SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua

Scientific Horizons, 24(2), 54-61

UDC 619:618.19-006.06:636.7

DOI: 10.48077/scihor.24(2).2021.54-61

PROGNOSTIC VALUE OF VASCULAR INVASION IN BREAST TUMOURS IN SHE-DOGS (PILOT STUDY)

Maksym Kovalenko*, Dmytro Bilyi

Dnipro State Agrarian and Economic University

49600, 25 Serhiy Yefremov Str., Dnipro, Ukraine

Article's History:

Received: 12.04.2021

Revised: 25.05.2021

Accepted: 18.06.2021

Suggested Citation:

Kovalenko, M., & Bilyi, D. (2021). Prognostic value of vascular invasion in breast tumours in she-dogs (pilot study). *Scientific Horizons*, 24(2), 54-61.

Abstract. Breast tumours are the most common neoplasm in intact she-dogs. However, breast tumours in she-dogs differ significantly in morphological features and biological behaviour, so the definition of prognostic factors is relevant. A study on tumour cell dissemination in breast tumours in she-dogs by migration of these cells into blood and lymphatic vessels depending on their histological characteristics and disease stage was conducted. The study of the problem was performed on the basis of the clinic of modern veterinary medicine "Best" in Zaporizhzhia. She-dogs with breast neoplasms of different ages and breeds were used as objects in the proven absence of signs of metastatic lesions of other organs and tissues, including lungs, abdominal organs and bones. The presence of cancer cells in the vessels was determined by the tumour clots formed by them, fixed to the endothelium. The study revealed the presence of angioinvasion regardless of the clinical stage of the tumour process. In this case, the dissemination of tumour cells by migration into lymphatic vessels was observed only in the second clinical stage of breast tumours. In patients with stage 1 breast cancer in the vast majority of cases (66.7% of patients) angioinvasion is registered in the micropapillary invasive carcinomas (ICD-O code 8507/2). Tumour cells in blood vessels were verified in simple cribriform carcinoma (ICD-O code 8201/3) in more than 80% of she-dogs with stage 3 breast cancer. In contrast to the above groups, in patients with stage 2 cancer, migration of tumour cells into both blood and lymphatic vessels was found. Most often the signs of angio- and lymphoinvasion were found in invasive carcinoma mixed type (ICD-O code 8562/3), tubulopapillary carcinoma (ICD-O code 8503/3), and tubular carcinoma (ICD-O code 8211/3) – in 34.1% and 36.8%, 19.3% and 26.3%, 17.0% and 10.5% of cases, respectively. The obtained results allow predicting the probability of penetration of tumour cells into blood and lymphatic vessels with a high degree of reliability, which in the future can better predict the biological behaviour of breast tumours

Keywords: dogs, neoplasia, breast, metastasis

*Corresponding author

INTRODUCTION

It is a proven fact that in breast tumours, lymph node involvement is one of the most important predictors of clinical outcome in humans and animals [1; 2]. Lymphovascular invasion, which means the identification of tumour cells in the space lined with endothelium, in the primary site of a tumour or around it, is considered by oncologists as an invasion of blood vessels [3]. However, data on the prognostic significance of vascular invasion, proliferation index and angiogenesis in relation to the development of metastases and survival in sick dogs remain ambiguous [4]. As a small number of tumour cells are able to survive outside their microenvironment, the mere presence of tumour cells in the blood vessel lumen does not guarantee the development of metastatic lesions; therefore, although vascular invasion in the form of tumour emboli is not uncommon, it is not known whether it affects the development of metastases and life expectancy in dogs [5-7]. In addition to it is important to remember that during histological examination vascular invasion sometimes may not be detected, mainly due to the rarity of tumour emboli in the tumour tissue array [8], and in 20% of cases of lymphovascular invasion it may differ in evaluation of the same material given by different pathologists [9].

The question of the probability of angio- and lymphoinvasion in neoplasms of different types remains debatable. In the study by Yoshimura et al. [10] vascular invasion was more common in simple solid carcinomas, and much less common in malignant myoepitheliomas and biphasic carcinomas; in another study by Yoshimura *et al.* [11] vascular invasion was registered in 40.5% of simple solid breast cancers. However, in another study, it was registered only in 15.1% of malignant breast tumours [12], which corresponds with the results obtained by Seung *et al.* [13] – 14.8%; according to Pastor et al. [14] it was registered in 28.6% of cases and according to the study by Monteiro et al. [15] – in 33.3% of cases. Probably, the unresolved issue is due to the lack of a unified methodological approach.

