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Medial iliac lymph node as a target for metastasis in bitches with malignant mammary neoplasms

João Pedro Scussel Feranti[1,2] Gabriela Pesamosca Coradini[2] Marcella Teixeira Linhares[2] Rammy Vargas Campos[2] Felipe Baldissarella Gavioli[2] Bernardo Nascimento Antunes[2] Marília Teresa de Oliveira[1] Maurício Veloso Brun[2*]

[1]Curso de Medicina Veterinária, Universidade Federal do Pampa (UNIPAMPA), Uruguaiana, RS, Brasil.
[2]Departamento de clínica de pequenos animais, Universidade Federal de Santa Maria (UFSM), 97105-900, Santa Maria, RS, Brasil. E-mail: mauriciovelosobrun@hotmail.com. *Corresponding author.

ABSTRACT: The regional lymphatic connections of the mammary glands in canines are poorly investigated, both in healthy animals and in those with mammary neoplasms. The objective was to establish the occurrence of metastases in the medial iliac lymph nodes (MILN) in bitches with mammary neoplasm, in addition to evaluating the effectiveness of the technique of lymphatic labeling of the MILN, administering methylene blue (MB) dye intrauterinely or intradermally. Thirty-two bitches with tumors in different mammary glands were included in the study. In 13 bitches (GU group), the MILNs were stained with intrauterine MB, followed by unilateral lymphadenectomy, ovariohysterectomy (OVH) and unilateral mastectomy. Nineteen bitches (GI group) underwent OVH, followed by MILN staining with intradermal MB, lymphadenectomy, and unilateral mastectomy. In GU, 11 bitches (84.62%) exhibited MB-stained MILNs, but staining was not observed in two (15.38%). Metastasis to MILNs was observed in three bitches (9.38%) from the GI and two (6.25%) from the GU. Intrauterine and intradermal staining of MILNs in bitches with mammary tumors was efficient, but intradermal administration of MB produced better results. Given metastatic spread to MILNs in approximately 15.63% of patients, we recommend that screening for MILNs should be considered as part of the staging of animals with malignant mammary neoplasms.
Key words: cancer, canine, lymphadenopathy, mammary neoplasms, methylene blue.

Linfonodo ilíaco medial como alvo de metástase em cadelas com neoplasmas mamários malignos

RESUMO: As conexões linfáticas regionais das glândulas mamárias em caninos são pouco investigadas, tanto em animais sadios quanto naqueles com neoplasmas mamários. Objetivou-se estabelecer à ocorrência de metástases nos linfonodos ilíacos mediais (MILN) em cadelas com neoplasma mamário, além de avaliar a eficácia da técnica de marcação linfática dos MILN, administrando o corante azul de metileno (MB) por via intrauterina ou intradérmica. Trinta e duas cadelas com neoplasmas malignos em diferentes glândulas mamárias foram incluídas no estudo. Em 13 cadelas (grupo GU), os MILNs foram corados com MB intrauterino, seguido de linfadenectomia unilateral, ovariohisterectomia (OVH) e mastectomia unilateral. Dezenove cães (grupo GI) foram submetidos a OVH, seguido de coloração MILN com MB intradérmico, linfadenectomia e mastectomia unilateral. No GU, 11 cães (84,62%) exibiram MILNs corados com MB, mas a coloração não foi observada em dois (15,38%). Metástases para MILNs foi observada em três cães (9,38%) do GI e em dois do GU (6,25%). A coloração intrauterina e intradérmica de MILNs em cadelas com tumores mamários foi eficiente, mas a administração intradérmica de MB produziu melhores resultados, observando a coloração de 100% dos linfonodos no GI e 84,62% no GU. Dada a disseminação metastática para MILNs em aproximadamente 15,63% dos pacientes, recomendamos que a investigação dos MILNs deve ser considerada como parte do estadiamento de animais com neoplasmas mamários malignos.
Palavras-chave: câncer, caninos, linfadenopatia, neoplasmas mamários, azul de metileno.

INTRODUCTION

Most mammary gland tumors are malignant and metastases to regional lymph nodes are observed in 25% at diagnosis, in addition to occasional metastases to the lung parenchyma (BESERRA et al., 2013). There are several characteristics of neoplasms that are predictive of prognostic factors for the patient, such as tumor size, presence of adhesions in adjacent structures, ulceration, as well as the status of satellite lymph nodes (HÖRNFELDT & MORTENSEN, 2023).

