

3 - Experimental model in rat for sentinel node biopsy¹

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Oliveira RS, Paiva GR, Ferreira LM, Wagner J, Nogueira AS. Experimental model in the rat for sentinel node biopsy. Comparison between dextran and phytate. Acta Cir Bras [serial online] 2003 Vol 18 Special Edition. Available on URL: <http://www.scielo.br/acb>.

ABSTRACT - Although sentinel node procedure has been used world wide, there are many aspects to be defined and better standardized. This study address if the experimental model in rats is appropriate for sentinel node biopsy. In this model, the lymph nodes are showed by lymphoscintigraphy, they are dyed by patent blue and identified by intraoperative gamma probe detection. It isn t necessary to use magnification for the procedure. The model demonstrated that sentinel node biopsy in rats is feasible. So, besides allowing researches in this field, the model is useful for training and diffusing this technique.

KEY WORDS Lymph node biopsy. Experimental model. Rats.

Introduction

The fear of micro metastasis has justified radical lymph node dissections on the purpose to promote cure. Nevertheless, the real benefit of such a procedure is yet to be known and its universal adoption may be of damage especially for those without lymphatic invasion.¹

The sentinel lymph node biopsy has emerged by the end of the 20th Century as a less aggressive procedure to detect micro metastasis in tumor drainage node chain. Following the procedure, only patients with positive sentinel lymph node (those in which metastasis are detected) undergo radical dissections and patients without metastasis are preserved, avoiding complications as infection, sensibility disturbances and edema.^{1,2}

Although the signification of sentinel lymph node (the first of a chain which drains the primary tumor) has been previously proposed, the procedure has deserved respect and became the spot of interest after the description of the lymphatic mapping by the end of the eighties by Morton et al.³ In the beginning it was used in melanoma and now has been applied in other solid tumors, especially those, which spread through lymphatic vessels, like breast cancer.

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The so called sentinel lymph node procedure involves three phases as follows: (1) pre-operative lymphoscintigraphy, (2) biopsy of the sentinel node by means of intra-operative gamma detection and blue dye lymph mapping and (3) examination of histological sections of the node.

The pre-operative lymphoscintigraphy is fundamental and leads to the identification of the lymph node chains that may present metastatic disease and where the sentinel node must be searched. For the exam, a radiotracer is injected intradermal around the tumour or just near the scar of the tumor resection.⁴ The most used tracer is the meta stable shape of technetium (Tc^{99}) that is routinely used in most nuclear medicine procedures since it presents proper amount of energy to be detected in a gamma camera.⁶ Other radiopharmaceuticals can be used like colloid sulphur, human albumin, phytate and dextran. In Brasil dextran 500 and phytate are the available radiopharmaceuticals pattern.^{7, 8} It is established that there is a narrow relation between the size of the radiopharmaceutical and its properties of diffusion and of delay in the lymph node, the bigger the particle, lesser the diffusion and higher the retention in the lymph node.⁹

The lymphatic mapping with the blue dye simulates the anatomy of the lymphatic way the tumor cell could have followed from the primary lesion and dyes the sentinel lymph node leading to its identification.⁵

The intra-operative gamma detection by means of a portable gamma radiation probe permits easier localization of the sentinel lymph node in a less aggressive dissection and complements the blue dye mapping in its search.^{4, 10} Most of times it is possible to make the search, with the probe intra-operatively, of the same radiopharmaceutical injected for the pre-operative lymphoscintigraphic exam.

Sentinel node biopsy is included as part of melanoma staging (AJCC 2002).¹ It will probably have a great development during the 21st Century and will determine a special approach for solid tumors. Molecular biology (RT-PCR) will contribute for the detection of micro metastasis and the robotics will allow sentinel node biopsy from difficult anatomic sites.¹¹

Although the sentinel node biopsy has been adopted world widely, aspects as which would be the best radiopharmaceutical are yet to be defined and standardized. Experimental models could contribute for advances in this technique.

Proposition

To report a model for experimental sentinel node biopsy in rat.

