Turkish Journal of Veterinary & Animal Sciences

Volume 45 | Number 4 Article 3

1-1-2021

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TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2021) 45: 609-620

doi:10.3906/vet-2011-8

Clinical and hemostasis predictors of mammary gland tumors in bitches

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Received: 02.11.2020 • **Accepted/Published Online:** 06.07.2021 • **Final Version:** 25.08.2021

Abstract: This study presents the results of the study of clinical and hemostasis predictors of mammary gland tumors in bitches, which allow to determine the risk groups for this disease. The highest incidence rates were found in German and Eastern European Shepherds (22.1%), Dachshunds (12.8%) and Yorkshire terriers (9.5%) when kept in private households: 97.1%, 96.3% and 97.3%, respectively. Significantly increases the likelihood of mammary gland cancer in excess body weight – by 30%–100%, in the case of mixed feeding – up to 39.3%, the use of hormonal contraceptives – by 50%–60%. Adverse prognosis correlates with an increase in the size, nature of the surface and the relationship with the surrounding tissues of the lesion, localization in 4th and 5th pairs of the mammary glands. Progression of the disease is accompanied by the development of hypercoagulable syndrome, which is characterized by an increase in fibrinogen and soluble fibrin due to activation of external and internal coagulation pathways, as evidenced by the prolongation of activated partial thromboplastin and prothrombin time. The results of the screening can be used for prevention and early diagnosis of the disease, as well as the development of promising treatment protocols.

Key words: Dogs, neoplasms, mammary gland, coagulation potential, risk factors

1. Introduction

Mammary gland cancer is a complex heterogeneous group of tumors for which it is important to find appropriate animal models in order to discover new biomarkers and therapeutic strategies. Occurring spontaneously, as in humans, in 50% of cases they have signs of malignancy. The most reliable models for this pathology are dogs that have a significant number of human-like traits (from pathohistological to molecular levels), which is confirmed by the results of genome sequestration [1].

Over the past four decades, there has been an increase in the number of similarities between mammary gland dogs and human breast tumors: molecular, histological, morphological, clinical, and epidemiological, leading to comparative cancer studies [2]. The imaging methods used are used to determine the location of the tumor, quantify the mass of neoplasia, visualization of genes and proteins, tumor microenvironment, proliferation and metabolism of cancer cells, response to treatment [3].

Solid tumors are abnormal formations that consist of several cell types and extracellular matrix. An important aspect of the biology of tumors is their understanding not only as clonal monocultures of cancer cells, but also as disorders of organs acting at the level of the organism as a whole [4].

For many years, veterinary oncologists have discussed the problem of predicting the behavior of mammary gland tumors in dogs. Transformation of neoplasia characteristics into prognostic information is an important tool for planning, development and clinical implementation of innovative treatment protocols. Collection, analysis and interpretation of information regarding the prediction of mammary gland neoplasms in small domestic animals are difficult due to the use of different methodological approaches for their evaluation [5].

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Progress in the personalization of treatment is hampered by the lack of cancer models that take into account individual heterogeneity within and between types of cancer. Tumor diseases that occur in nature in domestic animals are heterogeneous, so they allow us to answer questions about these strategies and optimize their interpretation for human patients [6].

Research using dogs is an ideal solution to the problem in animal models of controversial human diseases, as evidenced by more than 400 similar diseases of dogs and humans. There are several hundred isolated populations of dog breeds, each with significantly less genetic variability than humans, which simplifies disease mapping and pharmacogenomics [7]. In this case, long-term goals for predicting and evaluating these types of tumors can be achieved using a large natural resource of dogs at minimal cost [8]. Shorter overall life expectancy and rapid disease progression are factors that justify the superiority of the companion animal model [9].

Despite the large number of studies on the spontaneous manifestation of mammary gland cancer in bitches, there is no single statistical basis that allows the analysis of clinical cases. Low statistical reliability and underestimation of negative results are associated, first of all, with different methodological approaches, insufficient sampling (median n = 33), significant fluctuations in the duration of survival and disease progression, the high proportion of studies with controversial randomization (44%), sample reporting (94%), as well as the definition of the primary result (72%) [10]. The issue of neoplasms in animals up to 12 months of age remains insufficiently studied, which does not allow a full assessment of sexual, breed and genetic susceptibility, as well as the influence of oncogenic factors [11].

It has been shown that it is advisable to use indicators of the hemostasis system as objective criteria for assessing the malignancy risk of progression and the effectiveness of treatment of mammary gland tumors, which in most cases are accompanied by a hypercoagulable state [12].

Thus, mammary gland neoplasms are the most common tumor lesions in dogs, so the study of prognostic factors of the disease is important for standardization and individualization of diagnostic measures and treatment protocols.

Given the above, the aim of the study was to determine a set of predictors of development and progression of mammary gland tumors in bitches, which will increase the efficiency of predicting their biological behavior.