The lymphatic system plays a crucial role in local control of tumorigenesis and in the transport of tumor cells, which is the most common process of metastatic spread (BRENET et al., 2013). While many studies investigate the mammary lymphatic system of dogs, this system has been actually poorly assessed regarding the communication between mammary

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lymphatic chains, both in healthy animals and in those with mammary gland tumors (COLLIVIGNARELLI et al., 2021).

Although, lymphoscintigraphy is considered the gold standard for lymphatic mapping, it can be studied in different ways, with its advantages and disadvantages. It is cited as methods of lymphatic mapping of the mammary glands, CT lymphography, radiographic lymphography, scintigraphy, as well as the use of vital dyes such as patent blue and methylene (BARBAGIANNI & GOULETSOU, 2023; BEER et al., 2023; FERANTI et al., 2018; SOULTANI et al., 2021).

SOUZA et al. (2016) evaluated intra-abdominal inguinoiliac lymphadenectomy in healthy animals after intradermal lymphatic staining around the inguinal mammary gland with methylene blue (MB) and indocyanine green; however, they did not succeed in harvesting the lymph nodes. The relationship between medial iliac lymph nodes (MILNs) and drainage of uterine horns and corpus was assessed by JUSTINO et al. (2014) with the aim of helping establish an early diagnosis of uterine disorders, highlighting the fact that the iliac lymph node chain receives most of its drainage via the uterine horns.

Pelvic lymphadenectomy is considered the most accurate staging procedure for prostate cancer in humans and some studies have addressed the therapeutic possibility of this technique, despite the lack of consistent data. It is widely known; however, that lymph node status and distant metastases are strongly associated and; consequently, lymph node involvement is an important indicative sign of metastatic potential in cancers (DE NARDI et al., 2016; JEUDY et al., 2008; PEREIRA et al., 2003).

The present study determined the presence of MILN metastases in female dogs with mammary tumors to assess the efficiency of MILN staining with intrauterine or intradermal administration of MB.

MATERIALS AND METHODS

Dog population

Animals with mammary gland tumors for which mastectomy was recommended as treatment option were selected. A total of 32 female dogs, with malignants neoplasm (ulcerated or not) in different mammary glands, were included in the study. Animals with mammary neoplasms only in the cranial thoracic and/or caudal thoracic breasts were excluded from the study. Of all dogs evaluated and excluded, none had sonographic evidence of metastases in MILNs. As a way of assisting in the inclusion and exclusion of patients, a cytological examination of the primary tumor was carried out in all patients using fine needle aspiration biopsy.

Prior to the surgical procedure, the patients underwent clinical, laboratory, and imaging evaluations. Preoperative tests included complete blood count and biochemistry tests for determination of creatinine, urea, albumin, alanine-aminotransferase (ALT), and alkaline phosphatase (ALP), in addition to screening of metastization by chest radiographs in three positions and by abdominal ultrasound. Surgically neutered animals or those whose abdominal ultrasound and chest radiograph suggested metastization were excluded from the study.

Anesthetic and analgesic protocol

Fifteen min after premedication (0.3 mg/kg of methadone intramuscularly (IM)), Ringer's lactate solution (5 ml/kg/hr intravenously (IV)) was administered as fluid therapy, and propofol (4 mg/kg IV) was used for anesthetic induction. Anesthetic maintenance consisted of inhaled isoflurane using 100% oxygen in a circuit without rebreathing.

The dogs were placed in dorsal recumbency and the dorsalis pedis artery was punctured using a 24G catheter connected to a pressure transducer for invasive measurement of systolic blood pressure (SBP), mean arterial pressure (MAP), and diastolic blood pressure (DBP) in a multiparameter monitor. The parameters were recorded every 10 min. If SBP and heart rate increased by more than 20% of the baseline value, fentanyl sulfate (5 µg/kg IV) was used for rescue analgesia.

At the end of the surgery, an infiltrative local block with bupivacaine (4 mg/kg) was performed around the surgical wound for postoperative analgesia. The following medications were used: meloxicam (0.1 mg/kg once daily for 4 days), tramadol (5 mg/kg four times a day for 5 days), and dipyrone (25 mg/kg three times a day for 3 days), the first dose of which was given intravenously, with subsequent doses administered subcutaneously.

Surgical procedures

All patients were positioned in dorsal recumbency and had a urinary catheter (Foley 10 Fr) placed for bladder emptying, which was maintained throughout the intraoperative period. Pre-surgical asepsis was performed with 2% chlorhexidine followed by 0.5% chlorhexidine-alcohol. All surgical procedures were always performed by the same surgeon and anesthetist with expertise in the proposed technique.

