National equipment of intraoperatory gamma detection in the identification of sentinel lymph node in animal model[1]

Equipamento nacional de detecção gama intra-operatória na identificação de linfonodo sentinela

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ABSTRACT
Purpose: To investigate a national equipment of intraoperatory gamma detection in the identification of sentinel lymph node. **Methods**: Thirty young adult male rats were used. After anesthetized, animals were divided into two groups of 15 animals each. Animals from group A received dextram 500 - Tc^{99} radiopharmaceutical and patent blue V and those from group B received only patent blue V to map the lymphatic drainage. The presence of radiation in the background area, in the area of injection and of the *ex vivo* sentinel lymph node of group A were measured. After the exeresis, each lymph node in group A and in group B was mixed forming a new random sequence and the radioactive reading of each lymph node was carried out, using both pieces of equipment. **Results**: The hottest sentinel lymph node was identified by the national equipment when radiation was measured in the area of limphatic drainage after the Dextran 500 was injected. Also, the ex vivo sentinel lymph node. The national equipment has also detected radiation in the lymph nodes that had not received radiopharmaceutical, leading to false positive, checked by the application of Mann-Whitney tests and Student's paired t-tests. The Cronbach alpha has shown high internal consistency of data 0,9416. **Conclusions**: The national equipment of intraoperatory gamma detection identifies the LS and showed false positives LS and needs improvement.
Key words: Sentinel Lymph Node Biopsy. Equipment. Research Design. Rats.

RESUMO
Objetivo: Investigar o equipamento nacional de detecção gama intra-operatória na identificação de linfonodo sentinela. **Métodos**: Foram utilizados 30 ratos machos, adultos jovens. Depois de anestesiados, os animais foram distribuidos em dois grupos de 15 animais cada. O grupo A recebeu radiofármaco dextran 500 – Tc^{99} e azul patente V e o grupo B, somente azul patente V para realização do mapeamento linfático. Foi realizada a medição da captação radioativa da região de fundo, do sítio de injeção e do linfonodo sentinela *ex vivo* do grupo A. Após a exérese, cada linfonodo do grupo A e do grupo B foram misturados formando uma nova seqüência aleatória e procedeu a leitura da radioatividade de cada linfonodo com os dois equipamentos. **Resultados**: O linfonodo sentinela hipercaptante foi identificado pelo equipamento nacional durante as medições da captação radioativa na região do sítio de injeção e linfonodo sentinela ex vivo. O equipamento nacional detectou radiação mesmo nos linfonodos que não receberam o radiofármaco, causando falso positivo, verificado na aplicação dos testes de Mann-Whitney e t pareado de Student. O alfa de Cronbach mostrou alta consistência interna dos dados (0,9416). **Conclusões**: O equipamento nacional de detecção gama intra-operatória identifica o linfonodo sentinela e mostra falsos positivos e necessita de aprimoramento.
Descritores: Biópsia de Linfonodo Sentinela. Equipamento. Projetos de Pesquisa. Ratos.

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Introduction

The use of intraoperatory gamma detection has already been established in oncology to identify sentinel lymph nodes in melanoma[1-4], in breast cancer[5-10] and its application for other tumors such as vulva[8-10]. Also, its use in head and neck[11] cancer is being studied. Today the sentinel lymph node biopsy procedure is considered to be essential and indispensable. Besides its high sensitivity to detect lymph nodes, the IGD allows for lower dissection, making the procedure less invasive. The SLB has reduced the number of unnecessary radical lymphadenectomies, thus reducing the surgical morbidity[3,7,4,12-16].

The sentinel lymph node (SL) is defined as the first lymph node of the lymph nodal basis to which the drainage of the primary tumor occurs[17,18]. The SLB in melanoma was established by Morton *et al.*[19], at first only using the vital blue dye that allows for the microstaging of solid tumors. These tumors, in the beginning, spread preferably through the lymphatic system. The IGD has already been included in the official staging of melanoma and of breast cancer in accordance with the AJCC/UICC (American Joint Committee on Cancer Staging System)[20].

The combination of the lymphatic mapping with the vital blue dye and the intraoperatory gamma detection in the SBL has been shown as a more sensitive combination of methods to detect SL[4,7,8,10,21-23]. The accuracy to find the SL with this association is high, ranging between 98-100%[24-30].

Today, there are several pieces of equipment commercially available to carry out the intraoperatory gamma detection such as Neoprobe®, Europrobe®, Navigator®. However, most pieces of equipment are imported and expensive, which a lot of times limits their use to large centers[31,32]. They basically have a probe with a crystal or small gamma camera in one end, which is connected to a portable counter. In our country, Oliveira Filho *et al.*[14] carried out a clinical investigation of the selective lymphadenectomy with the SLB, using vital blue dye (patent blue V) and IGD, confirming the data in the literature and its technical reproductibility.

