Morphological study of Lymphatic Drainage and Lymph Nodes of Mammary Glands in Doe

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Abstract
Direct relations between human and animal cancers are not completely understood, but the fact that metastasis is identical it’s well recognized. The investigation of lymphatic metastasis in mammary neoplasia or in malignant melanoma in rabbits requires a good knowledge of the topography of main superficial lymph nodes that drains superficial structures. This study aims to perform a detailed description of the lymphatic drainage and the topography of lymph nodes that drain the mammary glands in doe. Using colored solution injection technique, we study the topography of sentinel lymph nodes that drain the mammary glands in ten rabbits. Macroscopically and histological analyses were performed. Lymphatic drainage of the thoracic and cranial abdominal mammary gland (T and A1) was achieved by ventral accessories axillary lymph nodes, in a variable number; from one lymph node in two subjects, two lymph nodes in five subjects, even three lymph nodes in three subjects. This group of ventral accessories axillary lymph nodes represents a distinct group of axillary lymph center in rabbits, being localized at the dorsal border of pectoral ascendant muscle on the lateral thoracic vein trajectory. Caudal abdominal and inguinal mammary glands (A2 and I) drainage was achieved by mammary lymph nodes, belonging inguinofemoral lymph center. They are willing in dorso-lateral part of last mammary gland, embedded in adipose tissue, two lymph nodes on each side. Results presented in this study encourage the use of induced mammary tumor in rabbits as an animal model to study the lymphatic metastatic spread in mammary neoplasia. Also, the data obtained can be used to differentiate the lymph nodes which drain superficial structures in rabbits and improvement of surgical treatment in case of developing mammary tumors.

Keywords
lymph node, topography, doe

INTRODUCTION
The lymphatic system is a less approached subject by most researchers due of the effort required to identify its components: lymph vessels and lymph nodes. It’s very hard to identify a lymph vessels that is sinuous, tiny, and filled with colorless lymph, as well as the lymph nodes that drain different regions, especially the superficial ones (Quere I., 2010). These aspects are encountered both in humans and animals. Also, the lymphatic reaction and metastasis have the same characteristics (Tuohy et al 2009). Recently, the directives for research prohibit the use of pets as experimental model, but rodents and lagomorphs are accepted. Previous studies conducted on canines, define the lymphatic system in two territories: superficial and profound (Stan, 2008; Suami et al., 2012). Taking into consideration that the occurrence of mammary tumor has the same characteristics both in woman and bitch (Sorenmo et al., 2011), together with the fact that metastasis is achieved through the lymphatic
system (Stan, 2012), our studies focused on identifying the lymphatic drainage of mammary glands for the doe. We considered appropriate the anatomic description of superficial drainage in doe, due to the fact that in this species it's possible to experimentally induce different tumor types: melanoma, esophageal or rectal carcinoma and not in the last-mammary tumors (Baker, 1985; Anja-A et al., 2003; Chen et al., 2008). Also, in this species it is very easy to follow the lymphatic reaction in the case of infectious disease (Kanter et al., 1990; Shin et al., 2003; Catarina Hadamitzky and Reinhard Pabst, 2008). All this knowledge is useful in establishing the scientific basis for clinical management in situations of metastasis, lymphedema and in infectious disease.

**MATERIAL AND METHODS**

The study was conducted on a lot consisting of 10 does, according to the guidelines of Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. The animals were accommodated in optimal conditions, having their vital constants permanently monitored. The research method consisted in injecting coloring solution, blue dye Evans 0.1% in the mammary glands parenchyma (Fig.1). It were injected cranial thoracic mammary gland (T)1, cranial abdominal (A1) and inguinal (I) mammary gland.

Injection was performed under general anesthesia (Xilazin 5mg/kg/bw and Ketamin 2mg/kg/bw). To facilitate the diffusion and penetration of the colorant into the lymphatic vessels, a gentle massage of the injected area was performed. At 24h post injection the subjects were euthanatized by intravenous administration of potassium chloride 2meq/kg/bw.