The patients were randomly (by draw) divided into two groups (GI and GU). In GU (13 animals), lymphatic staining with 1% MB® (Injectcenter, São Paulo, Brazil) injected into the uterus (intramurally into the uterine corpus) (Figure 1A), followed by MILN excision, ovariohysterectomy (OVH), and unilateral mastectomy, was performed after ventral midline celiotomy and exposure of the uterine corpus. In GI (19 animals), OVH by ventral midline celiotomy was initially carried out, followed by lymphatic staining with 1% MB given intradermally (around M5) and subsequent MILN excision, celiorrhaphy, and later unilateral mastectomy.

All animals received 0.5 mg/kg of 1% MB. Lymphadenectomy (left or right) varied, depending on the involvement of the mammary chain. In those animals with tumor in the right mammary chain, the right iliac lymph node was excised, whereas those with tumor in the left mammary chain had the left MILN excised. After MB administration, in both groups, a maximum period of 20 minutes was waited to conclude the effectiveness of lymph node marking.

Soon after MB injection in both groups, the blue-stained (or unstained) MILN was identified (Figure 2A,C), displacing the descending colon laterally, and the lymph node was dissected next to the ureter, below the retroperitoneal adipose tissue, at the bifurcation of the iliac artery, using gauze and a Crile curved hemostatic forceps (Figure 2B,D).

All excised tissues (ovary, uterine horns and corpus, MILN, mammary chain) were harvested and sent for histopathological analysis immediately after the surgical procedure and for establishment of any correlation between the histological findings on intracavitary lymph nodes (in case any of them contained tumor cells) and those of mammary gland tumors and of extracavitary regional lymph nodes.

RESULTS

Patient characteristics

A total of 32 female dogs aged 9.6±2.3 years, weighing on average 10.9±4.4 kg were included. The mammary tumors affected heterogeneous sites. Single tumors were observed in 24 and multiple tumors in eight animals. Among all patients, the inguinal mammary gland (M5) was the most commonly affected site (40.5%), followed by caudal abdominal (M4) (33.3%), cranial abdominal (M3) (14.3%), and caudal thoracic (M2) (11.9%) glands. Sixteen neoplasms were ulcerated.

MILN staining results

In GU, right after administration of the dye, it was possible to visualize the blue-stained lymphatic vessels of the uterine corpus and mesometrium (Figure 1B) and, 1 min after MB administration, we sought to identify the MILN on the side affected by the tumor. Among 11 animals in GU (84.62%), the iliac lymph nodes were remarkably stained in blue; however, they were unstained in two animals, but excised after their detection. In GI, in which the dye was injected intradermally around M5, the blue-stained MILN

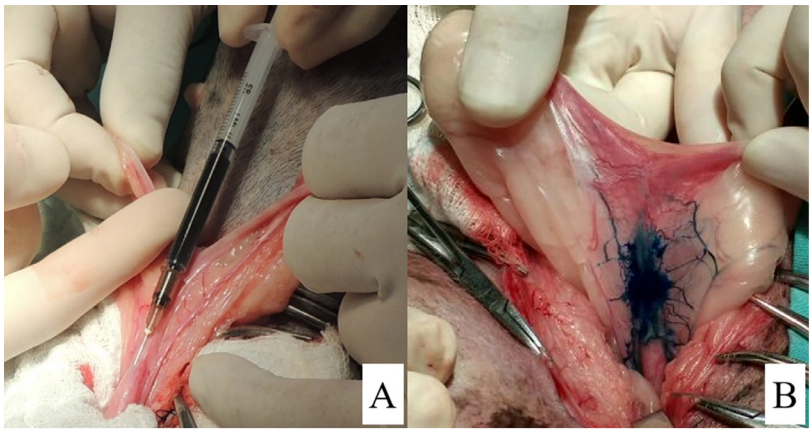

Figure 1 - (A) Exposure of the uterine corpus for insertion of insulin needle coupled to a 1-mL syringe for intraparenchymal administration of 1% MB. (B) Uterine staining and impregnation of lymphatic vessels with 1% MB after intrauterine, intraparenchymatous injection.

