Correlation between Ultrasonographic Features and Morphological Pattern after Blue Dye Injection of Normal Superficial Lymph Nodes in Carnivores

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Abstract. The present study performs a description of ultrasound anatomy of superficial lymph nodes using gray scale ultrasound, Doppler techniques (color Doppler and power Doppler), compared with a morphological identification method - injection of coloring solutions (Blue Dye), with lymphatic tissue tropism. Eleven subjects were examined (nine female and two male). A total of 58 lymph nodes were assessed belonging to the axillary, superficial inguinal, popliteus, superficial cervical and submandibular lymph centers. Ultrasonographic criteria followed were: shape, size, echogenicity, ecotexture, capsule appearance, hiperecoic band of the hilum and angioarchitecture. For comparison, subcutaneous injection of Blue Dye Evans, 0.5% in 4 subjects was performed. Ultrasound examination detected right and left inguinal, and popliteus lymph nodes in all subjects, axillaries lymph nodes in 10 subjects, cervical superficial lymph nodes in 9 subjects and submandibular in 7 subjects. Normal lymph nodes present oval (fusiform) shape, with a corticomedullary homogenous ecotexture, well defined capsule. The ecogenicity was hypoecoic or isoecoic to the surrounding tissues, with a hiperecoic hilar band. The distribution of vascular flow within the lymph node, assessed by color Doppler techniques, was hilar in 78% of subjects. Power Doppler technique detected with high accuracy the presence of vascular signal in small lymph vessels. Subcutaneous injection of Blue Dye, identified in all subjects the axillary, inguinal superficial, popliteus and cervical superficial lymph nodes. Compared with Blue Dye, ultrasound examination revealed with about the same accuracy the lymph nodes, with 96% specificity and 87% sensitivity.

Keywords: lymph nodes, topography, ultrasound, Doppler ultrasound, blue dye Evans, anatomy

INTRODUCTION

Although technological progress takes its toll on methods for noninvasive investigation of human and animal body, in terms of lymphatic system a perceived shortage on its evaluation methods exists (Olivier, 2004). The lymphatic system is involved in many metabolic processes, immune response and tumor metastasis (Baba A. I., 2007). The in vivo evaluation of these components, vessels and lymph nodes is not easy and is not specific enough, in case of disease. Clinical examination and laboratory evaluation are nonspecific in lymphatic illness. Therefore it is necessary to know the normal aspects made by intravital noninvasive methods, to which pathology may be reported (Badea et al., 2006; Stan et al., 2005). Nowadays, in human medicine, ultrasound is the first method of intention, due to its noninvasiveness (Badea et al., 2010).
There are sporadic descriptions in literature of lymph nodes imaging in veterinary medicine. Compared to human, in which ultrasound is a method of first choice in lymphatic evaluation, veterinary medicine lacks this current practice.

In human cancers prognostic significance of lymph nodes metastasis is well known. Introducing the sentinel lymph node concept made a major change regarding both therapeutic and prognostic attitude (Badea et al., 2006). Furthermore, in recent years in woman breast cancers, histopathologic evaluation of presumptive metastatic lymph nodes is made by biopsy under ultrasound guidance (Frati et al., 2011). Thus, it’s possible to avoid lymphoscintigraphy based on radiant products. Classical methods for identifying lymphatic system are based on using dye solutions and radiopharmaceuticals (Pereira et al., 2003). Most used dye solution was ink of China, isosulphan blue and blue dye Evans. The possibility of anaphylaxis if ink of China is used is high, therefore most researchers have conducted their studies on cadavers. Intravital evaluation of lymphatic drainage is made by lymphoscintigraphy using usually radiant $^{99m}$Tc sulphur colloid and a dye solution injected prior of lymphoscintigraphy. Separately, the two techniques do not provide sufficient accuracy in identifying the sentinel lymph nodes, but applied together an increased detection capability of sentinel lymph nodes is achieved.

