ORIGINAL ARTICLE

# Characterizing an ideal experimental canine model for penile sentinel lymph node identification

## Caracterização de modelo experimental ideal para a identificação de linfonodo sentinela peniana

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## ABSTRACT

**Objective:** Develop and evaluate a canine experimental model of penile sentinel lymph node (SLN) detection using a combination of patent blue dye (PB) and technetium$^{99m}$ phytate ($^{99m}$Tc). **Methods:** The experiment sampled healthy adult male mongrel dogs (N=19; 10-20 kg). After approval by the local ethics committee, we injected$^{99m}$Tc in the glans penis along the midline raphe. After 10 min, PB was injected in the same region. Ten minutes later, we identified hot spots within the inguinals and iliac artery areausing a gamma probe. We dissected identified sites to quantify the radiation at the SLN *in vivo* and *ex vivo* and accounted for the stained and unstained nodes. We analyzed the results for statistical significance and determined the level of agreement between the two methods. **Results:** SLNs were detected in 94.76% of the cases using PB and $^{99m}$Tc. There was no difference (p>0.05) between the SLNs-count on the left and the right side. However, PB and $^{99m}$Tc correlated well on the right side (kappa index: 0.642) and perfectly on the left side (kappa index: 1), indicating a high level of consistency. **Conclusion:** The experimental canine model of penile SLN, in our study, was shown to be feasible.

**Keywords:** Penis. Sentinel lymph node. Dogs.

## RESUMO

**Objetivo:** Desenvolver e avaliar um modelo experimental de identificar linfonodo sentinela (SLN) em cães utilizando Azul Patente (PB) e Fitato de Tecnécio ($^{99m}$Tc). **Métodos:** Foram selecionados 19 (10-20 kg) cães de rua, adultos e masculinos para serem submetidos ao protocolo experimental após aprovação pelo comitê de ética local. Foi administrado por injeção na ráfe mediana da glande $^{99m}$Tc e após 10 min, PB foi administrado no mesmo local. Dez minutos depois, os animais foram submetidos à técnica de identificação de pontos radiologicamente quentes usando um transdutor-gamma. Tais pontos foram dissecados para quantificar a radiação nos SLN *in vivo* e *ex vivo* e posteriormente comparados aos linfonódos corados e não corados. Em seguida, analisamos a significância estatística e determinamos o nível de concordância entre ambas as técnicas. **Resultados:** Identificamos SLN em 94,76% dos casos usando PB e $^{99m}$Tc sem ter obtido nenhuma diferença estatística em quantidade identificado no lado direito, quando comparado ao lado esquerdo. Porém, o PB e $^{99m}$Tc correlacionaram bem no lado direito (Estatística Kappa: 0,642) e perfeitamente no lado esquerdo (Estatística Kappa: 1), indicando um nível de consistência alto. **Conclusão:** O modelo experimental proposto que identificou SLN em cães utilizando PB e $^{99m}$Tc, provou-se aplicável.

**Palavras-chave:** Pênis. Linfonodo sentinela. Cães.

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## INTRODUCTION

In Brazil, penile carcinoma (PC) is a rare neoplasm; representing only 2% of all malignant tumors diagnosed in men. According to figures published by Instituto Nacional do Câncer (INCA) in 2013, 396 deaths were linked to PC whose main prevalence was in the north and northeast region.[1] Usually, PC disseminates via the lymphatic system by embolism rather than by lymphatic permeation. Furthermore, distant metastases are extremely rare and result from vascular dissemination.[2]

Tumor staging is vital in programming treatment options, especially in patients with clinical disease but negative lymph node involvement. Prophylactic inguinal lymphadenectomy been shown to increase cure indices up to 80%, but it is marred with high levels of morbidity, psychological distress and functional disability. Unnecessary performed lymphadenectomy is associated with high levels of complications, among them hemorrhage, site infection, deep vein thrombosis and lymphedema.[3] However, advances in sentinel lymph node (SLN) detection procedures have made it possible to identify patients with lymph node involvement. Thus, they are likely to benefit from lymphadenectomy.

Intra-operative gamma imaging (IGI) is widely used for SLN detection in patients with melanoma[4,5,6] and breast cancer.[7,8] Due to its minimum invasive nature while at the same time highly sensitive, it is considered indispensable in SLN biopsy.

