

MAMMARY ADENOCARCINOMA WITH TRANSCELOMIC METASTASES IN A GERMAN SHEPHERD FEMALE DOG

ADENOCARCINOM MAMAR CU METASTAZE TRANSCELOMICE LA O FEMEĂ CIOBĂNESC GERMAN

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ABSTRACT | REZUMAT

The study concerns a single case, a 9-year-old, unsterilised bitch, from the German Shepherd breed, which had a history of false lactation, heartworm disease, babesiosis, and progressive weight loss for 3 weeks, with a "suddenly" relaxed abdomen, without other associated clinical symptoms. At the clinical examination, the female presented a good general condition, normothermic, with a relaxed abdomen (pear appearance) and a nodular mammary formation, without ulceration with a diameter of 6 cm a straight breast chain, and an inguinal gland - M₅, associated with regional lymphadenopathy. Laboratory and imaging investigations (radiography and ultrasound) revealed anaemia, hemoperitoneum, irregular contour of the splenic capsule, and thickened wall of the gallbladder; radiographic (thoracic) and ultrasound (cardiac, abdominal) no cardiac changes (primary to the hemoperitoneum) or lesions secondary to breast neoplasia were detected. Exploratory laparotomy was used to establish the diagnosis, identifying multiple, non-pedunculated nodular formations of different sizes (1-7 mm), disseminated in the abdominal serosa, with carcinomatous appearance, the animal being euthanised at the request of the owner. Postmortem, the bitch was diagnosed with generalised peritoneal, perivascular, and hepatic carcinomatosis, starting with invasive micropapillary adenocarcinoma. We emphasise the increased attention that should be paid to breast tumours (some may have increased invasiveness, but without drawing the clinician's attention to their aggressiveness through the present characteristics) and the complex investigation of the patient. Lesions related to breast carcinomas, although very common, can sometimes be confusing in diagnosis and can lead to a delay in therapy.

Keywords: female dog, trancelomic metastases, breast adenocarcinoma

Studiul vizează un singur caz, o cățea, în vârstă de 9 ani, nesterilizată, din rasa Ciobănesc German, care a prezentat în antecedente lactație falsă, dirofilarioză, babesioză, slăbire progresivă timp de 3 săptămâni, cu abdomen destins "brusc", fără alte semne clinice asociate. La examenul clinic, femela prezenta o stare generală bună, normotermică, abdomen destins (aspect de pară) și o formațiune mamară nodulară, fără ulceratie, cu un diametru de 6 cm, lanț mamar drept, glanda inghinală - M₅, asociată cu adenopatie regională. Investigațiile de laborator și imagistice (radiografie și ecografie) au relevat anemie, hemoperitoneu, contur neregulat al capsulei splenice și perete îngroșat al colecistului; radiografic (toracic) și ecografic (cardiac, abdominal) nu au fost depistate modificări cardiace (primare hemoperitoneului) sau leziuni secundare neoplaziei mamare. S-a recurs la laparotomie exploratoare pentru stabilirea diagnosticului, identificându-se formațiuni multiple nodulare, nepediculate, de diferite dimensiuni (1-7 mm), diseminate la nivelul seroaselor abdominale, cu aspect carcinomatos, animalul fiind eutanasiat la cererea proprietarului. Postmortem, cățeaua a fost diagnosticată cu carcinomatoză peritoneală generalizată, perivasculară și hepatică, având punct de plecare adenocarcinomul de tip micropapilar invaziv. Pornind de la acest studiu de caz, subliniem atenția sporită care ar trebui acordată tumorilor mamare (unele pot avea o invazivitate crescută, dar fără a atrage atenția clinicianului asupra agresivității acestora prin caracteristicile prezente) și investigației complexe a pacientului. Leziunile conexe carcinoamelor mamare, deși foarte frecvente, uneori pot crea confuzie în diagnostic și pot conduce la o întârziere a terapiei.

