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Morphological Identification of Lymph Node Vasculature Using Contrast Enhanced Ultrasonography (CEUS)

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Abstract. Small superficial normal canine lymph nodes often appear as vascular at classic Doppler technique evaluation. The aim of present research is to describe the characteristics of lymph nodes vasculature after administration of the contrast agent. Contrast enhanced ultrasonography (CEUS) was performed on a total of 6 subjects, after intravenous administration of SonoVue (Bracco – Italy), containing stabilized micro bubble of sulphur hexafluoride. The sentinel lymph nodes of the mammary gland, the axillary and superficial inguinal lymph nodes were examined. In advance, for comparison, the same lymph nodes were evaluated using color Doppler and power Doppler. Imaging characteristics followed after contrast agent administrations were: the beginning of contrast agent in lymph node penetration (time to wash in), maximum intensity time of visualization of contrast agent and time when wash out is complete. Besides these periods were followed, echogenicity, ecotexture of lymph nodes, during phases mentioned above. Also the way of lymph nodes parenchyma filling was followed, during the same periods. In normal lymph nodes, contrast wash in phase was viewed at 7 sec from the time of injection, with a maximum intensity of micro bubble visualization, at 13 sec. Normal lymph nodes have a central artery with an hilar centrifugal branching oriented towards the capsule. Lymph node angioarchitecture was best visualized after intravenous contrast agent administration, compared with Doppler techniques. The results of this preliminary study shows that CEUS is a noninvasive method witch can be successfully applied to describe the lymph node vasculature in carnivores, making an improved visualization compared with conventional technique.

Keywords: lymph nodes, canine, ultrasonography, CEUS, micro bubble, contrast agent

INTRODUCTION

Classic methods for identification of lymphatic system in carnivores are based on invasive methods (Pereira CT, 2008; Oz et al. 2012). In recent years one of the noninvasive method to assess the anatomical pattern of lymph nodes is ultrasonography (Tawni et al. 2012; Nyman et al. 2005)). Among these methods, CEUS using different sonographic agents have gained more interest during the past years (Cosgrove D., Eckersley R., 2006, Severet al. 2012).

Based on our previous research in which we demonstrated the great variability of lymphatic drainage of the mammary glands in the bitches, (Stan, 2009, Stan et al. 2010), we hypotheses that contrast agent, could detect the sentinel lymph node vasculature more accurate. SonoVue is a second generation of contrast agent, containing microbubble of an inert gas-sulphur hexafluoride, stabilized by a phosphor lipidic membrane) useful for the detection of hepatic
lesion when is intravenous injected (Badea et al. 2007, Sparchez et al. 2009). CEUS with intravenous administration has the capacity to show the microvasculature and perfusion of various parenchymas (Lorrie Gaschen 2010, Badea et al. 2006, 2010). Goldberg et al., showed that micro bubbles administrated peritumourally in a swine melanoma model were seen to enter in lymphatic channels and sentinel lymph node were identified by sonography (Goldberd et al. 2004). More recently, Sever et al., shown that with CEUS, sentinel lymph nodes were correctly identified in more than 90% of female breast cancer patients (Sever et al.2009).

MATERIALS AND METHOD

The study was conducted on a group of six female dog, of different breeds and ages between 2 to 8 years, weighing between 5 and 12 kg. Specimens were consulted, being declared clinically healthy. The study enrolled only subjects without overt disease.

SonoVue (Baracco, Italia) is second generation of contrast agents, was intravenous administrated. It contains sulfur hexafluoride micro bubble, stabilized by a phospholipids membrane with dimensions less than 5 microns, which, when injected intravenously leads to an increase of echoes wherever it may be. Prior to examination, each subject was fitted with a catheter on the radial vein of the left thoracic member.

Palpation and clinical identification of the target lymph nodes was followed by grey scale ultrasound examination and Doppler techniques, using a 12 MHz transducer. All the data. To get optimal protection during ultrasound examination with contrast agents, the animals received neuroleptanalgezy by taking Acepromazine (0.5 mg / kg / bw) and ketamine (3mg/kg/bw).

Contrast agent was reconstituted by adding 5 ml saline followed by gentle swirling of the bottle, so that each ml would contain on average (1-5) x108 micro bubbles. It has been shown that micro bubbles with an average size between 3 and 99 μm have the best acoustic efficiency.

Amount of contrast agent administered was 0.5 mL of reconstituted solution, followed by a bolus of 10 ml saline. The soft parts program and a linear probe of 4MHz were used, and dedicated software with pulse inversion specific to contrast agents (contrast software).

