Original article

Effect of increased $^{99m}Tc/^{99}Tc$ ratios on count rates in sentinel node procedures: a randomised study

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Abstract. The aim of this study was to evaluate the count rates of sentinel lymph nodes (SLNs) in patients with breast cancer in the operating theatre, using ^{99m}Tc-Nanocoll with different ratios of technetium-99m to technetium-99. After written informed consent had been obtained, we tested different ratios of $^{99m}Tc/^{99}$Tc-Nanocoll in a double-blinded randomised study performed in 161 patients. Twenty-five MBq/µg ^{99m}Tc-colloid albumin was prepared in vacuum. In 87 patients (group A) a 2-h elution was used and in 74 patients (group B) a 24-h elution was used. Patients were subcategorised into subgroups 1 and 3, in which an SLN procedure for breast carcinoma was performed simultaneously with lumpectomy, and subgroups 2 and 4, in which an SLN procedure was performed 2–3 weeks after prior excision biopsy. All patients were injected along the lateral border of the areola (two injections: 50 MBq/0.3 ml intradermally and 50 MBq/2 ml intraparenchymally). Ex vivo measurement of count rates was performed with a gamma probe. Comparing groups A and B in respect of registered counts per second (cps) of excised SLNs, a significant difference was found ($P<0.004$). When comparisons were made between subgroups 1 and 2 (2-h elution) and between subgroups 3 and 4 (24-h elution) in respect of registered cps of excised SLNs, no significant difference was found (subgroup 1 vs 2, $P=0.825$; subgroup 3 vs 4, $P=0.915$). Use of a 2-h elution in vacuum yielded a significantly higher count rate of maximum specific activity of ^{99m}Tc-colloid albumin in SLNs than was achieved using a 24-h elution in vacuum. SLN procedures performed 2–3 weeks after prior excision biopsy proved reliable as compared to SLN procedures performed simultaneously with lumpectomy.

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Introduction

The concept of a specific lymph node, a sentinel lymph node (SLN), in the first basin to drain lymphatic flow from a primary tumour, and thus the primary site of metastasis, was first described by Cabana in 1977 [1]. Krag et al. in 1993 were the first to describe radiolocalisation and surgical resection of an SLN in breast cancer using a gamma probe [2]. In the meantime, a number of studies have been published on the sentinel node concept [3, 4, 5, 6]. The technique uses an agent, either a vital dye or a radiopharmaceutical, that enters the lymphatic drainage of the breast after injection and concentrates in one or more SLNs [7, 8]. As various radiopharmaceuticals, injection techniques and volumes are introduced, more detailed knowledge of the outcome of these diverse methods is required for optimisation [9, 10, 11, 12, 13]. In Europe, technetium-99m labelled colloid albumin (Nanocoll) is used [14, 15], whereas in the United States the most common radiopharmaceutical for SLN detection is ^{99m}Tc-labelled sulphur colloid [16, 17, 18]. There are still some goals to be achieved, especially concerning the prevention of false-negative biopsies [19, 20, 21] and improvement of the yield of successful visualisation and localisation [22, 23]. In previous studies we presented the effect of labelling high specific concentrations of

^{99m}Tc-colloid albumin in vitro and in vivo. Dynamic light scattering tests for measurement of the particle sizes of colloid albumin combined with mathematical calculations of the number of atoms of ^{99m}Tc and ^{99}Tc labelled to colloidal albumin showed that several ^{99m}Tc and ^{99}Tc atoms can be labelled to one colloid albumin particle [23]. Based on these studies, we hypothesised that an increase in the ratio of ^{99m}Tc to ^{99}Tc atoms will result in an increase in the number of ^{99m}Tc atoms labelled to colloid albumin, and correspondingly a lower density of ^{99}Tc atoms, thereby resulting in higher count rates in SLNs. This hypothesis is linked with the fact that a higher number of ^{99m}Tc atoms labelled to equal concentrations of colloid albumin will lead to a higher labelled density of ^{99m}Tc atoms to a colloid particle.