Thus, despite the significant number of publications on predicting the course of the disease in breast tumours and the main stages of cancer cell dissemination, the mechanisms of angio- and lymphoinvasion, which are predictors of the aggressiveness of breast tumours, remain insufficiently studied in oncogenesis. Notably, in practice, in the vast majority of cases, the prognosis of the disease and the choice of treatment measures is made without considering the vascular invasion, which causes insufficient effectiveness of the protocols.

The purpose of this study was to determine the role of angio- and lymphoinvasion as predictors of malignancy at different stages of breast tumours in she-dogs.

LITERATURE REVIEW

Breast tumours, most of which are malignant, are the most common neoplasms in intact she-dogs, mostly middle-aged and older (8-11 years) [16; 17]. The incidence rate fluctuates significantly, depending on the spay at an early age. The main cause of death from breast cancer in she-dogs is metastasis to the lungs. Breast carcinomas have heterogeneous clinical behaviour in the rate of metastasis, recurrence, impact on life quality and expectancy [4].

Recurrence of breast cancer may occur partially due to a lack of understanding of the early stages and mechanisms involved in the breast cancer metastasis, especially the effects of neoplastic inflammatory cells, including tumour macrophages, on the invasion process [18].

In the study by Diessler *et al.* [4] vascular invasion was negatively correlated with life expectancy and lymph node involvement; similar results are presented by Rasotto *et al.* [19]. The same data were obtained in the study of breast tumours in women; in addition, the study by Mohammed *et al.* [20] shows that vascular invasion is more common in tumours larger than 1.5 cm, and that oestrogen-negative and progesterone-negative tumours are more likely to have significant vascular invasion than hormone-positive tumours. Lymphovascular invasion is also more common in Her2-positive tumours [21]. Studies by Monteiro *et al.* [15; 22] showed that vascular invasion correlates with the number of tumour-associated macrophages, which is confirmed by the data obtained by Seung *et al.* [21]. Separation of tumour cells from the main tumour mass and their intravasation into blood vessels usually occurs in areas of the largest accumulation of macrophages [23]; the study by Carvalho *et al.* [24] shows that high expression of COX-2 is correlated with the tumour emboli formation. However, according to studies by Santos *et al.* [25], vascular invasion does not depend on the degree of nuclear pleomorphism, one of the criteria for malignancy [19], which in its urn correlates with life expectancy.

Despite the large number of publications of lympho- and angioinvasion for breast tumours in dogs, does not reveal unambiguous data on the prognostic value of this process. Therefore, further research is needed to target the biological behaviour of tumour tumours that are healthy to invade blood vessels.

MATERIALS AND METHODS

The research was conducted in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals [26] and the Law of Ukraine "On Protection of Animals from Cruelty" [27]. Ethics Committee approval was received for this study from the Animal Researches Committee of the Dnipro State Agrarian and Economic University, Ukraine (Approval number: 2019/02). The research was conducted on the basis of "Best" veterinary clinic (Zaporizhzhia). The objects of study were dogs of different breeds and crossbreeds, the average age was 8.6 years (from 3.5 to 16), in which during 2019-2020 were diagnosed with breast tumours.

At the same time, clinical and special (X-ray, ultrasound diagnostics, determination of hemostasiological status) research methods in such patients ruled out the presence of metastatic foci in other organs and tissues. The clinical stage of the tumour process was determined by the TNM classification according to Owen [28]. This study included dogs with I-III stages of the tumour process.

In the first stage, the tumour was removed from she-dogs by electrosurgery in the amount of regional or unilateral mastectomy. Pieces were excised from the tumour node(s), unaffected breast tissue and regional lymph nodes: axillary and additional lymph nodes – for lesions of I or II milk package, inguinal – for lesions IV or V, axillary, additional and inguinal – for lesions of III milk package. To facilitate the identification of lymph nodes in the surgical material was injected intradermally 0.2-0.4 ml of 0.05% aqueous solution of methylene blue before 7-10 minutes surgery in areas I or V of milk packets. Pieces of tissue were fixed for 48-72 hours in 10% neutral buffered formalin according to Lily. Subsequently, the tissues were passed through ascending alcohols, chloroform, chloroform-paraffin and paraffin and poured into paraffin. Sections 5-6 μm thick were made on a rotary microtome MPS-2 (Micromed Ukraine). Paraffin sections were dewaxed in xylene and stained with hematoxylin and eosin according to the method of Horalskyi *et al*. [29]. Microscopy was performed using an Olympus BX43 microscope (Olympus, Japan). Tumours were histologically verified according to the classification of Goldschmidt *et al*. [30] and coded according to the World Health Organisation's International Classification of Disease for Oncology system (ICD-O) [31] to facilitate comparison with existing human and animal cancer registries. The presence of cancer cells in the form of tumour emboli in the lumen of blood and lymphatic vessels was also identified.

