The use of ^{99m}Tc-phytate for sentinel node mapping in melanoma, breast cancer and vulvar cancer: a study of 100 cases

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Abstract. Sentinel node mapping reduces surgical morbidity and allows the use of more accurate tumour staging techniques. Radionuclide studies are preferentially performed using small colloids, which have limited availability in our country. The possibility of using phytate for sentinel node mapping was raised because of the similarity between its biodistribution and that of nanocolloids in the reticulo-endothelial system. In this paper we evaluated the use of ^{99m}Tc-phytate for sentinel node mapping, correlating the histopathological results with the status of the rest of the lymph node chain in different malignant tumours. A total of 100 patients were studied. group 1 consisted of 62 patients with breast cancer, group 2 of 20 patients with melanoma and group 3 of 18 patients with vulvar carcinoma. Lymph node scintigraphy was carried out after injecting ^{99m}Tc-phytate subdermally, and the sentinel node projection was marked on the skin. After 18–24 h, intraoperative sentinel node localisation was performed using a gamma probe (combined with visual localisation using patent blue dye) in 75 patients, and lymph node dissection was then carried out. Radionuclide scintigraphy identified the sentinel node in 98% of all studies. Intraoperative detection using the gamma probe was equally efficient: group 1=93% (38/41), group 2=95% (18/19) and group 3=100% (15/15). The sentinel node was involved in 41%, 31% and 20% of cases in groups 1, 2 and 3, respectively. Among the patients with positive nodes, the sentinel node was the only one affected in 53% of group 1, 50% of group 2 and 67% of group 3 cases. The method's negative predictive value was 91% in group 1 and 100% in the other groups. One false-negative study occurred in a patient who had a multifocal tumour and an intraparenchymatous lymph node; another occurred in a patient with a macroscopically affected node found during surgery. There were no side-effects related to the ^{99m}Tc-phytate. It is concluded that scintigraphic and intraoperative sentinel node identification was satisfactorily performed using ^{99m}Tc-phytate. The results were comparable to those previously described in the literature using other radiopharmaceuticals. Easy availability and low cost justify the use of phytate in our practice.

Keywords: Sentinel node – ^{99m}Tc-phytate – Breast cancer – Malignant melanoma – Vulvar cancer

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Introduction

The sentinel node (SN) corresponds to the first lymph node receiving lymphatic drainage from a specific region. It represents the initial site of tumour implantation, bearing in mind that tumour spread normally occurs in a sequential manner along the lymphatic system. Therefore, tumour presence in the SN allows one to accurately predict whether the rest of the lymph node chain is affected. The possibility of performing adequate staging and reducing both morbidity and cost (when compared to dissection of the entire lymph node chain) has led to the widespread acceptance and development of new SN sampling techniques.

The initial studies on the subject were performed without using radionuclide techniques. In 1977, Cabanas detected the first lymph node draining from a penile carcinoma and found a lower mortality rate among patients in whom this node was tumour-free. In 1992, Morton et

al. applied the SN concept in patients with cutaneous melanoma, injecting blue dye inside the lesion before surgery to map the lymphatic drainage and visually identify the first coloured lymph node.

The use of dyes is straightforward and has yielded good results [1, 2, 3], although some limitations do exist. Firstly, direct visual analysis implies that the route of lymphatic drainage for a specific tumour is exactly known, which is not always true in melanomas of the trunk. In the same way, it is sometimes difficult to detect lymph nodes in atypical locations. The time necessary for the dye to appear in the node is also variable. Therefore, there is a risk of inadvertent node dissection before dyeing of the SN or after transport to a large number of lymph nodes has occurred.

In 1993, Alex and Krag proposed the intradermal injection of sulphur colloid (labelled with technetium-99m) around the melanoma, with performance of serial scintigraphic images to determine which lymphatic chain is responsible for drainage and to detect the SN. Marking the lymph node's cutaneous projection during scintigraphy provides a reference for the surgeon, permitting less aggressive incisions and dissections. The development of a portable gamma probe led to the more widespread use of radionuclide techniques (sometimes combined with the use of dyes) for intraoperative determination of SN location. Use of such a probe and lymphatic scintigraphy increases the detection rate and allows for quicker and less morbid intraoperative sampling. The most frequently studied tumours with this method are melanoma and breast cancer; occasionally it is also used to study carcinomas of the vulva, lung, and oropharynx [4, 5].

