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Investigation of serum survivin in dogs suffering from cancer: a multicenter study

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ABSTRACT

Background: In contrast to human medicine, only a small number of serum tumor markers are established in veterinary medicine even though they are a non-invasive diagnostic tool.
Objectives: This study examined whether survivin could be suitable as a potential canine serum tumor marker.
Methods: This study measured the serum survivin concentrations of dogs with mammary tumors (n = 33), squamous cell carcinoma (n = 9), soft-tissue sarcoma (n = 18) and multicentric lymphoma (n = 22), using a commercially available, competitive immunoassay kit (BlueGene). The serum survivin concentrations were compared with those of a healthy control group (n = 20) and a control group of dogs with non-neoplastic diseases (n = 17).
Results: Dogs with malignant tumors had serum survivin concentrations between 15 and 5,906 pg/mL (median, 72 pg/mL), those in the healthy group ranged from 7 to 99 pg/mL (median, 21 pg/mL) and those in the group of dogs suffering from non-neoplastic diseases from 15 to 93 pg/mL (median, 42 pg/mL). The differences in the survivin concentrations between the healthy dogs and dogs with malignant tumors and between the dogs with non-neoplastic diseases and those with malignant tumors were significant ($p < 0.001$ and $p = 0.006$, respectively).
Conclusions: The serum survivin concentrations in dogs with malignant tumors, with some exceptions, are higher than in dogs with benign tumors and dogs that do not suffer from a malignancy. Therefore, survivin can provide information on the presence of malignant tumors and be used as a tumor marker in dogs.

Keywords: Biomarker; dog; malignancy; serum; survivin

INTRODUCTION

The existence of embryonic molecules in an adult organism most likely indicates tumor tissue [1]. Therefore, molecules expressed during embryonic development, such as carcinoembryonic antigen (CEA) or alpha-fetoprotein (AFP), are used as serum tumor markers [2-4].

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S; Investigation: Estaller A; Methodology:
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Another embryonically expressed molecule is survivin [5], which plays an essential role in regulating mitosis and cytokinesis by inhibiting apoptosis [6-8]. The association of survivin expression with the inhibition of apoptosis in fetal development has been demonstrated in mouse embryos [9], but no apparent function has been detected in adult tissues, as corroborated by the very low serum concentrations in adults [7]. Survivin expression in adulthood occurs mainly in the presence of malignant neoplasms because the inhibition of apoptosis is a driving factor in the survival of neoplastic cells [10]. Survivin expression has been investigated in humans, with elevated expression found in various malignancies, such as breast, lung, esophageal, stomach, pancreatic, liver, bladder, and hematological cancers [10,11]. Therefore, survivin is considered a tumor-specific molecule [12]. Its expression in neoplastic cells is induced by lymphocyte activation, revealing a link between tumor cells and the immune system [13]. Clinical studies in humans have linked high survivin concentrations to poor responses to numerous cancer treatments and poor prognosis [14-16].

Compared to humans, only a small number of diagnostic tumor markers have been established in veterinary patients. Research into biomarkers in veterinary medicine has become a focus and could have the potential for veterinary oncology. Most studies examined the appearance of survivin in tissues, which usually requires invasive investigatory methods [17,18]. Only a few studies have evaluated the survivin concentrations in the sera of tumor-bearing dogs [19,20]. Thus, this study compared the serum concentrations of survivin in tumor-bearing dogs with those of healthy controls and dogs with non-tumorous diseases to determine if survivin could serve as a serum tumor marker in dogs.

MATERIALS AND METHODS

Animals
One hundred and nineteen dogs were classified into 7 groups: healthy, non-neoplastic diseased, mammary adenocarcinoma, mammary adenoma, squamous cell carcinoma, soft-tissue sarcoma, and lymphoma. All dogs were diagnosed between January 2018 and December 2019.

The dogs in the healthy control group (n = 20) had been presented to the veterinary clinic for small animals at the University of Göttingen (Germany) for a general examination. The dogs in this group were free of any signs of illness for a minimum of 2 months before the clinical examination and 4 weeks afterward. A complete blood count was performed. All blood work parameters had to be within the laboratory reference interval to be eligible for the control group. All dogs in the control group were subjected to abdominal ultrasonography and thoracic radiography and did not show any signs of disease.

The dogs of the non-neoplastic diseased group (n = 17) were presented to the veterinary clinic for small animals at the University of Göttingen (Germany) because of various diseases. All dogs underwent a clinical examination, diagnostic imaging (thoracic radiography and abdominal ultrasonography), and laboratory work. All dogs had confirmed non-neoplastic systemic diseases.