RESULTS

According to the data obtained, a high level of malignancy in breast tumours should be assumed in the case of tumour cells dissemination in the first stage of the disease (Table 1). Among them, infiltrative tubular carcinoma was verified in one third of cases, but the maximum risk of disease progression was found in patients with tubulopapillary invasive carcinoma, as in this case the probability of diagnosing tumour cells in the lumen of blood vessels is two times higher. Notably, in both cases only signs of angioinvasion were registered.

Table 1. *Angioinvasion in the first stage of malignant tumours of the breast in she-dogs*

Histologic type	Detection frequency	
	n	%
Tubulopillary invasive carcinomas (ICD-O code 8503/3)	4	66.7
Tubular carcinoma (ICD-O code 8211/3)	2	33.3
Total	6	100

Most often, the presence of tumour cells in the vessels is detected in stage 2 breast cancer (Table 2). Only in such patients, in contrast to she-dogs with stage 1 cancer and stage 3 cancer, lymphoinvasion was verified although the presence of cancer cells in lymphatic vessels is registered only in certain types of tumours. About a third of cases of angio- and lymphoinvasion were registered in she-dogs with invasive carcinoma mixed type on the background of approximately the same frequency of tumour emboli detection. The high risk of dissemination of tumour cells into vessels was found in tubulopapillary carcinoma, but it is slightly lower – by a factor of 1.8 and 1.4, respectively. In 10% of cases, cancer cells in blood and lymph vessels were verified in tubular and solid carcinomas. In micropapillary invasive and simple cribriform carcinomas, tumour emboli were registered in both angio- and lymphoinvasion in about 5% of patients. In the case of ductal carcinoma, the probability of cancer cells dissemination through lymphatic vessels, compared with blood ones, is 5 times higher.

Table 2. *Vascular dissemination of cancer cells in the second stage of breast cancer in dogs*

Histologic type	Tumour cells (microemboli)			
	Blood vessels		Lymphatic vessels	
	n	%	n	%
Carcinoma mixed type (ICD-O code 8562/3)	32	36.4	7	36.8
Tubulopapillary carcinoma (ICD-O code 8503/3)	17	19.3	5	26.3
Tubular carcinoma (ICD-O code 8211/3)	15	17.0	2	10.5
Solid carcinoma (ICD-O code 8230/3)	8	9.1	2	10.5
Micropapillary invasive carcinomas (ICD-O code 8507/2)	4	4.5	1	5.3
Simple cribriform carcinoma (ICD-O code 8201/3)	4	4.5	1	5.3

Table 2, Continued

Histologic type	Tumour cells (microemboli)			
	Blood vessels		Lymphatic vessels	
	n	%	n	%
Ductal carcinoma (ICD-O code 8500/3)	1	1.1	1	5.3
Anaplastic carcinoma (ICD-O code 8021/3)	3	3.4	–	–
Total	88	100	19	100

Among the studied stage 2 breast tumours there are histological types that are characterised by invasion only into blood vessels on the background of significant fluctuations in their registration frequency. In particular, compared with anaplastic carcinoma, in which cancer cells in blood vessels are verified relatively rarely (1.1%), the probability of angioinvasion is 2.1 times higher in carcinomas of mixed type, 4.1 times higher in mucinous carcinoma, and 6.2 times higher in tubular carcinoma.

Certain patterns of tumour cells dissemination in stage 3 breast cancer have been understood (Table 3). In contrast to stage 2 cancer, tumour cells in conglomerates were verified in a small number of histological types and only in blood vessels. In the vast majority of cases, angioinvasion is found in simple cribriform carcinoma (83.3%), while in anaplastic carcinoma the probability of detecting tumour cells outside the neoplasm is 5 times lower.

Table 3. *Angioinvasion in the third stage of malignant neoplasms of the breast in she-dogs*

Histologic type	Detection frequency	
	n	%
Simple cribriform carcinoma (ICD-O code 8201/3)	5	83.3
Anaplastic carcinoma (ICD-O code 8021/3)	1	16.7
Total	6	100

Histologically, in anaplastic carcinoma of any stage only angioinvasion was recorded, whereas in stage 2 simple cribriform carcinoma, the presence of tumour cells in the lymphatic vessels was detected.

DISCUSSION

The analysis of reports and results of our research on the pathogenetic mechanisms of breast tumours in she-dogs proves their multi-vector nature and the need for further, more detailed study of carcinogenesis mechanisms in order to improve diagnosticating and identifying therapeutic targets for better treatment [32]. Vascular invasion, as a predictor of breast malignancy in she-dogs, allows predicting an unfavourable prognosis based on the correlation of high levels of tumour-associated macrophages with the clinical stage (P<0.001), tumour type (P=0.016), tumour size (P=0.013), the presence of tumour emboli in lymphatic and blood vessels (P=0.031), proliferation rates (P=0.009), lymph node metastases (P=0.003), vascular microdensity (P=0.008), invasive profile (P=0.002), and unfavourable prognosis (P<0.018) [15].

