
Chapter 8

Thesis summary, future perspectives and conclusion

Summary

The sentinel lymph node (SLN), the first draining node within a lymph node basin, is the first to receive lymphatic drainage from a tumor site. The tumor status of the SLN is of particular interest for clinicians, since it has an important prognostic value for the patient and because of its impact on the treatment regime. Before a surgical sentinel lymph node procedure is being performed, patients are subjected to an elaborate diagnostic pathway. In agreement with SLN criteria on breast carcinoma, patients are proposed for surgery after a malignancy of the breast has been diagnosed by physical examination, mammography, and ultrasound and cytology evaluation. Usually surgical excision of the primary tumor and a SLN procedure are performed in a single session. Ever since the development of the diagnostic procedure for SLN's, no radiopharmaceutical has been registered exclusively for this purpose. Nanocolloid [1], Sulphur colloid [2], Antimone sulphide [3], and the recently commercial available cysteine-Rhenium [4] labeled colloid are all being used for a diversity of SLN procedures. These pharmaceuticals are in fact registered and used clinically for either lymph-edema, liver scintigraphy, or bone marrow scintigraphy, respectively. In Europe, the most frequently used radiopharmaceutical for this purpose is Nanocoll[®], whereas in the United States filtered Sulphur colloid and in Australia Antimone sulphide is generally being used. The reason for the discrepancy is the fact that Antimone sulphide is not registered in Europe and in the United States, Sulphur colloid is not registered in Europe and Australia whereas Nanocoll[®] is neither registered nor available in the United States and Australia.

This thesis focuses on the optimization of a radiopharmaceutical labeling procedure, that leads to maximal specific activity of ^{99m}Tc-Nanocoll[®] with radiochemical purities exceeding 95% [1]. The thesis also describes several studies that were conducted using the optimized radiopharmaceutical. The contents and major findings of the thesis are summarized below.

In chapter 2, two pilot studies are described in which batches of ^{99m}Tc-colloid albumin with different specific activities were used to detect axillary sentinel lymph nodes. In the first pilot study 28 patients were given ^{99m}Tc-colloid albumin, which was derived from a stock that was made by labeling 1.3 GBq ^{99m}Tc to 500 µg colloid albumin. In the second pilot study, 20 patients were included, which were all injected with ^{99m}Tc-colloid albumin that was derived from a stock that was made by labeling 1.3 GBq ^{99m}Tc to 125 µg colloid albumin. For labeling of the colloid albumin, a 24 h eluate was used for both groups, and all patients from both groups were injected with identical

doses and volumes using a standardized technique. Surgery took place at 20-24 h after injection of the radiopharmaceutical. In 10/28 patients of the first group, detection of the SLN proved to be difficult (mean count rate of 94 CPS, corrected for radioactivity decay), whereas in three patients the SLN could not be detected. In the second pilot experiment, a high detection rate was achieved (18/20 patients; mean count rate of 490 CPS). In two patients of this group, the SLN could not be detected (both of these patients had previously undergone excision biopsy). At time of surgery, the count rate of the SLN was increased with a magnitude of approximately five in the second group as compared to the first group. From this pilot study, it can be concluded that a high specific activity of ^{99m}Tc labeled colloid albumin favors the target to non-target ratio, eventually facilitating detection at surgery. Although no significantly shorter surgical procedures were detected in the group of patients that were injected ^{99m}Tc -colloid albumin with a high specific activity, the procedure was reported to be more efficient and effective as compared to procedures that were performed using ^{99m}Tc -colloid albumin with a lower specific activity. The other studies that are presented in this thesis were inspired on the results of this pilot study. In the following chapters of the thesis, the in-vitro and in-vivo evaluation of an optimized protocol for labeling of colloid albumin with ^{99m}Tc is described which has led to a higher specific activity of the radiopharmaceutical. Furthermore, the step by step description of methodological aspects of extracting colloid albumin of the original vial Nanocoll[®] is described in this chapter.

It also explains ITLC chromatography of radiochemical purity of labeled ^{99m}Tc -colloid albumin with and without involved oxygen.

Chapter 3 describes an in-vitro study that was carried out in order to identify the particle size of Nanocoll[®], the amount of colloid albumin particles that are contained in a vial, and the number of technetium atoms that can be labeled to a single colloid particle. Also, the maximal specific activity of Nanocoll[®] that can be obtained from an in-vitro labeling procedure using a 24 h ^{99m}Tc eluate is determined. Additionally, a clinical study using various specific activities of the tracer in 5 groups of patients (including double blind randomized groups) is presented in chapter 3. The particle sizes that were calculated from the in-vitro experiment were within a range of 7-23 nm, with a mean of 12 nm, which was at odds with the product specification of Nanocoll[®], as provided by the manufacturer.

