Lymph node Staging methods in mammary tumors in female dogs: palpation x sentinel node biopsy

Métodos para Estadiamento Linfonodal em tumors mamários em cadelas: palpação x pesquisa do linfonodo sentinela

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ABSTRACT
Breast cancer is one of the most common cancers in both women and female dogs. The lymphnode condition is one of the most important prognostic factors in both species. The main goals of this search are to determine the role of inguinal and axillary palpation preoperatively, to find the sentinel node detection rate using the blue dye technique and its role in predicting metastasis. Fifty female dogs having mammary tumors were included in this study. The protocol included data collection, clinical examination, blood sampling and radiologic exams before surgery. Blue dye subcutaneous injection was performed just after anesthesia and ten minutes before skin incision. Mastectomy was performed according to the previously established hospital protocol. Eighty-six percent of dogs presented with malignant tumor. Lymph node palpation offered high accuracy, low sensitivity, high specificity, low positive predictive value and high negative predictive value for metastasis detection. The inguinal sentinel lymph node was detected in 92.3% of the female dogs and the axillary sentinel lymph, in 78.9%. Twenty-five percent of all detected lymph nodes contained metastatic carcinoma. Sentinel node detection rate was high both inguinal and axillary, although sentinel metastasis were rare in both stations. The procedure did not cause any additional morbidity. Thus, the optimal protocol according to this study, is lymph node palpation and, in positive cases, the sentinel node biopsy using the blue dye technique.

Keywords: Mammary tumor, staging, Sentinel Node, blue dye.

RESUMO
O câncer de mama é a segunda neoplasia maligna mais incidente em mulheres e a primeira em cadelas, crescendo em importância no cenário mundial. Para otimizar o tratamento das cadelas, é importante acessar a condição dos linfonodos, pois a positividade dos mesmos afeta negativamente a sobrevida. Os objetivos deste trabalho são esclarecer o papel da palpação linfonodal inguinal e axilar no pré-operatório; elucidar a taxa de detecção do linfonodo sentinel, bem como sua capacidade em predizer metástases linfonodais inguinais e axilares em cadelas portadoras de câncer de mama e utilizar as metástase linfonodais para predizer metástases viscerais pulmonares. Foram incluídas 50 cadelas portadoras de tumores de mama atendidas no Hospital Veterinário da UFV no período de 01 de junho de 2014 a 01 de dezembro de 2015. O protocolo de atendimento incluiu coleta de dados, exame físico, radiológico, hematológico e cirurgia, conforme protocolo vigente desse serviço, acrescida da pesquisa do linfonodo sentinela pela técnica do azul patente. Das cadelas analisadas, 86% apresentavam neoplasias malignas e 14% benignas. A palpação linfonodal ofereceu alta acurácia, baixa sensibilidade, alta especificidade, baixo valor preditivo positivo e alto valor preditivo negativo, sendo útil para a exclusão de metástase linfonodal tanto na cadeia inguinal quanto na cadeia axilar. A pesquisa do linfonodo sentinel apresentou alta taxa de detecção e baixa positividade em ambas as cadeias
linfonodais. Sugere-se o seguinte protocolo: palpação linfonodal em todos os casos, seguida de pesquisa de linfonodo sentinel na nos casos positivos (palpáveis).

**Palavras-chave:** Câncer de mama, estadiamento, Linfonodo Sentinel, azul patente.

**1 INTRODUCTION**

Mammary tumors are the second most frequent tumors in dogs according to CASSALI et al. (2017). The increasing incidence of cancer in dogs is explained by their longer life expectancy nowadays. There are no consistent evidence-based treatment protocols due to the lack of consensus on female dog’s mammary lesions histopathological classification. The most recent staging protocol proposed by SORENMO et al. (2013), cited by CASSALI et al. (2017), is shown in table 1.

<table>
<thead>
<tr>
<th>Tumor (T)</th>
<th>T0</th>
<th>No evidence of primary tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>Tumor &lt; 3 cm</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>Tumor between 3 and 5 cm</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>Tumor &gt; 5 cm</td>
</tr>
</tbody>
</table>

| Regional lymph nodes (N) | N0 | No lymph node metastasis |
|                         | N1 | Inguinal or axillary lymph node metastasis |

| Metástases (M) | M0 | No distant metastasis |
|               | M1 | Distant metastasis, including non regional lymph nodes |

**Clinical Staging**

<table>
<thead>
<tr>
<th>Stage I</th>
<th>T1</th>
<th>N0</th>
<th>M0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage II</td>
<td>T2</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>Stage III</td>
<td>T3</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>Stage IV</td>
<td>anyT</td>
<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td>Stage V</td>
<td>anyT</td>
<td>anyN</td>
<td>M1</td>
</tr>
</tbody>
</table>

From: CASSALI et al., 2017.

