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Anatomic and imaging study of the lymphatic vascularization of the mammary gland in the domestic cat

SUMMARY OF THE PH.D. THESYS

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INTRODUCTION

The mammary gland of the cat has recently become an increasingly explored organ from an anatomical and histological point of view. This aspect is due to the resemblance of this gland to the one encountered in the human species, especially from a histological point of view.

Mammary cancer holds a preponderant place in the pathology of the mammary gland in both veterinary and human medicine.

Most of the neoformations of the mammary gland, whether benign or malignant, occur on a certain terrain, in which certain general or local conditions generate “risk factors” for mammary cancer. The lymphatic system is the main way for tumor dissemination.

All these aspects impose a very precise knowledge of the mammary gland’s morphology, especially of the lymphatic vascular system of the mammary glands, knowledge that helps elucidate the causes of a potential pathology linked to this organ.

PURPOSE OF THE RESEARCH

The study that we have initiated has the purpose to describe, interpret and elucidate certain aspects regarding the topography, morphology and drainage of the mammary lymphatic circulatory system in the domestic cat.

In order to attain this goal, we have investigated the lymphatic circulatory system using four relevant methods: injecting Evans blue dye, followed by dissection, radiographic indirect lymphography with contrast medium, ultrasonography and computed tomography with contrast agent.

The objectives of our research are focused on the morphological and clinical importance of this anatomic and imaging study. The achievement of these objectives is imposed by the present requirements of clinical anatomy.

Objectives:

- establishing the topography of the lymphatic vessels of the mammary gland in the domestic cat, and that of its afferent and efferent lymph nodes;
- detecting all the lymph nodes that drain the lymph from the mammary glands;
- establishing the lymphatic drainage possibilities for each mammary gland (T1, T2, A1, A2);
- identifying possible connections between adjacent *mammae* and other connections as well;
- identifying possible connections between the lymph nodes that drain the mammary glands;
- finding the optimal moment for radiological exposure after administering the contrast agent, both for the CT scan examination and for the radiographic indirect lymphography;
- establishing the shape, size, topography, vascularization and normal echo-structures of lymph nodes, using the ultrasonography method.
INJECTION OF THE DYE IN VIVO FOLLOWED BY STRATIGRAPHIC AND REGIONAL DISSECTION

MATERIAL AND METHOD

The study was carried out at the Comparative Anatomy Department of the Faculty of Veterinary Medicine of Cluj-Napoca during the period from 2008 to 2012.

The biological material consisted of 22 common mixed breed cats, clinically healthy, coming from an urban background. The 22 cats of different ages (1-5 years), with a body weight between 1.5 and 6 kg were split into four groups, according to the mammary gland injected with the dye. Group I is composed of 5 cats that had their cranial thoracic mammary gland (T1) injected, group II is composed of 5 cats that had their caudal thoracic mammary gland (T2) injected, group III contains 7 cats had their cranial abdominal mammary gland (A1) injected, and group IV is composed of 5 cats in which we have studied the caudal abdominal mammary gland (A2).

In order to investigate the mammary lymphatic drainage with this method, we have used 2.5% Evans blue dye as colorant substance. The injection was performed slowly, using a quantity of 0.4 ml, with a 26-G needle in the subareolar region and in the mammary parenchyma, choosing the four cardinal points adjacent to the mammary gland (cranial, caudal, lateral and medial). Euthanasia was induced at 24 or 48 hours after the injection, after a prior neuroleptanalgesia followed by the intravenous administration of the product T-61. The following stage was the stratigraphic and regional dissection by incising the skin along the median plane (linea alba) starting with the injected region and continuing cranially both cranially and caudally.

RESULTS AND DISCUSSIONS

Regarding the results obtained after the investigation of the lymphatic drainage of the cranial thoracic mammary gland, we can assert that all subjects present an exclusively cranial lymphatic drainage at the level of the axillary lymph nodes (Raharison and Sautet, 2007, 2006; Sugimura et al., 1960; Vollmerhaus and Roos, 1997). The first lymph node reached by the lymph is the cranial accessory axillary lymph node. The results we obtained for this gland indicate that both the main axillary lymph node and the cranial sternal lymph node play a role in the lymphatic drainage of the T1 gland.