The outcome of the clinical study in terms of detection rate and ease, were similar for groups in which Nanocoll[®] was used that was labeled under nitrogen using 5.2 GBq ^{99m}Tc (from a 24 h eluate) per 500 μg of the product, as compared to Nanocoll[®]

that was labeled using 1.3 GBq ^{99m}Tc per 125 μg of the product. The double blind randomized series of patients were injected with 75 MBq, which was prepared using either 50 μg or 125 μg Nanocoll[®] under vacuum with 1.3 GBq ^{99m}Tc , obtained from a 24 h eluate. Surgery was performed at 24 h after injection of the radiotracer and the patients that were injected using the batches in which 1.3 GBq was labeled to 50 μg Nanocoll[®], demonstrated enhanced count rates of the SLN (corrected for radioactivity decay), with a magnitude of approximately 1.5 as compared to the group that was injected with batches in which 1.3 GBq ^{99m}Tc was labeled to 125 μg Nanocoll[®]. From this study, it is concluded that labeling in nitrogen of the radiopharmaceutical using enhanced concentrations (amount of ^{99m}Tc per μg colloid albumin) by a magnitude of 4, results in an increase of ex vivo count rate of SLN's by a magnitude of 6 ($P < 0.002$) after excision. Enhancement of the amount of ^{99m}Tc per μg colloid albumin in the labeling mixture (in vacuum) by a magnitude of 9, results in an increase of count rate of SLN's that are measured ex vivo by a magnitude of 9 ($P < 0.001$). Measurements of CPS in all clinical studies were performed with standardized conditions.

In Chapter 4, data is presented from an in-vitro study that was performed to assess an optimal labeling procedure for ^{99m}Tc Nanocoll[®] using eluates that were obtained from a $^{99}\text{Mo}/^{99m}\text{Tc}$ generator at different time points after the previous elution. Based on thin layer chromatography studies of a large series of labeled concentrations of Nanocoll[®], the maximal labeling efficiency of ^{99m}Tc Nanocoll[®] at a radiochemical purity of $>95\%$ is determined under nitrogen or vacuum conditions. To evaluate the effects of differences in $^{99m}\text{Tc}/^{99}\text{Tc}$ ratios on the maximal specific activity that could be obtained, eluates were used that were extracted from the $^{99}\text{Mo}/^{99m}\text{Tc}$ generator at 2, 24 and 72 h after the previous elution. Another experiment demonstrated that there are no adverse effects on specific activity of reduction of $^{99m}\text{Tc}^{7+}$, which may occur when stannous chloride and colloid albumin are extracted simultaneously from the vials. Thus, $^{99m}\text{Tc}^{7+}$ did reduce to $^{99m}\text{Tc}^{5+}$ and $^{99m}\text{Tc}^{4+}$ to the same extent in all tested concentrations of Nanocoll[®], regardless of extraction of stannous chloride and colloid albumin from the vial. Additionally performed in-vitro experiments in which cysteine was added to the vials, showed an increase of the labeling efficiency up to almost 100%, which was the result of addition of electrons derived from cysteine to the reduced Tc^{5+} . The interaction of the electron with Tc^{5+} , leads to a further reduction to Tc^{4+} . Under nitrogen, nearly all $^{99m}\text{Tc}^{4+}$ atoms rapidly bind, together with oxygen atoms, to albumin.

This chapter also describes some clinical appliances of ^{99m}Tc Nanocoll[®]. In a randomized study in which 158 patients were included, uptake in the SLN's of