Costa *et al.*[31] have developed a piece of national intraoperatory gamma detection equipment, which has a radioguided surgical probe. They carried out tests with doctors that found the device friendly to use and aimed to get the product to the market at a lower cost. Most importantly is the acess to maintenance, as imported equipment has to be sent abroad for maintenance.

Consequently, it is imperative that experiments are made to demonstrate feasibility of this new equipment.

Methods

This study was approved by the Ethics Committee on Animal Experimentation of the Federal University of Sao Paulo–UNIFESP, and was conducted under the guidance and supervision of the Center for Radiation of the Federal University of Sao Paulo (UNIIFESP). For the continuation of the study, it was used equipment imported from Europrobe® brand, serial number: 00,139, model: 506,160,014. The equipment was made available by the Office of Diagnostic Imaging of the Samaritan Hospital. The equipment's national IPEN (patent MU8602566-0) model III, was handled in accordance with the instructions of the unit.

Thirty young adult 250 to 300g male Wistar EPM-1 rats (*Rattus novergicus: var. albinus, Rodentia, Mammalia*) from the Animal Colony of the Federal University of Sao Paulo were studied. All procedures were performed in accordance with the ethical principles defined by the Brazilian College of Animal Experiments (COBEA).

The animals, under the same environmental conditions, were submitted to lymphatic mapping with vital blue dye, intraoperatory gamma detection and sentinel lymph node exeresis.

Each animal was anesthetized with 25 mg/kg of tiletamine chloridrate and 25 mg/kg of zolazepam chloridrate with syringe and insulin needle, injected in the peritoneal cavity, in the lower left quadrant of the abdomen. In this dosage, the anesthesia lasts around 120 to 180 minutes.

The animals were dstributed into two groups of fifteen animals each. The animals of the first group (group A) were submitted to the injection of 0.1 ml (10uCi) of dextran 500 – Tc^{99} (IPEN) radiopharmaceutical in the plantar cushion of the right posterior limb. After that, 0.1 ml of patent blue V (Guerbet) was injected in the same place to carry out the lymphatic mapping.

First, the radiation in the left axillary region was measured (background reading) and then the one in the place of the injection. These measurements were always made both with the imported and the national devices.

Five minutes after the patent blue injection, a single incision was made from the skin in the popliteal region to the inguinal area to carry out the biopsy of the sentinel lymph node, checking if the lymph note was blue dyed by the patent blue or not. Then, the popliteal lymph node (sentinel) in the popliteal cavity was identified and removed.

During surgery, the two gamma radiation detectors were alternately used in sequence, one after the other. Individual cards were used for each of the animals to take notes of the data with reference to each piece of equipment and the radiation reading values in both. Use of the equipment under study was according to its instruction's manual.

After the exeresis, the dyed hottest sentinel lymph node of each animal from group A was identified with numbers (from 1 to 15) by the researcher and placed in separate recipients.

The other group of 15 animals (group B) underwent only the lymphatic mapping with vital blue dye and sentinel lymph node exeresis, following all the procedures used in group A, except for the injection of the radiopharmaceutical. After the exeresis of the sentinel lymph node of each animal in group B, they were identified with numbers (from 16 to 30) by the researcher and placed in separate recipients.

The 30 lymph nodes numbered from 1 to 30, were randomly distributed forming a new sequence. Another observer, who didn't know the random sequence of the lymph nodes, measured the radiation in each lymph node with both pieces of equipment.

After the exeresis of the SL, the animals were subjected to euthanasia with a hyper dose of anesthetic. The animals were disposed according to the protocol of the Radiological Protect Unit.

Statistical analysis

To check discrepancies between the results reached with the conventional equipment and the IPEN equipment, Mann Whitney tests were used. The discrepancies between the pairs of results measured with the conventional piece of equipment were compared to the IPEN piece of equipment were evaluate using the

Student´s paired t-test. The Cronbach Alpha was applied to check the internal consistency of the data. Five percent was used as the level of significance.

Results

The IPEN equipment detected radiation even in lymph nodes which had not received the radiopharmaceutical, leading to a false-positive, as verified by the Mann-Whitney's test and Student's paired-t testes). This was found when the values of gamma radiation measured with the pieces of intraoperatory gamma detection equipment of each *ex vivo* sentinel lymph node were analyzed (Table 1) – Cronbach Alpha showed a high internal consistency of the data (0.9188). In background area (Table 2), the Cronbach Alpha showed a low internal consistency of data (0.4690).

In the area of the injection, a discrepancy between the pairs of results of both pieces of equipment (p=0.0013) was observed, but Mann-Whitney's test could not detect differences between the two groups of results (Table 3). The Cronbach Alpha showed high internal consistency of data (0.9416).

