Chapter 1

General introduction and outline of the thesis

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In the Netherlands, approximately 13,000 women are diagnosed with breast cancer each year and approximately 1,300 are diagnosed with in situ breast carcinoma. For women, the life-time risk for developing breast carcinoma is 12-13%. The diagnosis of the malignancy is based primarily on the triple diagnosis (physical examination, imaging and cytology or histology) [1-3], although other criteria may apply for palpable abnormalities that are not suspicious for malignancy. The revised version of the NABON-note (National Breast Cancer Consideration Netherlands) describes how diagnosis, treatment, counseling and follow-up of breast pathology should ideally be organized, in compliance with the national guidelines for the management of breast tumors [4]. It also refers to the European Criteria of the European Society of Breast Cancer Specialist (EUSOMA) and the surgical quality criteria.

Being located between fascia and skin, the mammary gland drains its lymph to the axillary lymph nodes, similar to the fascia and skin, towards to the subareolar plexus. Lymph pools in this plexus, before it drains to the axillary area (figure 1). There may also be alternative lymph drainage routes to the inframammary region, or to diaphragm and liver. Other possibilities include drainage to the parasternal, or the supraclavicular region.

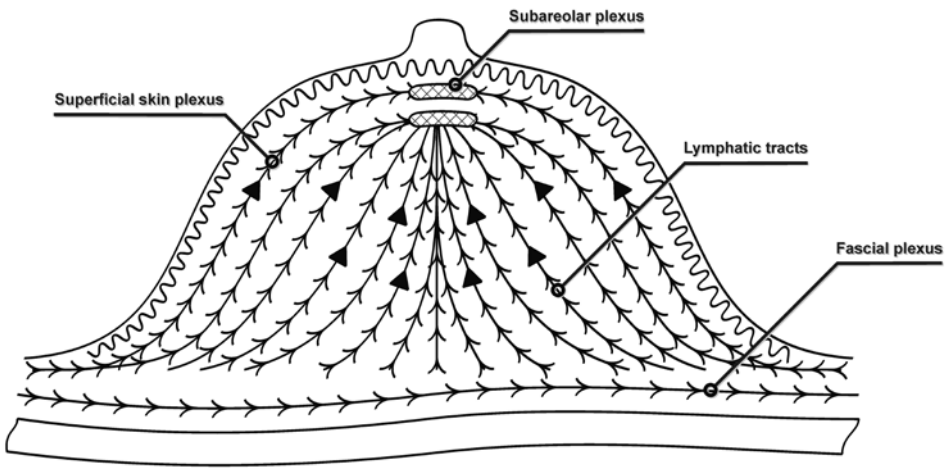

Figure 1: lymph drainage of the breast by Sappey. Lymph of the mammary gland drains to the subareolar plexus.

The sentinel lymph node (SLN) procedure is the preferred method for staging of lymph nodes for T1 and T2 breast tumors without clinical evidence of axillary metastases [5]. In the past decade, SLN procedure has been widely implemented in daily clinical practice and over 10,000 procedures are performed each year in the Netherlands, a number that is still increasing every year.

Various studies show that a SLN is detectable in more than 95% of the patients. The lack of metastases in axillary SLN's has been proven to be a reliable predictor for the absence of lymph node metastases in 95% of the cases [5-12]. A study by Veronesi et al. demonstrates that SLN procedure is a safe alternative for axillary lymph node dissection in T1 breast malignancy when there are no axillary lymph node metastases [13]. If a SLN procedure is not possible, a complete dissection of the axillary lymph nodes on both level I and II should be carried out [5]. Contra-indications for SLN procedures are axillary lymph nodes in which malignancy is suspected or proven, tumors >T2, disrupted lymph drainage after previous axillary surgery or disturbed drainage due to previous lumpectomy. Other groups are studying the whole of SLN after axillary lymph node dissection. Metastasis in SLN's in patients with invasive breast cancer requires further treatment of the axilla, except in cases of submicrometastases (ITC) [5]. There are no general guidelines for patients with relatively large or multifocal tumors with a diameter up to 5 cm, for patients with previous excision, or for patients treated with neoadjuvant therapy. The SLN procedure is considered to be safe in multifocal tumors limited to a single quadrant of the breast. However, if preoperative imaging studies suggest multifocal abnormalities, the reliability of the SLN procedure is uncertain [5].
Theoretically, in ductal carcinoma in situ (DCIS) there is a no indication for SLN biopsy since there is no association with lymphatic metastases [14]. However, in situ carcinomas have been shown to contain invasive properties by post-operative histopathology in 10-29% of the cases [15]. In large DCIS, a SLN procedure may be considered.