Cuvinte cheie: cățea, metastaze trancelomice, adenocarcinom mamar

Mammary gland tumours account for approximately 50% of the total neoplasms affecting female dogs, and, of these, half (41-53%) are malignant in nature (1, 4, 14). Approximately half the malignant mamma-

ry tumours reach metastasis (6, 11).

The literature mentions the fact that the primary spot where mammary tumours metastasize is the lung (60-80% of the studied metastases, when metastasis occurs haematogenically), but this can occur in other organs, as well: lymph nodes (metastasis occurs lymphatically), the adrenal glands, the kidneys, the heart, bones, the liver, the brain, the eyes, the nose, the spleen, the uterus, serous membranes (8, 19, 20, 21, 22, 23).

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It is known that ovarian hormones (oestrogen and progesterone) play an important part in the development of mammary tumours, as does the age when ovariohysterectomy is performed.

According to the literature, female dogs sterilised before reaching puberty are at a 0.5% risk of developing mammary tumours, whereas females sterilised after the first oestrous cycle are at an 8% risk, and those sterilised after the second oestrous cycle are at a 26% risk (24). Recent studies mention a lower frequency of mammary tumours in dogs under 6 years old; however, with an approximately 60% increase in female dogs aged between 8 and 13 (most females being sterilised at the time of examination) (3, 25).

In the study conducted by Hashimoto et al. in 2002 on a sample of 201 female dogs with mammary tumours, the histopathology examination results revealed the presence of mammary adenocarcinomas in 37.3% of all cases (10). A mammary adenocarcinoma will develop around the mammary gland canals (26), but the growth model is rather tubular than papillary. This is the most common carcinoma type specific to canine mammary glands (15).

MATERIALS AND METHODS

The study in question describes a 9-year-old German Shepherd female dog, weighing 40 kg, with tumoral mammary lesions and peritoneal effusion.

The investigations took place at the Faculty of Veterinary Medicine within Spiru Haret University Bucharest / The Institute for Diagnosis and Animal Health, the female dog being subjected to the following protocol:

- ❖ Case history data collection;
- ❖ The clinical examination focused on the current general state of health and identified a peritoneal effusion (puncture, collection, and cytology examination) and a primary mammary tumour, which was subjected to a morpho-clinical assessment (identifying the number of affected mammary glands, the degree of necrosis/ulceration, and detecting affected lymph nodes).
 - ❖ Additional examinations:
 - Abdominal ultrasound scan;
 - Cardiac ultrasound scan;
 - Pulmonary X-ray with a latero-lateral view;
 - Laparocentesis;
 - Laboratory tests (cytology examination, complete blood count, biochemical examination, Snap 4Dx test);
 - Exploratory laparotomy;
 - As part of the necropsy, biological samples were collected in order to place a definitive diagnosis.
 - Anatomopathological examination: conducted macroscopically and histologically on the samples collected after the necropsy. The tumoral tissue fragments collected for the histology examination were placed in a 10% formaldehyde solution, and processed via the classical histopathological method by way of paraffin immersion and microtome sectioning, where-

as staining was performed via the haematoxylin-eosin (HE) bichromatic method and the Pappenheim method.

RESULTS AND DISCUSSION

A non-sterilised 9-year-old German Shepherd female dog, called Lassie, weighing 40 kg, was brought for a consult at the clinic of the Faculty of Veterinary Medicine within Spiru Haret University Bucharest, following an abdominal distension occurring "out of the blue", with a presumptive pyometra diagnostic.

The clinical examination revealed that the female dog displayed a single tumoral mammary lesion, 6 cm in diameter, without ulcerations, non-adherent, hard upon palpation, located on the right mammary chain (M_5 - inguinal mamma), regional adenopathy (reactive inguinal lymph node - prominent upon inspection - 2 cm in diameter, hard upon palpation, with no associated ulceration), a pear-shaped abdomen (succussion-based palpation revealed a wave-like sensation, indicating a peritoneal effusion), and a good general state of health, being normothermic (38.3°C).