A randomised study was used in order to test our hypothesis. In this study, special attention was paid to optimisation of the localisation of SLNs in the operating theatre. We present the results of an optimised protocol for detecting SLNs in patients with breast cancer. A maximal specific activity of ^{99m}Tc-Nanocoll in vacuum [24] and different specific ratios of ^{99m}Tc/^{99}Tc were used, the ratio being varied by eluting a ^{99}Mo/^{99m}Tc generator at either 2 h or 24 h after the previous elution (2-h and 24-h elution).

Materials and methods

In vitro labelling. To label ^{99m}Tc-colloidal albumin, 5 ml saline was added to the original vial of Nanocoll. When the colloid was in solution, 10% of the colloidal albumin (0.5 ml solution) was removed from the original vial and transferred to a vacuum vial. For all patients a specific concentration of 1.3 GBq ^{99m}Tc in 4.5 ml saline was labelled to the colloid solution in vacuum. Patients were randomly assigned to a 2-h or a 24-h elution.

Stability and radiochemical labelling efficiency. Quality assessment was performed using ascending chromatography on ITLC-SG, with 95% methyl ethyl ketone as mobile phase (Rf=0: ^{99m}Tc-colloid; Rf=1: free $^{99m}TcO_4^-$). To comply with quality requirements, the radiochemical labelling efficiency needed to exceed 95%. A Canberra chromatograph (acquisition 180 s, 140 keV, 15% window) with Canberra automatic software (S-505C quality assurance software) was used.

Randomisation. All patients who presented to the Department of Nuclear Medicine between October 2001 and October 2002 were randomised to one of the two groups in a blinded manner according to the dice principle: an odd number represented series A (2 h elution) and an even number, series B (24 h elution).

In vivo study design. After written informed consent had been obtained, a double-blinded randomised series of 161 patients was tested. Inclusion criteria were: clinical tumour size ≤30 mm; no palpable nodes in the axillary region and presence of a carcinoma as proven by thick needle biopsy or by excision biopsy performed 2–3 weeks previously. We employed 25 MBq/μg ^{99m}Tc-colloid albumin prepared in vacuum using a 2-h or a 24-h elution.

Baseline characteristics. The baseline characteristics of patients are shown in Table 1.

Injection technique. In both series the nuclear medicine physician administered the radiopharmaceutical to patients along the lateral border of the areola (two injections: 50 MBq/0.3 ml intradermally using a G27 needle and 50 MBq/2 ml intraparenchymally using a G23 needle).

Imaging. Anterior and lateral gamma camera (General Electric Millennium VG) views (180 s, 140 keV, 15% window) were obtained 2 h and 4 h after injection using simultaneous cobalt-57 flood source transmission scanning. Four hours after tracer administration, the SLN was marked on the skin in anterior and lateral positions.

Probe handling. A physicist performed quality control of the probes (Europrobe, 16 mm CsI) in a 2-weekly cycle, checking constancy of sensitivity with a ^{57}Co point source.

Surgery. Surgery was performed 20–24 h after tracer administration. Ten minutes before surgery, 1 ml blue dye was injected along the lateral border of the areola intradermally. In order to validate our technique, all nodes were measured ex vivo with the same procedure. Holding the probe upward, the surgeon fixed each re-

Table 1. Baseline patient characteristics in series A and B, and in the subgroups 1–4[a]

Characteristic	Group A			Group B		
	Whole group (*n*=87)	Subgroup 1 (*n*=57)	Subgroup 2 (*n*=30)	Whole group (*n*=74)	Subgroup 3 (*n*=46)	Subgroup 4 (*n*=28)
Age range (yr)	20–79	20–79	39–73	35–85	36–85	35–72
Mean age ±SD	56.0±11.5	55.0±12.4	57.9±9.5	59.0±11.4	59.9±11.8	57.5±10.7
Left/right (breast containing carcinoma)	46/41	27/30	19/11	46/28	29/17	18/10
Tumour size (mm): clinical range	3–30	3–30	5–25	4–30	7–30	4–30
Tumour size (mm): mean±SD	15.9±6.5	17.2±6.5	13.3±5.7	15.4±6.7	16.8±6.7	13.0±6.2
Elution of ^{99}Mo/^{99}Tc generator	2 h			24 h		

SD, Standard deviation

[a] Subgroups 1and 3: carcinoma proven by thick needle biopsy; subgroups 2 and 4: carcinoma proven by excision biopsy performed 2–3 weeks previously