Method description

Presentation of the model

The lymphatic system of the rat has already been studied and described.¹² In this experimental model young male rats (*rattus norvegicus*: albinos variety, rodentia, mammalia) with weight varying from 300 to 350gr, from the wistar epm-1 line of the federal university of são paulo - escola paulista de medicina were used and kept in twin cages under the same conditions of temperature (20 - 21 °c), humidity (ambiental humidity) and noise (acoustic isolation), receiving commercial ration (purina labina) and water *ad libitum*”.

Procedure

The animals were anesthetized with chloridrate of tilethamine and chloridrate of zolazepan either each in the concentration of 25mg/kg of weight by means of injections with an insulin type syringe and needle in the left distal aspect of the peritoneal cavity. This concentration granted about 120 to 180 minutes of anesthesia and in those cases it was necessary, additional doses of 10 mg were injected.

Lymphoscintigraphy

For lymphoscintigraphy, 0.1ml of the radiopharmaceutical carrying 15 μ ci of ^{99}Tc was injected intradermal into left footpad of the rats. The exam was carried in a mobile gamma camera at the Hospital Israelita Albert Einstein (apex spx 4m-ge) (Figures 1, 2A and 2B).

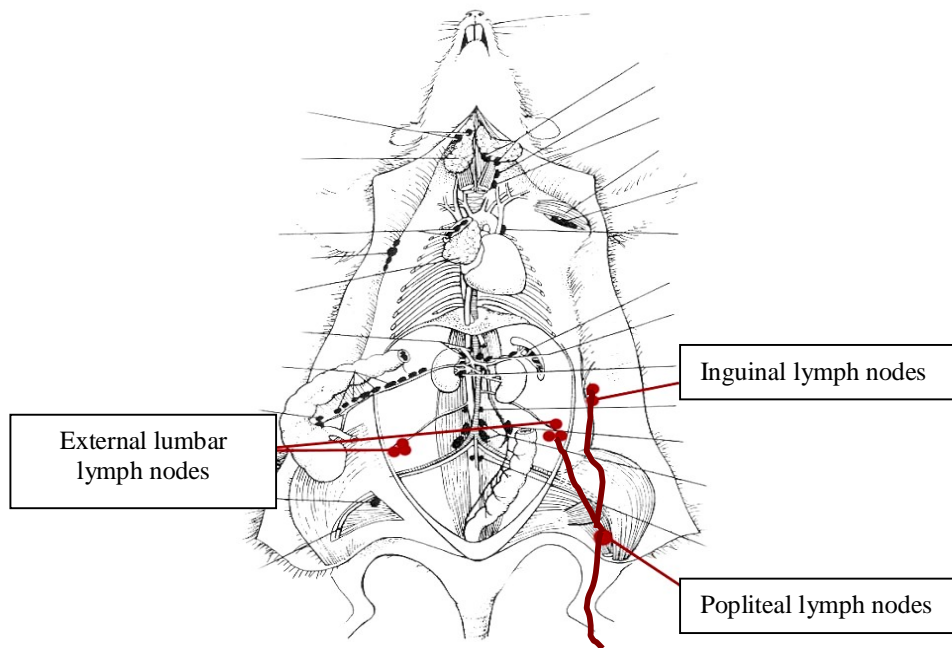


FIGURE 1 - Anatomy of lymph nodes in rats (modified from Tilney, 1971). Lymph nodes of posterior foot were stressed.