Even if the literature that we have consulted makes no reference to the ventral cervical lymph node, we wish to mention that we have underlined it in all the subjects of group I, as being lightly colored. This aspect makes it responsible for the cranial thoracic mammary gland’s lymphatic drainage.

When referring to the caudal thoracic mammary gland T2, our research has proved that this gland can present both an exclusively cranial lymphatic drainage and a double direction lymph drainage - cranially and caudally. The exclusively cranial lymphatic drainage was encountered in 4 cases and the double cranial and caudal one, in a single case.

In the case of the cranial abdominal mammary gland, the results of our research have shown that this gland has two lymph drainage possibilities. In 6 of the subjects we have noticed a double lymphatic drainage, both cranial and caudal. In a single subject, the lymph drainage of the A1 drainage was exclusively cranial. This result contradicts the aforementioned authors which state that the A1 gland never drains in an exclusively cranial direction (Raharison and Sautet, 2007, 2006). The cranial lymphatic drainage is
made through the axillary lymphocenter, the first relay being the accessory axillary lymph node and the caudal drainage going through the superficial inguinal lymphocenter, its first lymphatic relay being the caudal epigastric lymph node.

Referring to the cranial drainage of the A1 gland, it is worth mentioning that both our study and other authors have underlined the fact that the cranial sternal lymph node is involved in the drainage of this gland (Raharison and Sautet, 2007, 2006).

The present research has proven that, besides the superficial inguinal lymphocenter, the caudal drainage of the cranial abdominal mammary gland is also ensured by the ilio-femoral lymphocenter (when it exists), by the ilio-sacral lymphocenter and even by the lumbar lymphocenter.

The results regarding the caudal abdominal mammary gland A2 show a single course of lymphatic drainage: in all subjects, the gland was drained exclusively in a caudal direction. Our result is backed by all the authors that we have consulted (Meier, 1989; Raharison and Sautet, 2007, 2006; Sugimura et al., 1960, 1958; Vollmerhaus and Roos, 1997). The drainage of the A2 gland is similar with that of the A1 gland, but our study has shown a very important aspect that has not been mentioned by the authors that have published studies on this field, namely that the lymphatic drainage of the caudal abdominal mammary gland is also made through the popliteal lymphocenter in clinically healthy cats. This aspect is very important when discussing distal metastases of mammary neoplasms.

**LYMPHOGRAPHY OF THE MAMMARY GLANDS IN THE DOMESTIC CAT**

**MATERIAL AND METHOD**

The biologic material was represented by 11 common mixed breed cats, clinically healthy and well kept, coming from a rural background. The 11 cats of various ages (2-6 years), with a body weight of 2.5 to 4 kg were split into two groups according to the physiological state of the mammary gland. Group I was represented by 5 pregnant cats, and group II, by 6 lactating cats.

Indirect lymphography does not imply a direct approach of the lymphatic vessels and of their lymph nodes, the injection of the contrast agent being made in the interstitial conjunctive tissue. The lymphographies were made in the Radiology Laboratory of the Faculty of Veterinary Medicine of Cluj-Napoca. We have used the product Optiray 350 as contrast agent.

After the induction of the neuroleptanalgesia by administering Acepromazine 10%, 0.5 mg/kg body weight and Ketamine 10%, 20 mg/kg body weight, we have proceeded to the injection of the contrast agent. The injection was made with a 27-G needle in the subareolar area and in the mammary parenchyma. The injection rhythm was slow, trying to maintain the same pressure on the whole duration of the injection of a mammary gland. A quantity of 0.5 ml of Optiray was used for each studied mammary gland, with the mention that certain deviations from this dose will be specified in the “Results” chapter.

After the administration of the contrast medium, followed the radiological exposure, with the cats placed in a dorso-ventral decubitus and the exposure made ventro-dorsally and latero-laterally. The exposure was made sequentially, in the
following manner: at 1, 5, 10, 15, 20 minutes and at 24 hours de ore after the injection of the contrast agent.