The anamnesis revealed that, two years before, the female dog had been diagnosed with dirofilariasis (as per a cytology examination and a quick Snap 4Dx test, a condition left untreated) and babesiosis (treated with an antidote - Imizol); these two medical conditions were identified following the cytology examination (cytosmear), used to detect haemoparasites. During the period before the mammary lesion occurred, the female had gone through two nervous lactations, with a progressive growth of its mammary gland, within approximately three weeks, whereas in the third week its abdomen "suddenly" approximately tripled in size. Throughout this period, the female dog behaved normally, ate, urinated, defecated, and showed no behavioural changes.

The chest X-ray examination emphasised age-related changes, such as cardiac lesions (right atrial dilation), which caused changes in the tracheal topography (dorsally deviated trachea), condrocostal degenerations, as well as a small (limited) radiopaque area, ventrally located and specific to small-sized pleural effusions, whereas no metastasis-specific changes were present in the lungs (Fig. 1).

The abdominal ultrasound scan identified the presence of a large amount of ascitic fluid, with rich cellularity, which prevented viewing the stomach and the intestines; a peritoneal paracentesis was performed, which helped drain from the abdominal cavity approximately 1000 ml of haemorrhagic fluid, most likely from diffuse bleeding. After laparocentesis, we were able to locate the urogenital apparatus structures, which displayed no changes (Figs. 5 and 6); however, the spleen, as per the ultrasound, showed a homogeneous structure, a slightly irregular outline in the splenic capsule area (Fig. 3), the liver, as per the ultrasound - relatively small-sized, with a hyperechogenic homogeneous structure, and visible liver lobes, as a result of cavity effusion, the gallbladder displayed a

- the SNAP 4Dx test – a chromatography ELISA test – was positive for the heartworm disease antigen (dirofilariasis), whereas the other specific tests (*Anaplasma phagocytophilum*, *Ehrlichia canis*, Lyme disease) were negative.

- the serum albumin (ALB) value was low - 1.9 g/dl (2.2-3.9) - leading to a colloid osmotic pressure decrease, culpable for the emergence of ascites (albumin regulates 80% of the colloid osmotic pressure).

The echocardiographic examination was the next step in determining the aetiology of ascites and the dyspnoea secondary to it; the heart appeared slightly hypovolemic and hypoxic, with a slightly increased right atrium size and no other changes ascertained. The adult form of the *dirofilaria immitis* parasite was not revealed by the echocardiogram, but we were able to determine the dirofilariasis staging as a class I risk, as well as the fact that ascites was not of cardiac origin. Blood pressure was 198/120.

After the paraclinical investigations, the recommendation was for a support therapy, such as benazepril hydrochloride (Cibacen, 5 mg tablets), orally administered in a single 7.5 mg/day dose for 5-7 days to decrease high blood pressure, and exploratory laparotomy to place a diagnostic.

During the procedure (exploratory laparotomy), we ascertained multiple small-sized (1-3 mm) nodule formations (with a wall-like appearance), either adherent or fluctuant (in the case of individual ones), non-pedicated, laid out to form areas or isolated, disseminated throughout the area of abdominal serous membranes, as well as on the adjacent muscles, for which a suspected diagnostic of abdominal carcinomatosis was placed. The owner was informed about the laparotomy results and requested that euthanasia be performed. The laparotomy result is opposed to the ultrasound scan result due to the small sizes of said formations (1-3 mm) and the ascitic fluid with rich cellularity. A necropsy examination was performed upon the female dog's death. The outer inspection revealed it had been properly maintained; the abdomen increased in size, whereas the examined connective tissue did not show any modifications.

The examination of the head, the cervical area, and the spine did not reveal any modifications. Upon opening the cranial cavity, the brain hemispheres were congested, the hypophysis was hyperaemic, whereas the other structures showed no other changes.