The dogs in the tumor groups had been presented by their owners to the veterinary clinic for small animals at the University of Göttingen (Germany), the veterinary clinic for small animals in Hofheim (Germany), or the veterinary clinic for gynecology and obstetrics at the

University of Gießen (Germany) because of symptoms caused by their respective tumor-causing diseases. All the dogs underwent the required diagnostic procedures, such as thoracic radiography, abdominal ultrasonography, lymph node evaluation, and laboratory work to confirm their cancer diagnoses and exclude other diseases. The tumor volume was estimated by multiplying the height × width × depth. The histopathological diagnoses were established by Board-certified pathologists based on the incisional or excisional biopsy samples of the respective tumor. The tumor diagnoses were mammary tumor (adenocarcinoma n = 26; adenoma n = 7), squamous cell carcinoma of the skin and mucosa (n = 9), soft-tissue sarcoma (n = 18) of different locations like the skin and the spleen, and multicentric lymphoma (n = 22).

This study also documented which of the tumor group dogs were still alive after an observation period of 1 year after the cancer diagnosis. The experimental procedures were approved by the regional authorities for Consumer Protection and Food Safety Acts in Niedersachsen, Germany (reference No. 33.9-42502-05-17A148).

Blood sample collection and processing

The blood samples used for all measurements were collected from the cephalic veins before any treatment. The serum for survivin measurements was left to clot for 2 h at room temperature. After centrifugation at 1,000 × g for 15 min the serum was pipetted into Eppendorf tubes, frozen, and stored at −80°C until the measurement.

Immunoassay

The survivin concentrations were measured using a commercially available immunoassay kit (Canine Survivin enzyme-linked immunosorbent assay [ELISA] kit; BlueGene, China [Art. Nr.: E08S0218], competitive enzyme immunoassay technique) with non-diluted serum in duplicate, adhering to the manufacturer's instructions.

Briefly, the serum samples were added to the wells of a precoated 96-well microtiter plate, then covered and incubated for 1 h at 37°C. This was followed by a series of 5 manual washing steps to remove all the unbound antibodies. A solution containing an enzyme-conjugated detection antibody, which binds specifically to the antigen, was applied, followed by another incubation with coverage for approximately 15 min at 37°C. The substrate formed a colored solution when catalyzed by the addition of an acid solution, stopping the antigen–antibody reaction. The absorbances were measured spectrophotometrically in a Magellan optical emission spectrometer at 450 nm. A standard curve was determined using the program CurveExpert Professional (Hyams Development, https://www.curveexpert.net/) to calculate the serum survivin concentrations in pg/mL. The detection range of the ELISA in the serum samples, as per the manufacturer, was 25–1,000 pg/mL. The manufacturer reported mean intra- and inter-assay coefficients of variation of 4.4% and 6.6%, respectively.

Histopathology

The histology was performed using routinely embedded paraffin sections after hematoxylin and eosin staining. The diagnoses were made by board-certified pathologists.

Cell culture

For this procedure, 2 tumor cell lines were investigated: CMT-U309 (mesenchymal tumor; Eva Hellmén, Universität Uppsala) and P114 (anaplastic carcinoma; Gerard Rutteman, Universität Utrecht). The tumor cells were cultured in Dulbecco's modified Eagle's Medium (Biochrom/

Millipore, Bestell-Nr. FG0445) over 144 h. Dichloroacetic acid (DCA), an apoptosis inducer, was added to a one-cell culture preparation. As a negative control, a second preparation was free of additives. As a second positive control, cisplatin, a cancer medication that interferes with the growth of cancer cells, was added to a third preparation. Survivin was measured in the supernatants of all the preparations after 24, 48, 72, 120, and 144 h of incubation, and its concentration in each cell was calculated.

Leucocyte correlations
The link between survivin expression and leucocytes was investigated by calculating the following: the ratio between the serum survivin concentrations and the leucocyte counts, the counts of the different leucocyte subtypes, and the ratio between neutrophils and lymphocytes.

Statistical analysis
The statistical evaluation was performed using Prism 8 (GraphPad Software, USA). The data were tested for normal distributions using the Pearson's, the Shapiro-Wilk and the Kolmogorov-Smirnov tests. The non-normally distributed data were compared using the Mann-Whitney (2 groups) or the Kruskal-Wallis (multiple groups) tests. Linear regression analyses were performed to analyze the dependency of survivin on the tumor volume. The receiver operation characteristic (ROC) curves were calculated to discriminate healthy dogs from dogs with tumors. A p value < 0.05 was considered significant.

RESULTS

There was no predominance of any breed in the dog population in this study. **Table 1** lists the sex distribution and the age distribution in the different groups. The healthy control group consisted of 20 dogs. Dogs with non-neoplastic diseases (n = 17) suffered from hypoadrenocorticism (n = 1), chronic renal failure (n = 1), an allergy (n = 2), gastroenteritis (n = 6), cystitis (n = 4), and endometritis (n = 3). The mammary tumor group consisted of 26 adenocarcinomas and 7 adenomas. Dogs with mixed mammary tumors were not included in this study. The squamous cell sarcoma group consisted of nine dogs, and the tumors originated from the mandible, oral mucosa, tonsils, nasal septum, and toes.