REZULTS AND DISCUSSIONS

In our study, the obtained results referring to the cranial thoracic mammary gland show that it presents a cranial lymphatic drainage, with the first relay being the accessory axillary lymph node, situated at the ventral border of the latissimus dorsi muscle. The study shows that the drainage is made through a single main lymphatic vessel in 10 out of 11 cats, and in a single cat we have visualized 2 main lymphatic vessels.

The best results from the radiological point of view were obtained when the exposure was made immediately after injecting the contrast agent.

In the case of the pregnant cats group, the caudal thoracic mammary gland T2 drained of lymph by a single main vessel in 5 out of 5 cats. In four cats, this gland presents an exclusively cranial drainage thorough the axillary lymphocenter. In a single cat, we have underlined a double drainage of this gland, visualizing through the lymphography a lymphatic vessel leaving the mammary gland at the level of its caudal pole, heading towards the caudal plane of the body. Other authors have not confirmed this type of drainage, asserting that it is made exclusively cranially, through the axillary lymphocenter (Papadopoulou et al., 2009; Patsikas et al., 2010). For the lactating cats group, we mention that the obtained results confirm only the cranial drainage of the caudal thoracic mammary gland T2.

In the case of the cranial abdominal mammary gland A1, our results from the pregnant cat group indicate that this gland can have an exclusively caudal drainage in 2 cats and a double drainage both cranial and caudal in 3 cats. In the 6 lactating cats group, the cranial abdominal mammary gland has presented a lymphatic drainage that is exclusively caudal.

We have underlined connections between the cranial abdominal mammary gland A1 and the caudal abdominal mammary gland A2 in 3 out of the 5 cats of the pregnant group and in 3 out of the 6 cats of the lactating group. This aspect is infirmed by the researchers that have made lymphographic studies of the mammary circulatory system in the domestic cat (Papadopoulou et al., 2009; Patsikas et al., 2010).

Regarding the lymphatic drainage to the caudal abdominal mammary gland A2, this is made exclusively in the caudal direction, an aspect demonstrated in all the 11 subjects of the two groups. The first lymphatic relay is represented by the caudal epigastric lymph node, and, because of the internodal connections, the efferent lymphatic vessel of the epigastric lymph node becomes afferent for the mammary lymph node.

ULTRASONOGRAPHY OF THE LYMPHATIC CIRCULATORY SYSTEM OF THE MAMMARY GLANDS IN THE DOMESTIC CAT

MATERIAL AND METHOD

The study was performed during the period May-July 2011 in the Semiology, Ethology and Medical Imaging Department of the Faculty of Veterinary Medicine of Cluj-Napoca, under the supervision of the medical staff specialized in ultrasonographic examinations of the 3rd Medical Clinic of Cluj-Napoca.

The biologic material is composed of 10 clinically healthy cats coming from an urban background.
For each cat we have aimed to explore from an ultrasonographic point of view the mammary parenchyma and the lymph nodes that perform the cranial and caudal lymphatic drainage of the thoracic and abdominal mammary glands. We have focused on the topography, ultrasonographic qualities, number and size of the lymph nodes that are semilogically approachable and lend themselves to the echographic method.

We have used three echographic methods: B-mode, Doppler and ultrasonography with SonoVue contrast agent.

According to the chosen ultrasonographic method, we have focused on: echogenicity, shape, edges, size, acoustical transmission of the lymph nodes, and their relation to the adjacent tissues and their blood flow. Regarding contrast agent ultrasonography, there is very great importance attached to the instant when the contrast agent appears into the lymph node, to the moment of the maximum intensity of the contrast medium visualization and also to that of its disappearance form the lymph node.

RESULTS AND DISCUSSIONS

The results of the B-mode ultrasonographic exam have underlined all lymph nodes as oval-shaped or elongated, which indicated that the ratio between the longitudinal axis and the transversal axis is in favor of the former. The lymph nodes have presented an isoechogenic or slightly hypoechogenic parenchyma compared to the hyperechogenic hilum of the lymph node. The capsule covering the parenchyma has presented leveled borders in all examined subjects. Our results indicate the examination of healthy subjects from the point of view of the lymph nodes and they are in concordance with the results reported by other authors on the normal ultrasonographic characteristics of lymph nodes (Badea et al., 2004, 2006; Fritsch and Gerwing, 2000; Nyman and O’Brien, 2007; O’Brien and Barr, 2009).