2. Materials and methods

The study of risk factors for mammary gland tumors was conducted in cooperation of Dnipro State Agrarian and Economic University, Bila Tserkva National Agrarian University and clinics of veterinary medicine in the city of Dnipro during 2017–2019.

The research was conducted following the requirements of the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986) and the Law of Ukraine "On Protection of Animals from Cruelty" (2006), as confirmed by the conclusion of the Bioethics Commission of Dnipro State Agrarian and Economic University.

The neoplastic process in the breast was studied by clinical and pathomorphological, anatomical and topographic, breed, age and sex characteristics and reproductive status, its clinical features depending on the size of benign and malignant tumors according to the international classification [13]. The Melbourne pain scale (MPS), which is based on 6 criteria (total score from 0 to 27): physiological parameters, palpation response, activity, posture, vocalization, and mental status, was used to determine the intensity of the pain response [14]. The pain reaction was considered as weak with a total score of 0 to 7, moderate – from 8 to 15, strong – from 16 to 27. These patients also underwent biopsy and blood sampling for hemostasiological analysis. A total of 791 dogs, including 603 purebreds and 188 half-blood, were examined.

We analyzed the risk of developing mammary tumors with different modes of hormonal contraception: oral – Pillkan 5 or Pillkan 20 (Ceva Sante Animale, Libourne, France) at a dose of 5 mg of megesterol acetate per 2.5 kg of body weight for 8 days and injection – Depogeston (Bioveta, Ivanovice na Hané, Czech Republic) at a dose of 50–100 mg of medroxyprogesterone aceticum per animal once subcutaneously. Regular hormonal contraception meant the use of pharmacological agents before each estrus (2 times a year), irregular – their sporadic use (1 time within 1–1.5 years).

The coagulation potential was determined based on fibrinogen content according to Belicer et al. [15], soluble fibrin by Vareckaja et al. [16], the duration of activated partial thromboplastin (a set of reagents from the company Simko Ltd., Lviv, Ukraine) and prothrombin time according to Quick [17].

To determine the pathomorphological structure of mammary gland tumors, a biopsy was performed, followed by verification of the histological type according to the improved classification of Goldschmidt et al. [18].

Statistical processing of the results was performed using Statistica 10 (StatSoft Inc., Tulsa, OK, USA). Bonferroni corrected ANOVA was used to determine the probability of sample difference.

3. Results

Breed susceptibility to mammary gland cancer has been established (Table 1): the vast majority of bitches with mammary cancer are German and Eastern European shepherds (22.1%), in other breeds the incidence rate is significantly lower: in Dachshunds – 12.8%, Yorkshire

Table 1. Frequency of registration of mammary gland tumors depending on the breed and breeding use of bitches.

Breed	Breeding		Home maintenance		Total	
	n	%	n	%	n	%
German and Eastern European shepherd	5	2.9	170	97.1	175	22.1
Dachshund	4	3.7	97	96.3	101	12.8
Yorkshire terrier	2	2.7	73	97.3	75	9.5
French bulldog	4	6.3	60	93.7	64	8.1
Rottweiler	3	7.1	39	92.9	42	5.3
Russian and English spaniel	2	5.3	36	94.7	38	4.8
Shih tzu	1	3.6	25	96.4	28	3.5
Poodle	1	3.8	25	96.2	26	3.3
Pug	1	5.3	18	94.7	19	2.4
Other breeds (registration frequency < 1%)	2	5.7	33	94.3	35	4.4
Half-breed	-	-	188	100	188	23.8
Total					791	100

terriers – 9.5%, French bulldogs – 8.1%. The frequency of registration of mammary gland tumors in bitches of other breeds is less than in shepherds (22.1%, $p < 0.001$). It should be noted a significant number of breeds in which isolated cases of mammary gland cancer were diagnosed: within 1% of the total number within the Dnieper region, as well as mestizos: registered in almost a quarter of cases (23.8%). Therefore, despite significant fluctuations in the incidence of mammary glands tumors in dogs, the most susceptible breeds have been identified, for which medical examination it is necessary to study the reproductive system, in particular the mammary glands.

Analysis of the relationship between breeds and reproductive use of bitches shows that in breeding kennels, even among favorable breeds of dogs, the level of registration of mammary gland tumors is from 2.7 to 7.1%, i.e. do not exceed 10%. The lowest incidence among breeding bitches (within 3%) was found in Yorkshire terriers (2.7%), as well as German and Eastern European shepherds (2.9%), the maximum – French bulldogs (6.3%) and Rottweilers (7.1%). At the same time, in the case of keeping dogs in private households, mammary gland neoplasia is diagnosed in 92.9%–97.3% of cases. Such patients make up the vast majority of all cases of mammary gland cancer in bitches. This situation may be partly related to genetic susceptibility in some lines, but this idea needs to be studied in detail.