In total, 18 dogs suffered from mesenchymal tumors. The tumors were classified as fibrosarcoma of the hypoderm and mucosa (n = 7), hemangiopericytoma of the skin and hypoderm (n = 5), and hemangiosarcoma of the spleen (n = 6). Finally, the lymphoma group consisted of 22 dogs, mostly suffering from multicentric lymphoma, and in some isolated cases, from gastrointestinal lymphoma and cutaneous lymphoma.

Table 1. Number, sex, and age of all dogs investigated in this study

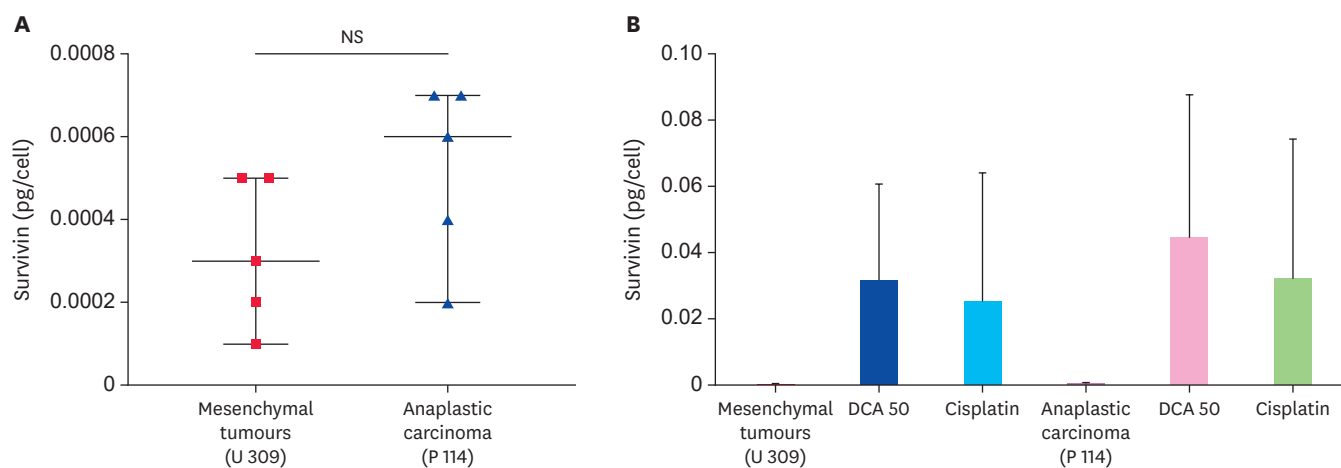| Category	No. (f/m)	Age (yr) min	Max	Mean
Controls	20 (11/9)	0.5	10	4.3
Non-neoplastic	17 (9/8)	0.5	13	6.2
Adenocarcinoma	26 (26/0)	6.5	15.5	11
Adenoma	7 (7/0)	5	12	8.5
Squamous cell carcinoma	9 (5/4)	6	15	10.5
Mesenchymal tumors	18 (9/9)	5	11.5	9.8
Lymphoma	22 (13/9)	5	14	9.2

Fig. 1. Survivin concentration of different tumor cell cultures. (A) Survivin concentration in the supernatant was measured, and the concentration per cell was calculated. There is no significant difference in the cell concentration of survivin between the mesenchymal and carcinoma cells (p = 0.152). (B) Survivin concentration in different tumor cell cultures in the supernatant 12 h after DCA 50 or cisplatin (10%) addition. The concentration in the supernatant increased significantly after DCA 50 and after cisplatin (10%) exposure.
NS, not significant; DCA, dichloroacetic acid.

The survivin concentrations were measured in 2 cell cultures: one of a mesenchymal origin and the other of an epithelial origin, both of which produced survivin. The average survivin concentration per mesenchymal and epithelial cell was 0.0003 pg/cell and 0.006 pg/cell, respectively. There was no significant difference between the 2 cell cultures (p = 0.152; **Fig. 1A**). An approximate 10-fold increase in survivin concentration per cell was measured 12 h after adding DCA 50, resulting in average cell concentrations of 0.035 and 0.042 pg/cell for the mesenchymal and epithelial cell lines, respectively (**Fig. 1B**). At 12 h after the addition of cisplatin, the increases in the concentrations of survivin in the supernatants were measured. There were no differences in the survivin concentrations between the DCA and cisplatin groups.