Mechanical index was below 0.2. The followed imaging characteristics after injecting the contrast agent were: -the time at which the contrast agent enters the lymph node (time to wash in) -the time of maximum intensity of contrast agent visualization -the time at which the lymph nodes’ wash is complete (time to wash out) Besides these periods were followed: -the lymph nodes’ ecogenicity and ecostructure during the above listed phases -lymph angioarchitecture during the same period, while following these vascularization parameters:

- the dislocation of central hilar vessels;
- the presence of vessels with a disorderly, aberrant distribution;
- presence of subcapsular vessels that arise from the capsule, which do not communicate with the central longitudinal or side dishes;
- presence of adjacent or outsider perlimfondale lymph vessels;
- avascular appearance in large lymph nodes

RESULTS AND DISCUSSIONS

All the lymph nodes evaluated before the contrast administration in grey scale mode, presented an oval shape, an axis ratio less than 0.5, homogenous ecostructure, were hipoecogenous with a smooth hyperecogenous band in the hilum region.
The vascularization evaluated with Doppler methods presented as being ordered, with a starting point in the hilum area.

Axillary and inguinal superficial lymph nodes were watched after SonoVue administration for 3 minutes. Obtained data was noted and the evaluation was registered. The visual effect of the contrast agent going into the lymph nodes took place after an average of 7 seconds after intravenous administration. The maximum intensity of lymph node filling took place, in average 14 seconds after administration.

After 2 minutes, the lymph node was completely washed. There was no contrast agent remaining in the lymph node parenchyma. On 5 subjects of the present lot, completely filled lymph nodes after administration of contrast agent appeared. In one case, the incomplete filling of
the proper axillar lymph node was noted. In complete filling the propagation of SonoVue in lymph nodes occurred at the hilum, making it highly hiperecogen, following centrifugal propagation in medullar and cortical parenchyma, then up to the capsule.

Lymph node angioarchitecture was best visualized after contrast agent administration. Immediately after entering the lymph node, there was a clear view of the central artery, with its centrifugal branching through small, but well defined blood vessels, arranged perpendicular to the longitudinal axis of the lymph. Viewing these vessels with a route ordered by the hilum took very little, followed by a homogeneous distribution of contrast agent in the parenchyma, which sold out in time, followed by lymph washing, opposite from the filling.

After intravenous administration of contrast agent, no sign of intolerance has been shown at any subject. Amount of contrast agent administered was 0.5 mL of reconstituted solution, followed by a bolus of 10 mL saline. The small parts program and a linear probe of 4MHz were used, and dedicated software with pulse inversion specific to contrast agents (contrast software). Mechanical index was below 0.2.

Fig. 3 Wash out fazes at 30 sec after SonoVue administration, and complete wash out at 1min30sec

Axillary and inguinal superficial lymph angioarchitecture has proper characteristics which we revealed by color Doppler ultrasound. Blood vessels are visualized starting in the hilum area, going through the capsule, being parallel with the longitudinal lymph ax or parallel with the skin surface. Out of the longitudinal vessels branches with radial distribution into capsule detach. All blood vessels in normal lymph nodes come from the hilar vessels of the lymph node, presenting an ordered character. The accuracy of the method consists in obtaining two-dimensional information in real-time regarding the characteristics of blood flow in lymph nodes.

Applying the power Doppler imaging method, in the evaluation of the first lymph nodes that drain mammary glands, following the principles of this technique (Cosgrove D., Eckersley R., 2006, Lorrie Gaschen, 2010), we analyzed the power and intensity of Doppler signal without pointing out blood flow direction or speed. Exploring the superficial inguinal lymph nodes using the power mode, coloring of the image, on relatively large areas, was given by the high degree of sensitivity of this technique, which detects vascular presence and flows much slower in tiny small vessels, compared with color Doppler technique.

Also, using power Doppler technique, we were able to identify specific tortuous vascular structures of lymph nodes, regardless of the angle of approach of vessels.
The presence of color attests the presence of blood vessels and hue defines the number of reflectors participating in Doppler signal generation. The major disadvantage of this exploration, for the neighboring lymph nodes of pulsating blood vessels, or near the heart, is the tissue movements that can induce multiple artifacts. Using the two methods (power and color Doppler) we have defined the quantity (vascular signal on a larger or smaller area) and quality (normal vs altered aspect) differences of the blood flow in lymph nodes.

Identification of metastases in sentinel lymph node in cutaneous melanoma and human breast cancer, proved to be very useful in disease staging and determining prognosis. Furthermore, therapeutic attitude is based on spreading metastasis, primary in the first draining lymph node—the sentinel lymph node (Sever et al. 2009, Takei et al. 2012).