The accurate assessment of the lymph node status is critical for staging, since lymph node metastasis changes overall survival, as described by ANGELIM & COELHO (2012) who reported 85% death within two years from the diagnosis among the animals which presented lymph node metastasis. The gold standard for lymph node status staging is radical lymphadenectomy and sampling all lymph nodes for histopathology. Female dogs´ mammary glands lymphatic drainage is to axillary and inguinal lymph nodes of the same side. No contralateral drainage was described for any gland, according to CASSALI et al. (2017).

There are five pairs of mammary glands in a female dogs. The cranial and
caudal thoracic pair of mammary glands drain to the ipsilateral axillary lymph nodes, while the caudal abdominal and inguinal pair of mammary glands drain to the ipsilateral inguinal lymph nodes, as described by PATSIKAS et al. (2006) and FOSSUM et al. (2008). The caudal abdominal pair of glands drain to axillary, inguinal or both lymph node stations at once, according to FOSSUM et al. (2008), and EL KHATIB et al. (2011). The axillary lymph node station usually contains one lymph node ranging from 0.5 to 5 cm size. The inguinal lymph node station may contain two or more lymph nodes ranging from 0.5 to 2 cm size.

According to FEITOSA et al., 2016, the lymph node stations palpable on routine physical examination in dogs are maxillary, superficial cervical, superficial inguinal and superficial popliteal. The axillary lymph nodes, as well as the parotid and retropharyngeal ones, are palpable only when altered by a disease. There is a different meaning between a palpable inguinal lymph node and a palpable axillary one. Nonetheless, this difference has never been studied in female dogs with mammary tumors.

PINHEIRO et al. (2003) performed the first study about sentinel lymph node detection in female dogs in Brazil. The method was double staining with both technetium Tc99m and blue dye injected in 17 bitches with no mammary lesion. The detection rate was 90.9% with the blue dye and 97.9% with the technetium Tc99m. EL KHATIB et al. (2011) injected methylene blue in 10 bitches with mammary neoplasia and had 100% detection rate. BESERRA et al. (2011) reproduced the experiment of EL KHATIB et al (2003) with similar results. The technique was then validated for dogs but there was no guideline for routine use.

The main goal of this research was to determine the optimal protocol for lymph node staging in clinical practice in female dogs presenting with mammary tumors. To address this issue, the accuracy of lymph node palpation in predicting metastasis from mammary tumors and the accuracy of the sentinel node detection using the blue dye technique were performed. The role of lymph node metastasis in predicting pulmonary spread of mammary tumors was also described.

2 MATERIALS AND METHODS

All female dogs presenting mammary tumors attended routinely in the Veterinary Hospital of the Institution from June, 1st of 2014 to December, 1st of
whose owner signed the informed consent protocol were included in this study.

A protocol including clinical and oncological information was filled and a complete clinical examination was performed. Blood samples and x-ray exams were taken for preoperative evaluation. All the surgical procedures followed the hospital’s routine. Anesthesia was performed using midazolam 0.2 mg/Kg, followed by propofol 6 mg/Kg, both trough the intravenous route, orotraqueal intubation, epidural blocking with bupivacaine 0.2 mg/Kg plus methadone 0.3 mg/Kg and maintenance with a mixture of oxygen and isoflurane.

The 2.5% blue dye solution was injected subcutaneously in peritumoral area in dosis of 0.5 mL for animals weighting less than 8 Kg, 1 ml for animals weighting 8 to 15 Kg and 2 mL for animals weighting more than 15 Kg. In case of multiple tumors, the biggest lesion was chosen for blue dye injection and TNM staging. The skin incision was performed ten minutes after blue dye injection.

Radical or regional mastectomy was performed according to tumor extent. Contralateral lesions were not operated at the same procedure. Inguinal lymph node station was dissected in all cases. Axillary lymph node station was dissected when the tumors was in cranial thoracic mammary gland, caudal thoracic mammary gland or cranial abdominal mammary gland.

