

# Lymph Drainage of the Mammary Glands in Female Cats

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**ABSTRACT** The mammary gland is a common site of neoplasms in the female cat. All the malignant tumors metastasize to a lesser or a greater extent through the lymphatic system. However, the anatomical knowledge of this system is not sufficiently well known in cats to develop a reasoned model for the extirpation of these glands in case of malignant tumors. A study of the lymph drainage in 50 female cats was done by indirect injection *in vivo* of India ink inside the mammary parenchyma. After a waiting interval, mammary glands were extracted and the thoracic cavity opened. All the lymph nodes were examined after clearing. The success rate of the colorations of lymph nodes and lymph vessels was 91.8%. Out of the 100 observed mammary chains, the two intermediate mammary glands (T2, A1) may drain caudally to the superficial inguinal lymph center and/or cranially to the axillary lymph center. The T1 gland always drains exclusively cranially and A2 exclusively caudally. The two mammary glands (T1 and A1) often drain towards the sternal cranial lymph nodes, but 100% of the T2 drain towards it. This research assumes that the limit between the two directions of drainage can exist only between glands T2 and A1. The results obtained with the study of the 1st, 2nd, 3rd, and 4th mammary glands permit production of new and more complete data of functional significance that will eventually aid block dissection surgical technique in the removal of malignant tumors in cats. *J. Morphol.* 000: 000–000, 2005. © 2005 Wiley-Liss, Inc.

**KEY WORDS:** mammary gland; female cats; lymph drainage

The mammary gland is a common site of neoplasm in cats (Dorn et al., 1968). Mammary tumors occur in older spayed or intact female cats and rarely in castrated males (Cotchin, 1957; Misdorp, 1964). The most frequent types of malignant tumors include carcinomas, sarcomas, and mixed malignant mammary tumors (Lombard, 1940; Nielsen, 1952). A mammary tumor appears between the age of 10 and 12, and when it occurs before the age of 6 it is often a benign form (Pellerin, 1977). It is known that all malignant tumors metastasize to a lesser or a greater extent through the lymphatic system (MacEwen et al., 1984). This fact explains the importance of this system in the neoplastic processes of mammary glands.

Female cats usually have eight mammary glands, which are arranged in two rows, one on each side extending from the caudal part of the pectoral region

to the inguinal region (Barone, 1996). Generally, the anterior pair of glands is affected and metastases frequently involve the regional lymph nodes and lungs (Hayden and Nielsen, 1971). Earliest surgical excision is the most effective therapy for any mammary tumor. In the cat, few surgical techniques are used in the treatment of mammary tumors (Hayes and Mooney, 1985). Surgical removal of all the glands on the affected side is a common method of treatment. Block dissection surgical techniques involve the removal of the affected mammary glands together with their lymphatic connections with other glands, the lymph nodes toward which these glands drain, and the other glands with which they are connected (Mann, 1984). The application of this technique is very limited in female cats due to the lack of sufficient data on the anatomy of the lymphatic system draining the glands. The few works, when available, are based on a very limited number of animals (Sugimura et al., 1956; Meier, 1989).

For these reasons, we decided to investigate the lymphatic drainage and the lymphatic connections between mammary glands in cats, with the aim of developing a reasoned model for the extirpation of these glands in cases of malignant tumors.

## MATERIALS AND METHODS

A total of 50 female cats (*Felis catus*) ages between 3 months and 8 years and live weight between 0.5 kg and 3 kg were used in this study. Nine of them were obtained from the Society for the Protection of Animals (France) and 41 were from Antananarivo (Madagascar). Animals from France were abandoned cats with health problems other than neoplastic diseases and were to be sacrificed. Cats from Madagascar were obtained during a rabies prevention campaign in the capital and were intended to be killed as part of the preventive measures. This experiment was started after both parties' full authorization for the use of these animals in our studies.

The mammary glands are referred to, depending on their position, as cranial thoracic (T1), caudal thoracic (T2), cranial abdominal (A1), caudal abdominal (A2), and inguinal (I). The inguinal gland, when existing, is rudimentary, and hence was not consid-

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TABLE 1. Summary of the number of female cats with the mammary glands injected with India ink

Female cats	One injection		Two injections	
	R	L	R + L	Total
Mammary chains				
T1	4	2		6
T2	—	1		1
A1	1	1		2
A2	—	6		6
T1 + T1			4	4
T2 + T2			13	13
A1 + A1			13	13
A2 + A2			5	5
Total	5	10	35	50

R, right; L, left.

ered for injections. These glands represent different functional states (lactation, gestation, resting). These different functional states were not taken into account due to the small number of studied animals.