Through an incision along the white line, the skin was removed carefully, following the trace of the lymph vessels from the injection side to the lymph nodes which drained the injected mammary gland. The lymph nodes were photographed and after excision they were fixed in formaldehyde 6%. After that, the lymph nodes were embedded in paraffin, followed by sectioning in pieces, each measuring from 6 to 8 microns.

The sections were colored with haematoxylin/eosin stain. Histopathological examination was performed at the department of Pathological Anatomy of IRGH Cluj-Napoca.

**RESULTS AND DISCUSSION**

At the injection site, the dye diffused very well in the mammary parenchyma, achieving an excellent emphasis of the lymph vessels, with a plexiform aspect.

At the periphery of the mammary glands, these vessels group into vessels with higher calibre which penetrate in depth. These vessels were unseen at superficial dissection.

In the ventral axillaries zone, the first seen lymph nodes were well coloured and with sizes varying from 3 to 5 mm. At six subjects, two lymph nodes were observed, at four subjects, it was
observed three lymph nodes which drained the cranial mammary glands (Fig. 2).

These were placed on the trajectory of the lateral thoracic vein, at the dorsal side of the ascendant pectoral muscle. The afferent vessels leave the lymph node and groups into a common vessel, with ascendant dorsal trajectory. The shape of the lymph nodes was oval in seven subjects and round for three subjects. In the subjects that showed five pairs of mammary glands (four subjects), the first two pairs of mammary glands were drained via the ventral axillaries lymph nodes, and for six subjects which presented four pairs of mammary glands, the first three pairs were drained through the same lymph nodes. As for the drainage of the last mammary gland, it was done via the mammary lymph nodes, which belong to inguinal femoral lymph center. Counting two at eight subjects, and three for two subjects, the mammary lymph nodes were arranged in the adipose tissue from the inguinal zone, at the dorsal-lateral margin of the inguinal mammary gland (Fig. 3). Shape of the lymph nodes was oval in six subjects and round in four subjects. Concerning the second to last mammary gland, depending on the number of mammals of the subject, the drainage was realized both through the ventral axillaries lymph node and the mammary lymph nodes in two subjects which presented four pairs of mammary glands.

Regarding communication between lymphatic vessels of contralateral mammary glands, we haven't seen lymph vessels which pass the white line. Between the ipsilateral mammary glands, we detected a few tiny lymphatic vessels which pass from the parenchyma of a mammary gland to another.

Histological examination of ventral axillaries lymph nodes and mammary lymph nodes has emphasized the cellular architecture (Fig. 4). Lymph nodes were well delineated by a fibrous capsule. They contain a large number of compact lymphatic follicles at lymph node periphery and less lymphatic follicles in the center. The examined follicles were in majority secondary follicles representing antigenically stimulated germinal centers. The germinal centers of the secondary follicles have shown a diversified population of cells, most of them being lymphocytes, some of them macrophage and numerous eosinophilic cells (Fig. 5).

The value of the toponography of the lymph nodes that drain the mammary glands is of great importance in the management of mammary gland disease, but is often underestimated (Tuohy et al. 2009; Stan F. 2010; Sorenmo et al., 2011). Also, identifying the lymphatic path ways to the lymph node is an essential requirement for understanding the lymphatic distribution of the
inflammatory or malignant process of a certain territory (Stan F. 2008). It is well known that the pathological process of a certain territory have an impact, firstly, on the lymphatic circulation together with the reaction of draining lymph nodes (Suami et al., 2013).

That is the justification for the necessity of an appropriate experimental model which will lead to solving the mechanisms of metastasis and to create a new concept of therapeutical management (Shin et al., 2003). In the last years, as experimental model were used mostly the dogs and the rats (Pereira et al., 2005).
et al., 2003; Catarina Hadamitzky and Reinhard Pabst, 2008; Stan, 2012, Suami et al., 2012), but the new Directive (2010/63/EU) of the European Parliament and of the Council on the protection of animals used for scientific purposes, prohibit using pets as research subjects. So, researcher’s attention directed to the Lagomorpha and other Rodenta species.