The chest examination did not reveal any topographic changes of the organs within the thoraco-pleuropulmonary segment, but we did identify zonal haemorrhagic infiltrates and serosanguinolent effusions (approximately 100 ml). There were no changes in the trachea and bronchia. The lung was slightly collapsed, displayed an oedema and hyperaemia, with fibrotic foci within the structure of the diaphragmatic lobes; there were visible crepitant areas (compensatory emphysema), more prominent across the apical lobes. The heart contained approximately 15 ml of reddish pericardial fluid and displayed a right cardiac dilation.

The aorta and the pulmonary arteries were ectatic,

whereas the other anatomical components did not show any modifications.

The abdominal cavity examination revealed large amounts of sero-haemorrhagic effusions (approximately 4 litres of dark red fluid), which displayed numerous multiple nodule formations (with a wall-like appearance) of various sizes (1-7 mm), either adherent or fluctuant (in the case of individual ones), non-pedicated, laid out to form areas or isolated, and disseminated throughout the area of abdominal serous membranes (with a carcinomatous appearance), as well as on the adjacent muscles, with more pronounced metastases on the omentum, the inner wall of the abdominal muscles, and the serous membrane of the organs making up the digestive tract. We also ascertained splenic congestion and hyperplasia, a hepatorenal degeneration with more prominent hyperaemic areas within the renal cortical region.

The examination of the female genital apparatus revealed numerous isolated or multiple tumoral formations with a carcinomatous appearance, present across the serous membranes of the fallopian tubes and the uterine horns, the ovaries being polycystic.

The examination of the inguinal mammary gland (M_s), on the right chain, revealed it to be hypertrophied (6 cm in diameter), whereas its cross-section displayed greyish-yellowish greasy-looking areas alternating with cystic degeneration areas, with cu haemorrhagic infiltrates, all of which were accompanied by satellite lymph node reactivity (visible upon inspection – 2 cm diameter, hard upon palpation, non-adherent, without any related ulceration).

Summing up the necropsy data, it revealed hemoperitoneum and peritoneal carcinomatosis with multiple metastases across the serous membranes and within the regional muscles; slightly collapsed lung with fibrotic areas; right cardiac dilation; splenic congestion; liver degeneration.

Following the histopathology examination (HE staining) of the inguinal mammary gland (M_s) and the regional lymph node, the diagnosis placed was highly invasive adenocarcinoma (numerous mitoses and atypical cells); with the lymph node featuring lymphoid depletion and numerous carcinomatous cell areas, with a micropapillary appearance and poorly differentiated, hyperaemia, and corticomedullary haemorrhagic microfoci (Fig. 5, 6).

The liver displayed granulovacuolar hepatocyte degeneration, interlobular hyperaemia areas, Disse space oedema, and a microfocus with poorly differentiated neoplastic cell infiltrations.

The entire peritoneum was enveloped in highly invasive (stage IV) carcinomatous metastases – omental metastases, serous membrane and muscle metastases, perivascular and liver metastases, whose possible starting point was the invasive micropapillary adenocarcinoma within the mammary gland, the metastases having formed aided by the lymphatic and transcoelomic pathways (Fig. 7, 8).

In the retrospective study performed in 2015 by Salas Y. et al., on the epidemiology of mammary tu-

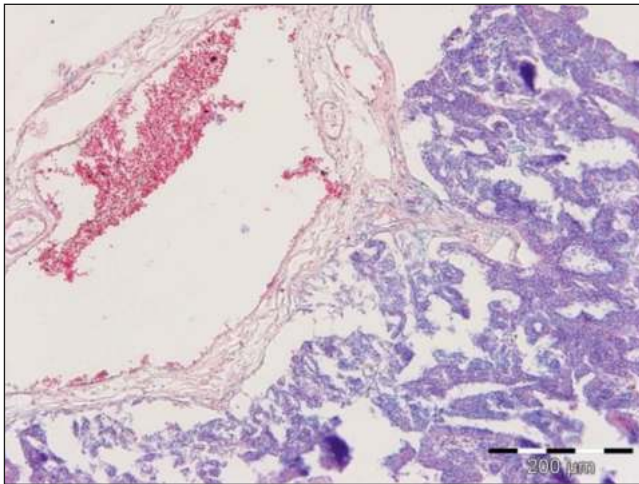


Fig. 5. Histopathology examination of the mammary gland, micropapillary mammary adenocarcinoma. Papanheim×100 staining