The results of studies presented by Rasotto *et al.* [33] also show that carcinomas and malignant myoepitheliomas are characterised by more aggressive behaviour, manifested by a higher incidence of vascular and lymph node invasion, a higher rate of visceral metastases, and a risk of death due to disease progression affected by Ki-67 overexpression.

The predictive significance of lymphoinvasion in breast tumours in she-dogs is confirmed by its correlation with the expression of DERL-1, a transport protein for the export of incorrectly folded proteins from the endoplasmic reticulum (ER) and apoptosis inhibitor [34].

Angioinvasion, which accompanies oncogenesis, is facilitated by circulating myeloid suppressor cells, which change the molecular pathways in tumour cells significantly due to increased expression of angiogenic factors and subsequent induction of angiogenesis by endothelial cells, epithelial mesentery, epithelial-mesenchymal transition, as well as increased migration of tumour cells [35].The data obtained are confirmed by the results of studies by Santos et al. [7], according to which highly proliferative tumours and tumours with invasive growth are characterised by a significant metastatic ability and, accordingly, an unfavourable prognosis on the background of short survival time and recurrence-free periods.

To understand the mechanisms of cancer cells dissemination, we consider the studies aimed at clinical aspects, the study of malignant transformation, histogenesis, epithelial-mesenchymal interactions to be relevant. They can standardise the criteria for diagnosis and breast tumour treatment. Individualisation of protocols will allow for more adequate disease management, thereby improving the she-dogs' survival and quality of their life [36].

The correlation of vascular invasion (r=0.76, P=0.043) with overexpression of vimentin filaments,

which participate in the mechanisms of epithelial-mesenchymal transition, established for breast tumours in dogs, confirms the obtained data on cancer cell dissemination into blood and vessels in malignant tumours [37].

Areas of tumour invasion show a higher level of hematoxylin-eosin staining and complete loss of α-SMA and p63 immunoreactivity, which in combination with the clinical stage and histological grade can be used for individual assessment of breast tumour [38]. The results are consistent in breast tumour with the correlation of lymph node status with histological grade, vascular invasion, proliferation index, expression of VEGFR-2, and microvascular density [4].

In this case, overexpression of vascular growth factor, which accompanies the progression of the tumour process, stimulates the proliferation, migration and survival of endothelial cells. The inverse correlation of VEGFR-1 with metastases to regional lymph nodes was proven. However, its prognostic significance for tumour cell dissemination and termination of disease remains unclear [39]. Along with the lesion size, histological grade, ERα-negativity, high Ki-67 proliferation index and absence of EGFR, the migration of tumour cells and lymph node involvement are of clinical importance [40].

In contrast to the tumour size and the histological type, which according to Chocteau *et al.* [41] correlate with early cancer mortality, but do not affect the conditional survival of patients significantly, lymphovascular invasion and regional lymph node involvement reflect the life expectancy of animals after treatment. In order to randomise patients in clinical experiments evaluating the efficacy of adjuvant chemotherapy, a histological classification based on lymph node status and lymphovascular invasion was proposed [42].

It is advisable to supplement the histological determination of the tumour emboli presence in blood and lymphatic vessels with a multi-marker study of seven RNA markers based on polymerase chain reaction with reverse transcription, which increases the sensitivity of detection of circulating tumour complexes up to 77.5% [43]. That means that the presence of circulating tumour cells in the peripheral blood is a prognostic factor for the survival of patients with breast tumours; the use of mRNA markers allows determining their spatio-temporal location, which makes it possible to assess the level of aggression and biological behaviour of the tumour more accurately [44].

Thus, the activation of the metastatic cascade, which is clinically manifested by the presence of tumour emboli in blood and lymphatic vessels, is proliferation, dedifferentiation, and loss of intercellular contacts. However, the complex molecular pathways and gene expression changes associated with these mechanisms in breast tumours are still largely unclear, so such researches remain relevant.

CONCLUSIONS

The determination of the tumour clinical stage does not always fully reflect the tumour aggressiveness. Detection of angio- and lymphoinvasion in tumours allows predicting the biological behaviour of the tumour and evaluating the therapeutic efficacy of conservative protocols more accurately. The presence of tumour emboli in the vessels increases the risk of both metastasis and recurrence (due to "contamination" of the surgical wound). Therefore, the most aggressive is stage 2 of neoplasia process, which is characterised by the maximum risk of metastasis and recurrence.