Anatomically, dogs are comparable to humans with regard to lymphatic drainage. However, the superficial inguinal lymph nodes in dogs are referred to as mammary lymph nodes as they drain the inguinal teats. They are located 3-4 cm cranially to the pubic bone on the dorso-lateral side of the mammary glands. Usually, two 0.5-2 cm long lymph nodes are observed, but in some cases, three to four nodes can be identified. The afferent lymphatic vessels of the skin and subcutaneous connective tissue of the abdomen, pelvis and hind legs are similar in males and females.

In an experimental study on female dogs, Pinheiro and contributors[9] found that patent blue dye (PB) and technetium$^{99m}$ (Tc) are both efficient in localizing SLNs in the subareolar area, whether used alone or in combination. Using similar tracers, we intended to elucidate on inguinal lymphatic drainage in dogs. Therefore, our objective was to develop and evaluate an experimental canine model of penile sentinel lymph node detection using a combination of patent blue dye and technetium$^{99m}$ phytate.

## METHODOLOGY

After approval (06/08) by the animal research ethics committee of the Universidade Federal do Ceará (UFC), animals donated by the Municipal Zoonosis Control Center of Fortaleza (Ceará, Brazil) were sampled. The process randomly selected 19 adult male mongrel dogs (*Canis familiaris*) each weighing approximately 10-20kg. The animals were transferred and housed under controlled and comfortable environs (25°C, relative humidity approx. 50%) of the Saul Goldenberg Laboratory (GEEON/UFC), with acess *ad libitum* to food and water. The circadian cycle was adhered to. Fasting was carried out 24 hours prior to the experiment.

During the experiment, a subcutaneous dose of 0.25mg/mL atropine (0.05mg/Kg) was administered to each dog, 15 min before an intramuscular injection of anesthesia (50mg/mL-ketamine hydrochloride (15 mg/Kg) + 20 mg/mL 2% xylazine hydrochloride (1.5mg/Kg). A peripheral vessel was catheterized for constant saline administration, using a 19G or 21G catheter inserted in the forepaw. Clinical parameters were constantly monitored through the experiment. Thereafter, using an insulin needle, 0.2 mL saline containing 5µCi technetium$^{99m}$ was injected into the dermis of the penis glans along the mid rafe. After 10 minutes, we quantified $^{99m}$Tc uptake by mapping the lymph-node mesh at the inguinal groove using a gamma probe, bilaterally. Next, patent blue dye was injected at the site of previous $^{99m}$Tc injection and after five minutes, $^{99m}$Tc uptake at the inguinal groove was quantified once again, bilaterally. This time, our objective was to identify the site with the highest uptake of $^{99m}$Tc. Upon its identification, incisions measuring approximately 3 cm were made, guided by the intense presence of PB in the afferent lymphatic system until the SLN (or SLNs) was localized and subsequently confirmed by IGI. The identification of SLN paved way for the *in vivo* quantification of $^{99m}$Tc uptake and the level of staining. This was followed up by total lymph node dissection and resection for *ex vivo* quantification of $^{99m}$Tc uptake. A radiation level that was five or more times higher than the basal radiation was considered significant.

After quantifying the level of radiation and verifying that no more SNLs were existent at the inguinal lymph node groove, we performed hemostasis and sutured up the dissected region. After the experiment, the animal was euthanized at the surgical theatre with an intravenous bolus injection of 10% potassium chloride.

## STATISTICAL ANALYSIS

The statistical analysis was performed with the software SPSS 20.0 (*Statistical Package for the Social Sciences*) license nº.10101131007. The data were analyzed with the McNemar test, and Kappa agreement coefficients were calculated to determine the level of consistence between the two methods.

## RESULTS

Table 1 shows lymph node $^{99m}$Tc uptake 5, 10 and 15 minutes after injection with technetium$^{99m}$ and the respective standard deviations (SD). As shown in Table 2, the two sides (left/right) did not differ significantly with regard to $^{99m}$Tc uptake, PB staining, *in vivo* radiation count, *ex vivo* radiation count, or number of nodes stained with PB ($p>0.05$). The level of agreement between the two methods was 94.7% *in vivo* and 100% *ex vivo*.

The correlation between $^{99m}$Tc uptake and PB staining was evaluated with the Kappa test. Using $^{99m}$Tc as standard, a non-perfect correlation *in vivo* and a perfect correlation *ex vivo* was observed for the right side. The test was not applicable to the left side since all nodes were detected with the $^{99m}$Tc method (Tables 3 to 6).

