Short Communication

Changes in C-reactive protein and haptoglobin in dogs with lymphatic neoplasia

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Abstract

Acute phase proteins (APP) are regarded as a useful diagnostic tool in humans with lymphomas, leukaemias and multiple myeloma. C-reactive protein (CRP) and haptoglobin concentrations were measured in dogs with malignant multicentric (high grade) lymphoma (n = 16), acute lymphoblastic leukaemia (ALL) (n = 11), chronic lymphocytic leukaemia (CLL) (n = 7) and multiple myeloma (n = 8). Twenty-five healthy dogs served as controls. Measurements of the CRP plasma concentration were performed using a commercial ELISA and haptoglobin was measured with an assay based on its haemoglobin binding capacity.

Global group comparisons using Kruskal–Wallis-test revealed significant group differences for both APPs (P < 0.0001). Median CRP concentrations were increased in all groups with neoplastic lymphatic disorders (lymphoma: 37.2 mg/L, ALL: 47.8 mg/L, CLL: 35.5 mg/L, myeloma: 17.6 mg/L) compared to controls (1.67 mg/L; P < 0.001). Compared to the healthy controls (median = 0.59 g/L), haptoglobin was especially increased in dogs with ALL (6.8 g/L, P < 0.0001) followed by dogs with malignant lymphoma (3.8 g/L, P < 0.0001), CLL (3.2 g/L, P = 0.0008), and multiple myeloma (3.0 g/L, P = 0.0163). For both APPs, a wide range of values was found in all patient groups. The results indicate that particularly severe and acute lymphatic neoplasia, such as high grade lymphoma and ALL, cause significant acute phase reactions in dogs and must be included in the differential diagnoses of increased blood levels of these APPs.

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Acute phase proteins (APP) are widely used as non-specific markers of inflammation in humans and in dogs (Jergens et al., 2003; Petersen et al., 2004; Vermeire et al., 2004; Agrawal, 2005). Over the last few years numerous articles have been published on the diagnostic and prognostic use of APP in haematopoetic neoplasia in humans and the results of these studies have shown that the blood levels of the APPs C-reactive protein (CRP) and/or alpha-1-acid glycoprotein (AGP) are correlated with the stage and/or the presence of clinical symptoms in Non-Hodgkin lymphoma (Pedersen and Sørensen, 2003), Hodgkin lymphoma (Wieland et al., 2003) and chronic myeloid leukaemia (Le Coutre et al., 2002).

In humans, it appears that blood APP levels can be used as prognostic markers. In multiple myeloma, CRP, AGP, and alpha-1-antitrypsin are negatively related to the survival of the patients (Alexandrakis et al., 2003). Furthermore, APPs can be used to monitor the clinical course and efficacy of therapy and are helpful in monitoring for recurrences. This has been proven for ceruloplasmin in cases of acute myeloid leukaemia (Kovtunova et al., 2003) and AGP and CRP in cases of chronic myeloid leukaemia, where elevation of AGP levels was present prior to haematological progress (Le Coutre et al., 2002). In addition, CRP has been used as an indicator of sepsis especially...
during leukopenic periods due to chemotherapy (Bayer et al., 2000). A further aspect in which AGP has been investigated is the possible alteration of pharmacokinetics of antineoplastic drugs by their high affinity for this serum protein. This could particularly affect the treatment of chronic myeloid leukaemia using a tyrosine kinase inhibitor (Le Coutre et al., 2002; Gambacorti-Passerini et al., 2003; Larghero et al., 2003).

Few studies are available in relation to the alterations of APPs in dogs with neoplasia. Studies on APPs in haematological neoplasia in both dogs and cats are restricted to AGP and malignant lymphoma (Ogilvie et al., 1993; Hahn et al., 1999; Correa et al., 2001). The aim of the present study was to examine two further important APPs, C-reactive protein (CRP) and haptoglobin, in dogs with different malignant, lymphatic blood disorders.

CRP and haptoglobin values were measured in dogs with malignant multicentric (high grade) lymphoma (n = 16), acute lymphoblastic leukaemia (ALL) (n = 11), chronic lymphatic leukaemia (CLL) (n = 7), and multiple myeloma (n = 8) at the time of diagnosis. The diagnosis of multicentric high grade lymphoma was based on lymph node cytology (>80% blasts) or histology. All dogs were classified as stage III–V according to the WHO-classification (Owen, 1981).

Diagnosis of ALL was based on examination of bone marrow aspirates and blood films and the lymphatic origin of the blasts confirmed by immunochemical or flow cytometric analyses. Chronic lymphocytic leukaemia was diagnosed based on characteristic blood cell count and film alterations (persistent absolute marked lymphocytosis based on small lymphocytes). Multiple myeloma was diagnosed based, firstly, on bone marrow infiltration with plasma cells (>20%) and/or abnormal plasma cell morphology as well as, secondly, monoclonal gammapathy or characteristic lytic bone lesions. Twenty-five clinically healthy adult dogs with unremarkable blood picture served as controls.