Histological types of breast tumours in she-dogs with a high risk of disease progression were determined (infiltrative tubular invasive and cribriform carcinomas), due to their angio- and lymphoinvasion at different disease stages. Verification of tumour emboli in mastopathy proves the potential aggressiveness of its biological behaviour. The results obtained prove the expediency of a comprehensive pathomorphological assessment of breast tumours with specification of structural disorders, in particular the determination of angio- and lymphoinvasion for more accurate disease prognosis.

REFERENCES

[1] Szczubiał, M., & Łopuszynski, W. (2011). Prognostic value of regional lymph node status in canine mammary carcinomas. *Veterinary and Comparative Oncology,* 9, 296-303. doi: 10.1111/j.1476-5829.2011.00268.x.

[2] Itoh, T., Uchida, K., Ishikawa, K., Kushima, K., Kushima, E., Tamada, H., Moritake, T., Nakao, H., & Shii, H. (2005). Clinicopathological survey of 101 canine mammary gland tumours: Differences between small-breed dogs and others. *Journal of Veterinary Medical Science*, 67, 345-347. doi: 10.1292/jvms.67.345.

[3] David Nathanson, S., Leonard-Murali, S., Burmeister, C., Susick, L., & Baker, P. (2020). Clinicopathological evaluation of the potential anatomic pathways of systemic metastasis from primary breast cancer suggests an orderly spread through the regional lymph nodes. *Annals of Surgical Oncology*, 27, 4810-4818. doi: 10.1245/s10434-020-08904-w.

[4] Diessler, M.E., Castellano, M.C., Portiansky, E.L., Burns, S., & Idiart, J.R. (2017). Canine mammary carcinomas: Influence of histological grade, vascular invasion, proliferation, microvessel density and VEGFR2 expression on lymph node status and survival time. *Veterinary and Comparative Oncology*, 15, 450-461. doi: 10.1111/vco.12189.

[5] De Matos, A.J., Lopes, C.C., Faustino, A.M., Carvalheira, J.G., Dos Santos, M.S., Rutteman, G.R., & Gärtner, M. (2006). MIB-1 labelling indices according to clinico-pathological variables in canine mammary tumours: A multivariate study. *Anticancer Research*, 26, 1821-1826.