Analysis of anamnestic data of cancer patients indicates the presence of a correlation between body weight and the likelihood of neoplastic processes in the mammary gland of bitches ($r = 0.95$, $p = 0.001$). The minimum incidence of mammary gland cancer has been established in individuals whose body weight does not exceed the average parameters of the breed. Exceeding this indicator within 20% increases the risk of tumors by 30%, 20%–50% – 80%, more than 50%–100%. It can be assumed that excess weight causes hormonal and metabolic imbalances, which play a key role in the mechanisms of oncogenesis.

Given the widespread uncontrolled use of hormonal contraceptives, their possible impact on the risk of mammary gland tumors has been identified (Table 2). The results show that compared to bitches who used regular or intermittent hormonal contraception, the absence of it reduced the risk of the disease by 50% and 60%, respectively. The ratio of the incidence of benign and malignant neoplasms ranged from 2%, i.e. did not depend on the mode of application of means of prevention of sexual seeking behaviour. The obtained results do not allow to state that violations of the hormonal status caused by the simultaneous action of several factors (hormonal contraception and obesity) significantly increase the risk of mammary gland tumors.

One of the prognostic factors in the development of mammary gland tumors is the feeding regime of animals (Table 3). The maximum risk should be assumed in the case of a mixed type of feeding (39.3%), which combines the use of commercial and prepared feed, which exceeds the corresponding indicators for use: commercial feed (17.6%), cooked beef feed (18.6%), chicken (24.5%). The ratio of the number of malignant tumors to benign is

Table 2. The risk of mammary gland cancer depending on hormonal contraception.

Hormonal contraception*		Animals with mammary gland tumors					
Mode	Drug	Total		Benign		Malignant	
		n	%	n	%	n	%
Oral regular	Pillkan 5 / Pillkan 20	163	20.6	75	9.5	88	11.1
Oral periodic		202	25.5	95	12.0	107	13.5
Parenteral regular	Depogeston	156	19.7	70	8.8	86	10.9
Parenteral periodic		194	24.5	101	12.7	93	11.8
Were not applied		76	9.6	44	5.6	32	4.0
Total		791	100	385	48.7	406	51.3

*: regular contraception - 2 times a year; irregular - once every 1–1.5 years.

Table 3. The effect of diet on the risk of mammary gland neoplasms in bitches.

Feeding ration		Animals with mammary gland tumors					
	Total		Benign		Malignant		
	n	%	n	%	n	%	
Commercial feed		139	17.6	86	10.9	53	6.7
Prepared feed-based	Chicken	194	24.5	86	10.9	108	13.6
	Beef	147	18.6	77	9.7	70	8.9
Mixed type of feeding		311	39.3	136	17.2	175	22.1
Total		791	100	385	48.7	406	51.3

for feeding on the basis of: commercial feed – 6.7:10.9%; prepared from chicken – 13.6:10.9%; beef – 8.9:9.7%, and mixed type – 22.1:17.2%.

One of the main predictors of mammary gland cancer in dogs is their pathomorphological structure. Malignant neoplasms of the mammary gland in bitches are characterized by a wide variety of histological types, which complicates the prediction of the disease. Malignant epithelial tumors were most often diagnosed (33.1% of the total number of neoplasms), while the frequency of verification of special types of epithelial neoplasms and osteosarcoma was lower – 9.7% and 8.7%. In most cases, among the epithelial types of malignant neoplasms of the mammary gland in dogs, carcinomas of complex, mixed type and in situ are registered: in 6.8, 5.7 and 5.3% of the total number of cancer animals. Inflammatory carcinoma (5.3%) and osteosarcoma (7.3%) are often diagnosed among specific epithelial tumors.

Among benign types of mammary gland tumors in dogs, the most common are complex adenoma, fibroadenoma and benign mixed neoplasia with rates of 25.5%, 23.8%, and 22.3%, respectively, accounting for more than 70% of cases. The incidence of other benign neoplasms (simple, intraductal papillary and ductal adenomas) ranged from 4% to 5%.

Analysis of clinical signs of mammary gland tumors in dogs indicates a change in the nature of their surface, depending on the stage and size of the lesion, which indirectly indicates the transition from benign to malignant (Table 4). In the benign course within stages 1–3 of the disease there is a change in the ratio of neoplasms with a smooth and bumpy surface: at a small size (up to 3 cm) it was 88.2:11.8%, 3–5 cm – 70.5:29.5%, and more than 5 cm – 58.9:41.1%. At the same time, areas of necrosis were registered, starting from the average size of tumors (3–5 cm) with an increase in their frequency with an increase in tumors to 12.3%. A similar situation with invasion into the surrounding tissues. In particular, the number of cases of involvement in the neoplasia of the skin, compared with small tumors (< 3 cm) – 5.9%, increased to 16.4%. The spread of the tumor to adjacent muscles was found only at their average and giant size against the background of an increase in the frequency of invasion with disease progression to 10.1%. At the same time, the pain response

intensifies, but a significant difference is found between the group T3/T4 and T1 ($p < 0.001$) and T2 ($p < 0.05$). The size of the tumor, the relationship with the surrounding tissues and the degree of expression of the inflammatory reaction allow to determine the extent of surgery.