Nanocoll® that was labeled in a specific concentration of 26 MBq/μg under vacuum conditions, with ^{99m}Tc from either a 2 h or a 24 h eluate, were compared. To assess the clinical effects of variations of the ^{99m}Tc/⁹⁹Tc ratio in the eluate on the count rate in SLN's, an experiment was performed in 3 groups of patients. The first group (n = 33) received ^{99m}Tc-Nanocoll® that was labeled under nitrogen with 8 MBq ^{99m}Tc/μg colloid albumin, using a 72 h eluate. The second group (n = 74) was given 10.4 MBq/μg colloid albumin, labeled under nitrogen using ^{99m}Tc from a 24 h eluate, whereas patients of the third group (n = 103) were injected with 26 MBq/μg colloid albumin that was labeled under nitrogen using a 2 h eluate. A statistically significant higher ex vivo count rate in SLN's was detected between the second as compared to the first group (P = 0.005; approximately magnitude), but also an even more statistically significant difference between the first and the third group was observed (the count rate was higher by a magnitude of 17 in the first group). The patient group that was given 26 MBq/μg colloid albumin that was labeled in nitrogen using a 2 h eluate, showed no statistically significant differences as compared to an additional group of patients that was injected with colloid albumin, labeled using identical concentrations of ^{99m}Tc, but in vacuum (P = 0.481). However, technically it is much easier to perform the labeling procedure in nitrogen than in vacuum. It is therefore concluded that ^{99m}Tc-Nanocoll®, labeled in nitrogen with a mixture of 26 MBq ^{99m}Tc (from a 2 h eluate) per μg colloid albumin, is the optimal tracer in our experiments that can be obtained for detection of SLN's.

Chapter 5 reports on a randomized clinical trial in which we have injected at total of 161 patients with the ^{99m}Tc-Nanocoll® that was labeled in vacuum using a 26 MBq ^{99m}Tc per μg colloid albumin mixture from either a 24 h (n=74) or a 2 h (n=87) eluate. The two groups were both further divided in subgroups that had underwent lumpectomy within several weeks before the sentinel lymph node procedure (n=28 from the 24 h and n=30 from the 2 h eluate group), or that had the lumpectomy and sentinel lymph node procedure during one single surgical procedure (n = 46 for the 24 h, and n = 57 for the 2 h eluate group). All groups were normally distributed. We encountered significant differences in ex vivo count rates of the SLN's between the 2 h and 24 h eluate groups (P<0.004). However, the study showed no differences in the success rate of the procedure between the groups that had both the SLN procedure and the lumpectomy simultaneously as compared to the group that had the lumpectomy several weeks before the SLN procedure. Also no differences in ex vivo count rate of the excised sentinel lymph nodes were detected between these groups using the radiotracer that was made using either the 24 h (P=0.915), or the

2 h ($P=0.825$) eluate.

We conclude that surgical SLN procedures using ^{99m}Tc -Nanocoll[®], when labeled with a high specific activity obtained from either a 2 h or 24 h ^{99m}Tc eluate, shows success rates of SLN procedures that are similar for both patients that are subjected to simultaneous lumpectomy as compared to patients that had lumpectomy within the weeks before the SLN-procedure.

In Chapter 6, a study is presented in which we evaluated the value of ^{99m}Tc -sestamibi in 101 patients that were previously diagnosed with a breast lesion by the Dutch screening program for breast carcinoma. All ^{99m}Tc -sestamibi scintigraphies were assessed in a blind setting by two experienced nuclear medicine physicians, without prior knowledge of the outcome of physical examination, mammography or ultrasound of the mamma. In 35/101 patients, ductal or lobular carcinomas of the breast were diagnosed, whereas in 5/35 patients also showed axillary uptake. Histopathology of SLN's after SLN procedures revealed metastases in the axillary lymph nodes in 15/35 patients, of which 10/15 were classified as being a micro metastasis. None of these micro metastases was detected on the ^{99m}Tc -sestamibi scintigraphy. In the other 5 patients, uptake in the axilla was detected by scintigraphy.

Scintigraphy of the mamma proved to have a sensitivity of 82% and specificity of 93%. The positive predictive value is 90% and a negative predictive value is 87% in this patient population. The sensitivity of 33% in patients with axillary lymph node metastases that are diagnosed with scintigraphy is by far inferior to the sensitivity of the histopathology and immunohistochemistry of 100%.

In Chapter 7, results are presented from a questionnaire concerning the techniques that are used for the SLN procedure in the Netherlands and developments between the techniques that were used in 2005 and in 2009. For this survey, 70 (2009-71) departments of Nuclear Medicine were questioned. The percentage of response was 89% in 2005 and 93% in 2009. Notable differences were detected in both 2005 and 2009 in the volumes that were added to vials of Nanocoll[®], but also variations in specific activity of the radiopharmaceutical during the week were observed. In 2005 and 2009, there proved to be differences in quality control of the labeling procedure and approval of a pharmacist before use of the radiopharmaceutical in humans. In both 2005 and 2009, the patient dose varied widely, as did the number of injections per patient and the administered volume per injection. The number of hospitals in which a second dose was administered in case of non-visualization of the SLN, was unchanged between 2005 and 2009. An increase was observed between 2005 and

2009 in the amount of quality control procedures of probes, although the spread is considerable in terms of frequency. In 2005, almost one quarter of the responders claimed that histology was performed on frozen tissue slices of the SLN at the time of surgery, whereas in 2009 this had increased to almost half. The survey in 2005 and in 2009 demonstrated a multitude of protocols and a range of nuclear medicine techniques that are being used in SLN procedures. Clearly, a Dutch standard on the technical aspects of SLN procedures is still lacking. Standardization of these techniques in a national protocol is recommended and should be integrated in the next update of the Dutch guidelines on breast carcinoma.