[6] Betz, D., Schoenrock, D., Mischke, R., Baumgärtner, W., & Nolte, I. (2012). Postoperative treatment outcome in canine mammary tumours. Multivariate analysis of the prognostic value of pre- and postoperatively available information. *Tierarztliche Praxis. Ausgabe K, Kleintiere/Heimtiere*, 40, 235-242.
[7] Santos, A.A., Lopes, C.C., Marques, R.M., Amorim, I.F., Gärtner, M.F., & de Matos, A.J.F., (2012). Matrix metalloproteinase-9 expression in mammary gland tumours in dogs and its relationship with prognostic factors and patient outcome. *American Journal of Veterinary Research*, 73, 689-697. doi: 10.2460/ajvr.73.5.689.
[8] Sarli, G., Preziosi, R., Benazzi, C., Castellani, G., & Marcato, P.S. (2002). Prognostic value of histologic stage and proliferative activity in canine malignant mammary tumours. *Journal of Veterinary Diagnostic Investigation*, 14, 25-34. doi: 10.1177/104063870201400106.
[9] Regan, R.C., Rassnick, K.M., Balkman, C.E., Bailey, D.B., & McDonough, S.P. (2010). Comparison of first-opinion and second-opinion histopathology from dogs and cats with cancer: 430 cases (2001–2008). *Veterinary and Comparative Oncology,* 8, 1-10. doi: 10.1111/j.1476-5829.2009.00203.x.
[10] Yoshimura, H., Nakahira, R., Kishimoto, T.E., Michishita, M., Ohkusu-Tsukada, K., & Takahashi, K. (2014). Differences in indicators of malignancy between luminal epithelial cell type and myoepithelial cell type of simple solid carcinoma in the canine mammary gland. *Veterinary Pathology*, 51, 1090-1095. doi: 10.1177/0300985813516637.
[11] Yoshimura, H., Michishita, M., Ohkusu-Tsukada, K., & Takahashi, K. (2011). Increased presence of stromal myofibroblasts and tenascin-C with malignant progression in canine mammary tumours. *Veterinary Pathology,* 48, 313-321. doi: 10.1177/0300985810369901.
[12] Pinho, S.S., Matos, A.J., Lopes, C., Marcos, N.T., Carvalheira, J., Reis, C.A., & Gärtner, F. (2007). Sialyl Lewis x expression in canine malignant mammary tumours: Correlation with clinicopathological features and E-Cadherin expression. *BMC Cancer,* 7, 124. doi: 10.1186/1471-2407-7-124.
[13] Seung, B.J., Cho, S.H., Kim, S.H., Lim, H.Y., & Sur, J.H. (2020). Quantitative analysis of HER2 mRNA expression by RNA in situ hybridisation in canine mammary gland tumours: Comparison with immunohistochemistry analysis. *PloS One*, 15, article number e0229031. doi: 10.1371/journal.pone.0229031.
[14] Pastor, N., Ezquerra, L.J., Santella, M., Caballé, N.C., Tarazona, R., & Durán, M.E. (2020). Prognostic significance of immunohistochemical markers and histological classification in malignant canine mammary tumours. *Veterinary and Comparative Oncology*, 2020, 1-10. doi: 10.1111/vco.12603.
[15] Monteiro, L.N., Rodrigues, M.A., Gomes, D.A., Salgado, B.S., & Cassali, G.D. (2018). Tumour-associated macrophages: Relation with progression and invasiveness, and assessment of M1/M2 macrophages in canine mammary tumours. *The Veterinary Journal,* 234, 119-125. doi: 10.1016/j.tvjl.2018.02.016.
[16] Sleeckx, N., de Rooster, H., Veldhuis Kroeze, E.J., Van Ginneken, C., & Van Brantegem, L. (2011). Canine mammary tumours, an overview. *Reproduction in Domestic Animals = Zuchthygiene*, 46, 1112-1131. doi: 10.1111/j.1439-0531.2011.01816.x.
[17] Sorenmo, K.U., Rasotto, R., Zappulli, V., & Goldschmidt, M.H. (2011). Development, anatomy, histology, lymphatic drainage, clinical features, and cell differentiation markers of canine mammary gland neoplasms. *Veterinary Pathology*, 48, 85-97. doi: 10.1177/0300985810389480
[18] Mi, S., Liu, Z., Du, Z., Yi, X., & Sun, W. (2019). Three-dimensional microfluidic tumour-macrophage system for breast cancer cell invasion. *Biotechnology & Bioengineering*, 116, 1731-1741. doi: 10.1002/bit.26961.
[19] Rasotto, R., Berlato, D., Goldschmidt, M.H., & Zappulli, V. (2017). Prognostic significance of canine mammary tumour histologic subtypes: An observational cohort study of 229 cases. *Veterinary Pathology,* 54, 571-578. doi: 10.1177/0300985817698208.
[20] Mohammed, R.A., Martin, S.G., Gill, M.S., Green, A.R., Paish, E.C., & Ellis, I.O. (2007). Improved methods of detection of lymphovascular invasion demonstrate that it is the predominant method of vascular invasion in breast cancer and has important clinical consequences. *The American Journal of Surgical Pathology*, 31, 1825-1833. doi: 10.1097/PAS.0b013e31806841f6.
[21] Seung, B.-J., Lim, H.-Y., Shin, J.-I., Kim, H.-W., Cho, S.-H., Kim, S.-H., & Sur, J.-H. (2018). CD204-expressing tumour-associated macrophages are associated with malignant, high-grade, and hormone receptor-negative canine mammary gland tumours. *Veterinary Pathology*, 55, 417-424. doi: 10.1177/0300985817750457.
[22] Monteiro, L.N., Dos Reis, D.C., Salgado, B.S., & Cassali, G.D. (2020). Clinical significance and prognostic role of tumour-associated macrophages infiltration according to histologic location in canine mammary carcinomas. *Research in Veterinary Science*, 135, 329-334. doi: 10.1016/j.rvsc.2020.10.010.
[23] Król, M., Pawłowski, K.M., Majchrzak, K., Dolka, I., Abramowicz, A., Szyszko, K., & Motyl, T. (2011). Density of tumour-associated macrophages (TAMs) and expression of their growth factor receptor MCSF-R and CD14 in canine mammary adenocarcinomas of various grade of malignancy and metastasis. *Polish Journal of Veterinary Sciences*, 14, 3-10. doi: 10.2478/v10181-011-0001-3.