In animals with malignant tumors of the mammary gland stages 1–3, the ratio of the number of neoplasms with a smooth surface to the hump with the progression of the disease varies from 84.6:15.4% to 35.4:64.6%, and in 4th stage is 16.7:83.3% (Table 5).

In this case, in contrast to dogs with benign neoplasms, already at the size of 3–5 cm, they are registered with approximately the same frequency, and later tumors with a bumpy surface begin to prevail. Necrotic defects were registered even at small neoplasia foci, although only in 3.8% of cases. The progression of the disease is characterized by an increase in their frequency in stages 1–3 to 45.6%, stage 4 – 66.7%. A sharp increase in the percentage of such patients with an increase in size to 3–5 cm is noteworthy. A similar situation is observed about the invasion of the surrounding tissues: the skin – 11.5%–77.8%, muscles 3.8%–83.3%. At the same time, with an increase in the size of mammary gland tumors in dogs, there is an increase in the intensity of pain in stages 1–3 from 4 ± 0.6 to 20 ± 3.1 points, but a statistically significant difference was found between groups T3/T4 (stages 1–3) and T1–T4 compared with T1 ($p < 0.001$) and T2 ($p < 0.05$). Clinical changes in mammary gland tumors have prognostic value only when used in a comprehensive assessment.

Monitoring the spread of mammary gland tumors in terms of age, depending on its stage and size of neoplasia foci revealed some patterns (Table 6). The maximum frequency of registration of small benign neoplasms, up to 3 cm in stages 1–3, is set at the age of 1–3 and 6–8 years, the minimum – up to six months and older 12 years, and malignant – 1–5 and younger 6 months, respectively.

Table 4. Clinical characteristics of different stages of benign mammary gland tumor in dogs (%).

Clinical criteria T1 (< 3 cm)		Stages 1–3		
		T2 (3–5 cm)	T3/T4 (> 5 cm)	
The nature of the surface	Smooth	88.2	70.5	58.9
	Hilly	11.8	29.5	41.1
Areas of necrosis		-	6.8	12.3
Invasion into the surrounding tissues	Skin	5.9	9.1	16.4
	Muscles	-	4.5	10.1
Intensity of pain reaction♦		2 ± 0.6	4 ± 1.5	15 ± 3.7***°

***: $p < 0.001$ compared with tumors T1, °: $p < 0.05$ compared with tumors T2;
♦: Melbourne pain scale (MPS) in points: weak 0–7, moderate 8–15, strong 16–27.

Table 5. Criteria for clinical evaluation of malignant neoplasms of the mammary gland in dogs (%).

Clinical criteria T1 (< 3 cm)		Stages 1–3			Stage 4
		T2 (3–5 cm)	T3/T4 (> 5 cm)	T1–T4 (various)	
The nature of the surface	Smooth	84.6	53.8	35.4	16.7
	Hilly	15.4	46.2	64.6	83.3
areas of necrosis		3.8	17.9	45.6	66.7
Invasion into the surrounding tissues	Skin	11.5	25.6	36.7	77.8
	Muscles	3.8	15.4	55.7	83.3
Intensity of pain reaction♦		4 ± 0.6	9 ± 3.5	20 ± 3.1***°	22 ± 4.4***°

***: $p < 0.001$ compared with tumors T1, °: $p < 0.05$ compared with tumors T2;
♦: Melbourne pain scale (MPS) in points: weak 0–7, moderate 8–15, strong 16–27.

Table 6. Stage of tumors depending on the age of patients (%).

Age, years	Stages 1–3						Stage 4
	T1 (< 3 cm)		T2 (3–5 cm)		T3 / T4 (> 5 cm)		T1–T4 (various)
	b*	m**	b*	m**	b*	m**	m**
< 0.5	5.9	3.9	2.2	5.1	4.1	3.8	-
0.5–1	5.9	7.7	6.8	7.7	6.9	8.9	-
1–3	23.5	34.5	15.9	23.1	15.1	10.1	5.6
4–5	17.7	23.1	34.1	15.4	26.0	12.7	11.1
6–8	23.5	11.5	18.2	25.6	16.4	27.9	22.2
9–11	17.7	11.5	11.4	12.8	16.4	17.6	44.4
≥ 12	5.9	7.7	11.4	10.3	15.1	19.0	16.7

b*: benign, m**: malignant tumor of the mammary gland.

At the same time, in 1–5-year-old dogs, the percentage of malignant tumors (3.9%–23.1%) exceeded the corresponding indicators for benign ones (5.9%–17.7%), while in 6–11-year-old bitches it was lower. In dogs, malignant neoplasms ranging in size from 3 to 5 cm in young animals (up to 0.5 years) were found in 5.1%, in 1–3 years – 23.1%, 6–8 years – in 25.6% of patients.

