplastic cohort. Musculoskeletal and neurologic disorders were only assessed in the non-neoplastic cohort. Changes in mammary

carcinomas were described previously, as well as those seen in certain mesenchymal tumours. As for splenic neoplasms it is described that hemangiosarcoma leads to elevated CRP level, which is in line with the present findings. In fact, splenic tumours assessed were all hemangiosarcomas. It appears that there is a high variability of CRP and albumin concentration in this patient cohort. All the patients were assessed in treatment. The high variability may potentially reflect disease activity although other causes cannot be ruled out. Comorbidities in this patient cohort have not been described and therefore the observed changes may be attributable to the hemangiosarcoma itself.

Image 2 shows that in the neoplastic cohort albumin concentrations are lower than in the healthy control or other cohort. The mean albumin level in the neoplastic cohort is 29.0 g/l whereas it is of 32.5 g/l in the healthy control group and of 32.8 g/l in the other group. CRP and albumin are extensively investigated in human and veterinary medicine. In addition to the aforementioned, CRP to albumin ratio prognostic scores appeared: the Glasgow Prognostic Score (GPS) [41] and the modified Glasgow Prognostic Score (mGPS). These scoring systems allow for a simplified approach, assigning a value of 0 or 1 to CRP and albumin if their value is within reference range or above (in the case of CRP) or below (in the case of albumin) of their respective lower reference limits. In the case of mGPS low albumin only matters if it is combined with a high CRP value. This way a score between 0-2 can be assigned to a patient. More mGPS score of 2 in the neoplastic group were observed. mGPS was also investigated in humans [74] and a score of 2 in both cases was found to carry an increased risk of death, although the odds ratios were somewhat lower (1.34 and 1.71 for mortality within 2 weeks or a month respectively). This held true for all cases suffering from solid tumours, haematologic malignancies and non-cancer cases alike.

It appeared that CRP was significantly higher in the active neoplastic disease group than in the remission group whereas for albumin such a difference could not have been demonstrated. Nevertheless, CAR values were higher in active neoplastic disease than in patients in remission. It seems from these results that in remission mostly CRP decreases if all neoplastic entities are considered. CRP and CAR could potentially be used to assess therapeutic efficacy.

It is noteworthy that CAR cutoff values have to be determined individually for any investigated group of diseases or disease entity. This partially reflects the different nature of the disease investigated. Yet, another confounding factor might be the low standardisation

of acute phase protein (in this case CRP) measurement in dogs. The lack of standardised methods may lead to discrepant results amongst investigators. This is less of a concern in the case of GPS or mGPS where only the reference range is used as a discriminator between patient groups.

This study is limited by several factors. One of them is the retrospective nature of it, limiting the possibility to measure outcomes in these patients, mostly attributable to them being assessed in various stages of their diseases and treatments.

Conclusion

In this retrospective study healthy dogs, dogs suffering from neoplastic diseases and dogs suffering from other diseases were investigated to assess systemic inflammation mostly in tumour bearing dogs and how CRP, albumin, and derived prognostic indices (CAR, GPS and mGPS) perform. Findings include that dogs suffering from neoplastic disease have significantly higher CRP and lower albumin concentration in plasma, leading to a significantly higher CAR score. Patients with higher GPS and mGPS scores were also found in higher proportion in patients with neoplastic diseases. Moreover, those patients with active neoplastic disease had higher CRP concentration and thus reached higher CAR scores than those in remission.

Prognostic value of the above-mentioned changes could not be estimated because the lack of survival data and the heterogeneity of the assessed patient population.

These analytes are readily available, easy to measure and appear to reflect systemic inflammatory activity. They are described as independent prognosticators in numerous human diseases, mostly neoplastic ones. Their assessment in canine disease is limited though. The value of the present study is that it expands the limited information available on these markers. The limitation is that it is retrospective and assesses patients in various stages of their disease, limiting comparability.

Further studies in individual disease entities appear to be justified.

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Thesis progress report for veterinary students

Name of student: Megane Pernollet……

Neptun code of the student: P30OVI……

Name and title of the supervisor: Márton Márialigeti DVM, department veterinarian……

Department: Department fo Clinical Pathology and Oncology……

Thesis title: C-reactive protein and albumin based predictive indices in canine cancer……

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	02	7	Literature review	
2.	2023	03	7	Literature review	
3.	2023	04	4	Data collection	
4.	2023	05	2	Data collection	
5.	2023	06	6	Data collection	

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

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	08	22	Statistical analysis	
2.	2023	09	5	Consultation	
3.	2023	10	3	Consultation	
4.	2023	10	17	Consultation	
5.	2023	10	31	Consultation	

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

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

I accept the thesis and found suitable to defence,

.....
signature of the supervisor

Signature of the student: …

Signature of the secretary of the department: ……

Date of handing the thesis in… 16/11/2023