From the Department of Clinical Science,
Intervention and Technology
Division of Radiology
Karolinska Institutet, Stockholm, Sweden

Utility of hybrid SPECT/CT in Sentinel Lymph Node mapping, and ^{18}F FDG-PET/CT for treatment response evaluation in cancer patients.

Stefan Gabrielson

Karolinska Institutet

Stockholm 2019

Cover image by Karin Fjellman
All previously published papers have been reproduced with permission from the publisher.
Published by Karolinska Institutet.
Printed by Eprint, Stockholm, Sweden

ISBN 978-91-7831-296-2

Utility of hybrid SPECT/CT in Sentinel Lymph Node mapping, and ^{18}F FDG-PET/CT for treatment response evaluation in cancer patients.

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Stefan Gabrielson

Principal Supervisor:
Professor Rimma Axelsson
Karolinska Institutet
Department of Clinical Science, Intervention and Technology
Division of Radiology

Co-supervisor(s):
Associate Professor Alejandro Sanchez-Crespo
Karolinska Institutet
Department of Oncology-Pathology
Division of Medical Radiation Physics

Associate Professor Jon Tsai
Karolinska Institutet
Department of Clinical Science, Intervention and Technology
Division of Surgery

Med. Dr. Fuat Celebioglu
Karolinska Institutet
Department of Clinical Science and Education, Södersjukhuset
Division of Surgery

Opponent:
Med Dr. Tore Bach-Gansmo
Oslo University Hospital
Department of Radiology and Nuclear Medicine

Examination Board:
Professor Lennart Blomqvist
Karolinska Institutet
Department of Molecular Medicine and Surgery
Division of Diagnostic Radiology

Professor Håkan Geijer
Örebro University
School of Medical Sciences
Division of Radiology

Associate Professor Mirna Abraham Nordling
Karolinska Institutet
Department of Molecular Medicine and Surgery
Division of Colorectal Surgery

“Life is short, and art long,
opportunity fleeting, experimentations perilous,
and judgment difficult.”

– Aphorisms by Hippocrates of Kos –

To my beloved family, Karin, Erik and Arvid

ABSTRACT

The Sentinel Lymph Node Biopsy (SLNB) method is currently well established in the staging of clinically node-negative breast cancer. However, there is some debate concerning the reliability of this method following previous breast surgery. The SLNB method may also be a valuable tool in the staging of oesophageal cancer or cancer of the gastro-oesophageal junction (GOJ), though there are also indications that the method may be less reliable in more advanced cases. Moreover, the impact of a history of neoadjuvant treatment with either chemo-radiotherapy or chemotherapy alone on lymphatic drainage patterns from the oesophagus or GOJ is not well understood. Therefore, there exists a need to further investigate the SLNB method in this patient group. The addition of neoadjuvant therapy in patients with cancer of the oesophagus or GOJ has been shown to improve long-term survival when compared to surgery alone, but there is a need for better diagnostic tools to evaluate the clinical effects of neoadjuvant therapy in this patient group.

This thesis had two main aims. The first aim was to evaluate the utility of hybrid SPECT/CT lymphoscintigraphy in patients with lesions of the breast, or lesions of the oesophagus or GOJ. The second aim was to evaluate the predictive value of ^{18}F-FDG PET/CT in regard to histological response following neoadjuvant treatment in patients with cancer of the oesophagus or GOJ.

Paper I: In this study including patients with benign breast lesions, and using SPECT/CT lymphoscintigraphy prior to, and six weeks following a diagnostic breast excision, with the non-operated breasts serving as a control group. We observed no statistically significant differences in reproducibility between the operated and non-operated breasts regarding SLN detection.

Paper II: In this study including patients with cancer of the oesophagus/GOJ and using hybrid SPECT/CT lymphoscintigraphy. SPECT/CT yielded a high number of detected Sentinel Lymph Nodes. Another aim was to investigate the overall performance of the SLNB method in this patient group, however the accuracy of the Sentinel Lymph Node Biopsy method in the current patient population was poor.

Paper III: In this study investigating the effect of neoadjuvant chemo-radiotherapy on tumour lymphatic drainage patterns in patients with cancer of the oesophagus or GOJ using sequential SPECT/CT lymphoscintigraphy before and following chemo-radiotherapy, but before surgery. The reproducibility of SLN detection was very poor. The SLNB method may be unreliable in patients with cancer of the oesophagus/GOJ with a history of previous

neoadjuvant chemo-radiotherapy or chemotherapy. Neoadjuvant chemo-radiotherapy in fact appears to have a considerable impact on lymphatic drainage patterns from the oesophagus or GOJ regarding SLN detection.