That is, neoplasms of this size were characteristic of middle-aged dogs. In the case of an increase in the size of tumors of stages 1–3, the shift of emphasis on their localization is established. In particular, the frequency of benign neoplasms larger than 5 cm gradually increases with a maximum in 4–5-year-old animals with a subsequent decrease in their registration, while the highest probability of malignant tumors occurs at the age of 6–8 years. Malignant neoplasms of stage 4 are diagnosed starting from one year, the frequency of their registration increases sharply in 9–11-year-old dogs (44.4%): twice, compared with 6–8-year-old animals (22.2%), with a subsequent decrease of 2.7 times in animals older than 12 years (16.7%). Thus, the incidence of mammary gland tumors is minimal in bitches before one and after twelve years.

The established features of tumor localization in dogs indicate that benign neoplasms of small size (< 3 cm) are located in most cases in 2–4th pairs, while malignancies are more often diagnosed in 3th and 5th pairs of mammary glands (Table 7). In benign neoplasms 3–5 cm in size, a similar situation is observed, while in malignant tumors in the vast majority of cases affect 4th and 5th pairs of mammary glands. In particular, their percentage is 9.1:10.3 for 1th pair, 22.7:12.8% – 2th pair; 22.7:15.4% – 3th pair. For tumors larger than 5 cm, the minimum frequency of their registration, regardless of the nosological profile, is characteristic of the first pair, the maximum – the 3th–5th pairs of the mammary gland. At the same time, if benign tumors are characterized by an increase in the number of cases with a maximum of 5th pairs, then for malignant – in 4th pairs against the background of a decrease in the frequency of localization in 5th pairs. In stage 4, malignant neoplasms in the vast majority of cases are located in 4th and 5th pairs of mammary glands: the total number of such patients is 72.1% of all dogs in this group.

Analysis of functional disorders of the hemostasis system in cancer patients is characterized by activation of coagulation cascades, the intensity of which correlates with the stage and size of the tumor, as well as the histological type of tumor (Table 8). In particular, compared with clinically healthy animals, the content of fibrinogen in benign tumors 1–3th stages ($p < 0.001$): < 3 cm increased up to 4.1 ± 0.09 g/L, from 3 to 5 cm – 9.4 ± 0.8 g/L, more than 5 cm – 11.5 ± 0.5 g/L, and malignant – 6.0 ± 0.03; 12.3 ± 0.7; 17.2 ± 1.1 g/L, respectively. At the same time, regardless of the stage and size of neoplasms, its concentration in malignant neoplasms of the mammary gland significantly exceeded the corresponding indicators of patients with benign tumors ($p < 0.01$).

Similar dynamics are also characteristic of the content of soluble fibrin. The progression of the disease was accompanied by its accumulation with the maximum level in bitches in 4th stage of cancer: 22.6 ± 2.3 mg/dL. In contrast to the fibrinogen content, the concentration of soluble fibrin in the malignant course differed significantly ($p < 0.001$) from the benign one only starting from stage T2 (3–5 cm).

The development of hypercoagulable syndrome is realized both externally and internally, as evidenced by changes in the duration of the activated partial

Table 7. Localization of mammary gland tumors in dogs (%).

Mammary gland pairs	Stages 1–3						Stage 4
	T1 (< 3 cm)		T2 (3–5 cm)		T3 / T4 (> 5 cm)		T1–T4 (various)
	b*	m**	b*	m**	b*	m**	m**
1	11.8	11.5	9.1	10.3	8.2	6.3	5.6
2	23.5	19.2	22.7	12.8	12.3	17.7	5.6
3	23.5	23.2	22.7	15.4	23.3	26.6	16.7
4	23.5	19.2	27.3	28.2	27.4	29.1	33.3
5	17.7	26.9	18.2	33.3	28.8	20.3	38.8

b*: benign, m**: malignant tumor of the mammary gland.

Table 8. Metabolism of fibrinogen in bitches depending on the size of neoplasms.

Indicator	Fibrinogen, g/L		Soluble fibrin, mg/dL	
	b*	m**	b*	m**
Clinically healthy	2.2 ± 0.1		0.1 ± 0.01	
Stages 1–3				
T1 (< 3 cm)	4.1 ± 0.09°°°	6.0 ± 0.03°°°♦♦	0.5 ± 0.01°°°	0.7 ± 0.08°°°
T2 (3–5 cm)	9.4 ± 0.8°°°	12.3 ± 0.7°°°♦♦	5.9 ± 0.4°°°	9.9 ± 0.6°°°♦♦♦
T3 / T4 (> 5 cm)	11.5 ± 0.5°°°	17.2 ± 1.1°°°♦♦	7.1 ± 1.2°°°	13.1 ± 1.2°°°♦♦♦
Stage 4				
T1–T4 (various)	-	25.7 ± 1.8°°°	-	22.6 ± 2.3°°°

b*: benign, m**: malignant tumor of the mammary gland; °°°: $p < 0.001$ relatively clinically healthy animals; ♦♦: $p < 0.01$, ♦♦♦: $p < 0.001$relatively benign tumors.