Discussion, future perspectives and conclusions

Since Morton et al. reported on the clinical appliance of the SLN procedure in 1992 [5], many studies have been focusing on this matter. The topic has increasingly gained interest of the scientific community, which has led to many publications and conventions on this topic worldwide. The technique that was proposed by Morton was introduced in 1995 in the NUGES (an alliance between departments of Nuclear Medicine from various institutions) affiliated hospitals. At first, the technique was used only for detection of SLN's of melanoma, but the procedure was later extended to mamma carcinoma patients in 1998. The Dutch multidisciplinary clinical consensus on the treatment of mamma carcinoma was released in 2002, with revisions in 2004, 2005, 2006 and 2008 [6]. However only one nuclear medicine physician participates in the committee that was involved in this consensus. For instance, the ROLL technique (radio-guided occult lesion localization) and the biopsy of SLN's under local anesthesia was part of a revision that was made several years after the initial version of the consensus. [7-15].

Radiopharmacy

Up to date no adequate studies have been performed to evaluate the possibility of high specific labeling of Nanocoll[®], a radiotracer that is widely used in Europe these days [1]. In the United States of America [2] and Australia [3] other radiopharmaceuticals are used, although the recently commercially introduced cysteine-rhenium-colloid [4] is used only in a small number of hospitals. None of these radiopharmaceuticals has been registered for the detection of sentinel lymph nodes, and none of the manufacturers intends to register the products. Nevertheless, both products have proved their value for the localization SLN's [2, 16-19]. This thesis

addresses a simple, reproducible and optimized protocol for high specific labeling of Nanocoll®. We conducted a survey in the majority of departments of Nuclear Medicine in the Dutch hospitals and encountered a large diversity of protocols that are used for localization of SLN's, but almost all hospitals report a high success rate of the procedure. For instance, the effects of differences in the injected volume and the injection technique have been matter of debate [20-25]. Standardization of the procedure should be initiated by organizations such as the Dutch Society of Nuclear Medicine (NVNG) [26]. Relevant reports that may support the composition of such a standardization protocol have been published previously [27-28].

Similar guidelines from other European countries may also help in the development of domestic guidelines for SLN procedure in breast cancer [29-32]. In the British guideline on SLN procedure in breast carcinoma, as compiled by the British Nuclear Medicine Society (BNMS), the use of Nanocoll® labeled with high specific concentrations of ^{99m}Tc is recommended [32]. Worldwide, no radiopharmaceuticals are registered for use in SLN procedures, but all radiopharmaceuticals are registered for other purposes [33]. One reason for the lack of registration of the radiopharmaceuticals is the fact that there is no commercial benefit from registration as compared to the actual situation. Expensive large scale clinical trials would be necessary for registration. However, legislation such as the Dutch law on pharmaceuticals, as well as the relatively high number of SLN procedures that are being performed essentially requires the development of a radiopharmaceutical intended for the detection of SLN's specifically.

Developments in imaging techniques

In the diagnosis of the SLN, a range of imaging techniques may be involved. Nuclear medicine techniques focus on the visualization of the SLN using a gamma camera, and may implicate dynamic imaging of the sentinel lymph node during several minutes starting from the moment of injection [34]. Another approach is the acquisition of planar images at 2-4 hours after injection with or without acquisition of SPECT images. Recently, a portable gamma camera was introduced to visualize the SLN during the surgical procedure. Unfortunately, the value of technique has proven to be limited due to the low resolution of the images and problematic fixation of the device during surgery.

Other recent publications have demonstrated the benefits of combined SPECT/CT for anatomic visualization of SLN's [35-37], and showed that the improved localization of SLN's prior to surgery was helpful to the surgeon, especially when the SLN was located at unusual sites. SPECT/CT may also be of value to localize the SLN in

cases in which the node is not or hardly visible on planar scintigraphy at 2 to 4 h after injection. However, physical localization during the surgical procedure is difficult for SLN's that are detected by SPECT imaging but not planar scintigraphy due to the threshold of the detector probes and thus the difficulty to discriminate the SLN from the background activity.