Literature review shows relatively homogeneous results in radionuclide-guided SN identification, despite differences in terms of radiopharmaceuticals, activity, volume and route of administration. It is generally accepted that radionuclide-labelled colloids with diameters between 20 and 500 nm should be used [6, 7]: larger particles are limited by their slow transport through the lymphatic system, while smaller particles are not phagocytised in the SN and ascend along the rest of the chain uneventfully. The use of albumin nanocolloids in Europe, antimonials in Australia and sulphur colloids (subjected to modified preparation or filtration) in the United States is noteworthy. However, the limited availability of the first two agents in Brazil, and the non-uniformity and technical difficulty in preparation of sulphur colloid, led us to seek an alternative.

Although direct measurement of particle size is difficult, we considered using ^{99m}Tc-phytate for SN mapping. This possibility was raised because of the similarities in the biodistribution of phytate and nanocolloids in the reticulo-endothelial system.

Materials and methods

Patients

One-hundred patients were studied between March 1998 and July 2000 and separated into three groups:

- Group 1: 62 patients, all women, aged from 34 to 81 years (mean 58 years). The patients presented breast cancer or suspicious mammographic findings, with tumour diameters of less than 5 cm, clinically negative axillae and no distant metastases (T1/T2N0M0). Patients with multifocal tumours and those who had previously received chemotherapy were excluded. There was no history of previous breast surgery.
- Group 2: 20 patients (14 women, 6 men) aged from 22 to 74 years (mean 51 years). The patients had undergone resection of malignant melanoma, on average 3 months before the study, and did not present clinically suspicious lymph nodes. The primary lesion was located on the trunk in five patients, on the upper extremities in five, and on the lower extremities in ten. The Clark index was II in two patients, III in five, IV in six and V in seven. According to Breslow's classification, tumour depths ranged from 1.1 to 7 mm (mean 4.1 mm).
- Group 3: 18 patients with vulvar carcinoma, 16 of whom had clinically negative nodes in the evaluated lymphatic basin. In the two patients with clinically positive nodes, SN mapping was only carried out on the contralateral side.

The study was approved by the institution's Ethics Committee. All patients were informed of the nature of the procedures and gave their consent.

Lymphatic mapping

Radionuclide. The phytate vial (IPEN, São Paulo), containing 20 mg phytate and 1 mg lyophilised tin chloride, was reconstituted into a volume of 3 ml with an activity of 222 MBq (6 mCi) of ^{99m}Tc, obtained in the form of sodium pertechnetate from a molybdenum-Tc generator (IPEN, São Paulo).

Administration. Subdermal injection of 0.8 ml of radiopharmaceutical, with an activity of 55–74 MBq (1.5–2 mCi), was performed. In group 1 patients, the injection was carried out at four or more distinct points on the cutaneous projection of the palpable breast tumour, in group 2 around the scar resulting from resection of the primary tumour, and in group 3 around the vulvar lesion. After the injection, local massage was carried out in all patients to accelerate lymphatic transport.

Imaging. All 100 patients underwent scintigraphy at the Brazilian Institute of Cancer Control (IBCC), using a large field of view scintillation camera, model SPX-6 (Elscint, Haifa), with a low-energy high-resolution collimator.

The dynamic phase was carried out only in patients with melanoma (group 2), with 30-s images being obtained over a 5-min period after the injection of radionuclide. In all groups, 5-min static images were obtained from 10 to 60 min after the injection, and late images at up to 4 h were performed if necessary.

In group 1, thoracic imaging was carried out in the anterior and lateral projections (of the affected breast side). Group 2 presented the most significant regional variations after static imaging, depending on primary tumour site and on the transport ob-

served during the dynamic phase. The images typically obtained included thorax/axilla and pelvis for trunk melanoma, pelvis for lower extremity lesions and thorax/axilla for upper extremity lesions. Images of the popliteal and cubital regions were also obtained in patients with distal extremity lesions. In group 3, imaging was carried out in the anterior and lateral projections of the pelvis.

Transmission images were also performed to improve body contour identification. The identified lymph nodes were marked over the skin with the patient placed in the same position as for the future surgical procedure.