Another subject of debate is the choice of site of dye or contrast injection (Frati et al., 2011). Patsikas and Dessiris made their own study regarding identification of healthy and neoplastic mammary draining lymph nodes using subareolar and peritumoral injection of ultrafluid lipiodol (Patsikas, M. N. and Dessiris, A., 1996). Perreira et al. have used both ink of China and 10% fluorescein, injected subareolar and in mammary gland parenchyma to identify the sentinel lymph nodes in the same condition (Pereira et al., 2003). All these researchers note that in condition of neoplastic disease of mammary glands, the pattern of lymphatic drainage is completed changed. Present research correlates two methods in describing the anatomy of superficial lymph nodes in carnivores. The morphological method is injection of blue dye Evans and the noninvasive imaging method is ultrasonography. Starting from the premise that both methods are addressed to superficial lymph nodes, and the fact that most researchers report a substantial increase of sentinel lymph nodes identification if two methods are used simultaneously, we hypothesized that ultrasonography and blue dye injection could make a better assessment on superficial normal lymph nodes of carnivores.

**MATERIAL AND METHODS**

The study was conducted on eleven subjects (nine female and two males) in two steps. The first part consisted in evaluation of superficial lymph nodes by ultrasound. Ultrasound was performed using a General Electric device, Logique 7, equipped with soft parts module. A linear transducer with 7-12MHz frequency was used. Ultrasound approach was made taking into consideration the anatomical projections of superficial lymph nodes, respectively submandibular region, lateral cervical region in the middle third, axillary region, inguinal region, and popliteal region. The sections obtained were transversal and longitudinal dependent on lymph nodes location. Ultrasound examination was done in stunning, after acepromazine administration (1mg/kg/bw). Subjects were positioned in supine position. In cases where it was possible, ultrasound examination was performed after clinical palpation of lymph nodes. This was easy for popliteus lymph nodes, inguinal lymph nodes in tiny subjects, and if the thoracic limb could be easily pushed forward the palpation was possible for the axillary lymph nodes. Superficial cervical lymph nodes were palpated in three subjects. Both, manual palpation and
transducer palpation, was performed for lymph node identification in the local area to assess the consistency and lymph node relationship with surrounding tissues. To obtain a good visualization, a mechanical toilet was made followed by the application of an appropriate amount of ultrasound gel, for getting a good contact between transducer and skin surface.

Followed ultrasound criteria were: shape, size, by measuring the two axes: transverse and longitudinal, their report, echostructure, parenchymal echogenicity, lymph capsule appearance and hilum appearance. Doppler technique watched vasculature aspects by following criteria: presence of vascular flow, its lymph node distribution.

In the second part of this study, six subjects were injected using 0.5 % blue dye Evans solution. Since the superficial lymph nodes draining the mammary glands, and given their frequent pathology, blue dye injection was made both periareolar and in mammary gland parenchyma in four adjacent points: cranial, caudal, medial and lateral.

A total amount of 0.4ml blue dye Evans was injected, 0.1ml per injection point. Three subjects were injected in cranial thoracic and cranial abdominal mammary glands, and three other subjects were injected in caudal thoracic, caudal abdominal and inguinal mammary glands.

All injections were made under anesthesia. After injection, for easier penetration and diffusion of the dye in the lymphatic vessels, a slight massage of the injected area was made. Twelve hours later, after general anesthesia, the subjects’ euthanasia was made and the stratigraphic dissection was performed. The following aspects were observed: lymphatic drainage of the injected mammary glands, their afferent lymph nodes, their size, macroscopic appearance after blue dye impregnation, location and number of lymph nodes that compound every lymph center. Ultrasound assessment results were compared with those obtained after blue dye administration.

RESULTS AND DISCUSSIONS

Ultrasound examination has been made to all the specimens on the superficial inguinal and popliteus lymph center without any difficulties. Superficial inguinal lymph center consisted of two lymph nodes at five subjects, three lymph nodes at four subjects and only one lymph node at two subjects. Popliteus lymph nodes were unique in the majority of the cases (nine subjects of eleven). The axillary lymph center was detected in ten cases of eleven, in six cases in its composition accessory and proper lymph nodes were found, and in four cases, it was composed only of one lymph node. Hence the superficial position and the caudal disposition from the inguinal mammary gland at 2-3 cm in front of the symphysis pubis, which most of the times was a reference of identification due to its hyperecogenous feature. Superficial inguinal lymph nodes needed a shorter detection time in most of the cases, compared with axillary lymph center. For the axillary lymph center, axillary accessory lymph nodes were easily detected when the thoracic limb was pushing forward because they are placed caudally by olecranon, right under the skin muscle of the thoraces. The olecranon was a point of reference.