Measurements were performed from lithium-heparinised plasma which was stored frozen at −70 °C until assayed. Both methods (see below) are recommended for assay of serum samples which, however, were not available from all patients. In a preliminary unpublished study we compared the use of these methods in measuring the concentrations of CRP and haptoglobin in plasma and serum prepared from the same blood collection taken from healthy dogs and dogs with different diseases (CRP, n = 31; haptoglobin, n = 36) which were not part of this study. The study showed that for both APPs the results in serum and plasma were highly significantly correlated (Spearman rank correlation coefficient: rs = 0.947 [CRP], rs = 0.987 [haptoglobin], P < 0.0001).

CRP concentration was measured using a commercial canine CRP ELISA kit (Tridelta Development Ltd.) as described by Jergens et al. (2003) except that an automated ELISA system was employed (Triturus Analyser, Grifols). Haptoglobin was assayed based on its haemoglobin binding capacity (Eckersall et al., 1999) and the test was calibrated with a bovine reference preparation with defined levels of haptoglobin (Dr. P.M.H. Heegaard, Danish Institute for Food and Veterinary Research, Copenhagen, Denmark).

Due to the lack of normal Gaussian distribution, values of different groups are presented as modified box plots indicating separately outside values (> upper quartile + 1.5 × inter-quartile range) and far outside values (upper quartile + 3 × inter-quartile range). The upper limit of reference ranges was defined based on the 95% percentile. Comparisons between different groups were performed globally using the Kruskal–Wallis-test and locally between individually groups using the Mann–Whitney-test (MWT). P-values <0.05 were considered as significant.

Global group comparisons using Kruskal–Wallis-test revealed significant group differences for both APPs (P < 0.0001). Median CRP concentrations were increased in all groups with neoplastic lymphatic disorders (malignant lymphoma: 37.2 mg/L, ALL: 47.8 mg/L, CLL: 35.5 mg/L, myeloma: 17.6 mg/L) compared to the controls (1.67 mg/L; P < 0.001 MWT) (Fig. 1). Local statistical comparisons between the groups with neoplasia did not reveal

![Fig. 1. Blood plasma concentration of C-reactive protein in dogs with high-grade multicentric lymphoma (n = 16), acute lymphoblastic leukaemia (ALL) (n = 11), chronic lymphocytic leukaemia (CLL) (n = 7) and multiple myeloma (n = 8) compared to controls (25 healthy dogs). Modified box plots: The box identifies the middle 50% of the data (25% and 75% quartiles), the horizontal line within the box represents the median, the upper and lower end of the vertical lines represent the extreme points with the exception of outliers which are indicated separately: outside values (> upper quartile + 1.5 × inter-quartile range) and far outside values (> upper quartile + 3 × inter-quartile range).](attachment:image.png)
any remarkable differences \( (P > 0.05_{\text{MWT}}) \). Compared to the 95% percentile of the healthy control dogs (9.6 mg/L), CRP concentrations were increased in 13/16 dogs with malignant lymphoma, 10/11 dogs with ALL, 7/7 dogs with CLL and 5/8 dogs with multiple myeloma.

With regard to the healthy controls (median = 0.59 g/L), haptoglobin levels were particularly increased in dogs with ALL (6.8 g/L, \( P < 0.0001_{\text{MWT}} \)) followed by dogs with malignant lymphoma (3.8 g/L, \( P < 0.0001_{\text{MWT}} \)), CLL (3.2 g/L, \( P = 0.0008_{\text{MWT}} \)) and multiple myeloma (3.0 g/L, \( P = 0.0163_{\text{MWT}} \)) (Fig. 2). The median values in the dogs with ALL were significantly higher than in dogs with other neoplastic lymphatic disorders \( (P < 0.05_{\text{MWT}}) \), whereas no significant differences were observed between the other patient groups. Compared to the 95% percentile of the controls (2.45 g/L), increased values were measured in 13/16 dogs with malignant lymphoma, 10/11 dogs with ALL, 4/7 dogs with CLL and 5/8 dogs with multiple myeloma.

The results of this study show that lymphatic neoplasia is associated with significant elevations of CRP and haptoglobin in dogs. With respect to CRP this result confirms studies in humans in which elevated CRP blood serum concentrations were reported for patients with malignant lymphoma (Pedersen and Sørensen, 2003) and multiple myeloma (Yag˘ci et al., 2003). This fact indicates that lymphatic neoplasia has to be considered as a possible differential diagnosis in cases of increased CRP and haptoglobin concentrations. It also reflects the recognised low specificity of APPs when related to a particular disease (Ceron et al., 2005). Due to the low specificity of increased blood levels of APPs such as CRP, caution has to be exercised when using APPs as tumour markers (Ogilvie et al., 1993), although the specificity may increase if results from a number of APPs are combined.