[24] Carvalho, M.I., Pires, I., Prada, J., Raposo, T.P., Gregório, H., Lobo, L., & Queiroga, F.L. (2017). High COX-2 expression is associated with increased angiogenesis, proliferation and tumoural inflammatory infiltrate in canine malignant mammary tumours: A multivariate survival study. *Veterinary and Comparative Oncology*, 15, 619-631. doi: 10.1111/vco.12206
[25] Santos, A.A., Oliveira, J.T., Lopes, C.C., Amorim, I.F., Vicente, C.M., Gärtner, F.R., & Matos, A.J. (2010). Immunohistochemical expression of vascular endothelial growth factor in canine mammary tumours. *Journal of Comparative Pathology*, 143, 268-275. doi: 10.1016/j.jcpa.2010.04.006.
[26] European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. (1986, March). Retrieved from https://rm.coe.int/168007a67b.
[27] Law of Ukraine No. 3447-IV "On Protection of Animals from Cruelty". (2006, February). Retrieved from https://zakon.rada.gov.ua/laws/show/3447-15#Text.
[28] Owen, L.N. (1980). Clinical stages (TNM) of canine mammary tumours. In L.N. Owen (Ed.), *TNM classification of tumours in domestic animals* (pp. 16-18). Geneva: World Health Organisation. Retrieved from http://apps.who.int/iris/bitstream/handle/10665/68618/VPH_CMO_80.20_eng.pdf?sequence=1.
[29] Horalskyi, L.P., Khomych, V.T., & Kononskyi, O.I (2015). *Histological techniques and methods of morphological studies in normal and pathological conditions*. Zhytomyr: Polissya.
[30] Goldschmidt, M., Peña, L., Rasotto, R., & Zappulli, V. (2011). Classification and grading of canine mammary tumours. *Veterinary Pathology,* 48(1), 117-131. doi: 10.1177/0300985810393258.
[31] Fritz, A., Percy, C., Jack, A., Shanmugaratnam, K., Sobin, L., Parkin, D.M., & Whelan, S. (2000). *International classification of diseases for oncology*. Geneva: World Health Organisation. Retrieved from https://apps.who.int/iris/handle/10665/42344.
[32] Bilyi, D.D., Gerdeva, A.A., Samoiliuk, V.V., Suslova, N.I., & Yevtushenko, I.D. (2020). A modern look at the molecular-biological mechanisms of breast tumours in dogs. *Regulatory Mechanisms in Biosystems*, 11, 3-12. doi: 10.15421/022001.
[33] Rasotto, R., Goldschmidt, M.H., Castagnaro, M., Carnier, P., Caliari, D., & Zappulli, V. (2014). The dog as a natural animal model for study of the mammary myoepithelial basal cell lineage and its role in mammary carcinogenesis. *Journal of Comparative Pathology*, 151, 166-180. doi: 10.1016/j.jcpa.2014.04.013.
[34] Klopfleisch, R., Schütze, M., Linzmann, H., Brunnberg, L., & Gruber, A.D. (2010). Increased Derlin-1 expression in metastases of canine mammary adenocarcinomas. *Journal of Comparative Pathology*, 142, 79-83. doi: 10.1016/j.jcpa.2009.06.006
[35] Mucha, J., Majchrzak, K., Taciak, B., Hellmén, E., & Król, M. (2014). MDSCs mediate angiogenesis and predispose canine mammary tumour cells for metastasis via IL-28/IL-28RA (IFN-λ) signaling. *PLoS ONE*, 9, article number e103249. doi: 10.1371/journal.pone.0103249
[36] Cassali, G.D., Damasceno, K.A., Bertagnolli, A.C., Estrela-Lima, A., Lavalle, G.E., Di Santis, G.W., De Nardi, A.B., Fernandes, C.G., Cogliati, B., Sobral, R., Costa, F.V.A., Ferreira, E., Salgado, B.S., Campos, C.B., D'Assis, M.J.M.H., Silva, L.P., Machado, M.C.A., Firmo, B.F., Nunes, F.C., & Nakagaki, K.Y.R. (2017). Consensus regarding the diagnosis, prognosis and treatment of canine mammary tumours: Benign mixed tumours, carcinomas in mixed tumours and carcinos. *Brazilian Journal of Veterinary Pathology*, 10(3), 87-99. doi: 10.24070/bjvp.1983-0246.v10i3p87-99.
[37] Rismanchi, S., Yadegar, O., Muhammadnejad, S., Amanpour, S., Taghizadeh Jahed, M., & Muhammadnejad, A. (2014). Expression of vimentin filaments in canine malignant mammary gland tumours: A simulation of clinicopathological features of human breast cancer. *Biomedical Reports*, 2, 725-728. doi: 10.3892/br.2014.312.
[38] Damasceno, K.A., Bertagnolli, A.C., Estrela-Lima, A., Ribeiro, L.G.R., Rabelo, B.S., Campos, C.B., Barros, A.L.B., & Cassali, G.D. (2012). Versican expression in canine carcinomas in benign mixed tumours: Is there an association with clinical pathological factors, invasion and overall survival? *BMC Veterinary Research*, 8, article number 195. doi: 10.1186/1746-6148-8-195.
[39] Capp, C., Wajner, S.M., Siqueira, D.R., Brasil, B.A., Meurer, L., & Maia, A.L. (2010). Increased expression of vascular endothelial growth factor and its receptors, VEGFR-1 and VEGFR-2, in medullary thyroid carcinoma. *Thyroid*, 20, 863-871. doi: 10.1089/thy.2009.0417.
[40] Nguyen, F., Peña, L., Ibisch, C., Loussouarn, D., Gama, A., Rieder, N., Belousov, A., Campone, M., & Abadie, J. (2018). Canine invasive mammary carcinomas as models of human breast cancer. Part 1: Natural history and prognostic factors. *Breast Cancer Research and Treatment*, 167, 635-648. doi: 10.1007/s10549-017-4548-2.
[41] Chocteau, F., Mordelet, V., Dagher, E., Loussouarn, D., Abadie, J., & Nguyen, F. (2020). One-year conditional survival of dogs and cats with invasive mammary carcinomas: A concept inspired from human breast cancer. *Veterinary and Comparative Oncology.* doi: 10.1111/vco.12655.
[42] Chocteau, F., Abadie, J., Loussouarn, D., & Nguyen, F. (2019). Proposal for a histological staging system of mammary carcinomas in dogs and cats. Part 1: Canine mammary carcinomas. *Frontiers in Veterinary Science,* 6, article number 388.