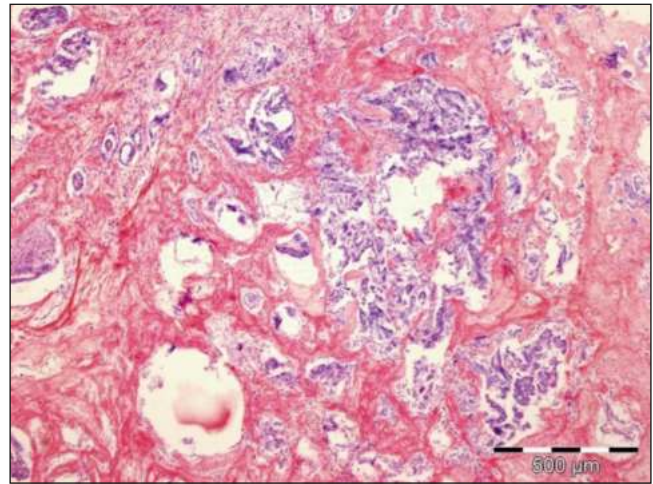


Fig. 6. Histopathology examination of the mammary gland, tubular-micropapillary neoplastic proliferation of the glandular acini. Papanheim×100 staining

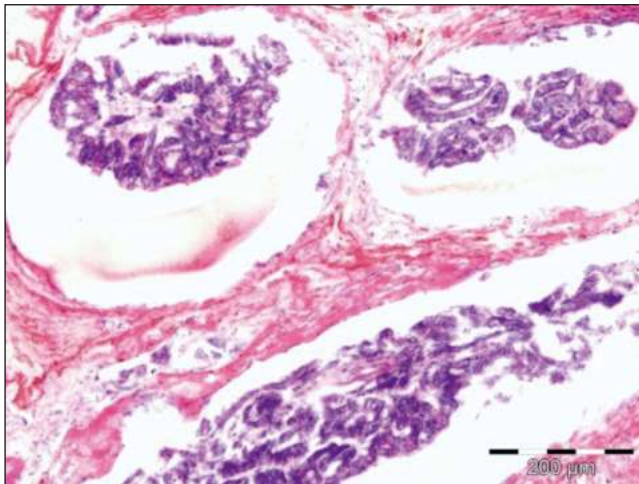


Fig. 7. Histopathology examination of the mammary gland. Acini with carcinomatous papillary tumoral proliferations, laid out onto a pronounced connective stroma. Papanheim×100 staining

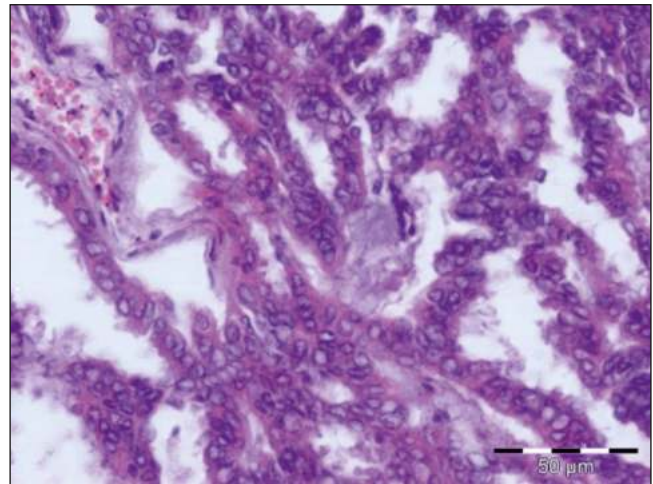


Fig. 8. Histopathology examination of the visceral pleura. Carcinomatous proliferation of neoplastic micronodules of a glandular and micropapillary variety. HE×400 staining

mours in dogs, it was ascertained that the most affected subjects are of pure breeds, whereas more than 80% of the female dogs were Poodles, Cocker Spaniels, and German Shepherds (18).