Intraoperative evaluation

Intraoperative SN localisation using the gamma probe and the blue dye was carried out in 75 patients (41 with breast cancer, 19 with melanoma, and 15 with vulvar carcinoma). The other 25 patients did not undergo the gamma probe study because the equipment was unavailable at the time. Surgery was carried out on average 18–24 h after scintigraphy.

Patent blue dye (diluted at 2%) was injected approximately 30 min before surgery. In breast cancer cases, 4 ml was injected around the tumour, whereas 2 ml was injected intradermally in patients with cutaneous melanoma and vulvar carcinoma. In surgery, the lymphatic chain was searched, and its course followed to the dye-stained SN.

We employed a GAMMED II-Eurorad gamma probe with a CdTe detector, adjusted to an energy of 140 keV. Before the incision, we assessed the number of counts over the skin markings performed during scintigraphy. These were then compared to the other areas within the same lymphatic basin, and counts of over 20 during a 10-s period were considered significant. The probe was then covered with a sterile protection and used by the surgeon to direct intraoperative SN mapping.

The number of counts in the lymph node in vivo and after its resection were measured. If activity in the surgical field was maintained at above 1/10th of the counts detected before SN removal, mapping and removal of the other lymph nodes demonstrating uptake were performed. After identification and resection of the SN, resection of or an increase in the primary tumour's margins, as well as radical node dissection, was carried out.

Histopathological analysis

In patients with breast and vulvar cancer, the resected SN was subjected to histopathological analysis. The SN was evaluated macroscopically, had its three dimensions assessed and was longitudinally incised. Imprinting of the two halves was carried out using haematoxylin-eosin (HE). In lymph nodes larger than 1 cm, frozen section analysis was also performed, preferentially on the half displaying positive imprinting. The half not subjected to frozen section (as well as any sentinel nodes smaller than 1 cm) was sent for inclusion in paraffin, and 3-µm slices were obtained and dyed using HE. In cases of melanoma, inclusion of the SN in paraffin, slicing and HE staining were carried out directly after macroscopic analysis.

Results

Scintigraphy

^{99m}Tc-phytate scintigraphy identified the SN successfully in 98% of cases. Identification occurred 30–40 min after the injection in patients of groups 1 and 3. In melanoma cases, a more rapid transport of the radionuclide was observed, which usually enabled identification of the node during the dynamic phase. The average number of lymph nodes found per patient was 1.3 in group 1, 1.7 in group 2 and 1.5 in group 3 (Figs. 1, 2 and 3).