At one, two or even three cm (in the case of two large sized subjects-one Rottweiler and one Pit-bull), ventrally from the origin of the thoracodorsal artery, with hypoeecogenous feature and vascular signal, proper axillary lymph nodes were detected. The popliteus lymph center was detected in all the subjects due to its facile approach in the popliteal space. Transversal and longitudinal sections were achieved. Lymph nodes were detected in the popliteal conjunctive tissues placed on the descendant branch of the caudal femoral artery. At ultrasound examination, this artery had a hypoeecogenous feature presenting a vascular signal at Doppler examination. In most of the cases (nine of eleven), the popliteus lymph center had one lymph node and in two
cases it had two lymph nodes. For the cervical superficial lymph nodes, approach was made on the median third of the neck, in the back side. The cervical superficial lymph node is placed in front of the supraspinatus muscle, close to the cervical superficial artery.

These lymph nodes are relatively easy to identify, if the cervical superficial area is appropriately prepared. They were identified at nine subjects, the majority being double (in seven of nine cases). The submandibular lymph center was identified by ultrasound in the submandibular triangle, ventral-caudaly from the angular process of the mandible. It was composed of two lymph nodes in four subjects and three lymph nodes in three subjects, right under the skin. In four cases, the submandibular lymph nodes were not detected. Caudal by the submandibular lymph node, the parenchymal structure of salivary glands was detected. On ultrasound examination these glands have a homogenous ecostructure, lightly hyperecogenous compared with lymph node ultrasound pattern. Cranialy by these lymph nodes the facial vein was detected, with hyperecogenous pattern.

![Image](image1.png)

**Fig. 1** Axillary lymph center composed by two lymph nodes and the macroscopic view after blue dye injection

The shape of axillary lymph nodes was oval, with a well-defined capsule which clearly delimits the lymph nodes from adjacent tissues. Lymph node parenchyma was homogenous, hypoeocogenous or isoecogenous with surrounding tissues. Centrally, lymph node hilus appears linear, hyperecogenous and with a slightly bold aspect, corresponding to blood vessels and supporting fibrous pocket. Axillary lymph node dimensions were variable, determined by measuring the two axes: transversal (short axes) and longitudinal (long axes), ranging between 0.3 / 1.2 and 0.9 cm / 2cm. Ratio between short and long axis was in favor of the longitudinal (less than 0.5, average 0.48.). Same oval shape and composition of lymph center was detected after injection of blue dye solution.

Using ultrasound examination, superficial inguinal lymph nodes appear as a group, composed by two lymph nodes, oval in shape and dimensions ranged by 0.4/1.5 to 1.7/3.5cm, well defined by a smooth capsule and homogenous ecostructure. Size of lymph nodes were determined by measuring the two perpendicular axes, transverse axis (short), and longitudinal axis (long), as in axillary lymph center.

In the popliteal region the structures located posterior to the knee joint were imaged respectively: aponeurotic plane, adipose tissue, vascular-nervous package, popliteal lymph nodes.
Ultrasound image guidance presents some particularities resulting from specific ultrasound approach, in the popliteal space. In cross section, in the top of the screen posterior structures will be located and in the bottom of the screen anterior structures will be located. In longitudinal sections the image remains in the same orientation, but in the left of the screen cranial structures will be located and in right side those located caudal. Compared with axillary and superficial inguinal lymph nodes, popliteal lymph nodes present a less oval shape, axis ratio was between 0.49 and 0.65 (average 0.59), with the same sonographic features as in axillary and superficial inguinal lymph nodes.

Fig. 2 Grey scale ultrasound of superficial inguinal lymph nodes and appearance after blue dye injection

Submandibular lymph nodes have dimensions between 0.55 / 1.4 cm, with an axis ratio below 0.5. They were found cranially by their enrollment next to the lingual vein, (which have an hypoecogenous feature) and salivary glands. Cervical superficial lymph nodes were rated on the same criteria as above described lymph nodes. Sizes ranged from 0.4 / 1.1 cm and 0.7 / 1.9 cm, with an axis ratio of 0.51. Ecostructure, echogenicity, characteristics of the capsule and hilum were framed in descriptions of other lymph nodes.