[43] da Costa, A., Lenze, D., Hummel, M., Kohn, B., Gruber, A.D., & Klopfleisch, R. (2012). Identification of six potential markers for the detection of circulating canine mammary tumour cells in the peripheral blood identified by microarray analysis. *Journal of Comparative Pathology*, 146, 143-151. doi: 10.1016/j.jcpa.2011.06.004
[44] da Costa, A., Kohn, B., Gruber, A.D., & Klopfleisch, R. (2013). Multiple RT-PCR markers for the detection of circulating tumour cells of metastatic canine mammary tumours. *The Veterinary Journal*, 196, 34-39. doi: 10.1016/j.tvjl.2012.08.021

ПРОГНОСТИЧНЕ ЗНАЧЕННЯ СУДИННОЇ ІНВАЗІЇ ЗА ПУХЛИН МОЛОЧНОЇ ЗАЛОЗИ У СУК (ПІЛОТНЕ ДОСЛІДЖЕННЯ)

Максим Станіславович Коваленко, Дмитро Дмитрович Білий

Дніпровський державний аграрно-економічний університет
49600, вул. Сергія Єфремова, 25, м. Дніпро, Україна

Анотація. Пухлини молочної залози є найпоширенішим новоутворенням у некастрованих сук. Однак пухлини молочної залози у сук відрізняються значним різноманіття за морфологічними особливостями та біологічною поведінкою, відтак визначення факторів прогнозу є актуальним. Проведено дослідження дисемінації ракових клітин за новоутворень молочної залози у сук шляхом міграції в кровоносні та лімфатичні судини залежно від їх гістологічної характеристики та стадії захворювання. Вивчення проблеми здійснювали на базі клініки сучасної ветеринарної медицини «Best» міста Запоріжжя. У якості об'єктів використовували сук із неоплазіями молочної залози різного віку та порід за доведеної відсутності ознак метастатичного ураження інших органів і тканин, зокрема легень, органів черевної порожнини та кісток. Наявність ракових клітин у судинах визначали за утвореними ними пухлинними згустками, фіксованими до ендотелію. У результаті дослідження встановлено присутність ангіоінвазії незалежно від клінічної стадії пухлинного процесу. Водночас дисемінація пухлинних клітин шляхом міграції в лімфатичні судини спостерігалась тільки за другої клінічної стадії новоутворень молочної залози. У пацієнтів із новоутвореннями молочної залози на першій клінічній стадії в абсолютній більшості випадків (66,7 % пацієнтів) ангіоінвазія реєструється в мікропапілярних інвазивних карциномах (код МКБ-О 8507/2). У сук із раком молочної залози третьої стадії більш ніж у 80 % тварин наявність ракових клітин у кровоносних судинах верифікували за простої крибриформної карциноми (код МКБ-О 8201/3). У пацієнтів з другою клінічною стадією новоутворень молочної залози встановлено міграцію пухлинних клітин як у кровоносні, так і лімфатичні судин. При цьому найбільш часто ознаки ангіо- та лімфоінвазії встановлено за інвазивної карциноми змішаного типу (код МКБ-О 8562/3), тубулопапілярної карциноми (код МКБ-О 8503/3) та тубулярної карциноми (код МКБ-О 8211/3) – в 34,1 і 36,8; 19,3 і 26,3; 10,2 і 10,5 % випадків, відповідно. Отримані результати дозволяють із високим ступенем достовірності прогнозувати імовірність проникнення проникнення пухлинних клітин у кровоносні та лімфатичні судини, що надалі може краще прогнозувати біологічну поведінку пухлин молочної залози

Ключові слова: собаки, новоутворення, молочна залоза, метастазування