The serum survivin concentrations in the group of healthy dogs (n = 20) ranged between 7 and 99 pg/mL (median, 21 pg/mL). The serum survivin concentrations were measured in the healthy group depending on age to exclude the possibility that the increased serum survivin concentrations in dogs with tumor diseases were due to their advanced age. No significant correlation was observed between the survivin concentrations of dogs aged 0.5–5 yr and older dogs aged 9–10 yr (p = 0.4; **Fig 2A**). Furthermore, the serum survivin concentrations were similar regardless of the gender of a dog in this group (p = 0.92; **Fig. 2B**). In the group of dogs suffering from non-neoplastic diseases (n = 17) the serum survivin concentrations were between 15 and 93 pg/mL (median, 42 pg/mL). A significant difference in the serum survivin concentrations was observed between the healthy dogs and those that suffered from non-neoplastic diseases (p = 0.018).

Dogs with malignant tumors (n = 75) had serum survivin concentrations ranging from 15 to 5,906 pg/mL (median, 72 pg/mL). The differences in the survivin concentrations between healthy dogs and dogs with malignant tumors and between dogs with non-neoplastic diseases and those with malignant tumors were significant (p < 0.001 and p = 0.006, respectively; **Fig. 2C**). Dogs with mammary gland adenocarcinoma (n = 26) had serum survivin concentrations between 25 and 1,113 pg/mL (median, 104 pg/mL). They differed significantly from the healthy control group and the group of dogs with non-neoplastic diseases (p < 0.001 and p < 0.001, respectively). The serum survivin concentrations in dogs with mammary adenoma were between 28 and 76 pg/mL (median, 45 pg/mL) and distributed
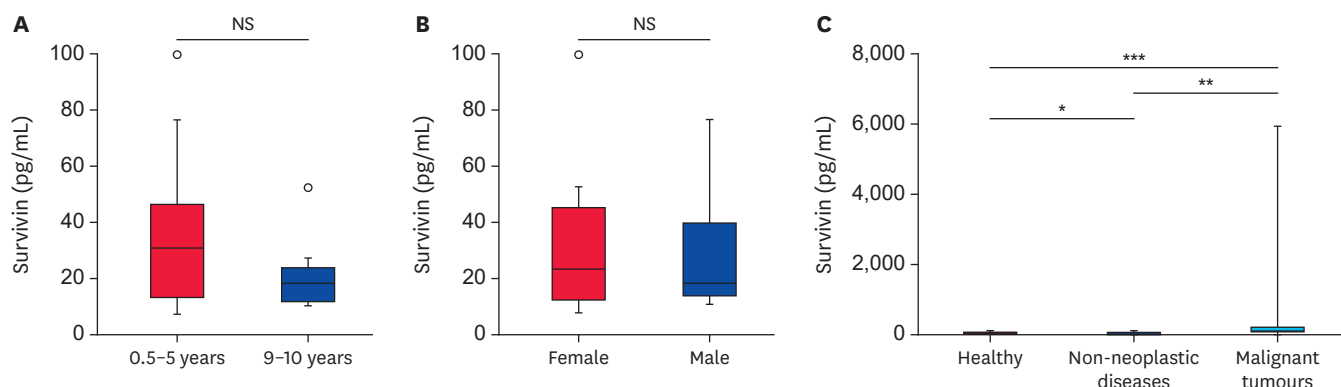
Fig. 2. Serum survivin concentrations in healthy dogs, dogs with non-neoplastic diseases, and dogs with malignant tumors. (A) Comparison of the serum survivin concentrations of healthy dogs depending on their age. The healthy control group (n = 20) consisted of 15 dogs aged 0.5–5 yr and 5 dogs aged 9–10 yr, serum survivin concentrations of healthy dogs were between 7 and 99 pg/mL with a median of 21 pg/mL. There was no statistically significant correlation between the survivin concentrations of the dogs aged 0.5–5 yr and older dogs aged 9–10 yr (p = 0.4). (B) Comparison of the serum survivin concentrations of healthy dogs depending on their gender. There were 11 female dogs and nine male dogs in the healthy control group. No significant difference in serum survivin concentration depending on the gender of a dog in this group (p = 0.92). (C) The serum survivin concentrations of healthy dogs (n = 20) were between 7 and 99 pg/mL with a median of 21 pg/mL. Non-neoplastic diseased dogs (n = 17) had serum survivin concentrations between 15 and 93 pg/mL with a median of 42 pg/mL. There was a significant difference between healthy dogs and those suffering from non-neoplastic diseases (p = 0.018). The dogs with malignant tumors (n = 75) had serum survivin concentrations between 15 and 5,906 pg/mL with a median of 72 pg/mL, significantly higher than the healthy group (p < 0.001) than the group of dogs with non-neoplastic diseases (p = 0.006).
NS, not significant.
*p < 0.05; **p < 0.01; ***p < 0.001.

normally. The comparison between dogs with adenocarcinoma and adenoma revealed a significant difference (p = 0.03; **Fig. 3A and B**).