Paper IV: In this study including patients with cancer of the oesophagus/GOJ. randomised to either neoadjuvant chemo-radiotherapy or neoadjuvant chemotherapy and using consecutive ^{18}F-FDG PET/CT examinations. Changes in PET parameters were studied in relation to post-operative histological response in the primary tumour. In particular, changes to the hitherto seldom-used Standardized Uptake Ratio (SUR) PET-parameter was of interest. When pooling the two treatment arms, there was found to be a statistically significant difference in reduction of SUR in patients with histological response compared to patients with little or no histological response. However, it was not possible to predict a complete histological response.

LIST OF SCIENTIFIC PAPERS

I. **Zetterlund L, Gabrielson S, Axelsson R, De Boniface J, Frisell J, Olsson A, Celebioglu F.**
Impact of previous surgery on sentinel lymph node mapping: hybrid SPECT/CT before and after a unilateral diagnostic breast excision.
The Breast 30 2016 30: 32-38.

II. **Gabrielson S, Tsai JA, Celebioglu F, Nilsson M, Rouvelas I, Lindblad M, Bjareback A, Tomson A, Axelsson R.**
Does detection of Sentinel Lymph Nodes with hybrid SPECT/CT improve the accuracy of Sentinel Lymph Node Biopsies in patients with cancer of the oesophagus or gastro-oesophageal junction?
Manuscript under submission.

III. **Gabrielson S, Tsai JA, Celebioglu F, Nilsson M, Rouvelas I, Lindblad M, Bjareback A, Tomson A, Axelsson R.**
Sentinel Lymph Node imaging with sequential SPECT/CT lymphoscintigraphy before and after neoadjuvant chemo-radiotherapy in patients with cancer of the oesophagus or gastro-oesophageal junction – a pilot study.
Accepted for publication in Cancer Imaging 2018-12-03.

IV. **Gabrielson S, Sanchez-Crespo A, Klevebro F, Axelsson R, Tsai JA, Johansson O, Nilsson M.**
^{18}F FDG-PET/CT evaluation of histological response after neoadjuvant treatment in patients with cancer of the oesophagus or gastroesophageal junction.
Acta Radiologica 2018 Aug 15:284185118791204. doi: 10.1177/0284185118791204. E-published ahead of print.

CONTENTS

LIST OF ABBREVIATIONS

^{18}F FDG	^{18}Fluorodeoxyglucose
^{18}F FLT	^{18}Fluorothymidine
^{99m}Tc	^{99m}Technetium
AC	Adenocarcinoma
ALND	Axillary lymph node dissection
ANOVA	Analysis of variance
AP	Anterio-posterior
CI	Confidence interval
CT	Computed tomography
DCIS	Ductal carcinoma in situ
EUS	Endoscopic ultrasound
FWHM	Full width at half maximum
GERD	Gastro-oesophageal reflux disease
GOJ	Gastro-oesophageal junction
Gy	Gray
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratio
IDC	Invasive ductal carcinoma
kV	Kilovolt
mAs	Milliampere second
MBq	Megabecquerel
nCT	Neoadjuvant chemotherapy
nCRT	Neoadjuvant chemo-radiotherapy
NPV	Negative predictive value
OR	Oestrogen receptor
OS	Overall survival
OSEM	Ordered subset expectation maximisation
pCR	Pathological complete response
PET	Positron emission tomography
PgR	Progesterone receptor

PPI	Proton pump inhibitors
PPV	Positive predictive value
ROC	Receiver-operated characteristic
SCC	Squamous cell carcinoma
SLN	Sentinel lymph node
SLNB	Sentinel lymph node biopsy
SPECT	Single photon emission computed tomography
SUR	Standardised uptake ratio
SUV	Standardised uptake value
ΔSUV	Difference in standardised uptake ratio
TNM	Tumour-node-metastasis
TRG	Tumour regression grade
VOI	Volume of interest

1 INTRODUCTION

1.1 CANCER OF THE OESOPHAGUS OR GASTRO-OESOPHAGEAL JUNCTION

1.1.1 Epidemiology

Cancer of the oesophagus and gastro-oesophageal junction (GOJ) causes more than 400,000 deaths yearly and is the ninth most common type of cancer globally(1).

The disease most commonly occurs in the sixth decade and the male to female incidence ratio is about 3:1 for squamous cell carcinoma and 6:1 for adenocarcinoma, although this ratio varies by region(2). Risk factors include obesity and prolonged gastro-oesophageal reflux disease (GERD) for adenocarcinoma and heavy tobacco and alcohol use for squamous cell carcinoma.(3).