Mammary gland tumors and sentinel lymph node samples were fixed in 10% formalin, and processed as routine for histological analysis. The slides were stained by hematoxylin and eosin (H&E) and evaluated through light microscopy by a single pathologist.

The detection rate of axillary and inguinal palpation was described, as well as sensibility, specificity, positive predictive value, negative predictive value and accuracy in finding metastasis. The detection rate of sentinel lymph node was described for both inguinal and axillary stations as well as the incidence of metastasis in those lymph nodes.

Sensitivity, specificity, positive predictive value, negative predictive value and accuracy were performed according to the ability of the sentinel node detection to predict metastasis. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy were performed according to the ability of the metastatic lymph node detected to predict lung metastasis.
3 RESULTS

Fifty female dogs were included in this study, seven had only benign lesions and 43 had at least one malignant lesion. The characteristics of the female dogs presenting mammary malignant tumors were summarized in table 2.

Table 2. Characteristics of the female dogs presenting malignant mammary tumors

<table>
<thead>
<tr>
<th>RACE</th>
<th>Percentage of dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undefined</td>
<td>27.9</td>
</tr>
<tr>
<td>Pinscher</td>
<td>16.3</td>
</tr>
<tr>
<td>Poodle</td>
<td>9.3</td>
</tr>
<tr>
<td>Daschund</td>
<td>9.3</td>
</tr>
<tr>
<td>Rotweiller</td>
<td>7.0</td>
</tr>
<tr>
<td>Yorkshire</td>
<td>7.0</td>
</tr>
<tr>
<td>Labrador</td>
<td>4.7</td>
</tr>
<tr>
<td>Cocker Spaniel</td>
<td>4.7</td>
</tr>
<tr>
<td>Other races</td>
<td>13.8</td>
</tr>
</tbody>
</table>

Average age: 10.26 years
Average weight: 12.96 Kg
Average body score: 7.2

<table>
<thead>
<tr>
<th>GINECOLOGIC HISTORY</th>
<th>Percentage of dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progestagen exposure</td>
<td>22.2</td>
</tr>
<tr>
<td>Regular cio</td>
<td>61.1</td>
</tr>
<tr>
<td>Pseudociese</td>
<td>27.8</td>
</tr>
<tr>
<td>Previous parturition</td>
<td>58.3</td>
</tr>
<tr>
<td>Castration</td>
<td>19.4</td>
</tr>
</tbody>
</table>

Twenty-six percent of the dogs presented one nodule, 27.9% presented two nodules, 30.2% presented three nodules, 7.0% presented four nodules and 9.3% presented five nodules. Moreover, 37.1% of the dogs presented T1 tumor (less than 3 cm), 23.3% presented T2 tumor (3 to 5 cm) and 39.5% presented T3 tumor (more than 5 cm). Pathological stage I was detected in 27.9% of cases, stage II in 11.6%, stage III in 27.9%, stage IV in 14.0% and stage V in 18.6%.

Ninety-one tumors were analyzed and showed the following distribution: 11% non-neoplastic lesions (hyperplasia, mastitis and cysts), 10% benign neoplasias (mixed benign tumor, adenoma and lipoma) and 79% malignant neoplasias (papillary carcinoma, carcinoma in mixed tumor, tubular carcinoma, solid carcinoma, sarcoma, osteosarcoma, cutaneous metastasis). The average tumor number was 1.82 tumors per animal. The average tumor size was 5.12±3.97 cm.

There were seven palpable inguinal lymph nodes, among which two presented metastasis. Thirty-two inguinal lymph nodes were not palpable, among which six presented metastasis. The sensitivity of inguinal palpation in detecting
metastasis was 25%, the specificity was 83.9%, the positive predictive value was 28.6%, the negative predictive value was 81.3% and the accuracy was 81.3%.

There were four palpable axillary lymph node, among which one was metastatic. Fifteen axillary lymph nodes were not palpable, among which three were metastatic. The sensitivity of axillary palpation in detecting metastasis was 25%, the specificity was 80%, the positive predictive value was 25%, the negative predictive value was 80% and the accuracy was 80%.

No adverse reactions were observed with the blue dye technique. The inguinal sentinel lymph node was detected in 92.3% of cases. The incidence of inguinal metastasis was 20.5%. None of the three cases in which the sentinel lymph node was not colored by the blue dye presented metastasis. The sensitivity of the sentinel node using the blue dye technique in detecting inguinal lymph node metastasis was 100%, the specificity was 9.7%, the positive predictive value was 22.2%, the negative predictive value was 100% and the accuracy was 28.2%.