**Table 1.** Description of measures of central tendency and dispersion for Tc$^{-99m}$ uptake 5, 10 and 15 minutes after injection with Technetium$^{-99m}$.

| | Mean | Max. | Min. | SD |
|---|---|---|---|---|
| Countat 5 min | 999 | 3180 | 250 | 846 |
| Countat 10 min | 1294 | 4000 | 280 | 1095 |
| Countat 15 min | 1611 | 4200 | 310 | 1203 |

**Table 2.** Comparison of the right and left side with regard to method (technetium$^{99m}$ and patent blue), and agreement between the methods.

| | Right | | Left | | | |
|---|---|---|---|---|---|---|
| Variables | N | % | N | % | c$^2$ | *p* |
| Sentinel lymph node | | | | | | |
| Stained*in vivo* | | | | | | |
| No | 1 | 5.3 | 2 | 10.5 | 0.362 | 0.547 |
| Yes | 18 | 94.7 | 17 | 89.5 | | |
| Sentinel lymph node | | | | | | |
| Stained*ex vivo* | | | | | | |
| No | 1 | 5.3 | 2 | 10.5 | 0.362 | 0.547 |
| Yes | 18 | 94.7 | 17 | 89.5 | | |
| Count*in vivo* | | | | | | |
| 0 - 500 | 1 | 5.3 | 2 | 10.5 | 0.49* | 0.738 |
| 501 - 1000 | 6 | 31.6 | 6 | 31.6 | | |
| 1001 - 2000 | 5 | 26.3 | 5 | 26.3 | | |
| 2001 - 3000 | 3 | 15.8 | 2 | 10.5 | | |
| 3001 - 4000 | 3 | 15.8 | 2 | 10.5 | | |
| > 4000 | 1 | 5.3 | 2 | 10.5 | | |
| Count*ex vivo* | | | | | | |
| 0 - 500 | 1 | 5.3 | 2 | 10.5 | 0.139* | 0.331 |
| 501 - 1000 | 6 | 31.6 | 6 | 31.6 | | |
| 1001 - 2000 | 3 | 15.8 | 6 | 31.6 | | |
| 2001 - 3000 | 4 | 21.1 | 1 | 5.3 | | |
| 3001 - 4000 | 0 | 0 | 2 | 10.5 | | |
| > 4000 | 5 | 26.3 | 2 | 10.5 | | |
| Agreement between methods | | | | | | |
| *In vivo* | | | | | | |
| No | 1 | 5.3 | 1 | 5.3 | 0.0 | 1.0 |
| Yes | 18 | 94.7 | 18 | 94.7 | | |
| Agreement between methods | | | | | | |
| *Ex vivo* | | | | | | |
| No | 0 | 0 | 0 | 0 | ** | ** |
| Yes | 19 | 100 | 19 | 100 | | |
| Total | 19 | 100.0 | 19 | 100.0 | | |

*Analyzed with Kendall's Tau B
**Not calculated, the variable was constant

**Table 3.** Level of agreement between the two methods of sentinel lymph node detection (technetium$^{99m}$ *vs* patent blue) *in vivo* at the right inguinal groove.

| | | | Detected | | Total |
|---|---|---|---|---|---|
| | | | No | Yes | |
| Stained | No | N | 1 | 1 | 2 |
| | | %N | 100.0% | 5.6% | 10.5% |
| | Yes | N | 0 | 17 | 17 |
| | | %N | .0% | 94.4% | 89.5% |
| Total | | N | 1 | 18 | 19 |
| | | %N | 100.0% | 100.0% | 100.0% |

*Kappa:* 0.642

**Table 4.** Agreement between the two methods of sentinel lymph node detection (technetium$^{99m}$ and patent blue) *in vivo* for the left side.

| | | | Detected | Total |
|---|---|---|---|---|
| | | | Yes | |
| Stained | No | N | 1 | 1 |
| | | %N | 5.3% | 5.3% |
| | Yes | N | 18 | 18 |
| | | %N | 94.7% | 94.7% |
| Total | | N | 19 | 19 |
| | | %N | 100.0% | 100.0% |

*Kappa* not applicable

**Table 5.** Level of agreement between the two methods of sentinel lymph node identification (technetium$^{-99m}$ and patent blue) ex vivo at the right inguinal groove.

| | | | Detected | | Total |
|---|---|---|---|---|---|
| | | | No | Yes | |
| | | %N | 100.0% | 0.0% | 10.5% |
| | Yes | N | 0 | 17 | 17 |
| | | %N | 0.0% | 100.0% | 89.5% |
| Total | | N | 2 | 17 | 19 |
| | | %N | 100.0% | 100.0% | 100.0% |