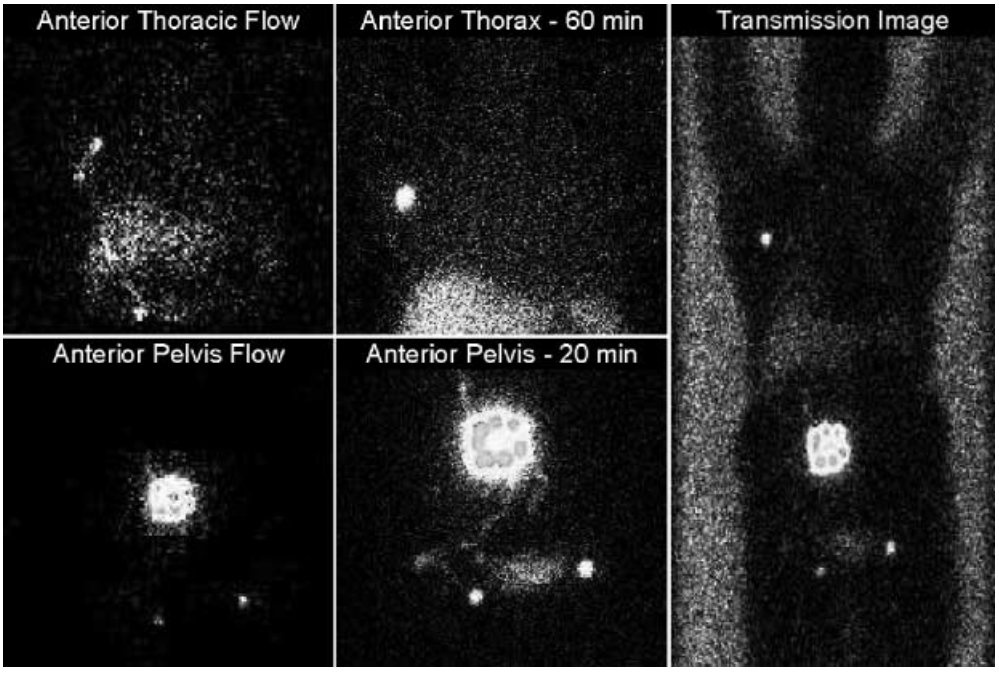

Fig. 1. ^{99m}Tc-phytate scintigraphy in a patient with melanoma on the abdomen. Rapid transport is observed to the right axillary chain and bilaterally to the inguinal chains, with uptake in the SNs of the three chains

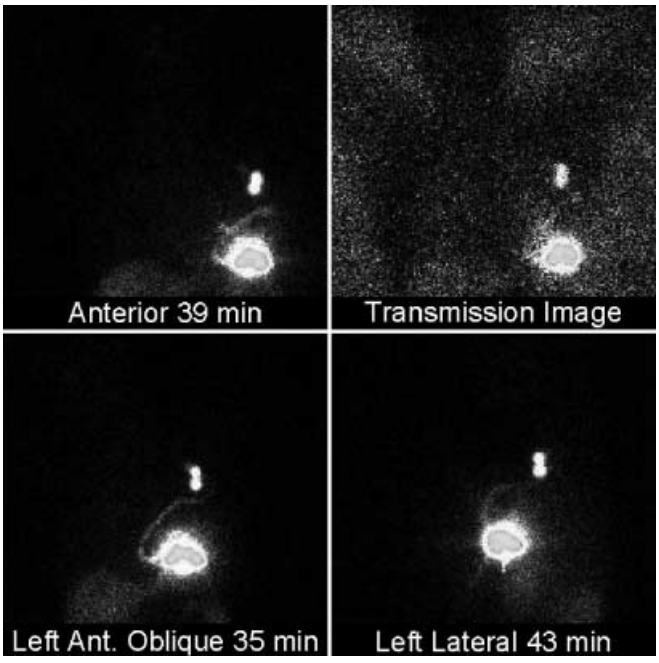

Fig. 2. ^{99m}Tc-phytate scintigraphy in a patient with left breast cancer. Transport of the radiopharmaceutical is observed towards two lymph nodes in the left axilla

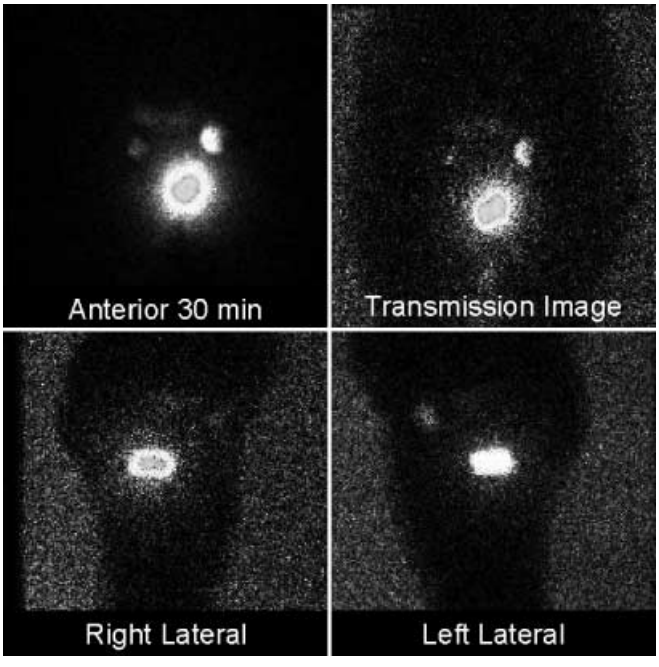

Fig. 3. ^{99m}Tc-phytate scintigraphy in a patient with vulvar carcinoma. Lymphatic transport is observed bilaterally to the inguinal chains

The SN was located in the axillary chain in all group 1 cases, and progression to the internal thoracic chain was not observed in any case. In group 2, patients with lower extremity melanomas presented SNs in the popliteal (one case) and inguinal regions (9 cases); in all five patients with upper extremity tumours, the SN was in the axillary chain. Drainage in the five patients with melanoma on the trunk was more variable: one case had bilateral axillary drainage, one had axillary and ipsilateral inguinal drainage, one had transport to the posterior cervical region, and two had exclusive drainage to the lymph node basin closest to the lesion. In vulvar carcinoma, the SN was always located in the inguinal chain. In lesions close to the clitoris, symmetrical and bilateral transport occurred towards the inguinal lymph nodes (eight cases).