The axillary sentinel lymph node was detected in 79% of cases. The incidence of axillary metastasis was 21%. None of the four cases in which the sentinel lymph node was not colored by the blue dye presented metastasis. The sensitivity of the sentinel node using the blue dye technique in detecting axillary lymph node metastasis was 100%, the specificity was 26.7%, the positive predictive value was 26.7%, the negative predictive value was 100% and the accuracy was 42.1%.

Among the six female dogs who presented lung metastasis, two presented axillary lymph node metastasis, one presented inguinal lymph node metastasis and three presented no lymph node metastasis. Therefore, the sensitivity of inguinal lymph node metastasis in predicting lung metastasis was 20%, the specificity was 79.4%, the positive predictive value was 12.5%, the negative predictive value was 87.1% and the accuracy was 71.8%.

For axillary lymph node metastasis the sensitivity in predicting lung metastasis was 40%, the specificity was 85.7%, the positive predictive value was 50%, the negative predictive value was 80% and the accuracy was 73.7%.

4 DISCUSSION

The 86% incidence of malignant lesions similar to the most recent data
published by CASSALI et al (2017) showing 90% malignant tumors in Southeastern and Northeastern Brazil. The wide variation found by OLIVEIRA et al. (2003), ITOH et al. (2005), OLIVEIRA FILHO et al. (2010), CAMPOS et al. (2011), PEDROSO et al. (2011), RIBAS et al. (2012) from 34 to 93% incidence of malignant lesions is probably due to the lack of homogenous criteria for pathological classification of mammary tumors in female dogs. Another possibility of bias for the malignant incidence is the sample origin: the samples from pathology services are more likely to be malignant since the assistant doctor may not send small non suspicious lesions. Late diagnosis is another point for the great malignant lesion incidence in Brazil, as reported by OLIVEIRA FILHO et al. (2010), PEDROSO et al. (2011) and RIBAS et al. (2012). The benign lesions grow and may evolve to malignant ones, for example in cases of carcinomas arising in the epithelial portion of benign mixed tumors after many years as found by CAMPOS et al. (2011) and PEDROSO et al. (2011).

The average 1.82 lesions per animal and of 74.4% multiple lesions is similar to the data of RIBAS et al. (2012) who found multiple tumors in 83% of the cases.

No studies reported lymph node palpation as a method for staging. The present study showed great negative predictive value for palpation in both inguinal and axillary stations, which means that the method allows the exclusion of metastasis when the node is not palpable on clinical examination.

The detection rate of inguinal sentinel node in this study was 92.3% and the axillary detection was 78.9%. BESERRA et al. (2011) and EL KHATIB et al. (2011) found more than 90% sentinel detection rate using blue dye and radioactive technetium (Tc99m), but both studies were performed in bitches without any mammary tumor. The lower detection in axillary station can be explained by the greater incidence of tumor in the inguinal mammary glands (as was shown in 65% of all female dogs). The drainage communication between the axillary and inguinal stations may be or may not be present in an individual. The incidence of inguinal sentinel node metastasis was 20.5% of the detected lymph nodes and the axillary was 21%. OLIVEIRA FILHO et al. (2010) found 29.5% of lymph node metastasis in necropsies, which is a different sample from the one in this study.

The great sensitivity of the sentinel node technique for both inguinal and axillary station allows the use of the method in series with the palpation, which
allows the exclusion of metastasis.

The inguinal station is resected in all standard mastectomies, as described by FOSSUM et al. (2008), but the axillary is not routinely accessed. Therefore, this metastasis rate is very important because those lymph nodes would not be resected otherwise. The variety of blue dye migration patterns within the cranial abdominal mammary gland also means a great role for sentinel node biopsy in such cases.

5 CONCLUSION

The sentinel lymph node biopsy using the blue dye in female dogs presenting with mammary tumors showed high detection rates and high sensitivity for both inguinal and axillary stations.

The clinical palpation of inguinal and axillary lymph nodes showed high negative predictive values, allowing the safe exclusion of metastasis on non palpable lymph nodes.

Therefore, the optimal protocol would be lymph node palpation first and, in the palpable cases, the sentinel node using the blue dye is recommended. This way, the result and the resources are optimally taken.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This project was approved by the Institutional Ethics Board on Animal Use for Research (protocols 41/2014 and 44/2016).
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