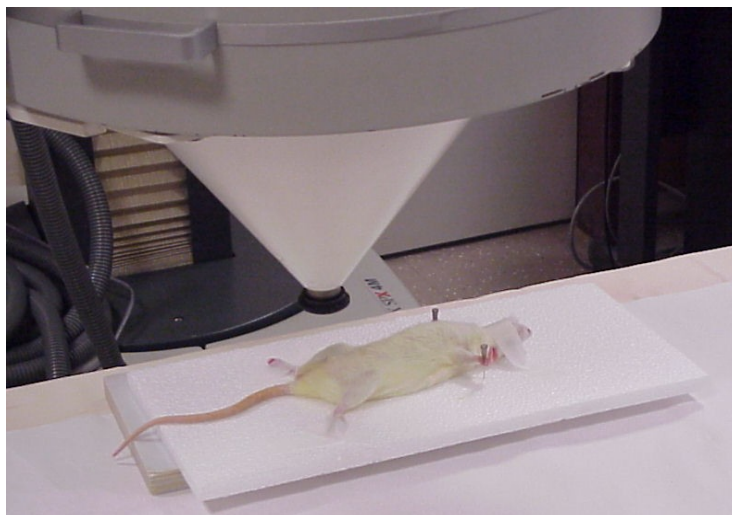


FIGURE 2A - Lymphoscintigraphy (APEXSPX 4M GE).

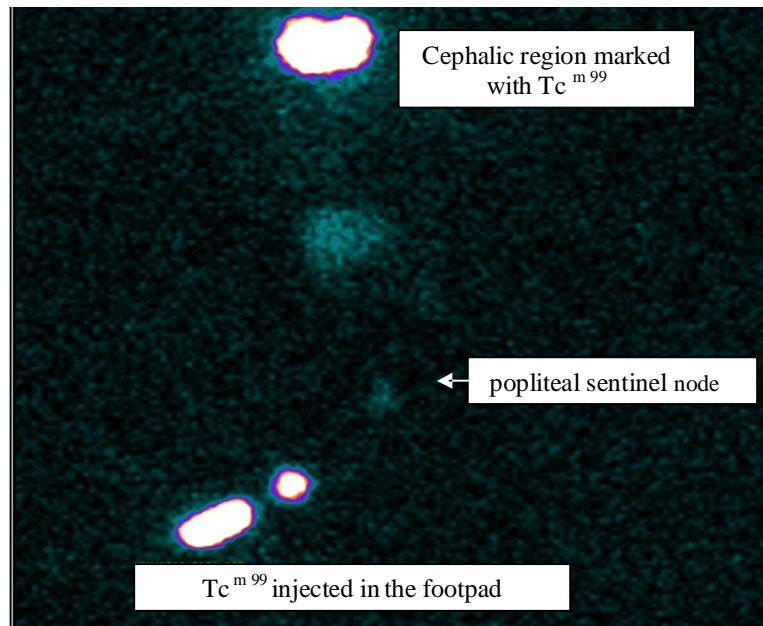


FIGURE 2B - Lymphoscintigraphy showing popliteal sentinel node.

Sentinel lymph node biopsy

One hour after the lymphoscintigraphy, a tonsure of the popliteal region of the right posterior limb was performed followed by the intradermal injection of 0.1ml of the blue dye (patent blue V, Guerbet) into right posterior footpad (Figure 3). After five minutes from the injection, an incision was made in the popliteal skin of the same limb to expose its deep aspect. The popliteal, the inguinal and the lombar lymph nodes were found, all dyed in blue (Figure 4). Then, the radioactivity in these lymph nodes was checked "*in loco*" as well as "*ex-vivo*" after the biopsy. The radioactivity of left foot pad (background) was measured too.

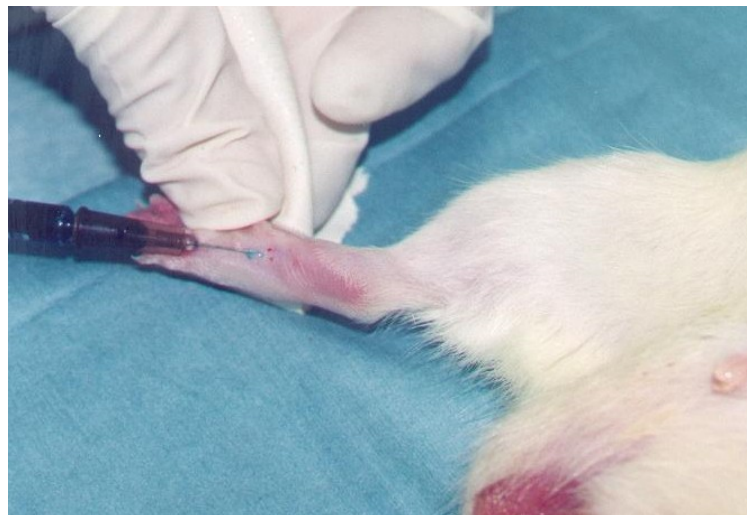


FIGURE 3 - Intradermal injection of blue dye into right posterior footpad.