1.1.1.1 Squamous cell carcinoma (SCC)

In global terms, this is by far the most common type of oesophageal carcinoma, predominant in southern Africa and East Asia. SCC accounts for roughly 90% of all cases worldwide(4). Conversely, the incidence of SCC in western countries has been continuously decreasing over the last few decades(5). SCC is caused by direct contact with carcinogens or prolonged abrasion or other trauma to the oesophageal mucosa. Apart from the most well-established risk factors in tobacco and alcohol use, the swallowing of chemical irritants and prolonged consumption of hot beverages may contribute to disease occurrence. Among the protective factors, above all smoking cessation and, to a lesser extent, dietary intake of fruit and vegetables reduce the risk(6).

1.1.1.2 Adenocarcinoma (AC)

Although internationally an uncommon form of oesophageal carcinoma, the incidence of AC in the western world has greatly increased over the previous decades. AC now accounts for the majority of cancer cases in certain European countries(2). AC, in contrast to SCC, is caused by prolonged exposure to gastro-oesophageal reflux and the resulting intestinal metaplasia of the normal stratified squamous cell epithelium in the mucosa to columnar epithelium with goblet cells(6). This pathological process gives rise to Barrett's oesophagus, which in turn, as a consequence of worsening dysplasia, may progress to adenocarcinoma at a rate of 0.5% per patient-year(7). The most obvious risk factor for the development of AC is prolonged gastro-oesophageal reflux disease (GERD), which in turn is associated with obesity and hiatal hernia. The role of symptomatic treatment of reflux with proton pump

inhibitors (PPI) remains controversial in the development of dysplasia and progression to carcinoma. Infection with Helicobacter pylori (HP) and a dietary intake of fruit and vegetables have been found to be protective. There is also some evidence that non-steroidal anti-inflammatory drugs (NSAID) may reduce the risk of disease occurrence(6,8). Due to the association to GERD, adenocarcinoma most often occurs in the lower third of the oesophagus or in the GOJ.

1.1.2 Histopathology

Squamous cell carcinoma occurs in the flat lining cells of the proximal oesophagus, and the upper and middle parts of the oesophagus are accordingly the most common sites of disease. This type of cancer is characterised by the proliferation of atypical, oftentimes pleomorphic squamous cells. The cancer may be well, moderately or poorly differentiated, reflecting tumour grade. The most common genetic alteration in SCC includes mutations of TP53, NFE2L2, MLL2, ZNF750 and NOTCH1(9).

As previously mentioned, adenocarcinoma may occur in cases of Barret's oesophagus. This condition with characterised by metaplasia of the mucosa originating in the GOJ end extending into the otherwise healthy mucosa of the oral oesophagus occurs in 5-8 per cent of patients with chronic GERD. This metaplasia is a process of replacing the normal stratified squamous epithelium of the distal oesophagus with columnar epithelium. This new tissue is in turn prone to develop dysplasia with associated pathological changes to glandular architecture culminating in carcinoma. (10). The most common genetic alteration in AC includes mutations of TP53, CDKN2A, ARID1A, SMAD4, and ERBB21(9).

1.1.3 Prognostic factors

Cancer of the oesophagus or GOJ is associated with a very poor prognosis and the 5-year survival rates are around 15%(11,12). Influencing factors are chiefly tumour grade and stage by TNM classification. This staging system classifies lesions based upon the depth of the tumour invasion (T stage), the status of loco-regional lymph node (N stage) and presence or absence of distant metastasis (M stage)(13).

One of the most important prognostic factors is lymphatic spread of cancer cells to loco-regional lymph nodes(14). This has necessitated an aggressive surgical approach to the disease. Extensive loco-regional lymphadenectomy is routinely preformed on patients with stage ≥ T2 cancers undergoing oesophagectomy. The distribution of loco-regional lymph node metastasis has been shown to be an independent prognostic factor in relation to 5-year

survival in patients with cancer of the middle-distal oesophagus(15).

1.1.4 Neoadjuvant treatment

Neoadjuvant treatment with chemotherapy (nCT) by itself or in combination with external beam radiotherapy (nCRT) is currently the main treatment course for >T2-stage or cN1-N3 lymph node-positive oesophageal cancer. There is evidence that this significantly improves long-term survival compared to surgery alone in adenocarcinoma(16,17). There is also evidence of the added benefit of nCRT in squamous cell carcinoma, though the effect of only nCT in SCC remains unclear(18). Typically, neoadjuvant therapy consists of cisplatin and fluorouracil with or without external beam radiation given in fractions. Definitive oncological treatment may be offered to patients considered otherwise unfit for surgery. The same may be offered to patients with cervical oesophageal cancer, where surgery is associated with significant co-morbidity.