*Kappa*: 1.00

**Table 6.** Agreement between the two methods of sentinel lymph node detection(technetium$^{99m}$ and patent blue) *ex vivo* for the left side.

| | | | Detected | | Total |
|---|---|---|---|---|---|
| | | | No | Yes | |
| Stained | No | N | 1 | 0 | 1 |
| | | % N | 100.0% | 0.0% | 5.3% |
| | Yes | N | 0 | 18 | 18 |
| | | %N | 0.0% | 100.0% | 94.7% |
| Total | | N | 1 | 18 | 19 |
| | | % N | 100.0% | 100.0% | 100.0% |

*Kappa*: 1.00

## DISCUSSION

The concept of sentinel lymph node (SLN) is well established,[10] although SLN biopsy in penile cancer remains controversial. This is largely attributed to the high rate of false-negative results associated with this procedure.[11-13] On the other hand, SLN biopsy is a widely validated procedure in breast cancer and skin melanoma. Basically, the pathophysiological condition of SLN reflects that of the lesion's entire drainage area.[14,15] Having this concept in mind, probably SLN in PC could help identify patients candidate for radical surgery. Consequently this would avoid unnecessary invasive procedures, reducing trans- and postoperative morbidity, besides improving patients' quality of life.

The administration of a radionuclide, usually by injection, and consequent lymphatic mapping using a gamma probe (radioguided surgery), offers the most efficient way of detecting SLN. Several authors[16,17] have evaluated the ability of $^{99m}$Tc-microaggregated albumin to identify SLNs by lympho-scintigraphy. A gamma probe was used to identify for exeresis of a respective SLN via a small incision. Currently, preoperative radionuclide lympho-scintigraphy is used to determine the location and quantify SLNs. Sometimes, PB dye is used in association to facilitate SLN identification and confirm its exact site.[18,15]

In our protocols, SLNs were detected in 94.76% of our cases, with no significant difference between the left and the right inguinal lymph node basins ($p$>0.05). As shown in Table 3 up to Table 6, PB and $^{99m}$Tc correlated well on the right side (kappa: 0.642) and perfectly on the left side (kappa: 1), indicating a high level of consistency. Our results are comparable to those reported by others. A review by Sapienza et al. on SLN detection in patients diagnosed with malignant melanoma using $^{99m}$Tc phytate and lympho-scintigraphy showed the technique was efficient in all studies (92 patients), with 98.8% intra-operative detection.[19] Similar results have been previously reported by us, though in dogs.[9] Thus, the concept of SLN shows its consistency in studies, whether in canines or humans.

Anatomically, lymphatic drainage is much more predictable in PC than in melanoma, since the former has only two potential drainage basins to consider: superficial and deep lymph node basin. The incidence of metastasis in patients with melanoma is approximately 1%, while that of PC is much less.[20,21] In a study on patients with PC, Perdona et al. found similar rates of metastasis when comparing bilateral dissection to SLN biopsy (39% vs. 36%).[22] However, the latter was associated with significantly less postoperative morbidity. In addition, other authors concluded in a recent review that when the metastases in the SLN are less than 2 mm, all other inguinal lymph nodes are almost certainly tumor-free and further dissection is unnecessary.[23]

The relevance of our study could be compared to that done by our group previously, which evaluated the ability of PB and $^{99m}$Tc phytate to identify SLNs in the subareolar region of the breast of female dogs.[9] In that work, the authors found out that both methods, whether alone or in association, were efficient. Up-to-date, this model is used by mastology residents at our institution to acquire skills in SLN detection in patients diagnosed with breast cancer. Furthermore, after evaluating and consequently validating the feasibility of our design on penile SLN - detection, this protocol could be applicable in the training of specialized surgeons. From an academic point of view, constant training could foster mastered skills, hence shortening surgeons' learning curve. Besides it could probably lead to a reduction in levels of false-negative results on inguinal SLN biopsy.

Studies involving SLN and PC are novel in literature data bases, thus, we are emphatic that our findings will elucidate continuous knowledge on this disease, which has a high prevalence in our region. Besides lower morbidity and improvement in quality of life of patients with PC, our protocols could serve as a benchmark for observation studies involving canine Sticker's sarcoma and its metastasis pattern.[24]

## CONCLUSION

Our results show that the experimental canine model of penile sentinel lymph node detection designed and evaluated in the present study is feasible. Patent blue dye and $^{99m}$technetium were both efficient at detecting penile sentinel lymph nodes, and were well correlated. The model may be used to test other methods of sentinel lymph node detection and adopted for clinical practice.

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