The value of the SLN procedure for multifocal tumors is still under debate. There are controversial opinions concerning the site of injection of the radiopharmaceutical (in or around the tumor vs. intracutaneously or subareolar). In a previous report by Roumen et al., it is demonstrated that drainage of the tracer in the lymphatic system after intra-parenchymal or subcutaneous periareolar injection is identical in the majority of the cases, but complementary in 9% of the cases [16, figure 2].

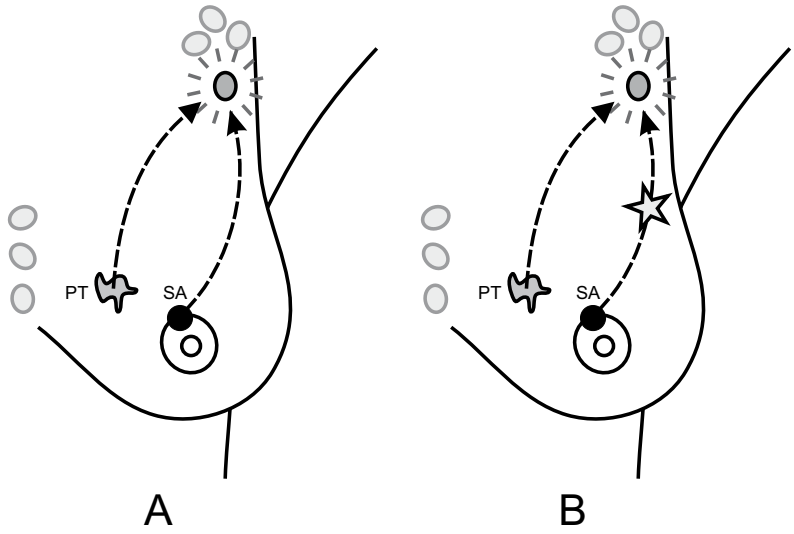

Figure 2: match or mismatch of focal accumulations after peri-tumoral or intra dermal injection of ^{99m}Tc Nanocoll®: Roumen et al. Eur J Surg Onco 1999; 25:349.

Some researchers, however, argue that each tumor has its own route of lymph drainage which necessitates injection of the radiotracer near the site of the tumor [17]. Other reports claim that this may lead to erroneous SLN localization resulting in an increase in the number of false negative SLN procedures [18-19]. Interestingly, other researchers state that the lymphatic drainage of the entire breast is uniform and that the radioactive tracer can be injected anywhere in the breast, and that multifocality of breast tumors is no contra-indication for a SLN procedure [20]. Intra- or peritumoral administration of the radiopharmaceutical leads to scintigraphic SLN localization in the parasternal area in nearly 20% of the cases [21].
The best results of the SLN procedure are obtained when pre-operative lymphoscintigraphy with radiocolloid tracer is combined with administration of patent-blue during the surgical procedure. The visualization with radiocolloid tracer is based on phagocytosis by macrophages in the lymph node(s), whereas the visualization using the blue dye results from simple flow via lymphatic vessels to lymph nodes. Veronesi et al. has demonstrated the occurrence of parasternal lymph node metastases in approximately 10% of patients with medially located tumors larger than

2 cm [22]. There is no consensus in the literature on the relevance of parasternal SLN biopsies in daily clinical care [23-26].