Fig. 3 Color Doppler technique on superficial cervical lymph nodes and their appearance after blue dye injection
Vasculature lymph nodes were assessed by ultrasound Doppler techniques. Information about the presence, direction and mode of vascular distribution into the lymph nodes was obtained. Thus, in lymph nodes bigger than 0.5 cm, vasculature was noted in 84% of lymph nodes, layout of the hilum, ordered toward the lymph nodes periphery.

Small lymph nodes, smaller than 0.5 cm showed no vascular signal (38% of cases). Easiest Doppler techniques were performed on the superficial inguinal lymph center. Power Doppler technique detected more accurately the vascular signals in small vessels of superficial inguinal lymph nodes, popliteus, and submandibular lymph nodes. This technique was applied with difficulty in the axillar lymph center.

Twelve hours later, a regional dissection was performed in order to visualize the lymphatic drainage of blue dye injected in mammary glands. According to it, lymphatic drainage of the mammary glands was performed in two ways: cranial and caudal by the axillary lymph center and superficial inguinal lymph nodes, dependent on the injected mammary gland.

Thus, cranial thoracic and abdominal mammary glands were drained cranial by axillary lymph nodes in five cases and in one case by axillary lymph node and superficial cervical lymph nodes simultaneously. In caudal direction lymph nodes were well impregnated with dye, superficial inguinal lymph nodes in all cases of the drainage of cranial abdominal mammary glands, and the inguinal one. Lymph node shape was oval having a well-defined capsule, crossed in 2-3 spots by afferent lymphatic vessels. In one case we saw a few lymphatic vessels crossing the white line establishing connections between contralateral superficial inguinal lymph nodes. No connections between healthy mammary glands on ipsilateral site were found. Popliteal lymph nodes and the submandibular ones were poorly impregnated with blue dye to a single subject.

In the present study it was demonstrated that the ultrasound can provide valuable information on normal appearance of superficial lymph nodes of carnivores. Lymph nodes are difficult to approach structures. Learning takes time and perseverance and not least experience (Badea et al., 2004, 2010). Description of a lymph node as normal or pathological requires knowledge about the region that it drains, their anatomical topography and normal appearance, and data on variability within the same species (Stan F., 2009). Being located in intermuscular connective spaces or embedded in adipose tissue, the difference in acoustic impedance is low. There are less echogenic interfaces and reflection is weak. Superficial lymph nodes evaluated in the present study belong to the lymphatic drainage of mammary glands in carnivores.

These were located in the subcutaneous tissue, relatively shallow, which facilitated approach on one hand, but on the other hand they were available only after adding the amount of ultrasound gel. Too superficial location presents its disadvantages in the examination process due to small surface that ultrasound must pass through. Aspect is related to the evaluation of submandibular lymph nodes. The most difficult to image were axillary lymph nodes due to their deepest position and embedding in adipose tissue, which echogenicity is similar to the lymph node. For deep located lymph nodes, ultrasound approach is hampered by tissue overlay, transmitted image quality also being affected. Deep located structures require long time for exploration and for each pulse ultrasonic reception.

In present study, lymph nodes shape was oval, elongated, axis ratio was <0.5 which corresponds with data obtained by other researchers (Mayer et al., 2010). In a study in which were compared normal and pathological lymph nodes, it was showed, that the ratio of the two axes is significantly increased in pathological lymph nodes, especially by increasing the value of short axis (DE Swarte et al., 2011). These researchers have found that inflammatory or metastatic
Lymph nodes tend to be rounded, without being able to make a distinction between the two categories based solely on this criterion. In human medicine, in skin malignant melanoma and breast cancer, ultrasound is the method of first choice in evaluation and staging. Badea et al. have shown that lymph nodes ultrasound in metastatic disease provides important data for staging. They showed that the shape, distribution mode of vascularization, echogenicity and ecostructure are profoundly changed (Badea et al., 2010; 2006). In our study only the normal lymph nodes were imaged. Their size was directly related to body weight.