The measurements of all lymph nodes are situated within normal limits, compared to physiological sizes of each separate lymph node. Physiological measurements of the lymph nodes in the domestic cat were available to us due to the results obtained following the method of injecting dye with tropism for the lymphatic system followed by dissection.

Regarding the number of lymph nodes composing a lymphocenter, it differed according to the subject and to the examined lymph node.

The vascularization of lymph nodes was examined through the Color Doppler method, which has performed the visualization of the normal vascularization of all the examined lymph nodes. The vascularization type found in them is normal based on the fact that the vessels have entered through the hilum, and spread to the periphery in an orderly manner.

The contrast medium ultrasonography has underlined best the angioarchitecture of the examined lymph nodes. For most of them, the time of penetration for the SonoVue contrast medium was 2-3 seconds, and the complete filling time was 4-5 seconds.

Knowing the normal echographic characteristics of the lymph nodes that drain the mammary glands is very important for the identification of pathological lymph nodes. Referring to subjects with mammary neoplasms, and not only, we will certainly encounter reactions of the lymph nodes that perform their lymphatic drainage.
COMPUTED TOMOGRAPHY (CT) OF THE LYMPHATIC CIRCULATORY SYSTEM OF THE MAMMARY GLANDS IN THE DOMESTIC CAT

MATERIAL AND METHOD
The study was undertaken during July 2010 - February 2011, in a private clinic.

For the investigation of the lymphatic circulatory system of the mammary glands using the contrast agent indirect lymphography method through computed tomography we have benefited of a biological material of 5 common mixed breed cats. All cats used for this method were clinically healthy at the time of the study.

In all cats we have investigated the mammary chain from one side of the sagital plane, examining thus 5 of each mammary glands (cranial thoracic, caudal thoracic, cranial abdominal and caudal abdominal). For each individual, we have aimed to assess the direction of the lymphatic drainage and the lymph nodes that perform that drainage.

The contrast agent that we have used is Optiray 350. The injection of the contrast medium Optiray 350 was made into the mammary parenchyma in one spot, and the used quantity was 0.5 ml for each examined gland. Computed tomography scans were taken at 1, 5, 10 and 15 minute after the administration of the contrast agent Optiray 350. The reconstruction of the tridimensional images was made in both coronal (antero-posterior) and sagital (latero-lateral) plane.

RESULTS AND DISCUSSION
Our research has demonstrated using the contrast agent computed tomography method the drainage of the cranial thoracic glands. This was performed in an exclusively cranial direction in all of the examined cats. The first lymphatic relay for the T1 drainage is the accessory axillary lymph node. The have seen it in all the subjects, thus agreeing with the aforementioned authors (Papadopoulou et al., 2009). Unlike them, we have succeeded in visualizing the second lymphatic relay of the lymphatic drainage of the T1 gland, represented by the main axillary lymph node.

From the caudal thoracic mammary gland, the lymph was drained exclusively in the cranial direction in all the specimens subjected to the CT scan examination. Our results have fitted into those obtained by other authors (Papadopoulou et al., 2009; Patsikas et al., 2010). The accessory axillary lymph node from the T2 gland represents its first lymphatic relay.

Regarding the cranial abdominal mammary gland, our research has proven its lymphatic drainage, visualizing on the CT images the lymph vessels that drain caudally, heading to the superficial inguinal lymphocenter. Reconstruction of the post-contrast acquired images has led to the observation of the mammary lymph node examination in all of the examined cats.

The obtained results referring to the caudal abdominal mammary gland also confirm through CT scan examination its exclusively caudal drainage through the superficial inguinal lymphocenter. In 100% of the subjects, the A2 glands drained themselves in the mammary lymph node that appears strongly opaque. In a single subject we have underlined the other component of the superficial inguinal lymphocenter, the caudal epigastric lymph node. It is situated at the base of the A2 mamma, having the same size are the mammary lymph node.
GENERAL CONCLUSIONS AND RECOMMENDATIONS

1. The method of investigating the lymphatic circulatory system of the mammary glands in cats using the Evans blue dye has offered the most complete data regarding the type of the lymphatic drainage and precious information regarding the number, shape, size and topography of the lymph nodes and of the lymphatic vessels.