After a general anesthesia by intramuscular administration of ketamine chlorhydrate (Imalgen 500 ND; 20 mg/kg of live weight) completed with morphine chlorhydrate (0.05 mg/kg of live weight in subcutaneous injection), 0.25 cm<sup>3</sup> per gland of black India ink was injected into the mammary parenchyma with a 0.45 × 12 mm (26 G × 1/2) hypodermic needle at different points around the teats. The use of India ink is a classical technique, the Gerota technique, used by Apostoleano (1925) and in the present for studying the lymphatic system: indirect injection in vivo. Only one mammary gland in each row was injected and in 15 animals only one mammary gland in one of the two rows was injected (Table 1). The cats were then sacrificed at 1 h 15 min to 26-h intervals using a general anesthesia (Imalgen and morphine chlorhydrate) followed by an intravenous administration of T61 (ND) (0.3 ml/kg). This waiting interval is the time of diffusion of the India ink to the lymph nodes. Different times were tested. The best results were observed between 15–24 h. The skin over the abdominal, inguinal, and thoracic regions was released laterally from the median line. Both rows of mammary glands were extracted, the thoracic cavity opened, all the lymphatic nodes were examined and indexed, and the presence of India ink in the lymph nodes verified.

For the lymph centers and their respective lymph nodes we adopted the anatomic nomenclature of the International Committee on Veterinary Gross Anatomical Nomenclature (Nomina Anatomica Veterinaria, N.A.V., 1994). We examined them using a binocular lens (Nikon SMZ-2T) and with microdissection of the axillary (*Ln. axillaris proprius*), accessory axillary (*Ln. axillaris accessorius*), first rib (*Ln. axillaris primae costae*), superficial inguinal (*Ln. inguinalis superficialis*), cranial epigastric (*Ln. epigastricis cranialis*), caudal epigastric (*Ln. epigastricis caudalis*), and sternal cranial (*Ln. sternalis cranialis*) lymph nodes.

Histological sections were prepared from 11 lymph node samples fixed in a 10% formalin solution and stained with hematoxylin–eosin, the most simple and efficient technique to illustrate the structure of the lymph node.

In order to study the interglandular connections, the lymph vessels were dissected under a binocular lens (Fig. 9). The injected glands, together with the neighboring glands, were examined for the deep lymph vessels after clearing (Spalteholz, 1914; cited by Tompsett, 1970) in different baths: hydrogen peroxide, ethanol for dehydration, xylol for transparency of tissue, and mixing of benzyl benzoate and methyl salicylate for conservation.

## RESULTS

In a total of 100 chains of examined mammary glands, no animal presented less than four glands

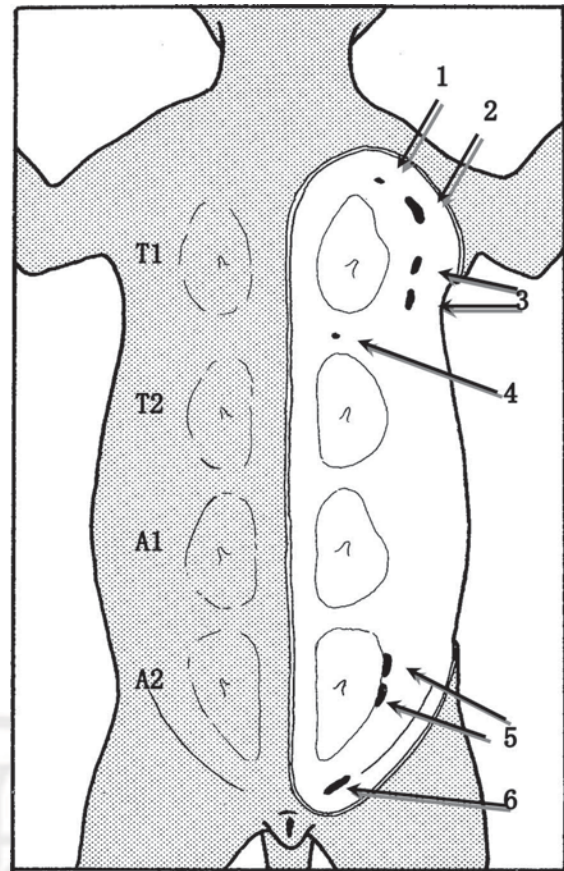


Fig. 1. Schematic topography of the mammary glands and the lymph nodes in the cat. 1, Axillary of first rib lymph node; 2, Proper axillary lymph node; 3, Accessories axillary lymph nodes; 4, Cranial epigastric lymph node; 5, Caudal epigastric lymph nodes; 6, Superficial inguinal lymph node. T1, Cranial thoracic mammary gland; T2, Caudal thoracic mammary gland; A1, Cranial abdominal mammary gland; A2, Caudal abdominal mammary gland.

per row (Fig. 1). However, nine out of the 50 animals were found to have one additional rudimentary gland at the inguinal region. Out of the 85 glands injected (Table 2) with India ink, the success rate for the pigmentation of the draining lymph nodes was 91.8% (78/85). The histological examinations (Figs. 6–8) proved the presence of India ink in the population of macrophages in the lymphatic sinuses of lymph nodes in the cortical region and/or in the medullar region.