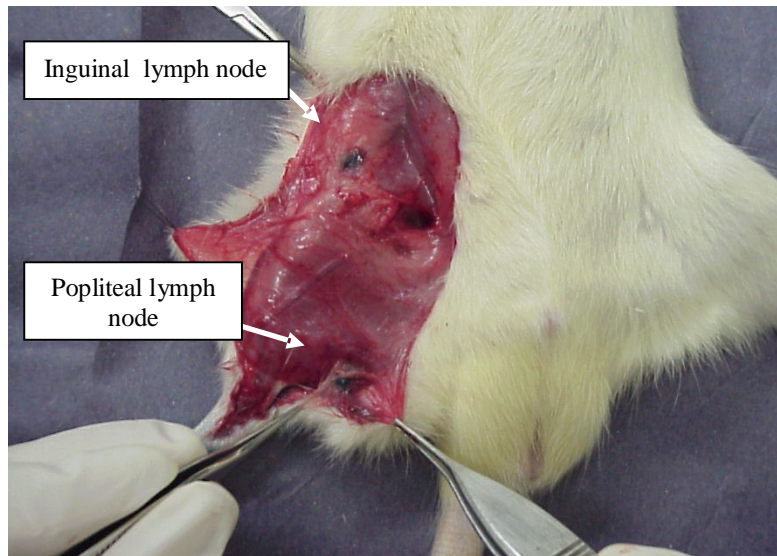


FIGURE 4 - Popliteal and inguinal lymph nodes dyed by patent blue.

Perspectives

Sentinel node biopsy plays an important role in the development of the oncologic surgery in the attempt of conducting less aggressive procedures with the same cure rates for the patients.

The experimental model in the adult rat evidences lymph nodes in the pre-operative lymphoscintigraphy, which dye blue with the proper injection of patent blue dye, retains the radiopharmaceutical to be detected by intra-operative gamma probe and may be dissected without magnification identifying sentinel lymph node.

The described experimental model demonstrated that sentinel lymph node biopsy in adult rats is feasible. Besides being useful for researches it can be used for training and diffusing this practice.

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Oliveira RS, Paiva GR, Ferreira LM, Wagner J, Nogueira AS. Modelo experimental em rato para biópsia de linfonodo sentinela. Acta Cir Bras [serial online] 2003 Vol 18 Edição Especial. Disponível em URL: <http://www.scielo.br/acb>.

RESUMO - Embora os procedimentos de biópsia de linfonodo sentinela estejam sendo reproduzidos em diversos centros oncológicos, existem muitos aspectos a serem melhor definidos e padronizados. Este trabalho tem por objetivo verificar se o modelo experimental em rato é apropriado para o procedimento de biópsia de linfonodo sentinela. No modelo experimental em rato, os linfonodos se mostram evidenciados na linfocintilografia pré-operatória, são corados pelo azul patente, captam o radiofármaco, são mensurados pelo detector portátil de radiação gama intra-operatória, e podem ser dissecados a olho nu em ratos adultos, permitindo identificação do linfonodo sentinela. O modelo demonstrou que a realização de biópsia de linfonodo sentinela em ratos é factível. Isto tem um grande significado, pois, além de permitir pesquisas relacionadas a este neste tema, ele se presta também para o ensinamento do procedimento, contribuindo para a divulgação da técnica.

DESCRITORES Biópsia de linfonodo sentinela. Modelo experimental. Ratos.

Conflict of interest: none
Financial source: none

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Data do recebimento: 22/ 04/2003
Data da revisão: 18/05/2003
Data da aprovação: 28/07/2003