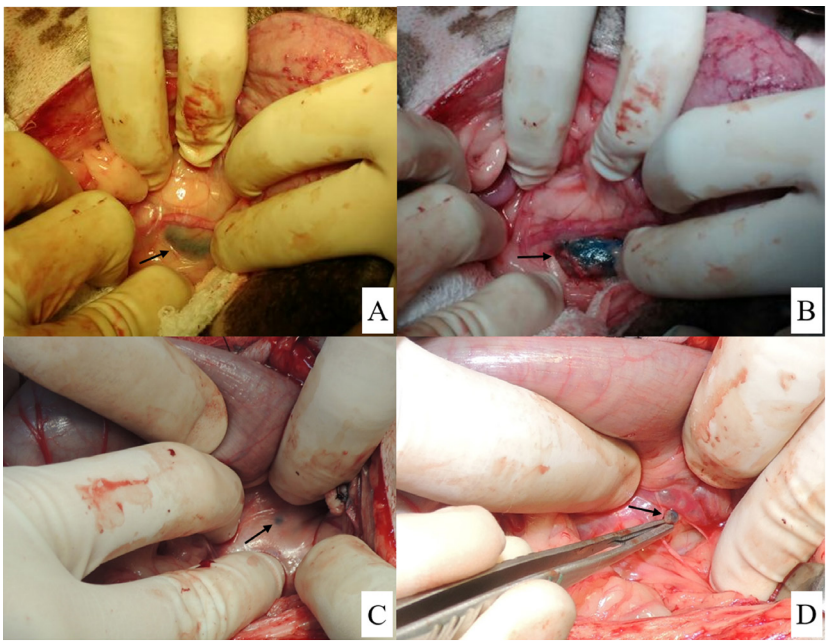

Figure 2 - Visualization of medial iliac lymph nodes next to the ureter after staining and impregnation with 1% methylene blue (MB) in different patients and at different sites. (A and B). Evident lymphatic staining with intrauterine injection of 1% MB in a dog with enlarged lymph node. (C and D) Lymph node of normal size visualized after intradermal injection of 1% MB.

could be seen in all animals (100%) 1 min after MB administration, even when the lymph nodes were enlarged, thus facilitating lymphadenectomy.

Among the animals in GU, five had ulcerated tumor at the time of the procedure, with enlarged MILN in those patients and in two which had no ulceration, totaling seven animals with MILN enlargement. In GI, 12 dogs exhibited ulceration in the mammary tissue, with MILN enlargement in these 12 patients and in another two which had no ulceration, totaling 14 dogs in GI with MILN enlargement.

Histological findings

Among the 32 dogs, in nine cases, the superficial inguinal lymph node was affected by neoplastic cells. Of these, there was metastasis of mammary tumor to MILNs in five (15.62%), however, MILNs were not enlarged in two patients, but the diagnosis was compatible with simple carcinoma metastasis. All lymph nodes with mammary tumor metastases were stained with MB, regardless of presentation. Of the 21 animals with MILN enlargement, there was mammary gland tumor metastasis in three, squamous cell carcinoma in one, and simple carcinoma in two.

Simple carcinoma was the most frequent type of neoplasm (15 animals), followed by complex carcinoma (10), complex adenoma (4), carcinosarcoma (3), squamous cell carcinoma (2), cribiform carcinoma (1) and malignant myoepithelioma (1).

Among the five patients with MILN metastasis, all had neoplasms in M5. In three of them, the neoplasm was detected only in M5, one of them had neoplasms in M2, M3, M4, and M5, whereas another patient had neoplasms in M4 and M5. Three of these animals had ulcerated neoplasms and tumor sizes in M5 varied (3, 4, 8, 10, and 12 cm in diameter).

Based on the clinical staging of canine mammary neoplasms modified by SORENMO et al. (2013), two animals in the present study were classified as stage I (S1), six as stage II (S2), 16 as stage III (S3), and eight as stage IV (S4). Of the eight patients classified as S4 prior to the surgery, five (62.5%) were diagnosed with MILN metastasis in the postoperative histopathologic analysis.

DISCUSSION

To the authors' best knowledge, there is no study to date that recommends MILN excision in cases

of mammary gland tumor. The results obtained herein indicated that MILN excision should be considered as part of the staging of animals with malignant mammary neoplasms and those requiring celiotomy, either for OVH or for other procedure. Studies could elucidate whether abdominal lymphadenectomy (of pelvic lymph nodes and/or of other abdominal lymphatic chains) should or should not be an essential part of mammary gland tumor management in dogs referred to mastectomy, and also whether abdominal/pelvic lymphadenectomy may increase the survival of patients. These issues are important and have to be investigated since, based on our findings, the removal of sentinel lymph nodes closer to the tumor might not be enough in most cases.