Thus, the large lymph nodes were the superficial inguinal ones, axillary and at least the cervical superficial lymph nodes. Even in the same lymph center, size varies, depending on the number of lymph nodes that compose it (Stan et al., 2010; Stan, 2009), and the presence or absence of components: proper axillary lymph node was larger in the absence of accessory lymph node. Size of lymph nodes evaluated in the present study, was determined by measuring perpendicular axes, in accordance with the requirements of human medicine.

In the absence of diseases, ratio of the transverse and the longitudinal axis must be less than 0.5. In the absence of accessories, axillary lymph nodes have sizes between 0.5 / 1.4 cm up to 1/2, 5 cm, and the accessories when they were present, had dimensions of 0.2 / 0, 7 cm to 0.7 / 1.5 cm. This is due to the lymph nodes’ need to drain from a large region. A correlation can be made from these determinations for the purposes of assessing lymph shape: ie. at ratio below 0.5 lymph node is oval shaped, normal, whereas increasing this value, tends to be rounded, which implies the presence of a disease. Lymph nodes visualized by injecting blue dye Evans showed the same size as the ultrasound assessment. Furthermore, as described at ultrasonography, oval shape was recovered at regional stratigraphic dissection (Stan et al., 2007; 2005).

Lymph nodes parenchyma evaluated in the present study was homogeneous, isoecogenous or reduced as compared with hilum or capsule lymph node echogenicity. Higher echogenicity in the hilum is the result of interlocking medullary sinuses at this level, each behaving as an acoustic interface, which partly reflects giving ultrasound hyperecogen appearance. Also, supporting conjunctive sheath has the same acoustic behavior. However, hilum echogenicity was determined in nine of eleven specimens.

In normal lymph nodes, echogenic capsule was delineating well the lymph nodes from the surrounding tissues, fact confirmed by dissection followed by ultrasound examination. Moreover, the fact that at regional dissection, lymph nodes were presented surrounded by adipose tissue, confirms the ultrasounds well visualization of lymph node capsule.

Lymph node vasculature was evaluated by applying Doppler methods. Applying this method in our study, we obtained dynamic images, focused and integrated on functional and anatomical criteria on blood flow characteristics: present of vascular flow and type into the lymph node distribution. The blood flow from the hilum, was noticed in 84% of examined lymph nodes. Hilum is actually normal blood vessel approaches, or in small lymph nodes, hilum is hardly detectable. It can be said in this context that normal lymph nodes vasculature may be more easily detected in larger nodes. In these lymph nodes, blood vessels were visualized at the hilum, with trajectory and direction toward the capsule, being parallel to the longitudinal axis of lymph node, or, parallel to skin surface. We can attribute this observation to the fact that normal superficial lymph nodes were evaluated in this study. Vascular signal was not detected in small superficial cervical lymph nodes and axillary accessories. This corresponds with data from the literature on human lymph nodes, upon which the vasculature of the hilum can be visualized only in node with transverse diameter greater than 5mm (DE Swarte et al., 2011; Mayer et al., 2010).
At power Doppler superficial lymph nodes evaluation, the color appearance of relatively large area image was based on high sensitivity of this technique, which was capable to detected much slower blood flow in small vessels compared with color Doppler technique. This technique has been applied successfully in the superficial inguinal and popliteus lymph node. In axillary, superficial cervical and submandibular lymph nodes, we encountered difficulties in applying this technique because their location near pulsation vessels, proximity of heart, and in case of axillary nodes respiratory movements which led to the unpredictable artifacts.

CONCLUSIONS

In this study we used as golden standard the morphological detection method of superficial lymph nodes by injecting blue dye solution. Basically, combining the two methods, imaging and morphological, we responded to the need for knowledge of normal feature of superficial lymph nodes in carnivores. As in human medicine, it is required to combine several methods to make a lymph node investigation. Ultrasound may be the method of first choice, because its noninvasiveness in lymph nodes evaluation. In veterinary medicine, cytological and histological assessment of presumptive pathological lymph nodes is made by surgical excision. Ultrasound guided biopsy can be easy and minimally invasive alternative in assessment of pathological carnivores lymph nodes.

REFERENCES