Table 2. Results in the different patient groups and subgroups[a]

	Group A			Group B		
	Whole group (*n*=87)	Subgroup 1 (*n*=57)	Subgroup 2 (*n*=30)	Whole group (*n*=74)	Subgroup 3 (*n*=46)	Subgroup 4 (*n*=28)
No. of second injections (SI)	6	4	2	4	3	1
No. of successful procedures (SP)	82 (95%)	54 (95%)	28 (94%)	72 (97%)	45 (98%)	27 (97%)
No. of failed procedures after SI	5	3	2	2	1	1
Removed nodes (SP)	130 (1–4)	86 (1–3)	44 (1–4)	119 (1–5)	75 (1–5)	45 (1–4)
Mean no. of removed nodes (SP)	1.59	1.59	1.57	1.65	1.67	1.67
Cps (SP)	93–6,213	125–6,213	93–5,410	78–6,902	78–4,812	80–6,902
Cps (mean±SD), first node[b]	1,738±1,596	1,812±1,652	1,600±1,505	1,070±805	1,011±891	1,202±806
IHC and HE staining neg.	49 (57%)	30 (53%)	19 (64%)	47 (63%)	29 (63%)	18 (64%)

SD, Standard deviation

[a] Group A, 2-h elution; group B: 24-h elution; subgroups 1and 3: carcinoma proven by thick needle biopsy; subgroups 2 and 4: carcinoma proven by excision biopsy performed 2–3 weeks previously

[b] $P<0.004$ for mean cps between A and B;$P=0.825$ for mean cps between subgroups 1 and 2; $P=0.915$ for mean cps between subgroups 3 and 4

moved node to the top after searching for the highest count rate. When the highest count rate was reached, a 10-s sample was registered. To correct for decay, for all patients the count rates measured ex vivo were normalised to 24 h after preparation.

Statistical analysis. All quantitative data are expressed as mean±SD. The Kolgomorov-Smirnov test was used to test data for normal distribution. Differences in data between subgroups were tested for statistical significance with the two-tailed *t* test in the case of a normal distribution. For all subgroups we used the ANOVA test to compare mean and variance of age and tumour size. *P* values <0.05 were considered statistically significant.

Results

Among 87 patients in group A (2-h elution), the SLN was successfully localised in 82 and 130 nodes (mean 1.59; range 1–4) were removed. The count rate in the nodes varied from 93 to 6,213 counts per second (cps). In five patients no focal uptake of ^{99m}Tc-colloid albumin was seen in the axillary region and the SLN procedure failed. Axillary lymph node dissection was performed and tumour-positive nodes were identified by immunohistochemistry (IHC) and haematoxylin-eosin (HE) staining in all five patients.

Among 74 patients in group B (24-h elution), the SLN was successfully localised in 72, and 119 nodes (mean 1.66; range 1–5) were removed. The count rates in the nodes varied from 78 to 6,902 cps. In two patients no focal uptake of ^{99m}Tc-colloid albumin was seen in the axillary region and the SLN procedure failed. Axillary lymph node dissection was performed and tumour-positive nodes (IHC and HE staining) were found in both patients.

When groups A and B were subcategorised into subgroups 1 and 2 (group A) and subgroups 3 and 4 (groups B), according to whether the SLN procedure was performed simultaneously with lumpectomy (subgroups 1 and 3) or 2–3 weeks after prior excision biopsy (subgroups 2 and 4), a normal distribution (one-sample Kolgomorov-Smirnov test) was found for age, tumour size and count rate. Similar high success rates for SLN localisation were observed in the four subgroups: subgroup 2, 94%; subgroup 4, 97%; subgroup 1, 95%; and subgroup 3, 98%. The overall success rate for group A was 95%, and for group B, 97%. When the mean cps of groups A and B were compared, a significant difference was found ($P<0.004$). No significant difference was found for mean cps between subgroups 1 and 2 ($P=0.825$) or between subgroups 3 and 4 ($P=0.915$). Table 2 shows the results for patient series A (2 h elution) and B (24 h elution) and for the subgroups 1–4.