Although there are numerous studies of rat’s lymphatic system, not always this species is suitable for studies regarding metastatic dissemination. (Shin et al., 2003; Suami et al., 2011a). Even the neoplastic pathology of rats presents certain similarities with human cancers, regarding the metastasis, the facts are truly changing. The basis of this affirmation is the observation that in rat’s carcinoma metastasis process is done hematogenously (van Es et al., 1999). In rabbit, like in humans, carcinoma metastasis process is achieved by lymphatic vessels (Chen et al., 2008).

Regarding the lymphatic vascularization of doe mammary gland and sentinel mammary lymph nodes there are few studies in the literature. Detailed studies of metastatic lymph nodes topography in esophageal cancer were made, having New Zeeland White Rabbits as experimental model (Mine and Nakamura 1983). Also, it was studied neck and head lymph nodes topography in carcinomas situated in this regions (Anja-A Dune et al., 2002). In otorhinolaryngology, rabbits are the most common experimental model (Baker et al., 1985) and in lymphedema, the rabbits are the preferred model (Kanter et al., 1990; Catarina Hadamitzky and Reinhard Pabst, 2008). Recently, Soto Miranda et al., (2013) have shown that the superficial lymphatic system of rabbits can be summarized into eight lymphatic territories, but they have not accomplished a detailed description of the lymph nodes which drain these territories. The emphasis was put on lymphatic vascularization. Instead, we have shown in previous research (Stan, 2008 and 2012) that the lymphatic vessels are in full interdependence with the lymph nodes arranged along them. We have shown that lymph node reaction take place after the reaction and proliferation of lymphatic vessels.

The present study describes the lymphatic drainage of doe cranial mammary glands through the ventral axillaries lymph nodes which belong to the axillaries lymph centre. Compared to carnivores, in which the axillaries lymph center contains two groups of lymph nodes, the proper and accessories axillaries, in doe, the axillaries lymph centre contains three groups of lymph nodes: proper axillaries, ventral axillaries, dorsal axillaries. In our opinion, this commentary is not differential, since in another study (Stan et al, 2008) we have shown the presence of an additional lymph node.
of the axillaries lymph center in dogs. From these groups, in doe, only the ventral axillaries lymph nodes are the sentinel for the cranial mammary glands. The next lymph nodes have direct afferent lymphatic vessels from the precedent lymph nodes and other drainage areas. Concerning the caudal drainage, we have shown the similarities with the caudal drainage in bitch of the mammary glands through mammary lymph nodes (superficial inguinal) with the same disposition with the ones from carnivores. Therefore, the lymph nodes that drain the mammary glands as well as lymphatic vascularization are equally important.

By describing the lymphatic pathways this study can offer another treatment option in mammary cancers more exactly, direct intra-lymphatic injection of chemotherapeutics, as previously stated by other researchers (Chen et al., 2008). Intra-lymphatic therapy can become an efficient path to treat tumors with predisposition towards lymphatic metastatic dissemination, especially of those with epithelial origin, like mammary gland, gastric, colon or pulmonary cancer. If the subcutaneous peritumoral administration of carboplatin-activated carbon suspension in VX2 induced mammary tumors (Chen et al., 2008) has shown a better anti-tumoral activity in metastatic lymph nodes, compared to systemic administration, we can state that a detailed description of mammary lymphatic drainage can offer the possibility of an efficient intra-lymphatic approach and therapy. Concerning the histological description of lymph node in doe, this is similar with the human one, both species showing similar characteristics regarding the type and location of lymphatic follicles in lymph nodes.

In conclusion, by describing the anatomy of the lymphatic drainage of mammary glands in doe, this study justifies using the doe as an experimental model for research focused on metastasis of mammary cancers. Pointing out the topography of the lymph nodes in doe, we provided exact data which can be of use to surgery or biopsy of the lymph node. Also, this study offers the possibility of another type of management for mammary tumor therapy, recommending usage of intra-lymphatic therapy in case of lymph node metastasis.

REFERENCES