thromboplastin and prothrombin pathways (Table 9). In both cases, their elongation is registered, compared with clinically healthy dogs. The increase in the size of malignant neoplasms was accompanied by a progressive increase in the duration of activated partial thromboplastin time in patients with stages 1–3 up to 55.7 ± 1.0 –68.9 ± 3.1 s ($p <$ 0.05–0.01) times, stage 4 – 81.5 ± 3.4 s ($p < 0.001$). In the case of benign tumors, this indicator does not depend on the clinical stage of the disease and the histological type of tumors. In contrast to the activated partial thromboplastin time, the prothrombin time was prolonged in all cases as the process progressed ($p < 0.001$): for foci up to 3 cm in benign course – up to 11.7 ± 1.2 s, malignant – 17.7 ± 0.9 s; 3–5 cm – 14.3 ± 1.2 and 21.0 ± 1.3 s; more than 5 cm – 17.0 ± 0.7 and 24.6 ± 1.3 s, respectively. In dogs with malignant neoplasms, the prothrombin time was significantly ($p <$ 0.01) higher than in patients with benign tumors.

Certain disorders of hemostasiological status suggest a high risk of thrombosis in dogs with cancer, thereby contributing to metastasis, which is the leading cause of death in such patients.

4. Discussion

Our results are consistent with the reports of other researchers on the negative trend to increase the incidence of malignant tumors (up to 70%) of the mammary gland relative to benign neoplasms (30%), the peak of their registration (60%) in 8–13-year-old bitches [19]. Some reports indicate a high prevalence of mammary tumors among certain breeds (Samoyeds, Dobermans, Schnauzers, Yorkshire terriers) due to genetic susceptibility [20], although our studies do not confirm this pattern.

The increase in the incidence of mammary gland cancer is probably due to the high intensity of life in the metropolis, the inability of animals to adapt to such conditions and, as a result of stress, as evidenced by the results of Cannas et al. [21] regarding the presence of a correlation ($p < 0.05$) between stress and the frequency

Table 9. The state of the external and internal blood clotting pathways for tumors.

Indicator	Activated partial thromboplastin time, sec.		Prothrombin time, sec.	
	b*	m**	b*	m**
Clinically healthy	46.1 ± 1.3		8.7 ± 0.9	
Stages 1–3				
T1 (< 3 cm)	49.5 ± 0.6	55.7 ± 1.0°	11.7 ± 1.2°°°	17.7 ± 0.9°°°♦♦
T2 (3–5 cm)	53.5 ± 1.1°	62.8 ± 3.6°°♦	14.3 ± 1.2°°°	21.0 ± 1.3°°°♦♦
T3 / T4 (> 5 cm)	59.7 ± 1.0°°	68.9 ± 3.1°°♦	17.0 ± 0.7°°°	24.6 ± 1.3°°°♦♦
Stage 4				
T1–T4 (various)	-	81.5 ± 3.4°°°	-	30.2 ± 1.5°°°

b*: benign, m**: malignant tumor of the mammary gland; °: $p < 0.05$, °°: $p < 0.01$, °°°: $p < 0.001$ relatively clinically healthy animals; ♦♦: $p < 0.05$, ♦♦♦: $p < 0.01$ relatively benign tumors.

of this pathology in dogs. At the same time, clinical signs according to the international classification of stages of malignant neoplasms (TNM: size of the primary tumor, presence or absence of metastases in regional lymph nodes and distant tissues) are of important prognostic value, but in combination with histological verification of tumors.

The incidence, macropathological changes and pathological type of neoplasia do not correlate with long-term survival, while the number of metastases, maximum tumor diameter, TNM stage and hormone receptor levels significantly affect the duration of the recurrence-free period [22].

The results of our study are consistent with the opinion of Sorenmo et al. [23] regarding the fact that malignant tumors are recorded in older animals (9.5 versus 8.5 years, $p = 0.009$), their sizes exceed benign ones (4.7 versus 2.1 cm, $p = 0.0002$) by against the background of a high likelihood of developing new primary neoplasias ($p = 0.0015$). Giant tumors (T3) in most cases are characterized by malignancy, lower progesterone receptor expression and high markers of cell proliferation, as well as a shorter survival period, which confirms the importance of tumor size as a prognostic indicator of mammary gland tumors in bitches [24], in particular for adenocarcinoma [25]. The most commonly diagnosed mammary gland cancer is stage 1 (31.75%), less often stages 2–5 [26].