Discussion

High specific concentrations of ^{99m}Tc-colloidal albumin have been found to result in a significant increase in the count rate measured ex vivo. Raising the ^{99m}Tc-colloid particle concentration in a smaller labelling volume and using a higher injected dose, as described by Valdes Olmos et al. [22], or labelling of high specific concentrations of ^{99m}Tc atoms to a minimum of colloid albumin particles without achieving a higher dose yielded similar results [23]. Both labelling procedures showed increased count rates, but different injection techniques were used, with intratumoural [22] or sub-areolar [23] administration.

Preliminary tests by other investigators on a higher specific activity mixture of sulphur colloid yielded similar results in vivo. Preparation of a higher specific activity was achieved by using only one-eighth of the sulphur colloid while the same activity (1 GBq) of ^{99m}Tc was used [24].

Eluting a ^{99}Mo/^{99m}Tc generator twice a day leads to a different ratio of ^{99}Tc/^{99m}Tc atoms. From the mother/daughter relationship of ^{99}Mo and ^{99m}Tc/^{99}Tc, it is known that the ^{99m}Tc/^{99}Tc ratio is 1:2 when eluate is obtained 24 h after a previous elution. The advantage of labelling ^{99m}Tc-colloid albumin using an elution taken 2 h after the previous one is the increased amount of ^{99m}Tc (^{99m}Tc/^{99}Tc ratio of 9:1). Raising the density of ^{99m}Tc atoms and correspondingly reducing the density of ^{99}Tc atoms in solution will increase the number of ^{99m}Tc atoms labelled to a colloid particle. Lymphatic transport of an equal concentration of colloid albumin particles labelled with a higher number of ^{99m}Tc atoms results in higher count rates in SLNs, measured ex vivo. Although the increased count rate in our study was statistically significant, it did not affect the number of successful procedures.

Overall, in the two series, a total of ten patients did not show any uptake of ^{99m}Tc-colloid albumin in an SLN (six patients in series A and four in series B). Administering a second dose appeared not to be helpful in seven patients (five in series A and two in series B), and in all of these patients, lymph node dissection revealed lymph node metastases. These findings suggest that non-visualisation of lymph node(s) 2 h after administration may indicate a high probability of nodal disease and that administration of further activity to identify an SLN may not be appropriate.

Colloid albumin (Nanocoll) is available as a kit containing human albumin particles and stannous chloride dihydrate. The preparation of ^{99m}Tc-colloid albumin involves the addition of sodium pertechnetate to a lyophilised vial of human colloid albumin particles, stannous chloride, glucose, polyoxamer 238, sodium phosphate and sodium phytate [25]. It is critical to exclude oxygen from the vial during the addition of the sodium pertechnetate, as the oxygen will form a stannous technetium colloid, which prevents the ^{99m}Tc from binding to the albumin particles [26]. Labelling a high specific activity of ^{99m}Tc-colloid albumin in vacuum requires experience. Extraction of the colloid albumin solution from the original vial, transfer to the vacuum vial and addition of the sodium pertechnetate to the vacuum vial have to be done within less than 1 min. Avoidance of contact with oxygen is critical while transferring the colloid albumin solution from the original vial into the vacuum vial.

According to whether it is administered into breast tissue or tumour tissue, injection of ^{99m}Tc-colloid albumin may lead to different adherence of in vitro stabilisers of ^{99m}Tc-colloid albumin. Several mechanisms may be responsible, including different reactions of normal breast tissue or tumour tissue with glucose, polyoxamer 238 or sodium phosphate (stabilisers of colloid albumin). Furthermore it is obvious that low ^{99m}Tc/^{99}Tc ratios of a 48-h (2:8) or 72-h (1:9) elution (for example the first elution on a Monday) might give rise to less satisfactory results.

There are still questions to be answered, especially concerning the stability of ^{99m}Tc-colloid albumin in vivo and the high amount of activity remaining at the injection site in relationship to the activity transported to SLNs via the lymphatics.

Conclusion

Use of a 2-h elution in vacuum yielded a significantly higher count rate of maximum specific activity of ^{99m}Tc-colloid albumin in SLNs than was achieved using a 24-h elution in vacuum. Performance of SLN procedures 2–3 weeks after prior excision biopsy proved to be reliable as compared to simultaneous performance of the SLN procedure and lumpectomy.

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