Although both devices determined which was the SL, no statistical significant differences were found between the gamma radiation values of the *ex vivo* sentinel lymph node by the two pieces of intraoperatory equipment (Table 4). The Cronbach Alpha showed high internal consistency of data (0.9251).

Discussion

The use of radioguided surgery in the intraoperatory location of the sentinel lymph nodes has brought new possibilities in the treatment of tumor metastases. There are criteria that guide the evaluation of radioguided probes in this type of application and define the minimum necessary requirements. The *Nema Standarts Publication Nu 3-2004 Performance Measurements and Quality Control Guidelines for Non-Imaging Intraoperative Gamma Probes* published by the *National Electrical Manufactures Association* aims at standardizing performance measurements of radioguided probes for intraoperatory use[31].

The intraoperatory gamma detection probe developed by Costa *et al.*[31] has shown that the physical characteristics have met the suggested proposals in international publications to be used as radioguided probes in the location of sentinel lymph nodes, marked with ^{99m}Tc.

Oliveira-Filho *et al.*[14] described that the sentinel lymph node is found in around 83% to 100% of the cases, varying according to the location of the region of the sentinel lymph node. In the inguinal cases, 100% of success was reached only

TABLE 1 – Comparison between the values of gamma radiation reading using the international and the IPEN intraoperatory gamma detection equipments of each extirpated sentinel lymph node

	International	IPEN	Paired-t test
n	30	30	
Average	29,6	40,2	
Standard Deviation	38,9	26,5	t = 3,18
Median	8,5	26,0	p=0,0034*
Minimum	0	13	
Maximum	116	93	
Mann-Whitney Test	U = 293,0	p=0,020	

Cronbach's Alpha = 0,9188

* Statistically significant difference

TABLE 2 – Comparison between the values of radiation reading in the background of injection by using the International and the IPEN

	International	IPEN	Paired-T test
n	15	15	
Average	3,7	23,5	
Standard Deviation	1,4	8,2	t = 11,03
Median	4,0	27,0	p<0,00001*
Minimum	2	11	
Maximum	6	33	
Mann-Whitney Test	U = 0,0	p<0,00001	

Cronbach's Alpha = 0,469

* Statistically significant difference

TABLE 3 – Comparison between the values of radiation reading in the injection area using the International and the IPEN intraoperatory gamma detection equipment

	International	IPEN	Paired-t test
n	15	15	
Average	2824,7	3901,5	
Standard Deviation	1799,8	2583,9	t = 3,99
Median	3639,0	4688,0	p=0,0013*
Minimum	557	729	
Maximum	5176	7142	
Mann-Whitney Test	U = 68,0	p=0,067-ns	

Cronbach's Alpha = 0,9416

* Statistically significant difference

TABLE 4 – Comparison between the values of radiation reading of the *ex vivo* SLN using the International and the IPEN intraoperatory gamma detection equipament

	International	IPEN	Paired-T Test
n	15	15	
Average	78	80	
Standard Deviation	45,9	30,4	t = 0,36
Median	76	76,5	p=0,7218-ns
Minimum	31	10	
Maximum	129	161	
Mann-Whitney Test	U = 112,0	p=1,00-ns	

Cronbach's Alpha = 0,9251

with the vital blue dye, but in other locations the lymph cintilography and the intraoperatory gamma radiation detector are important.

The internal consistency of each piece of equipment was studied by means of the Cronbach Alpha analysis. The high internal consistency of data of each extirpated sentinel lymph node was 0.9188 and the low internal consistency of data in the area of the background area was 0.4690 (Table 2).

The IPEN equipment detected radiation even in lymph nodes that had not received the radiopharmaceutical, leading to a false positive, as verified by the application of Mann-Whitney's test and Student's paired-t test. On the other hand, The IPEN equipment found radiation and the hottest sentinel lymph node by means of sound guidance and also the number scale showed a level above the *background* values.

Costa *et al.*[31] reported that because of the high penetrability of gamma rays,radiation can be detected in any part of the patient and not only at the desired area. This situation implies that the detection should not only present high energy resolution, but also the capability to spatially reject undesirable gamma rays which might be reflected over the detector. Therefore false positive readings can be found in normal areas with radioactivity, leading to a diagnostic error.

These results give basis to the possibility to use the national intraoperatory gamma detection equipment in human beings.

Conclusions

1. The national equipment of intraoperatory gamma detection identifies the LS;

2. The national equipment of intraoperatory gamma detection showed false positives LS and needs improvement.

Certainly, this study will serve as the basis to use the national equipment to identify the sentinel lymph node in human beings so that the procedure can become more accessible and less expensive.

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