Table 1. SN detection using scintigraphy and the gamma probe with ^{99m}Tc-phytate

	Scintigraphy	Gamma probe detection
Breast cancer	97% (60/62)	93% (38/41)
Melanoma	100% (20/20)	95% (18/19)
Vulvar carcinoma	100% (18/18)	100% (15/15)
Total	98% (98/100)	95% (71/75)

The only two cases in which SN identification was unsuccessful occurred at the beginning of the study, in patients with breast cancer, reducing the sensitivity in this group to 97% (60/62) (Table 1). In one patient visualisation of the node was hampered by the spreading of activity throughout the injection site, near to the axilla. In another obese patient with a tumour in the right superior-medial quadrant, the SN was not identified until 4 h after the injection. In the first case, the gamma probe was unavailable and the lymph node was found using the blue dye technique. In the second case, the SN was identified intraoperatively using the probe. There were no local or systemic side-effects related to the use of ^{99m}Tc-phytate.

Intraoperative detection

The SN was identified intraoperatively using the gamma probe in 71 patients: in 38/41 group 1 cases (93%), 18/19 group 2 cases (95%) and all 15 group 3 cases (Table 1). In the four cases in which the gamma probe failed to identify the SN, use of the blue dye did not permit identification of the node.

In two breast cancer patients in whom intraoperative identification of the SN was negative, histopathological analysis failed to identify tumour spread to the lymph nodes. In a third patient, analysis showed micro-metastases in four nodes. In these three patients, scintigraphy identified the SN in the axillary chain.

The melanoma patient in whom the SN was not localised by gamma probe or blue dye presented a tumour on the back (in the superior midline region); scintigraphy demonstrated drainage to the posterior cervical lymph

Table 2. Tumour spread to lymph nodes in patients with intraoperative SN identification after the administration of phytate

	Affected lymph nodes	Affected SN	Affected SN (and rest of the chain negative)	False-negative SN	SN accuracy	Negative predictive value
Group 1 (*n*=38)	17 (45%)	15	9	2	95%	91%
Group 2 (*n*=18)	6 (33%)	6	3	0	100%	100%
Group 3 (*n*=15)	3 (20%)	3	2	0	100%	100%
Total (*n*=71)	26 (37%)	24	14	2	97%	96%

nodes. Histopathological analysis failed to demonstrate metastatic spread to these nodes.

Tumour spread to the SN and lymphatic chains

Tumour spread to the lymphatic chains was detected using the gamma probe in 26 of the 71 patients (37%): 17/38 (45%) in group 1, 6/18 (33%) in group 2 and 3/15 (20%) in group 3. In these patients, histopathological analysis of the SN allowed us to predict the status of the rest of the chain in 95% of group 1 patients and in all group 2 and 3 patients, with a global accuracy of 97%. The negative predictive value of SN identification using phytate was 96% in all patients; specifically, it was 91% (21/23) in group 1 and 100% in groups 2 and 3 (Table 2).

The only two false-negative results occurred in group 1. One patient presented a multifocal breast tumour and we identified an affected lymph node inside the parenchyma. The other patient had a hardened and affected lymph node adjacent to the one identified as the SN by both ^{99m}Tc-phytate and blue dye.

Discussion

A high rate of SN detection was achieved using the gamma probe and ^{99m}Tc-phytate scintigraphy, with a satisfactory correlation between the status of the SN and the rest of the chain. The obtained results are comparable to those described in the literature.

In a recent review of 1,135 melanoma cases, SN detection using radioisotopes was achieved in 97% of patients, with a negative predictive value of 100% [8]. Our results were similar, with rates of 95% and 100%, respectively, even though tumours with an invasion depth of up to 7 mm were included in the study (the mean was 4.1 mm). For the purposes of technique validation, patients who would routinely be submitted to lymph node dissection and would not benefit from previous SN detection were included in the study group. Tumours with larger invasion depths showed a relatively high percentage of spread to the chain [9, 10, 11]. However, the efficiency of phytate in terms of SN detection was not affected in this group.