Nine dogs in this study suffered from squamous cell carcinoma with serum survivin concentrations between 17 pg/mL and 658 pg/mL (median, 45 pg/mL). Compared to the healthy controls, there was a significant difference in the serum survivin concentrations (p = 0.006). The difference compared with the non-neoplastic diseased dogs was not significant (p = 0.163; **Fig. 4**).
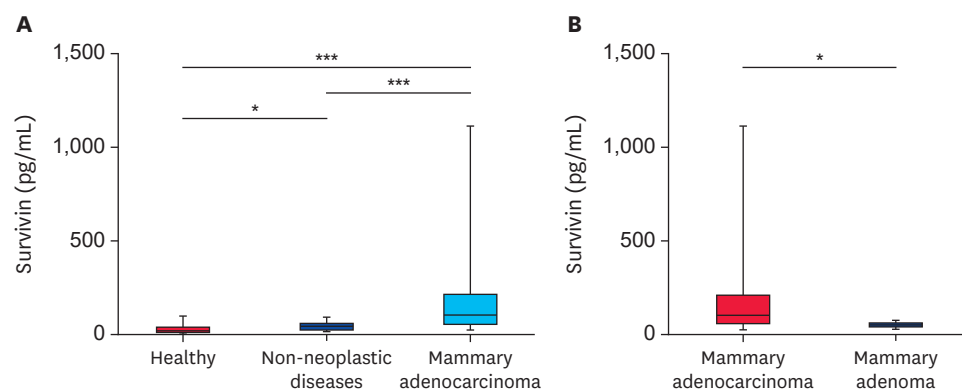

Fig. 3. Serum survivin concentrations in dogs with mammary adenocarcinoma. (A) The serum survivin concentrations in dogs with mammary adenocarcinoma were between 25 and 1113 pg/mL (median, 104 pg/mL) and differ significantly from those of the healthy control group (p < 0.001) and those of the non-neoplastic diseased dogs (p < 0.001). (B) Comparison of the serum survivin concentrations of dogs with mammary adenocarcinoma and dogs with mammary adenoma. Twenty-six dogs with adenocarcinoma and 17 dogs with adenoma of the mammary gland were included with concentrations between 28 and 76 pg/mL (median, 45 pg/mL). The serum survivin concentrations differ significantly (p = 0.03).
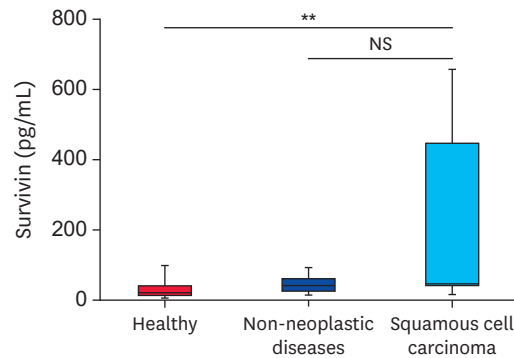*p < 0.05; ***p < 0.001.

Fig. 4. Comparison of the serum survivin concentrations of healthy dogs, dogs with non-neoplastic diseases, and dogs with squamous cell carcinoma. Nine dogs with squamous cell carcinoma could be included in this study. The survivin concentrations were between 17 and 658 pg/mL (median, 45 pg/mL). They differed significantly compared to the healthy control group (p = 0.006), but the levels were similar to the group of dogs with non-neoplastic diseases (p = 0.163).
NS, not significant.
**p < 0.01.

In total, 18 dogs in this study suffered from mesenchymal tumors (soft-tissue sarcoma). The serum survivin concentrations were between 18 and 5,960 pg/mL (median, 93 pg/mL). A significant difference was observed between the dogs with soft tissue sarcoma and the healthy control group and the dogs with non-neoplastic diseases (p < 0.001 and p = 0.018, respectively; **Fig. 5**). The survivin concentrations in dogs with lymphoma were between 15 and 2,349 pg/mL (median, 38 pg/mL). Compared to the healthy group, dogs with lymphoma had significantly higher serum survivin concentrations (p = 0.005), but there was no significant change in concentration compared to dogs with non-neoplastic diseases (p = 0.46; **Fig. 6**).

The serum survivin concentrations in the different groups were not distributed normally, except for the group of dogs with mammary adenoma. The serum survivin concentrations between the different groups of veterinary tumor patients did not differ significantly (p = 0.118; **Fig. 7**). The tumor volume had no influence on survivin concentrations according to linear regression analysis (p = 0.85; r = 0.0008; **Fig. 8A**), but there was an association in the 7 dogs suffering from mammary adenoma (p = 0.022; r = 0.78). The influence of the serum survivin concentrations on the survival time of the canine patients was also examined using
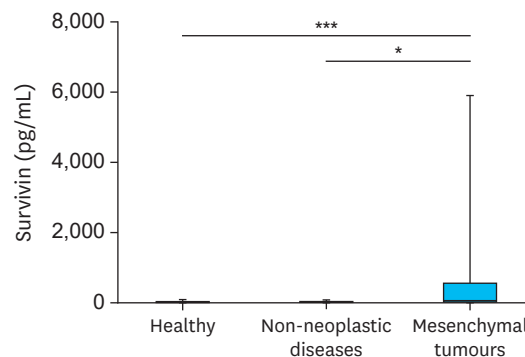