TABLE 2. Number of injected mammary glands of female cats

Injected mammary glands	Right	Left	Total
T1	8	6	14
T2	13	14	27
A1	14	14	28
A2	5	11	16
Total	40	45	85

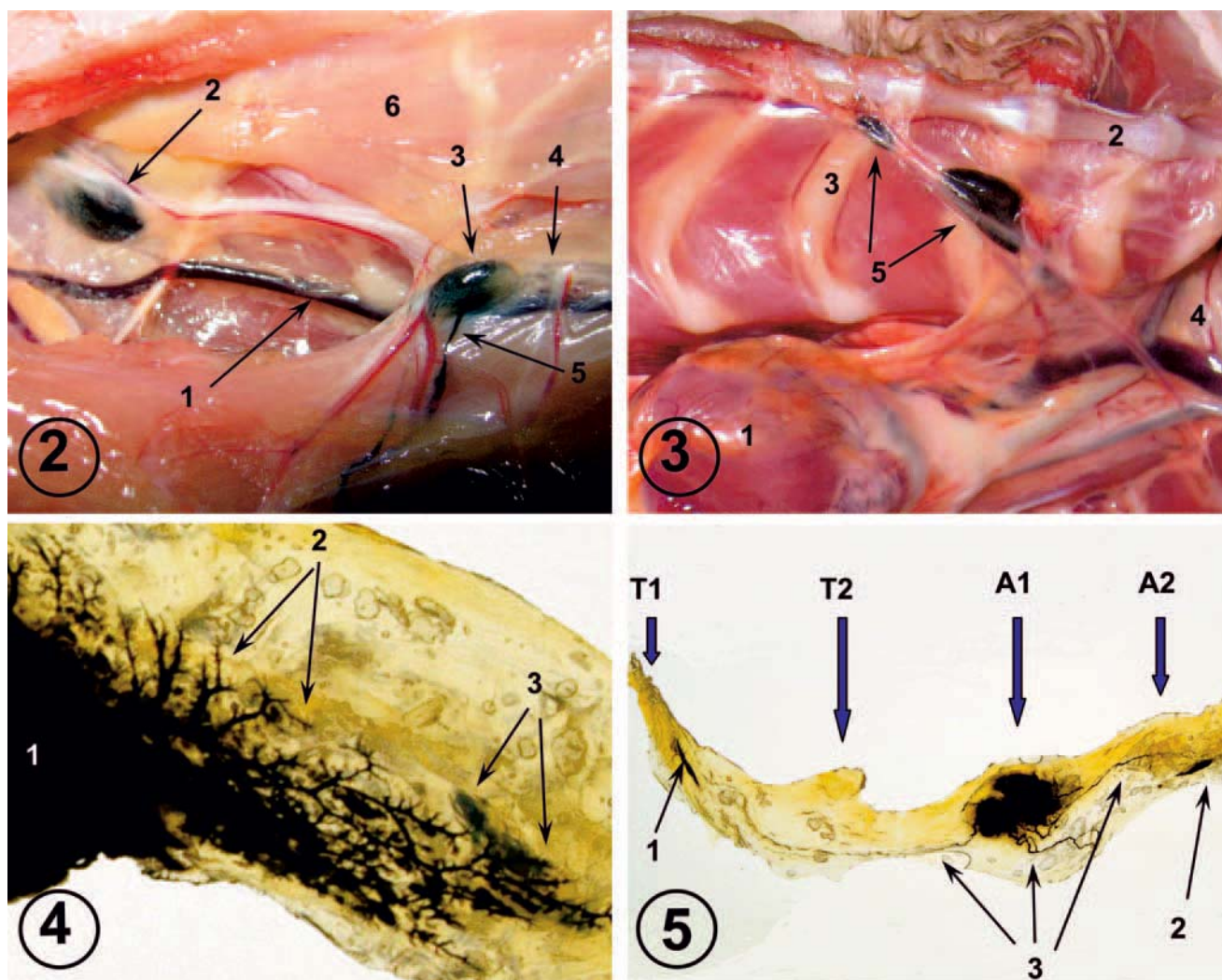


Fig. 2–5. Dissection of the axillary lymph center of a female cat. Dorsal decubitus. Lateral aspect. 1, Lateral thoracic vein; 2, Proper axillary lymph node; 3, Accessory axillary lymph node with India ink; 4, Accessory axillary lymph node not colored; 5, Afferent lymph vessel; 6, Deep pectoral muscles. Fig. 3. Dissection of the ventral thoracic lymph center of a female cat. Ventral aspect. 1, Heart; 2, Sternum; 3, Rib; 4, Trachea; 5, Cranial sternal lymph nodes. Fig. 4. Cleared preparation. Ventral aspect. Injected mammary gland A2 with India ink in a female cat. 1, Mammary gland A2; 2, Canals and acini filled with India ink; 3, Caudal epigastric lymph nodes. Fig. 5. Cleared preparation. Ventral aspect. Injected mammary gland A1 with India ink in a female cat. Drainage in two directions: cranially and caudally. 1, Accessories axillary lymph nodes; 2, Caudal epigastric lymph nodes; 3, Lymph vessels.