In 443 patients with breast cancer, the SN detection rate and negative predictive value were 93% and 96%, respectively [12], compared with 93% and 91% in our study. Our results showed a higher prevalence of tumours sized 2–5 cm (T2=57%), which might explain the relatively high percentage of tumour spread to the lymphatic chain (45%). Tumour size might also decrease SN detection by the gamma probe, as a previous study reported rates of 94% in tumours smaller than 1 cm, 85% in tumours sized 1–3 cm, 70% in tumours sized 3–5 cm and only 63% in tumours larger than 5 cm [13]. Our study's two false-negative results occurred in patients with stage T2 tumours and could be explained by the clinical data. One patient presented a multifocal tumour, which was only detected intraoperatively. Multifocality is a well-known cause of false-negative results because drainage of the injection site and drainage of other tumoural foci do not necessarily correspond [14]. In the other case, even though the axilla was clinically negative, a hardened and macroscopically affected lymph node was found during surgery.

Vulvar carcinoma has been less studied than melanoma and breast cancer, although the results have also been promising. In terms of SN detection using radionuclides, sensitivity and predictive values of up to 100% and 95%–100%, respectively, have been reported [15, 16, 17]. We found a satisfactory correlation between SN and chain status in the 15 patients studied, including the two who presented contralateral involvement (one case in which the whole chain was negative and one in which the SN and another two lymph nodes were affected). Whether unilateral (contralateral) SN evaluation is appropriate when one of the chains presents signs of tumour spread is debateable. We chose to do this (although staging remained unchanged) because demonstration of an unaffected chain would permit a less aggressive approach and could reduce the postoperative complications in one of the extremities. Clinical validation of the SN in this particular situation would require certification that tumour progression has not occurred via communication with the contralateral chain.

Lymphatic staging using the SN can sometimes be considered superior to dissection and histological study of the whole chain, since one can employ more sensitive techniques (such as immunohistochemistry) for detection

of micrometastases. Furthermore, prospective studies have confirmed that complete dissection of the chain does not affect survival rates in patients with melanoma and negative sentinel nodes [8, 9]. Prospective follow-up of 132 breast cancer patients during 39 months did not demonstrate local recurrence when lymphadenectomy was not performed in patients without SN involvement [18]. Follow-up data are still limited in patients with vulvar carcinoma.

The results reported in the literature are relatively similar, even though the employed techniques vary. The main variations concern radionuclide type, volume and route of administration. In cases of melanoma, the administration of small volumes (0.2 ml) via the intradermal route (around the lesion) is recommended. In breast cancer, volumes ranging from 0.2 [19] to 6 ml [20] are injected inside the tumour [21], around it [22] or in its projection on the skin [23]. As regards the radiopharmaceutical, colloid particles with a diameter of 20–500 nm are considered ideal, since transport is faster compared with larger particles and retention in the SN (through phagocytosis) is increased compared with smaller particles.

Although experience with nanocolloids and antimonials has been widely reported, these substances are virtually unavailable in Brazil. A similar problem exists in the United States, where these substances still require approval for use. Therefore, procedures such as filtration and modified preparations to reduce the sulphur colloid particles (which normally measure between 100 and 1,000 nm) have been developed [7].

Phytate labelled with ^{99m}Tc was initially described as a radiopharmaceutical for reticulo-endothelial system scintigraphy in 1973, forming colloid in vivo upon reaction with ionised calcium [24]. Like the uptake of nanocolloids, ^{99m}Tc-phytate uptake occurs predominantly in the liver (rather than the spleen) after intravenous injection, which indicates the formation of particles with a small diameter in the blood. Before injection, the addition of calcium to the labelled preparation results in the formation of larger particles and in a proportional increase in splenic uptake [25, 26].

The use of phytate in lymphoscintigraphy has been described previously [27, 28], although not specifically for SN detection. Alavi et al. considered ^{99m}Tc-phytate adequate for lymphoscintigraphy because lymph nodes were efficiently visualised 1 h after subcutaneous injection of the substance in animals, suggesting that the formation of colloid might occur by contact with calcium in the lymphatics [27]. On the other hand, Ege and Warbick [29] and Strand and Persson [30] demonstrated low uptake in the internal thoracic chain after injection under the xyphoid process in animals and patients, with worse results when compared with antimonial colloid.