Regardless of the observation period and location, in a study of a significant number of patients, the ratio of spontaneous malignant and benign mammary gland tumors in dogs is about 1:1, which coincides with the incidence in humans [27]. In contrast to several indicators that vary significantly depending on the region (breed, frequency of registration of neoplasms of different sizes), in the vast majority of cases, tumor foci are located in the fourth and fifth pairs of mammary glands, and risk factors are age ($p < 0.001$), overweight ($p = 0.048$) and failure to perform ovariohysterectomy ($p < 0.001$) [28]. In addition, we additionally established a correlation between tumor localization and their staging, in particular, verification in 72.1% of stage 4 neoplasms in caudal abdominal and inguinal pairs of the mammary gland.

The highest probability of developing tumors in the last pair of the mammary gland (fourth – in 27%, fifth – 60% of dogs), due to the duration of growth before diagnosis, age of patients and high prevalence of stray animals [29], consistent with Gomes et al. [30] data: the frequency of lesions of the inguinal mammary glands in dogs reaches 66.6%–70%.

Obesity is a growing concern for pet owners, and the increase in morbidity associated with it reflects a general trend that is also observed in humans. The results obtained on obesity as a risk factor for mammary gland tumors are consistent with data from other researchers, who indicate a high risk of neoplasia transformation of functional tissue in the presence of excess weight in both humans and dogs [31]. Against the background of a significant overall prevalence of obese dogs (21.6%), a significant difference in the incidence of cancer depending on the bodyweight of animals. The prevalence of overweight dogs varied depending on the specific histological types of cancer [32]. In mammary gland cancer, obese and overweight bitches showed a shorter survival rate than animals that were normal weight. A positive correlation was established between body composition (fatness) and histological class ($p < 0.01$). According to the presented results, bitches had a higher body weight in the home diet, compared with feeding commercial feed, against the background of no dependence on the incidence of mammary gland cancer [33].

Overweight animals can influence the development and behavior of mammary gland tumors due to the interaction of neoplasia-adipocytes and increased growth

of tumors associated with HR-receptors. Dogs with excess body weight are characterized by a younger age of patients (8.7 ± 1.9 years), a higher proportion of low-differentiated (grade III) tumors, a direct correlation between high levels of aromatase expression and HR ($p = 0.025$) [34]. In addition, in dogs, similarly to humans, overweight is marked by a significant increase in prostaglandin E_2 (PGE_2), which correlates with the expression of aromatase, transcription factor NF-κB and leptin, but in the absence of a relationship with pathomorphological changes in mammary tissues [35].

The presented explanations for obesity-related cancer emphasize the direct mutagenic effects of feeding components or hormonal imbalances. One of the main tasks should be to differentiate between factors that independently contribute to obesity or cancer and those that cause carcinogenesis indirectly. Against the background of obesity, according to the first hypothesis, chronic inflammation causes the initiation of the kynurenine pathway, which leads to activation of the aryl hydrocarbon receptor (AhR) and modulation of synaptic transmission in the brain. According to the second hypothesis, serine proteases are able to deplete the cells of neoplasia suppressors [36].

The established heterogeneity of the structure of mammary gland tumors confirms the opinion of other researchers on the significant variability of their biological behavior and the need to find new prognostic and therapeutic factors, taking into account the individual characteristics of each patient [37]. Morphological and biological heterogeneity based on molecular analysis and classification Goldschmidt et al. [18], as well as the frequency of registration of malignant types at 52% is confirmed by Im et al. [38].

The expediency of pathomorphological verification is confirmed by the importance of tumor size as a predictor of recurrence and metastasis. A 10-fold risk of death from oncogenesis has been shown to be associated with tubulopapillary, intraductal papillary carcinoma, and malignant myoepithelial tumors. The most unfavorable prognosis is characteristic of anaplastic carcinoma and carcinosarcoma: the frequency of metastases is 89% and 100%, respectively, as well as adenosquamous carcinoma: the probability of recurrence reaches 50% [39]. A significant number of osteosarcomas verified in our study are due to myoepithelial differentiation, with a key role in the chondrogenesis of regulatory proteins [40]. Kim et al. [41] found a significant correlation ($p < 0.05$) between the size and breed of dogs, histological type and molecular phenotype in Shih Tzu dogs. In our opinion, this dependence is debatable, due to different methodological approaches to the diagnosis of tumors.

The established prognostic significance of the pathomorphological structure of neoplasms is supplemented by publications of other researchers. In particular, Scarpa et al. [42] showed the importance of determining the presence of tumor cells in the area of the edges of the operating field: the recurrence rate of infiltrated or slightly contaminated was significantly higher compared to clean edges. In malignant types, some researchers indicate the prognostic value of tumor necrosis, the volume of which depends on the intensity of local and systemic inflammatory reactions and affects survival, in the absence of correlation with the pathological stage [43].

Clinical stage, histological class, the involvement of sentinel lymph nodes, and sterilization status can be used as single criteria as independent prognostic variables to determine survival as well as the likelihood of recurrence and metastasis in mammary gland tumors in dogs [44]. In particular, low probability of survival can be predicted in older animals, with invasive growth, significant tumor size, the presence of ulcerative skin lesions and regional lymph node involvement. [45].