Identification of metastases in sentinel lymph nodes in cutaneous melanoma and human breast tumors also proved very useful in staging the disease, adaptation to specific therapies and determining prognosis (Badea et al. 2007, Torres et al. 2011). However, sentinel lymph node detection methods are invasive. Indirect lymphography by peritumoral dye injection and dissection involves besides alleged surveying areas of lymph, biopsy or excision too. If the biopsy is done and histopathological examination reveals tumor cells, a second surgery to remove the lymph is required (Takei et al. 2012, Torres et al. 2011). In addition, the dye may identify other adjacent lymph nodes, which often lead to unwarranted excision, more than necessary.

The second method, which uses radioactive pharmaceutical substances with affinity for lymphatic tissue and intraoperative detection of lymph nodes also has disadvantages.

A major one is related to irradiation, the next being that radiopharmaceutical are made of tiny particles that can easily pass the sentinel lymph node lymph node and identify the next stop, which is not actually the sentinel. Moreover, none of those techniques can determine the absence or the presence of metastases in lymph nodes.

The technique that aims to revolutionize the methods for detecting sentinel lymph node due to efficiency, accuracy and especially its safety is identification of sentinel lymph nodes using ultrasound contrast agents (Gelb et al. 2011, Sever et al. 2010).

Ultrasound contrast agents are used in spherical micro particles with a diameter less than 7 microns, which are able to cause an amplification of the reflected signal, well above the surrounding tissue. Contrast agents from the second generation (SonoVue-used in our study) are small, contain an inert gas, sulfur hexaflouride, stabilized in a membrane lipoprotein.

They pass easily through the pulmonary capillaries circulation and are distributed and can be found in capillaries of various organs, increasing their echogenicity (Cosgrove D., Eckersley R., 2006, Sever et al. 2012, Gelb et al. 2011) In human medicine, contrast agents were used for the first time in evaluating liver tumors yielding results comparable to those obtained from CT examinations. The next step was relatively simple, namely the extension of the investigation performance in identifying sentinel lymph node in breast tumors.

Compared with conventional ultrasound techniques, ultrasound contrast agents are more specific in that they have the ability to detect small intraparenchimal vessels of lymph nodes. Free blood vessels in lymph nodes are too small to be detected with conventional Doppler methods, and sometimes may appear avascular or only a small central visible vessel. CEUS displays the signal from the contrast agent that enhances small lymph vessels’ image.

In the present research, superficial inguinal and axillary lymph nodes followed at the contrasts’ administration had a very short time until filling (6-7 sec), corresponding to an early arterial phase, which quickly passed from the parenchymal phase to the capillary with maximum visibility of 12-14 sec. Due to the application of mechanical index below to get signal from
contrast agent, a small-time agent broke, which explains the delayed washing phase. Slow decrease of intensity over time to 1 min and over is due to parenchyma filling and micro bubble persistence, because they are not immediately destroyed by appropriate settings of a now mechanical index.

There are authors who reported quantitative quantification of pixel intensity but these measurements are dependent on device settings related to the gain, depth, and software applications may vary, or, in this study we received several types of apparatus for carrying out examinations with contrast (Sever et al, 2010, Stan et al.2010). Therefore, we considered parameters related to time during the filling begins, the maximum time in which lymph node is fully loaded with contrast agent during washing are suitable reporting and comparison, and the conditions are not dependent device or software used.

In normal lymph nodes, lymph node contrast agent enters at the hilum to seeing the great central arterial vessel, which immediately branches centrifugal collaterals into the lymph node that go up to the capsule Appearance is homogeneous when lymph node maximum filling is reached and the lymph node is completely enhanced. Washing out phase begins in reverse to the filling phase, centripetal towards the hilum of the lymph with mentioned reminiscence in the parenchyma and in the hilus in larger vessels.

In the case of superficial inguinal lymph nodes, the motion artifacts have not affected the images’ quality due to their caudal sighting and the fact that the subjects were sedated. Doppler contrast agents and techniques, especially Power Doppler, are very sensitive to motion, and we had difficulties in exploring the axillary lymph nodes in one subject, the more they are located near the dorsal thoracic artery whose pulse causes artifacts.

A limitation of this study is the lack of cytological or histopathological evidence of normality for the evaluated lymph nodes. Of six evaluated cases, a histopathological exam was done to 4, which confirmed the histological normal structure of lymph nodes. However, all the subjects which have been submitted to contrast evaluation were declared clinically healthy based on history, clinical examination and abdominal ultrasound.

CONCLUSIONS

Contrast enhanced ultrasound is a practical method to assess superficial lymph nodes. Due to its unique characteristics which includes: real time lymph node visualization, possibility to repeat the technique, easy and repetitive measurements, no need for radiation product, CEUS, is a valuable tool in morphological assessment of superficial lymphatic drainage in carnivores.

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