In human clinical oncology there is considerable interest in the identification of novel tumour markers using the advanced techniques recently developed for examining the plasma proteome. This approach has shown that the plasma proteins most consistently identified as changing in concentration in response to neoplastic disease are all recognised APPs (Diamandis and Van der Merwe, 2005). Interestingly the absolute values of CRP in dogs are very similar to results reported for humans with the respective diseases. For example, we found CRP median values of 37.2 mg/L in dogs with high grade lymphoma and Pedersen and Sørensen (2003) reported CRP median values of 42.0 mg/L in humans suffering from high grade lymphoma – significantly higher than the results in humans suffering from low grade lymphoma (24.0 mg/L). In 66 human patients with multiple myeloma, Yag˘ci et al. (2003) found median CRP values of 21.0 mg/L (1.34–330 mg/L) compared to 1.05 mg/L (0.19–8.03 mg/L) in the healthy controls \( (P < 0.0001) \), whereas the median values in dogs with multiple myeloma and healthy dogs were 17.6 mg/L or 1.67 mg/L, respectively. Due to the fact that (in contrast to dogs) haptoglobin is not a frequently monitored APP in humans, studies on haptoglobin in people with neoplastic disease are not available for comparison.

The increased APP levels in individuals with lymphoma, leukaemia and myeloma may reflect the fact that interleukin (IL)-6 on the one hand plays a central role in normal B-cell maturation and in the pathogenesis of lymphatic neoplastic disorders including multiple myeloma and malignant lymphoma (Klein and Bataille, 1992; Legouffe et al., 1998), and on the other hand is one of the major regulators of the acute phase response (Heinrich et al., 1990; Petersen et al., 2004). The peri-tumoral inflammatory reaction leading to activation of macrophages and release of further cytokines is also most likely an important cause for increased APP concentrations (Ganz et al., 1983).

In this study, the most significant acute phase reactions were seen in high grade lymphoma and ALL, which – compared to the CLL and multiple myeloma – are aggressive disorders with a rapidly progressing course. Consistent with this finding, several studies in humans suffering from malignant lymphoma, have demonstrated that serum levels of proinflammatory cytokines such as IL-6 and tumour necrosis factor as well as APPs correlate with clinical features such as tumour stage and disease progression (Macia et al., 1996; Warzocha et al., 1997; Pedersen and Sørensen, 2003). Due to the limited number of canine lymphoma patients examined in this study and the fact that all our dogs with malignant lymphoma were classified as at advanced stages (III–V) of the disease we hesitated to

![Fig. 2. Blood plasma concentration of haptoglobin in dogs with high-grade multicentric lymphoma (n = 16), acute lymphoblastic leukaemia (ALL) (n = 11), chronic lymphoctic leukaemia (CLL) (n = 7) and multiple myeloma (n = 8) compared to controls (25 healthy dogs). Please see legend to Fig. 1 for the interpretation of the modified box plots.](image-url)
investigate the correlation between the stage of disease and the concentration of the APPs. Ogilvie et al. (1993) who measured AGP in 55 dogs with (advanced) stages III, IV and V of malignant lymphoma did not find significant group differences.

In humans suffering from multiple myeloma, APP levels (CRP, AGP, and alpha-1-antitrypsin) are negatively correlated with the survival time and can be used as a prognostic factor (Alexandrakis et al., 2003). Long term studies with greater case numbers would be useful to determine if APPs such as CRP can be used as prognostic factors in canine multiple myeloma as is the case in humans.

Overall, the increase in CRP was more significant than the increase in haptoglobin. For example, the median CRP value in dogs suffering from ALL was 29 times higher and the median haptoglobin concentration which was 12.5 times more than the respective median control values. This reflects the fact that CRP is regarded as a major acute phase reactant in dogs, whereas haptoglobin is generally classified as a moderately severe reacting APP in this species (Murata et al., 2004; Petersen et al., 2004). Together with serum amyloid A, a further important APP in dogs, CRP belongs to the rapidly reacting first line APPs (Petersen et al., 2004). These are induced by IL-1 type cytokines and are characterised by a dramatic increase in serum concentration within 4 h after the inflammatory stimulus and a rapid normalisation (Gabay and Kushner, 1999). The blood serum concentration of secondary APPs such as haptoglobin, which are primarily induced by IL-6 type cytokines (Nakagawa-Tosa et al., 1995) shows a later increase remaining elevated for up to two weeks after the end of the trigger mechanism (Gabay and Kushner, 1999; Petersen et al., 2004).

In summary, the results of this study show that particularly acute and severe lymphatic neoplasia are associated with high CRP and haptoglobin values in dogs. Whether concentrations of APPs in dogs with neoplastic lymphatic disorders can be used as prognostic factors as in humans has to be investigated in further studies.

References

Bayer, L., Schöntube, M., Dörffel, W., 2000. CRP, IL-6 und PCT als Infektionsparameter bei onkologisch kranken Kindern. (C-reactive protein, interleukin-6 and procalcitonin as parameters of infection in pediatric patients with oncologic diseases). Klinische Pädiatrie 212, 326–331.