Our results are consistent with the general patterns of disorders of the hemostasis system and endothelial function in mammary gland tumors and indicate their strengthening in the case of increasing the size of neoplastic foci, which may indicate an increase in the aggressiveness of tumors. In dogs in the presence of prothrombotic diseases, a significant decrease in fibrinolysis activity is registered, and their development against the background of a systemic inflammatory reaction, in particular in cancer patients, causes a maximum decrease in the level of fibrinolytic potential [46]. In particular, the expression of tissue factor, which plays an important role in the pathogenesis of this pathology, affects the intensity of thrombin formation – the main link in the mechanism of blood clotting [47]. Increased tissue factor activity has been shown to correlate with D-dimer levels in dogs with pathology accompanied by disseminated intravascular coagulation syndrome [48].

Even though the data obtained are consistent with the results of other researchers, according to which mammary gland cancer in bitches is accompanied by a hypercoagulable state, although the syndrome of disseminated intravascular coagulation has been diagnosed relatively rarely. The most common hemostatic disorders found in dogs with carcinoma are hypercoagulation, thrombocytosis in 46% of cases, hyperfibrinogenemia in 32% of patients with decreased levels of activator inhibitor plasminogen [49]. The increase in procoagulant status and facilitation of metastasis is caused by increased expression of tissue factor, which depends on the content of antithrombin and plasminogen in the absence of its correlation with stage and degree of malignancy [50].

The systemic inflammatory reaction that accompanies the neoplasia process can cause a hypocoagulable state characterized by ($p < 0.001$) a decrease in antithrombin (AT), FVIII and the ratio activated protein C (APC) [51].

In our opinion, further research in this direction is expedient, which will probably allow to study in more detail the connection of hemostasiological balance with other factors that cause malignancy of neoplasms [52].

In animals with mammary gland tumors there is hyperactivation of the hemostasis system due to thrombinemia, as evidenced by the appearance in the blood plasma of soluble fibrin, but the wide range of its parameters cannot be clear clinical and hemostasis criteria of the neoplasia, but only a pathognomonic sign of coagulation. hemostasis, regardless of their types. The high concentration of soluble fibrin in the blood is a consequence of the functioning of the tumor, which not only directly produces thrombin, but also affects the tissue factor, activating its conversion from prothrombin. It is also a response to the increased levels of interleukin-6, which is secreted by macrophages, which is confirmed by some authors.

However, the dynamic increase in soluble fibrin concentration, especially in the case of malignant tumors, undoubtedly indicates the permanence of the hypercoagulant process of tumor growth, which was characterized by an increase in plasma concentration of soluble fibrin in the absence of statistically significant difference

To assess the internal pathway and the general cascade of the blood coagulation system, it is advisable to determine the activated partial thromboplastin time, which, among global indicators of coagulation, is the most sensitive and allows to assess not only the degree of its activation but also the deficiency of plasma coagulation factors disseminated intravascular coagulation syndrome [53].

The established prolongation of the activated partial thromboplastin time may be due to a deficiency or defect of plasma coagulation factors, which is evidence of coagulopathy of consumption as one of the stages of disseminated intravascular coagulation syndrome. This, on the one hand, weakens the biological barriers and creates the conditions for tumor aggression; on the other hand, there is a risk of both thrombosis and bleeding during surgical removal of tumors. Therefore, the rate of activated partial thromboplastin time may be a significant paraneoplastic hemostasiological criterion in the diagnosis and prognosis and treatment of animals with breast neoplasms, especially of malignant origin.

Indicators of the prothrombin test in any case confirm the formation of hypercoagulable syndrome in mammary gland tumors of different genesis in dogs. Its reduction reflects the first stages of activation of hemostasis, which gradually leads to a deficiency of plasma coagulation factors or to their inhibition by the secretory products of tumor cells. Thus, the change in prothrombin time may also be a paraneoplastic criterion for mammary gland tumors in dogs.

Although the aggression of tumor cells to increase their range is due to their own proteolytic systems and including tissue plasminogen activators [54], but for the most part the latter are suppressed [55], as proved above, especially in the case of malignant neoplasms.

Thus, despite the urgency of the problem of tumors in dogs, there are no short-term prospects for its solution. It is important to screen the disease in different regions, the analysis of the results of which will form a unified methodological approach, to determine the role of causative factors in the initiation of the mechanism of oncogenesis. Analysis of monitoring studies of clinical and hemostasiological features of the course of mammary gland tumors in bitches will minimize the development of recurrences and metastases, primarily due to early diagnosis of the disease. The presence of disorders of hemostasiological status justifies the feasibility of use in the treatment of low molecular weight heparins and nonsteroidal antiinflammatory drugs.

Comprehensive assessment of clinical, pathomorphological and hemostasiological parameters at the initial examination of bitches with mammary glands tumors allows with a high level of reliability to predict the course of the disease and apply optimal treatment protocols.

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