The tumor status of axillary lymph nodes is an important prognostic indicator for breast malignancy and in case of initiation of adjuvant systemic therapy is always considered. On the other hand, dissection of lymph nodes may be part of local control. In the SLN procedure, selective removal of one or more lymph nodes to which the lymph vessels of the breast drain directly, is being performed. The SLN procedure predicts the likelihood of axillary lymph node metastases in the other axillary lymph nodes. When no metastasis is detected in the SLN, existence of metastases in other axillary lymph nodes is very unlikely, if not negligible, and thus total dissection of axillary lymph nodes is omitted. However, if the SLN contains tumor, additional axillary treatment is indicated. The chance of detection of metastases in axillary lymph nodes depends on the preciseness of the sampling procedure by the pathologist, and therefore a standardized methodology is used to investigate SLN's histopathologically [4, 5].
There are still some dilemmas and issues concerning the SLN procedure that need to be clarified. Particularly the definition of isolated tumor cells (ITC) and micro metastasis and its significance remains controversial [4, 5]. The International Union against Cancer (UICC) has defined micro metastasis on the basis of size: a lymph node with a tumor larger than 0.2 mm and less than 2 mm. The definition of ITC is less clear. The UICC defines this category as isolated tumor cells (solitary tumor cells or small clusters of cells with a diameter < 0.2 mm). With regard to staging, there is consensus on the meaning and impact of ITC (pN0) and macro metastases (pN1). There is still debate about the prognostic and clinical significance of micro metastases (pN1mi) in the SLN [4, 5]. In local tumor control, total dissection of the axillary lymph nodes is part of the treatment regime. One of the uncertainties of the SLN procedure is its effect on local tumor control. The SLN procedure provides reliable staging of the axillary lymph nodes in at least 95% of patients with primary tumors smaller than 5 cm without clinical suspicion of axillary lymph node metastases [4-6]. It is also known that the amount of tumor in the SLN is related to probability of other metastases in axillary lymph nodes. The Dutch guidelines on breast carcinoma describe the consensus and standardization of diagnostic strategies, as well as directives for treatment regimes. The SLN procedure is an important part in determining the extent of the disease.
The success rate of sentinel lymph node detection depends on various factors, such as tumor type, tumor volume, age of the patient, breast size, type of radiopharmaceutical, site of injection of the radiopharmaceutical, choice of the hand-

held probe, the surgeons experience and the clinical stage of the disease [27,28]. A combination of preoperative lymphoscintigraphy, intra-operative gamma probe guidance and blue dye administration will increase the success rate of the SLN procedure [29,30]. There are still questions to be answered and problems to be solved, especially concerning prevention of false-negative biopsies and improvement of visualization and localization of SLN's [31,32]. The implementation of nuclear medicine technology in the Netherlands has many variants. Many hospitals have their own procedures. In the literature, differences in the effects of the injected volume and the injection techniques have been described [5]. In the diagnosis of the SLN, a range of imaging techniques is used for the visualization of the SLN (gamma camera, SPECT, SPECT / CT, PET, PET / CT) [33-38]. The principle of the probe-guided detection of SLN's is to convert a radioactive signal in an audio signal. The difference between the target and non-target ratio is important to localize the SLN. Although various radiopharmaceuticals have been introduced, there is no consensus about the most effective radiopharmaceutical for optimal visualization.

Colloidal radiopharmaceuticals are efficiently phagocytized by lymph cells. The density of lymphatic tracts in breast parenchyma is lower than the density of cutaneous lymphatic tracts. This leads to slower migration of colloid particles to SLN's in breast carcinoma as compared to melanoma [39,40]. Colloid particles migrate into the lumen of the lymphatic tract through pinocytosis by the endothelial cells or through junctions between these cells. An increase of the physical pressure differential from the interstitium towards the lumen of the lymphatics facilitates uptake of the mapping agents and thus, sentinel node detection [39]. The balance between physical internal and external pressure in lymphatic tracts, for instance due to gentle massage at the injection site, may also be of importance for sentinel node detection [39,40].