An interesting fact pointed out by Ege and Warbick is that phytate was taken up only in the popliteal lymph node and displayed less transport to lumbar lymph nodes in rabbits (as compared with antimonial colloid) [29]. Kaplan et al. also considered phytate inadequate for study of the internal thoracic chain in patients owing to retention in the first lymph nodes, unsatisfactory transport to the higher chains, and the identification of, on average, only half of the nodes detected using antimonial colloid [28]. Although inadequate for use in these two studies, radiopharmaceutical uptake in a smaller number of lymph nodes is a desirable characteristic when considering SN mapping.

Despite the indirect data obtained from biodistribution studies, direct measurements of phytate particle size have infrequently been performed and have demonstrated that the presence of calcium has a strong influence. Electronic microscopy performed after in vitro phytate labelling (without the addition of calcium) showed particles with a modal diameter of 8 nm, although only 5%–10% of ^{99m}Tc was attached to them [29]. Another similar study showed that when phytate was reconstituted without the addition of calcium, particles with diameters ranging from 100 to 200 nm were found among smaller particles. The addition of calcium at a 2:1 molar ratio resulted in a predominance of particles with a diameter of 100–200 nm, with occasional 1-µm particles. Particles measuring up to 6 µm were detected when calcium was added at a 6:1 molar ratio [25].

Conduction and refraction studies of calcium and phytate solutions at different molar ratios demonstrated the formation of two different colloids, with satisfactory labelling efficiency of ^{99m}Tc. The first colloid was formed at a 1:1 molar calcium to phytate ratio, with particle diameters of less than 500 nm; the second was formed at a 6:1 molar ratio and had diameters above 1 µm [31]. Another in vitro study, carried out by filtering phytate (after incubation with blood) through a Sepharose column, resulted in diameters of less than 5 nm. However, this study did not specify the concentration, the molar calcium to phytate ratio during incubation or the employed anticoagulant (critical if calcium-chelating agents were used) [30].

The rapid migration of ^{99m}Tc-phytate in the initial phase, with SN detection during the initial minutes of scintigraphy, could be related to the migration of low molecular weight compounds before the formation of colloid. However, radionuclide concentration and uptake in the SN, with diminished transport to the rest of the chain, clearly indicates that colloid is being formed in the subcutaneous tissue or during its course through the lymphatic channels. The concentration of calcium is probably the main factor related to the formation of in vivo colloids, despite the possible interference of other local components. The vial use in this study contains 20 mg of phytate ($C6H9O24P6Na9+8{\cdot}H_2O$) [25] at a concentration and volume of 6.6 m*M* and 3 ml, respectively. At a subcutaneous concentration of calcium similar to that observed in experimental animals

(1.1–1.47 m*M*) [32, 33], the low molar calcium to phytate ratio must lead to the slow formation of colloid. The absorption of low molecular weight compounds into the blood (in the initial phase after radiopharmaceutical administration) would explain the rapid clearance of phytate from the injection site identified in quantitative studies [30].

An important limitation occurred when ^{99m}Tc-phytate was injected around the tumour (in five breast cancer patients, studied prior to this study), in that there was a significant reduction of migration. It is possible that local factors (calcium concentration, pH or other extracellular components) may lead to the formation of larger diameter colloids than the skin injection. Therefore, we excluded injection into the parenchyma, which has been related to failure to identify the SN in the internal thoracic chain (identified in up to 39% of cases after peritumoural injection but in only 2% after injection at the site of cutaneous projection of the tumour) [22]. If SN detection in the internal thoracic chain is found to significantly influence treatment and prognosis, this would be a limitation to be considered when using phytate.

In conclusion: Sentinel node mapping using ^{99m}Tc-phytate was performed satisfactorily in the different patient groups, with results comparable to those previously described in the literature. Despite the difficulty in determining actual particle size, ^{99m}Tc-phytate is a readily available and cheap radiopharmaceutical that can be considered a good option for SN mapping in Brazil and other countries that have similar difficulties in obtaining appropriate radiopharmaceuticals.

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