### Lymph Node Topography

Our investigation for the lymphatic nodes revealed the existence of all the different types except for the accessory lymph node of the first rib and the cranial epigastric lymph node, which were only rarely encountered (Figs. 1, 10).

The *Ln. axillaris proprius* was always present (100%), one lymph node being embedded in fatty tissues (Fig. 2) around the junction of *V. thoracica lateralis* with *V. axillaris*.

The *Ln. axillaris accessorius* was almost always present (97%). There were usually two lymph nodes (Fig. 2), rarely one or three lymph nodes, ellipsoidally shaped, and embedded in fatty tissues along the *A. and V. thoracica lateralis*.

The *Ln. axillaris primae costae* was rarely present (5%), being very small and situated along the *V. axillaris*.

The *Ln. epigastricus cranialis* was rarely present (7%), was very small, and situated on the *M. rectus abdominis* along the *V. epigastrica cranialis superficialis*.

The *Ln. inguinalis superficialis* was always present (100%). There was usually one, sometimes accompanied by two or three small lymph nodes. They were embedded in considerable fatty tissue near the junction of the *V. epigastrica caudalis superficialis* with the *V. pudenda externa*.

The *Ln. epigastricus caudalis* was almost always present (98%). There were usually two, rarely one or

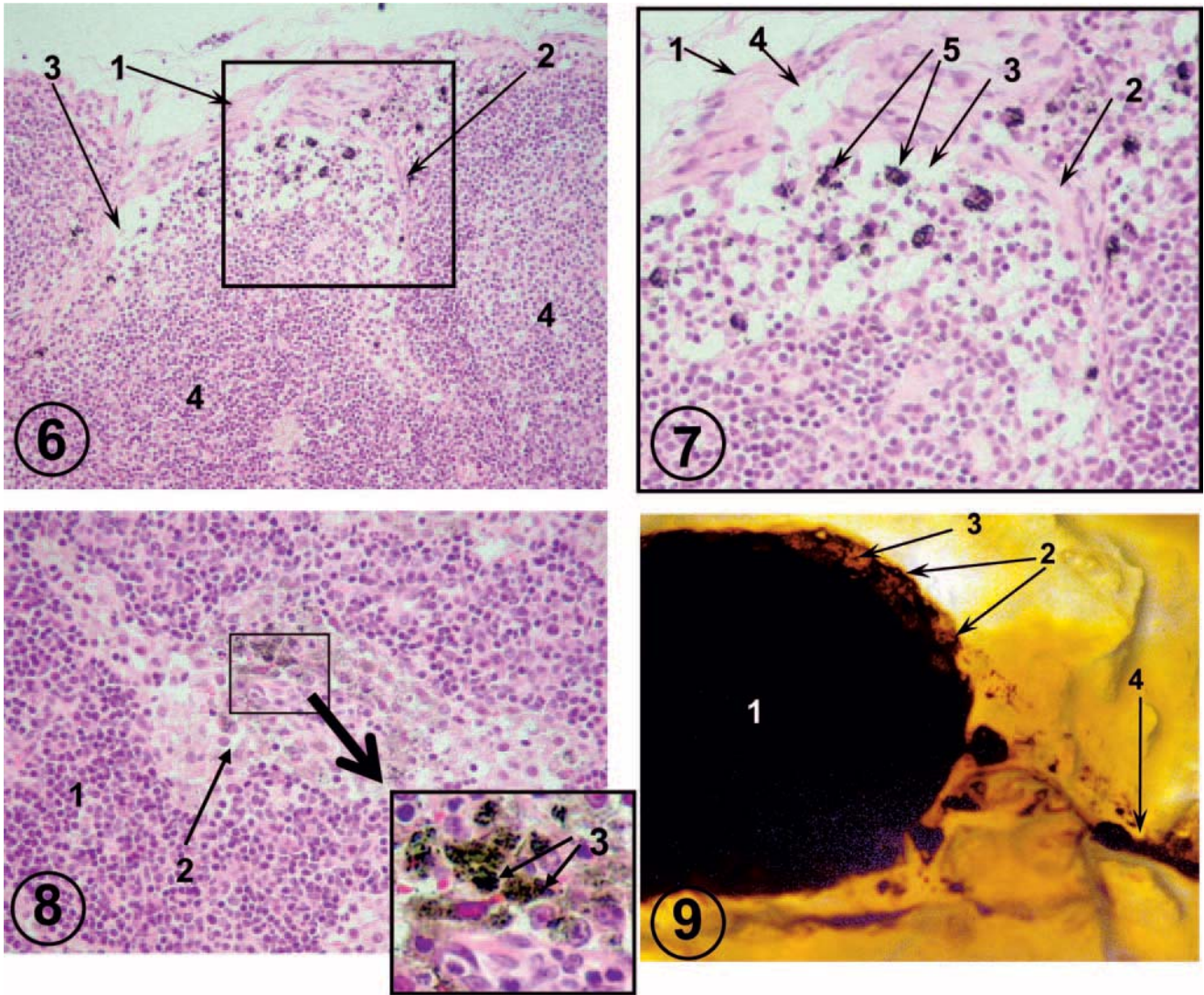


Fig. 6–9. Histological section of a lymph node in a female cat. Cortical region. 1, Connective tissue capsule; 2, Connective tissue trabeculae; 3, Subcapsular sinus; 4, Secondary follicle. H&E  $\times 20$ . Fig. 7. Enlargement of square in Figure 6. 