Fig. 5. Comparison of the serum survivin concentrations of healthy dogs, dogs with non-neoplastic diseases, and dogs with mesenchymal tumors. Eighteen dogs in this study suffered from mesenchymal tumors. The serum survivin concentrations were between 18 and 5,960 pg/mL (median, 93 pg/mL). There was a significant difference between the healthy group and the group of dogs with non-neoplastic diseases (p < 0.001 and p = 0.018).
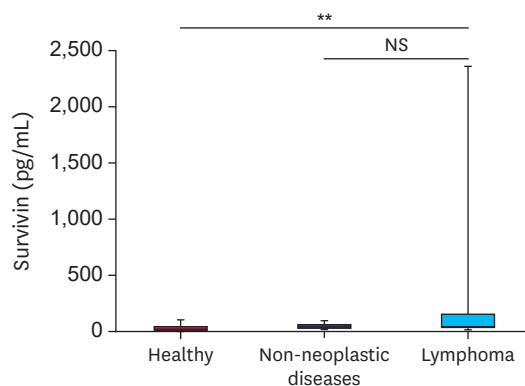*p < 0.05; ***p < 0.001.

Fig. 6. Comparison of the serum survivin concentrations of healthy dogs, dogs with non-neoplastic diseases, and dogs with lymphoma. The serum survivin concentrations in lymphoma patients were between 15 and 2,364 pg/mL (median, 38 pg/mL). Dogs with lymphoma showed significantly higher serum survivin concentrations than the healthy control group ($p = 0.005$), but there was no significant difference compared to the dogs with non-neoplastic diseases ($p = 0.46$).
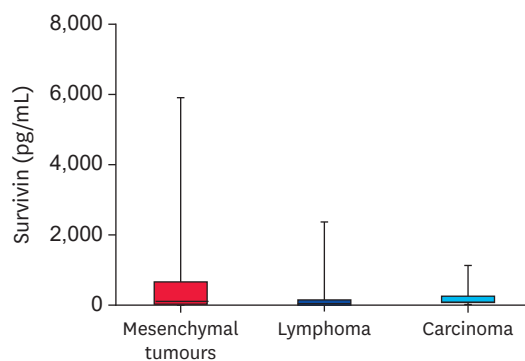NS, not significant.
**$p < 0.01$.

Fig. 7. Comparison of the serum survivin concentrations of dogs with epithelial tumors, mesenchymal tumors, and lymphoma. Comparing all 3 groups of tumors, no significant differences could be found ($p = 0.118$).
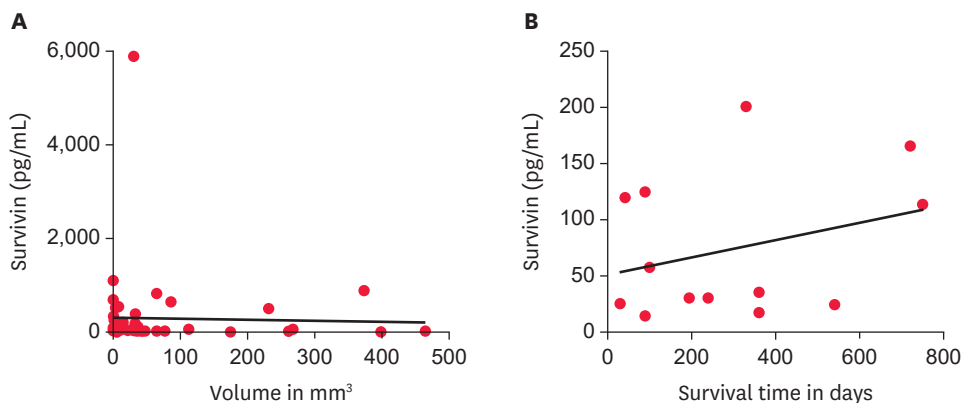
Fig. 8. Linear regression analysis. (A) The influence of the tumor volume (mm³) on the serum survivin concentrations was measured by linear regression analysis for epithelial and mesenchymal tumors. No significant relationship could be found ($p = 0.85$; $r = 0.0008$). (B) The influence of the serum survivin concentration on the survival time was calculated based on the data of 13 dogs with a linear regression analysis. No significant relation could be found ($p = 0.31$; $r = 0.09$).