In lymphoscintigraphy in lymph edema, the total clearance of colloidal radiopharmaceuticals from the upper limbs via lymph tracts to efferent lymph nodes, at 2 h after intra-dermal injection, is estimated as >15% of the initially injected dose [41]. In contrast, SLN's of melanoma have been shown to accumulate 2.1 ± 0.8 % of the initially injected amount of colloidal radiopharmaceutical per lymph node [42]. In single SLN's in procedures performed in patients with breast carcinoma, accumulation of less than 1% of the initially injected dose has been demonstrated [43]. In order to improve uptake we developed an optimal labeling procedure for ^{99m}Tc-colloid albumin. We hypothesized that an increased specific activity of ^{99m}Tc-colloid albumin would result in a better radio chemical purity (RCP) and a higher labeling efficiency. Labeling of a higher density of ^{99m}Tc atoms to individual colloid albumin particles should result in a better RCP and a higher labeling efficiency. An increased

specific activity of ^{99m}Tc-colloid albumin should lead to higher count rates during in-vivo measurements of radioactivity in SLN's. Furthermore it was hypothesized that different specific concentrations of ^{99m}Tc-colloid albumin, resulting from different labeling procedures, contain the same ratios of Tc with valence state Tc^{4+} and Tc^{5+}. It was also hypothesized that simultaneous extraction of colloid albumin and stannous chloride from an original vial for labeling procedures has no effect on the RCP in the final product (> 95%).
Regarding the in-vivo appliance of radiolabeled colloid albumin in SLN procedures, it was hypothesized that injection of ^{99m}Tc-colloid albumin with high specific activity increases the amount of probe detected radioactivity in SLN's. Higher ratios of ^{99m}Tc / ^{99}Tc atoms in the labeling mixture result in higher numbers of ^{99m}Tc atoms labeled to colloid albumin particles, and a correspondingly lower density or ^{99}Tc atoms, which results in higher count rates in SLN's.

This thesis addresses a simple, reproducible and optimized strategy for high specific labeling of Nanocoll® and results of several clinical studies using the optimized radiopharmaceutical were described. In **chapter 2**, two pilot studies are described in which batches of ^{99m}Tc-nanocolloid with different specific activities were used to detect axillary sentinel lymph nodes. 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 explaines instant thin layer chromatography (ITLC). **Chapter 3** shows an in-vitro study 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 is presented. 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, obtained from a ^{99}Mo/^{99m}Tc generator at different time points after the previous elution. In-vitro experiments were performed on the effects of specific activity of reduction of $^{99m}Tc^{7+}$, which may occur when stannous chloride and colloid albumin are extracted simultaneously. This chapter also describes effects of variations of the ^{99m}Tc/^{99}Tc ratio in the eluate on the count rate in SLN's. **Chapter 5** reports a randomized clinical trial in which we compared the ex vivo measured CPS rates in SLN of 24 and 2 h eluates, labeled in vacuum using a 26 MBq ^{99m}Tc per µg colloid albumin mixture. The patients were subdivided into subgroups that underwent either lumpectomy within several weeks before the SLN procedure, or, lumpectomy and the SLN procedure during one session.

Chapter 6 describes the sensitivity and specificity of ^{99m}Tc-sestamibi scintimammography in patients with non-palpable breast lesions diagnosed by the Dutch screening program for breast carcinoma. The value of ^{99m}Tc-sestamibi to detect axillary lymph node metastases was determined.
Finally, **chapter 7** presents a questionnaire concerning the techniques that are currently used in SLN procedures in the Netherlands and developments of the technique between 2005 and in 2009. In **chapter 8** and **chapter 9**, the thesis is summarized; discussed and general conclusions are drawn from the results of the studies that are presented in the thesis.

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