1, Connective tissue capsule; 2, Connective tissue trabeculae; 3, Subcapsular sinus; 4, Afferent lymph vessel; 5, Macrophages with India ink. H&E  $\times 45$ . Fig. 8. Histological section of a lymph node of a female cat. Medullar region. 1, Medullary cords; 2, Medullary sinus; 3, Macrophages with India ink. H&E  $\times 40$ . Fig. 9. Macrophotography of a lymph node of a female cat after clearing preparation. 1, Lymph node colored with India ink; 2, Subcapsular sinus; 3, Follicle; 4, Afferent vessel.  $\times 10$ .

three lymph nodes, ellipsoidally shaped, and embedded in fatty tissues along the *A. and V. epigastrica caudalis superficialis*.

The *Ln. sternalis cranialis* was always present (100%). There were usually two large lymph nodes (Fig. 3), situated dorsolaterally to the second sternbra, along the *A. and V. thoracica interna*.

#### Lymphatic Drainage of Mammary Glands

The lymphatic drainage of the mammary glands to the axillary and superficial inguinal lymph nodes (Fig. 11) demonstrated that gland T1 (14 injected including six unilaterally) always drained to the axillary lymph center in 11 out of the 14 cases. The

remaining three cases, which were negative for the pigment, correspond to glands for which the time interval (less than 6 h) between injection and sampling was insufficient.

Similarly, gland T2 (27 injected including one unilaterally) always (100%) drained to the axillary lymph center. Moreover, 6/27 drained caudally to the superficial inguinal lymph center (two on the right side and four on the left side).

Gland A1 (28 injected including two unilaterally) always drained to the superficial inguinal lymph center: 28/28. Moreover, 17/28 (Fig. 5) drained cranially to the axillary lymph center (nine on the right side and eight on the left side). Furthermore, gland A2 (16 injected including six unilaterally) always