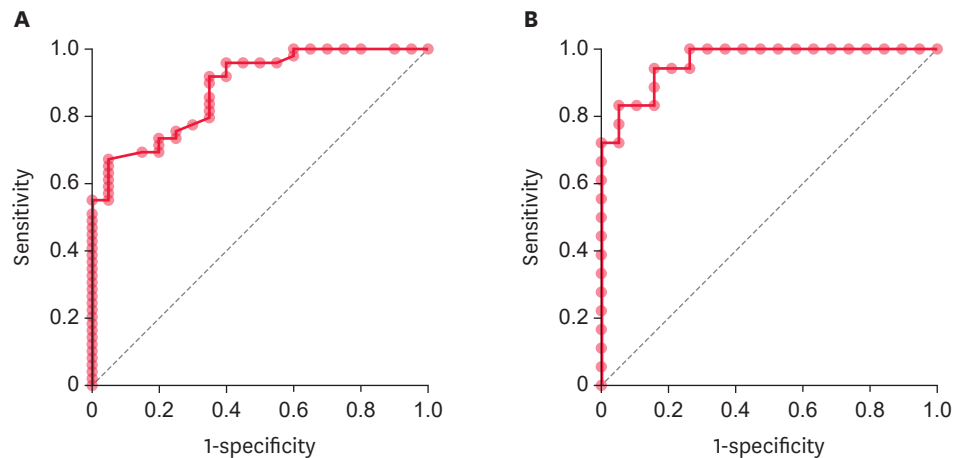**Fig. 9.** ROC curve analysis for discriminating healthy dogs from tumor patients. (A) The AUC was 0.88 ($p < 0.001$; 95% CI, 0.80–0.96). Using a cutoff value of 53 pg/mL, the sensitivity and specificity were 67% (CI, 0.53–0.78) and 95% (CI, 0.76–0.99), respectively. (B) The survivin-lymphocyte-ratio revealed an AUC of 0.96 ($p < 0.001$; 95% CI, 0.91–1.0). Using a cutoff value of 36, the sensitivity and specificity was 83% (CI, 0.6–0.94) and 95% (CI, 0.76–0.99), respectively.
AUC, area under the curve; CI, confidence interval.

linear regression analysis. No significant correlation was found based on 13 dogs, in which follow up information on the survival time was available ($p = 0.31$; $r = 0.09$; **Fig. 8B**).

Finally, a ROC curve was calculated to discriminate the healthy dogs from those with malignant tumors. The area under the curve (AUC) was 0.88 ($p < 0.001$; 95% confidence interval [CI], 0.80–0.96). Using a cut-off value of 53 pg/mL, the sensitivity and specificity were 67% (CI, 0.53–0.78) and 95% (CI, 0.76–0.99; **Fig. 9A**), respectively. The ROC curve was recalculated using the quotient of survivin and the lymphocyte count, which improved the ability to discriminate healthy dogs from those with malignancy. Using the survivin/lymphocyte ratio, the AUC was 0.96 ($p < 0.001$; 95% CI, 0.91–1.0). With a cut-off value of 36 pg/mL, the sensitivity and specificity were 83% (CI, 0.6–0.94) and 95% (CI, 0.76–0.99; **Fig. 9B**), respectively.

DISCUSSION

Serum tumor markers in human and veterinary medicine play an increasing role because of their advantages, including easy sampling and measurement, as well as rapid results [21,22].

This study focused in survivin because it is a known inhibitor of apoptosis, which is especially important for malignant tumor development [23]. Hence, this study investigated whether survivin provides information on the existence of a tumor in general, its tissue origin, and whether it is a malignant or benign tumor. This study also investigated whether the survivin concentrations may be used as a prognostic parameter by observing the survival time of the canine patients [24,25].

The survivin level was measured in the culture supernatants of epithelial and mesenchymal tumor cell lines. This corroborates the results of other studies, in which survivin production was also found in different tumor cell lines [23]. In these studies, the effects of survivin inhibition on the biological activities of canine histiocytic sarcoma cells were investigated. The addition of DCA, which is an inducer of apoptosis [26], increased the survivin

concentrations in the supernatants dramatically. Elevation of the survivin concentrations may be a consequence of the increased production by tumor cells, triggered by DCA, to protect themselves from apoptosis. The addition of cisplatin, a chemotherapeutic drug that binds to DNA, inhibits its replication, and induces cell necrosis, also led to an increase in the survivin concentrations in the supernatants. This may be a consequence of cell membrane destruction, cell death, and increased production. Furthermore, during the cisplatin treatment, the survivin concentrations decreased, suggesting that the drug sooner or later inhibits survivin expression [27].

All dogs in this study that suffered from a malignancy had measurable serum survivin concentrations. A study examining the diagnostic value of survivin in human breast cancer showed that 95% of the patients had moderate to high serum survivin concentrations of 50–200 pg/mL [28]. A veterinary study in dogs with mammary tumors reported a mean survivin concentration of 109 pg/mL [20].