Age is one of the predisposing factors in the occurrence of mammary tumoral lesions, the incidence increasing with age, as was also the case with the subject discussed in this study (9 years old). According to the various studies conducted on this topic, maximum frequency is reached among 9- to 11-year-old subjects (16). According to several data from the literature, the mammary glands most affected by neoplasias are the inguinal ones, females having one or two mammary glands affected by the tumoral process (22). Other studies state that the most frequent locations of mammary tumours are M4 and M5, more than 50% of mammary tumours have multiple locations (12). The results of the present study are similar to the literature data.

In regard to the TNM (tumour, lymph node, metastasis) classification, the female in the present study falls under stage IV ($T_4N_1M_1$), as per the classification drawn up by Mangol et al., 1998 (13). This classification system takes into account: the extent of the primary tumour (T), the state of lymph nodes (N), the absence or presence of remote metastases (M); these are added to digit-based notations to indicate the malignant tumour extent. The TNM system is particularly significant for the diagnostic accuracy, for the clinical progress, and for the forecast (2).

During its lifetime, the female dog in question had never been administered hormonal medication, had never given birth, and had experienced false lactations. The data in the literature acknowledge that both false lactations and the lack of sterilisation entail the risk of mammary tumour occurrence (5, 24).

Mammary adenocarcinomas in female dogs ex-

tend, by way of infiltration, to the skin and the adjacent tissues in approximately 80% of the cases, invading the lymphatic system, as well (2, 24).

Invasive micropapillary carcinoma (IMC) is a mammary cancer that forms metastases, particularly aided by the lymphatic pathways, most frequently in the area of adjacent lymph nodes, and is encountered both in female dogs and women. In dogs, IMC features larger tumour sizes and frequently associates regional metastases. In female dogs, this neoplasm reaches a high mortality rate (94%), as opposed to its mortality rate among women (4%) (9).

The literature data acknowledges the aggressiveness of IMC, reflected by both the neoplasm size and the degree of spread by way of infiltration into regional tissues (metastases being aided particularly by the lymphatic pathways), as well as the elevated mortality rate, this data bearing resemblance to our study.

CONCLUSIONS

The image-guided examination (X-ray, heart, and abdominal ultrasounds) performed on the female dog did not reveal any lesions secondary to mammary neoplasia and invalidated a cardiac aetiology – which favours ascites. The macroscopic and microscopic examination of the puncture fluid resulted from the abdominal paracentesis did not reveal any neoplastic cells. Exploratory laparotomy was the surgical/ investigation method, which helped determining the hemoperitoneum aetiology. The histopathology confirmed that all the metastases had, as their point of origin, the mammary gland, the diagnostic being that of highly invasive micropapillary mammary adenocarcinoma. The image-guided examinations are not always highly accurate and are sometimes limited in determining a diagnostic; they may emphasize morphological, functional and topographic changes, as they were identified in the present study, as well (left atrial dilation, slightly hypovolemic and hypoxic heart, dorsally deviated trachea), but there may be cases, such as the one under discussion, where the rich cellularity of the peritoneal effusion, combined with small-sized (1-3 mm) cell formations, may lead to confusion and difficulties in placing an image-guided diagnostic. Starting from this case study, we emphasize the increased attention that should be paid to mammary tumours (some may be highly invasive, without drawing the clinician's attention towards their aggressiveness by means of the displayed characteristics), and the need to implement a protocol for handling mammary tumour cases, in order to establish both diagnostics and therapies.

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