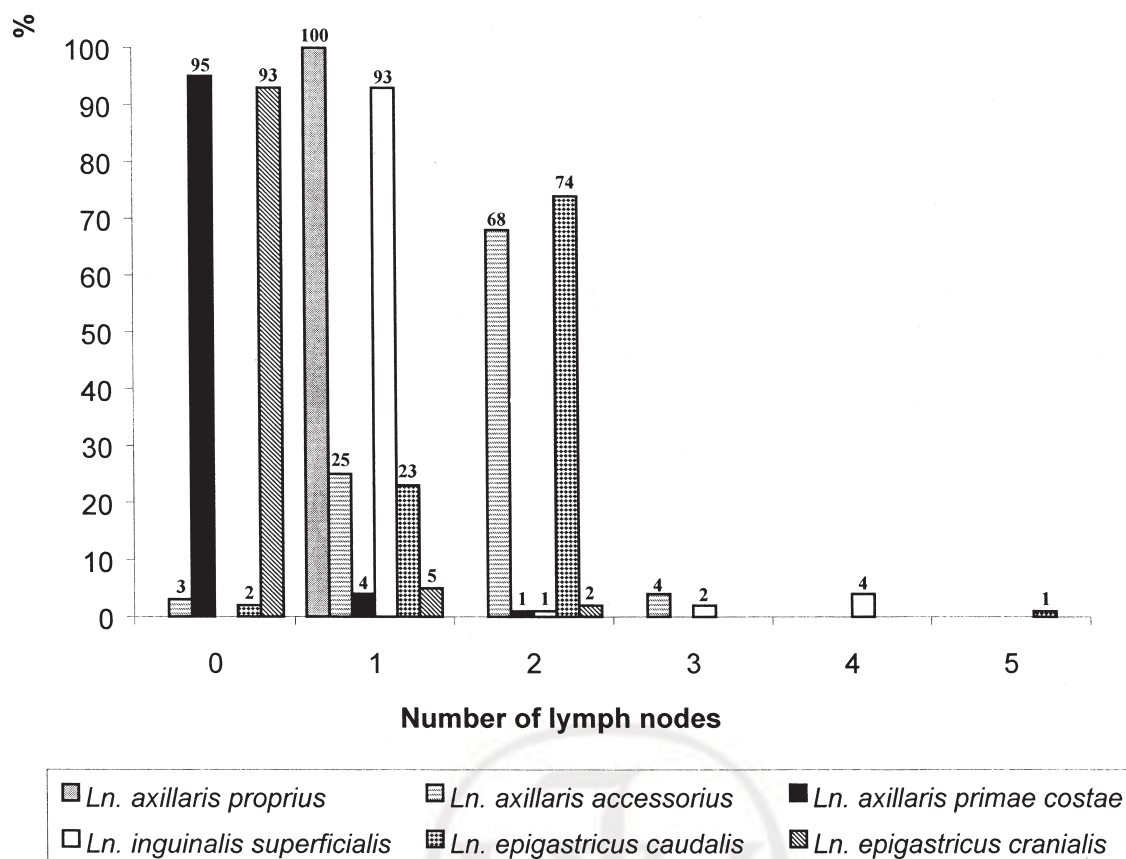


Fig. 10. Number of lymph nodes expressed in percentage of mammary chains of female cats.

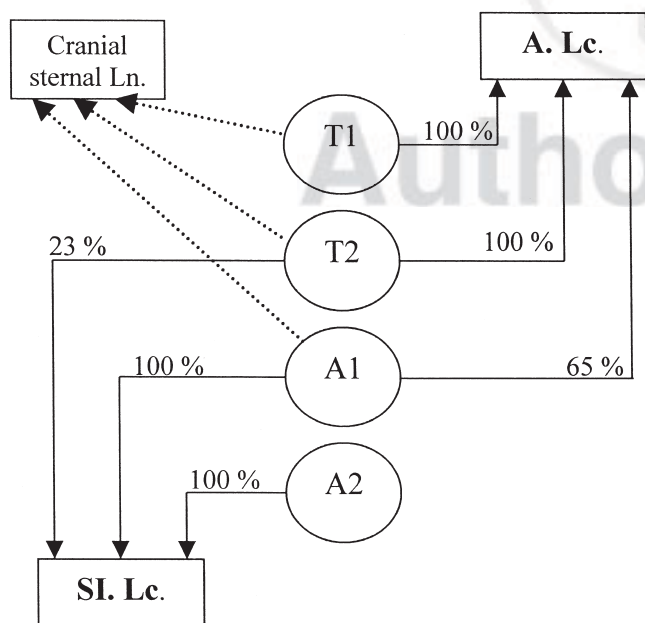


Fig. 11. Lymphatic drainage of the mammary glands of female cats. A. Lc., Axillary lymph center; SI. Lc., Superficial inguinal lymph center.

drained to the superficial inguinal lymph center (13/16). The remaining three cases correspond to a short time interval between injection and sampling.

Following the axillary and the superficial inguinal, the drainage of the cranial sternal lymph nodes was studied (Fig. 11).

Gland T1 (8 injected including 6 unilaterally): 7/8 drained to the cranial sternal lymph nodes.  
 Gland T2 (9 injected including 1 unilaterally): 9/9 drained to the cranial sternal lymph nodes.  
 Gland A1 (8 injected including 2 unilaterally): 5/8 drained to the cranial sternal lymph nodes.  
 Gland A2 (10 injected including 6 unilaterally): 0/10 drained to the cranial sternal lymph nodes.  
 Gland I: inguinal gland, when existing is rudimentary, and hence was not injected.

**Interglandular Lymphatic Connections**

Two types of interglandular lymphatic connections may exist. These are lymphatic connections between both series of mammary glands and lymphatic connections between adjacent mammary glands. Among the 15 cats receiving a unilateral injection of a single mammary gland, no animal presented a gland drained

TABLE 3. Comparison of the average number of lymph nodes corresponding to each row of mammary glands in female cats (according to different authors)

	Sugimura et al. (1956) 48 mammary chains	Meier (1989) 20 mammary chains	Present study 100 mammary chains
<i>Ln. axillaris proprius</i>	1.15	1	1
<i>Ln. axillaris accessorius</i>	3.21	1.35	1.73
<i>Ln. axillaris primae costae</i>	0.18	0.25	0.06
<i>Ln. inguinalis superficialis</i>	1.17	1	1.17
<i>Ln. epigastricus caudalis</i>	2.41	2.9	1.76
<i>Ln. epigastricus cranialis</i>	0.04	0	0.09

by the lymph nodes from the opposite side. Furthermore, in the 50 cats examined no indication was found for the existence of lymph vessels connecting the two rows of mammary glands.

Lymphatic connections between two adjacent glands (T1 and T2, T2 and A1, A1 and A2) were never observed.