In the healthy control group, low serum survivin concentrations were also found. The baseline production in adults was also described in humans [29]. Why healthy adult humans and animals produce survivin is unclear, but it is often found in tissues with high proliferation rates, such as mucosa [30]. Survivin is also found in animals suffering from non-neoplastic conditions, including inflammatory and non-inflammatory diseases. The survivin concentrations in humans and dogs with non-neoplastic diseases are significantly lower than those of individuals with tumors [20,31,32]. These results showed significant differences between the survivin concentrations of the malignancies groups compared to those of healthy dogs and dogs with non-neoplastic diseases, which confirmed the earlier findings. The non-significant difference between the dogs with squamous cell carcinoma and dogs with non-neoplastic diseases revealed the limitation of using survivin as a general tumor marker.

Compared with the other commercially available veterinary tumor markers like the plasma protein AFP and the glycoprotein CEA, survivin detects a greater variety of tumors, while AFP occurs at high levels only in hepatocellular carcinoma [33] and multicentric lymphoma [34], and CEA is a promising marker of canine mammary tumors [22].

Histopathology remains the gold standard for differentiating benign and malignant tumors and the malignancy grades [35]. A perfect tumor marker should differentiate between malignant tumors and benign processes [22]. This study only investigated a small group of dogs with adenoma. They had significantly lower serum survivin concentrations than the dogs with malignant tumors. This is in accordance with the results in humans, where the survivin concentrations were significantly higher in patients in the later stages of breast cancer (II–IV) [28]. These results demonstrate that survivin may potentially be a non-invasive tool to differentiate between malignant and benign canine mammary tumors.

Furthermore, in benign mammary adenomas, a correlation was noted between the serum survivin concentrations and the tumor volumes. The lack of a correlation between the serum survivin concentration and tumor volume in malignant mammary tumors may be explained by the different production levels in the tissue caused by different mechanisms, such as an enhanced number of mitotic tumor cells and an increased resistance to apoptosis [8].

According to the present study, survivin cannot be used to differentiate between different types of cancer because the concentrations in the different tumor entities do not differ

significantly. Similar results have been obtained in humans [36], in which high serum survivin concentrations occurred in patients with breast, colon, ovarian, and other cancers, but it was not possible to distinguish between the different tumor entities. To the best of the authors' knowledge, no veterinary studies have compared the survivin concentrations of different tumor entities. From these studies, survivin may be used to indicate the presence of a malignant tumor, but it is unable to differentiate between the tumor entities.

Tumor markers may provide relevant information concerning the outcome of a tumor disease for a patient. The prognostic relevance of survivin has been investigated in humans, associated higher survivin concentrations with a poor prognosis and lower survival rates [1,8,28]. In veterinary medicine, the prognostic relevance of survivin was investigated primarily for osteosarcoma and cutaneous and subcutaneous tissue tumors, showing mixed results depending on the tumor entity [17,18]. Based on the follow-up information in approximately 16% of the dogs in the present study, there was no correlation between the serum survivin concentration and the outcome of the disease. On the other hand, larger studies concerning this question are warranted.

According to the data, serum survivin concentrations above 53 pg/mL were associated with a malignant tumor with a sensitivity of 0.67 and a specificity of 0.95. The ROC curve is used in clinical biochemistry to determine the most appropriate cut-off for tests and distinguish between diseased and healthy patients. In addition, the AUC estimates the usefulness of the test in question [37]. Here, the AUC was calculated to be 0.88. A study examining serum survivin concentrations in human patients with different tumors produced an AUC of 0.66 [36]. In a human study on colorectal cancer, CEA had an AUC of 0.88 [3]. In a recent veterinary study, the ROC curve was also determined for CEA [38].

An increase in the AUC, as well as the sensitivity and specificity of the survivin concentrations was observed by calculating the ratio between survivin and the lymphocyte count, similar to other studies [39]. The basis for these calculations is the observation that inflammatory mediators in the tumor microenvironment, such as lymphocytes and other immune cells, support tumor cell proliferation, angiogenesis, invasion, and progression [40]. This can be used to develop a correlation between the tumor markers and immune cell numbers [39,40].

Some limitations of this study must be addressed. The differences in survivin concentrations among the malignant forms could not be determined because of the small number of dogs in the malignant tumor group. In addition, there were outliners in the lymphoma group (2,364 pg/mL) and in the mesenchymal tumor group (5,906 pg/mL), which cannot be explained. Therefore, further studies using larger numbers of dogs will be needed.

In conclusion, the serum survivin concentrations in dogs with malignant tumors are higher than those of dogs with benign tumors and dogs that do not suffer from any tumor. Using a cut-off value of 53 pg/mL, survivin may be used to differentiate dogs with malignancies from healthy dogs with a sensitivity and specificity of 0.67 and 0.95, respectively. The sensitivity and specificity of the test were increased using a survivin/lymphocyte ratio. On the other hand, survivin could not differentiate between tumor entities nor provide prognostic information.

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