Recent advances in PET/CT imaging have also led to the implementation of ^{18}F -FDG in SLN detection. In previous studies performed by van der Hoeven et. al., axillary metastases are demonstrated in a approximately 25 -50% using ^{18}F -FDG-PET [38]. Moreover, Ueda et. al. reported the detection of axillary metastases by PET/CT techniques with a diagnostic accuracy of 83%, a sensitivity of 58% and specificity of 95% [39]. On the other hand, Kim et al. reported a diagnostic accuracy of 94% with a sensitivity of 77% and specificity of 100%, for axillary lymph node metastases of breast carcinoma in a series of 137 patients [40]. Unfortunately, no cost-effectiveness study has been published to date to evaluate the cost of PET/CT against a possible reduction of SLN procedures.

Conclusion

In this thesis, a protocol for the optimization of the SLN procedure has been proposed. It was hypothesized and confirmed by in-vitro experiments that labeling of $^{99\text{m}}\text{Tc}$ -colloid albumin using higher concentrations of $^{99\text{m}}\text{Tc}$ per microgram colloid (MBq/ μg), results in a higher specific activity than the labeling in low concentrations $^{99\text{m}}\text{Tc}$ per microgram colloid (MBq/ μg). We have also performed a number of clinical studies and observed a clear enhancement of the ex vivo count rate of SLN's in patients that were given colloid albumin with high specific activity as compared to patients that received colloid albumin with a relatively low specific activity.

It was also hypothesized that labeling of $^{99\text{m}}\text{Tc}$ -colloid albumin at higher specific activity in a mixture containing a high ratio of $^{99\text{m}}\text{Tc}/^{99}\text{Tc}$, results in easier detection during the actual SLN procedure. We have performed both in-vitro experiments as well as a randomized clinical trial, which eventually led to confirmation of that hypothesis. In those clinical studies, a further increase in ex vivo count rate was observed in SLN's from patients that were given the $^{99\text{m}}\text{Tc}$ -colloid albumin labeled in a mixture containing a high ratio of $^{99\text{m}}\text{Tc}/^{99}\text{Tc}$.

It was also demonstrated by in-vitro studies that reduction of $^{99\text{m}}\text{Tc}^{7+}$ does result in either one of two valence states, i.e. $^{99\text{m}}\text{Tc}^{4+}$ and $^{99\text{m}}\text{Tc}^{5+}$, confirming our hypothesis of this process. Additionally performed in-vitro experiments in which cysteine was added

to the vials (all tested concentrations) proved this, using ITLC chromatography with trichloroacetic acid as the mobile phase. Thus, we conclude that the extraction of stannous chloride has no effect on the efficiency of the labeling procedure.

Furthermore, this thesis also states that labeling efficiency of ^{99m}Tc -colloid albumin is not influenced by the amount of colloid albumin that is present in the labeling mixture, since there is no difference in the efficiency between labeling that was performed in vials containing 5.2 GBq ^{99m}Tc and 500 μg colloid albumin, as compared to vials that contained 1.3 GBq ^{99m}Tc and 125 μg colloid albumin. Subdividing the vial of a commercial kit into smaller portions should therefore not have a negative effect on the detection or ex vivo count rate of SLN's, when labeled by high concentrations of ^{99m}Tc , as was demonstrated in the clinical study that was described in this thesis.

Another approach using nuclear medicine techniques for imaging of pathology in breast carcinoma proved to be less successful. Although ^{99m}Tc -sestamibi was originally developed as a tumor tracer, scintigraphy of the breast and axillary regions with this radiotracer seems insufficient for the evaluation of either primary tumors or lymph node metastasis in non-palpable breast carcinoma.

In the past decade, the SLN procedure has been widely implemented in daily clinical practice and <10.000 procedures are being performed each year in the Netherlands, and is still increasing every year. Our survey, covering Nuclear Medicine departments of 70 institutions in the Netherlands, all performing SLN procedures, revealed a diversity of protocols that are being used for the procedure. Standardization of the technical procedures of the SLN procedure should be pursued.

To date, development and registration of a radiopharmaceutical such as colloid albumin, specifically for imaging and probe guided detection of SLN procedures have been lacking. Given the increasing number of SLN procedures, it is of importance to develop such a radiopharmaceutical. Products of non-human origin, for example based on particles of glass (20 nm) have been suggested and may be developed for the purpose of SLN procedures in the future.

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