## DISCUSSION

This study, carried out on 50 female cats with their mammary glands at diverse functional states, permitted us to appreciate the existence of anatomical variations between individual animals. The two publications available, at the end of our research, were based on studies performed on a small number of animals, 24 cats for Sugimura et al. (1956) and 10 for Vollmerhaus and Roos (1997). Our unique approach of injecting one gland in a row at a time avoided confusions with the drainage of adjacent mammary glands. Vollmerhaus and Roos (1997) had partially circumvented the problem by injecting each gland with a different color. This approach may pose a difficulty, particularly when the draining lymph node presents a mixture of several dyes. Different from the methods used by Sugimura et al. (1956) and Vollmerhaus and Roos (1997), which involved the use of sacrificed animals, we injected live cats, an approach which allowed us to work in a relatively ideal situation, closer to the animals' physiological condition. The gross anatomical studies were also accompanied by histological examinations in order to minimize the risks associated with misinterpretations of data with microdissection in cases where an apparently negative lymph node could contain microscopic pigmentation (Figs. 6–9).

The total number of mammary glands (four on either side) found in this study was in agreement with other similar work (Silver, 1966; Christensen, 1979; Nickel et al., 1981; Barone, 1996; Vollmerhaus and Roos, 1997). We believe that four pairs of mammary glands can be taken as normal for cats. The five additional (5/100) glands found at the inguinal position were rudimentary and hence were not considered for injections.

Similarly, the number of lymph nodes in the different lymph centers is generally in accord with the literature (Table 3). Yet a few exceptions can be men-

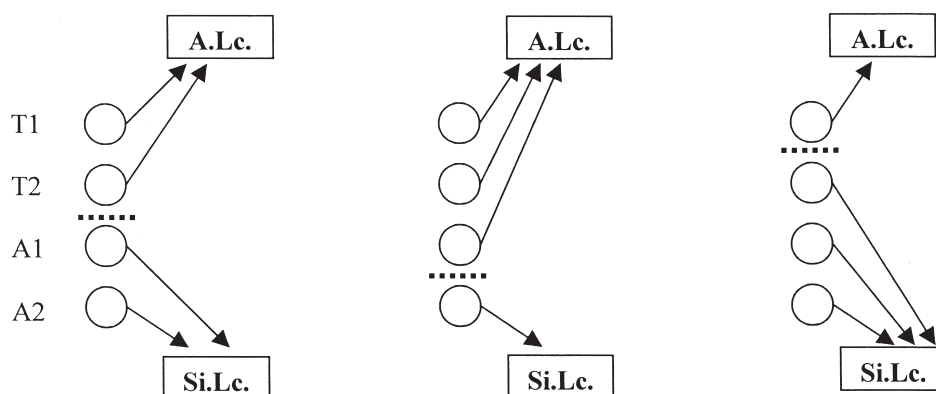
tioned in this study. *Ln. epigastricus cranialis* was found in 9% of the cats, while *Ln. axillaris accessorius* had an average number of 1.73 lymph nodes per mammary chain. The former lymph node was reported as being absent by Meier (1989), while the average number of the latter was 3.21 for Sugimura et al. (1956).

As noticed by Sugimura et al. (1956) and Meier (1989), the *Ln. axillaris primae costae* and the *Ln. epigastricus cranialis* cannot always be demonstrated when they are not colored by India ink—the problem of the “false negative.” Hence, the presented data do not always represent the actual frequency of these glands in the cat. Their frequency may be higher than reported here. On the other hand, no difference was noticed in the lymphatic drainage between the right and left rows of mammary glands.

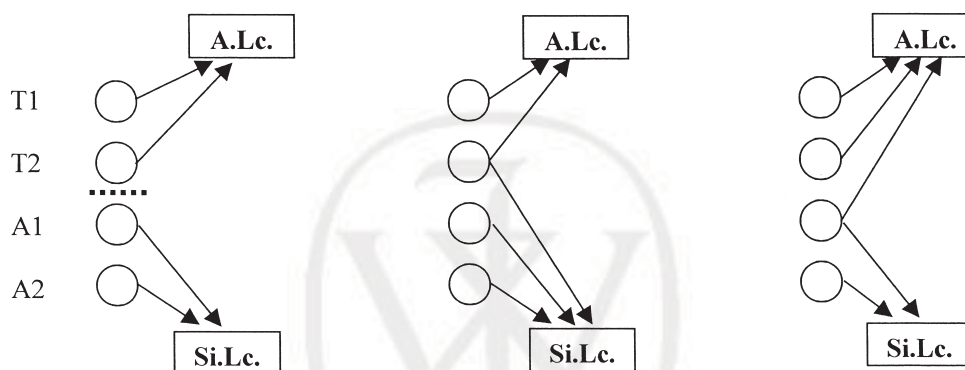
There is a general consensus among authors that the mammary gland chains in carnivores drain cranially towards the axillary lymph center and caudally towards the superficial inguinal lymph center (Sugimura et al., 1956; Meier, 1989; Ruberte et al., 1990; Ruberte et al., 1991; Patsikas and Dessiris, 1992; Sautet et al., 1992; Patsikas and Dessiris, 1996a b; Vollmerhaus and Roos, 1997; Pereira et al., 2003). Furthermore, it is believed that gland T1 drains almost exclusively towards the axillary lymph center and gland A2 drains always and exclusively towards the superficial inguinal lymph center.