The results obtained herein allow us to asseverate that abdominal lymphadenectomy should be considered as part of the staging of animals with malignant mammary neoplasms, since a considerable number of patients may present with intracavitary lymphatic metastases at the time of mastectomy.

Our recommendation for lymphadenectomy is valid regardless of the size, location, and characteristics of lymph nodes, because even if these structures are not neoplastic, lymphadenectomy may help with staging and selection of the best postoperative adjuvant therapy. Conversely, absence of gross findings in the lymph nodes does not rule out the presence of neoplasm.

Not all animals had MILN enlargement, not even those with mammary tumors larger than 5 cm in diameter and/or with ulcerated neoplasms. This underscores the importance of lymphatic staining for visualization and excision of lymph nodes, considering that lymph nodes were not enlarged in two patients but revealed tumor cells in the histopathological analysis.

Understanding of lymphatic flow in animals with metastatic mammary tumors is clinically important as the status of lymph nodes in the clinicopathological analysis is an important prognostic factor given the low survival rate among 85.7% of dogs with lymph node metastases (less than 2 years) (MARANHÃO et al., 2016). The lymph from cranial abdominal mammary glands may drain into the axillary lymph node or into the superficial inguinal lymph node, whereas the lymph from caudal abdominal mammary glands may drain into MILNs (MIYASHIRO et al., 2011), a finding observed in all animals subjected to intradermal staining around the inguinal mammary gland (GI), with blue staining of all lymph nodes, regardless of their size.

There are several methods for the identification of sentinel lymph nodes, such as the use of vital stains, radiopharmaceuticals, or the simultaneous use of both (BEER et al., 2023; CASTELO et al., 2010; PATSIKAS et al., 2006; PINHEIRO et al., 2003; REESE et al., 2016; SORENMO et al., 2013). MB, used in the present study, was chosen due to some characteristics, such as easy acquisition, low cost, and safe use in dogs. No reactions to MB, like the hypersensitivity described in other studies (REESE et al., 2016; SOUZA et al., 2016; SUAMI et al., 2008; SUAMI et al., 2013; SUAMI et al., 2016; SUGA et al., 2007; WAINSTEIN et al., 2015), were observed in the present study.

Sentinel lymph nodes may be stained using a broad array of techniques (CASTELO et al., 2010; COWELL et al., 2003; SUGA et al., 2007). Intratumoral or peritumoral injection of vital stains or radiomarkers is the most widely used technique in canine mammary neoplasms, as perineoplastic inoculation has yielded good results, with quicker contrast drainage (SOUZA et al., 2016). In the present study, both techniques revealed a high rate of staining, however, MILNs were stained blue immediately after intradermal administration in all animals from GI, but no staining occurred in three animals from the GU, probably because of lower uterine lymphatic drainage into iliac lymph nodes compared to the drainage of inguinal mammary glands rather than due to obstruction of lymphatic vessels in the region, as the histological analysis did not show presence of neoplastic cells in these three animals.

Bladder emptying in the preoperative period by placement of a urinary catheter and maintenance of the bladder empty in the intraoperative period were essential for proper manipulation of tissues and visualization of the MILN, since the lymph node is located at the bifurcation of iliac arteries, right below the urinary bladder. There was proper preoperative fasting and reduction of fecal matter, as the colon has to be displaced laterally for proper exposure of the lymph node, aiding with its detection.

In dogs with mammary tumors, lymphatic staining is important for the identification of possible metastases to cellular tissues, indicating the location of lymphatic vessels and regional lymph nodes, thus facilitating surgical excision of these structures with a safety margin. Moreover, lymphadenectomy plays a crucial role, even in the case of lymph nodes that are not enlarged or that have no gross findings, given that tumors have been identified in normal-appearing lymph nodes as well.

For those patients with mammary neoplasms >3 cm in M5 classified as S4, we currently recommend MILN excision. For other animals,

which will be subjected to mastectomy or celiotomy for OVH or other procedures, we recommend MILN excision, regardless of lymph node size, location, and presentation.

CONCLUSION

In conclusion, lymphatic staining of MILN with intrauterine and intradermal MB was efficient in female dogs with mammary tumors, but intradermal administration yielded better results. Therefore, iliac lymphadenectomy should be considered as part of the staging of animals with malignant mammary neoplasms.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was approved by the local Animal Welfare and Ethics Committee (process no. 043/2013), and an informed consent form was signed by the owners after full explanation about the techniques, their risks, and their consequences.

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