2. The method of injecting Evans blue dye, followed by stratigraphic and regional dissection underlines both the superficial lymphatic vessels and nodes, and the deep ones.

3. Unlike other methods, by using Evans blue dye and dissection, we have visualized all lymph nodes that drain a certain mammary gland, including the ones situated on the deep plane. We can state that this method surpasses the “barrier” of the first lymphatic relay.

4. The type of drainage for the cat’s mammary glands, as well as the visualization of the lymphocenters that drain them, can be easily and accurately made with the help of radiographic indirect lymphography, using Optiray 350 as contrast agent.

5. The classical indirect lymphography is the only method that has proved the glandular connection between the cranial abdominal mammary gland and the caudal abdominal mammary gland in 6 subjects.

6. It is very important clinically that the lymphatic relays draining the mammary glands in the domestic cat, namely the axillary, superficial inguinal, deep inguinal and the popliteal lymphocenters lend themselves to ultrasonographic evaluations.

7. B-mode ultrasonography, Doppler and contrast agent echography using SonoVue has permitted the evaluation of the lymph nodes, from the point of view of their topography, size, number, shape, echostructure and vascularization.

8. Topography, size and relation of the lymph nodes to the adjacent tissues are the most important characteristics which can be evaluated by the method of computed tomography with Contrast medium Optiray 350.

9. Regardless of the applied investigation method, the cranial thoracic mammary gland was always drained cranially, and the caudal abdominal mammary gland was always drained caudally.

10. Radiographic indirect lymphography and the Evans blue dye technique have underlined two possible situations regarding the lymphatic drainage of the caudal thoracic mammary glands: 1) exclusively cranial and 2) double drainage (cranial and caudal).

11. The cranial abdominal mammary gland can present an exclusively cranial, exclusively caudal or double lymphatic drainage.

12. The dissection preceded by the Evans blue dye technique is the only method that has demonstrated the lymphatic drainage of the caudal abdominal mammary gland at the level of the popliteal lymph node. We mention that this aspect hasn’t been underlined for healthy cats by any of the authors composing the bibliography that we have studied.

13. The general rule is that the cranial drainage of the mammary glands has a first relay in the axillary lymphocenter, and the caudal one, the superficial inguinal lymphocenter.

14. None of the methods has underlined any lymphatic communications between the two mammary chains.
**Recommendations:**

Based on our research, we recommend the investigation of the lymphatic circulatory system of the mammary glands in the cat through the medical imaging methods used in this study. The techniques that we have used are indicated in various lymph node pathologies, especially those of a tumoral nature, which can appear in the case of mammary neoplasia.

To investigate the direction of the lymphatic drainage of a mammary gland, we recommend the use of radiographic indirect lymphography with contrast agent and of computed tomography examination with contrast medium. The two methods identify the first lymph node that the lymph reaches, establishing at the same time its topography. The computed tomography method offers precious data regarding the relation between lymph nodes and adjacent tissues.

A disadvantage of the two methods is the necessity of neuroleptanalgesia induction in the examined patients. In the case of the radiographic indirect lymphography it is possible for a helper to restrain the examined individual. For the CT scan, this is not possible. Another disadvantage of the computed tomography medical exam is the high costs for a single examined body region.

Based on our results, we recommend the ultrasonographic method with or without contrast medium, to evaluate the topography, size, number, shape, and especially the echostructure and vascularization of the lymph nodes. The echostructure and vascularization of pathological lymph nodes are greatly modified by comparison to the normal echographic anatomy underlined by our study. The ultrasonography has the advantage that it is a non invasive technique, and its costs are very low compared to other medical imaging methods, both for the acquisition of an ultrasound machine and for the examination of the patient.

Based on our study, in case of mammary neoplasms, we recommend presurgical assessment of the *mammae* by means of the imaging techniques previously mentioned. After performing them, it can be decided whether to remove the affected mammary gland together with the sentinel lymph node. In case of the existence of interglandular communications, we recommend the removal of all the communicating glands, together with their corresponding sentinel lymph nodes.
SELECTIVE REFERENCES