Meier (1989) and Vollmerhaus and Roos (1997) described that T2 and A1 can drain either cranially or caudally, but this was not quantified. On the contrary, we demonstrate here that T2 always drains cranially and sometimes in both directions and that A1 always drains caudally and often in both directions. Moreover, we never encountered a gland T2 draining exclusively caudally and a gland A1 draining exclusively cranially. Hence, the direction of drainage of glands T2 and A1 appears contrary to the one reported in the literature, and thus remains a point of debate. In fact, this contradiction may raise the question of the limit between the two directions of drainage. For Vollmerhaus and Roos (1997) this limit can be either between T1 and T2, or between T2 and A1, or between A1 and A2. We assume that the only limit that can exist in certain animals is between glands T2 and A1 (Fig. 12).

The drainage of the three cranial glands to the cranial sternal lymph nodes coincided with the de-



Vollmerhaus et al. (1997)



Present study

Fig. 12. Comparison of the limits between the two directions of drainage for Vollmerhaus and Roos (1997) and present study. A. Lc., Axillary lymph center; Si. Lc., Superficial inguinal lymph center; dashed line, Limit between the two directions of drainage.

scription of Barone (1996). It was explained that the afferent vessels of the cranial sternal lymph nodes come from the pectoral area, the sternum, and the ventral walls and sides of the thorax (Fig. 3). Thus, they are likely to drain the cranial part of the mammary chains: 100% for T2, 87.5% for T1, 62.5% for A1, and 0% for A2 (Fig. 11).

The lymphatic connections between all the mammary glands were described in carnivores by Habel (1978) and Pierard (1972), and especially in dog by Ruberte et al. (1990), where they may exist via one or more lymph vessels connecting the lymphatic plexuses of the teats (*papillae*) of the three intermediate mammary glands. Mailot et al. (1980) mentioned such connections in cat between T1 and T2 and between A1 and A2 without any explanation. We never observed lymphatic connections between two adjacent glands in cat (T1 and T2, T2 and A1, A1 and A2).

The caudal drainage of gland T2 to the superficial inguinal lymph center was always mediated through a direct connection. Similarly, gland A1 was found to be drained cranially to the axillary lymph center directly, never through an intermediate gland T2.

In agreement with other works, we demonstrated that the drainage of the mammary glands to the lymph nodes is unilateral (Apostoleano, 1925; Stalker and Schlotthauer, 1936; Fidler and Brodey, 1967; Ruberte et al., 1990), but in contradiction with Patsikas and Dessiris (1996a), who found a connection between the superficial inguinal lymph nodes from either side in dog. No lymphatic vessel was found to connect both series of the mammary glands at the same time.

The detailed characterization of the mammary glands and their associated lymphatic drainage are essential in the successful treatment of mammary tu-

mors. We tried to produce new and more complete data that will eventually aid block dissection surgical technique in the removal of malignant tumors such as the one proposed in dog by Mann (1984) and Sautet et al. (1992). Thus, in the case of individual or simultaneous neoplasia of glands T2 and/or A1, these two glands should always be removed with the lymphatic nodes of which they are tributaries. If the tumor relates only to the mammary gland at the two extremities, procedures in the removal of malignant tumors in cats will be excision limited only to the affected glands and the lymph nodes that drain them.

The probability of success for all surgical interventions performed on any one of the three cranial mammary glands may be significantly reduced because of the drainage of these glands toward the cranial sternal lymph nodes, which are not easily accessible for excision (thoracotomy). This constitutes an important difference from the results obtained in dogs in earlier reports (Ruberte et al., 1990, 1991; Sautet et al., 1992). It would also be advisable to check if the unilaterality of the lymphatic drainage in the cat is not altered in neoplastic mammary glands, as recently demonstrated in dogs (Pereira et al., 2003).

## CONCLUSION

We stated precisely the number and the topography of the lymph nodes draining the mammary glands of female cats ( $n = 50$ ); demonstrated the existence of another route of lymph drainage from the caudal thoracic mammary gland and from the cranial abdominal mammary gland into two directions, cranially and caudally; demonstrated the importance of the lymph drainage of the three cranial mammary glands toward the cranial sternal lymph nodes; and also pointed out the absence of interglandular lymphatic connections. All these facts are of functional significant interest in cases of malignant mammary gland tumors on proposing a new block dissection surgical technique.

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