For locally advanced cancers, the CROSS-study showed a significant survival benefit of neoadjuvant chemo-radiotherapy for all types of carcinoma when compared to surgery alone with a median OS of 49.4 months in the nCRT surgery group and median OS of 24.0 months in the surgery group (HR, 0.657; 95% CI, 0.495 to 0.871; p=0.003)(16). In this patient group, and most definitely for SCC, the addition of neoadjuvant treatment prior to surgery is beneficial to outcome. The neoadjuvant treatment regimen consisted of five cycles of carboplatin + paclitaxel with concurrent external beam radiation therapy given in fractions.

Post-operative pathological staging of the specimen following nCRT has been shown to be an independently strong predictive factor in disease-free progression, as well as long-term survival of patients with cancer of the oesophagus or GOJ(19).Post-operative pathological staging consists of a semi-qualitative Tumour Regression Grading (TRG) of remaining cancer cells in the specimen, where a compete histological response (pCR) is defined as an absence of remaining cancer cells.

A more pronounced histological response in patients following nCT has been shown to correlate with clinical down-staging, which in turn is a strong predictive factor for long-term survival. Any association between overall survival and histological response may however be dependent on histologic type, i.e. squamous cell cancer (SCC) or adenocarcinoma (AC). The NeoRes randomised trial of neoadjuvant chemotherapy vs. chemo-radiotherapy was unable to show an clear link between pCR and short term survival, furthermore patients AC demonstrated a trend towards lower long-term survival, possibly as a result of mortality

related to the treatment, and the opposite in patients with SCC(20). The same results were later observed in regard to long term survival where no significant differences were observed between the two treatment arms(21).

It is known that pCR may be achieved in approximately 20% of all oesophageal cancers following neoadjuvant treatment, predominantly in SCC(22). It is debatable whether these patients benefit from oesophagectomy, which is a complex procedure with a high risk of post-operative complications and a substantial impact on quality of life in both the short and long term. In some parts of the world another strategy in the management of patients with limited SCC is to offer definitive chemotherapy, followed by active monitorisation and so-called salvage oesophagectomy in cases of residual cancer or local disease recurrence(23).

On the other hand, patients who show little or no histological response following neoadjuvant treatment arguably might not benefit from this course of action and may in fact be better suited for direct oesophagectomy.

1.1.5 Surgical treatment

In most parts of the world, oesophagectomy with two-field (D2) lymph node dissection is the standard surgical treatment for locally advanced cancer (≥T2) of the oesophagus or GOJ. For lower stage cancers or even premalignant lesions (≥T1a), organ-sparing endoscopic mucosal/submucosal dissection or radio-frequency ablation (in Barret´s oesophagus) may be considered definitive treatment(24). Oesophagectomy constitutes a significant trauma to the patient and the procedure is associated with considerable risks in both the short term, and significant impact on quality of life and organ function in the long term.

1.1.6 Staging methods

1.1.6.1 Video endoscopy

Definitive diagnosis of oesophageal cancer is achieved by means of endoscopic examination with high-resolution video oesophago-gastroscopy. Superficial lesions are assessed using the Paris classification, and multiple biopsies are gathered during the examination(25). Limitations in depth penetration means that there are limitations where more detailed staging in regard to tumour invasion depth and metastatic spread is required.

1.1.6.2 Endoscopic Ultrasound

Endoscopic ultrasound uses sonographic technology that can identify the layers of the oesophageal wall and may thereby assess depth of invasion, as well as identifying metastatic locoregional lymph nodes. The procedure can be performed using a conventional echoendoscope or a miniprobe. High-frequency (7.5-12 MHz up to 20-30 MHz) probes allow visual separation of the layers of the oesophageal wall (mucosa, submucosa, muscularis propria and adventitia). A cylindrical echoendoscope gives a full radial view of the oesophagus and surrounding mediastinum but does not allow for fine needle aspiration (FNA) to sample lymph nodes. However, this may be performed using a focused linear probe. Tumours typically appear as a hypoechoic lesion disrupting the layers of the oesophageal wall. EUS has an overall accuracy of 80–90% for T-staging but is less accurate for superficial tumours(25). The addition of EUS-evaluation following CT staging does not appear to significantly affect the accuracy(26). EUS is however a useful tool in the evaluation of loco-regional lymph node metastasis, in particular in combination with FNA, with an accuracy of 90%(25).

1.1.6.3 Computed Tomography (CT)

Following diagnosis, patients are routinely examined by means of CT of the cervix, thorax and abdomen for staging purposes. The technology is widely available and at a relatively low cost.

The limitations in spatial resolution when compared to high-frequency endoscopic ultrasound does not allow for visual separation of the oesophageal wall, thus severely limiting the ability to correctly T-stage tumours. The added value of CT lies in the evaluation of T4 tumours in relation to overgrowth to adjacent visceral structures and the detection of sites of nodal and distant metastasis. One prospective study showed that for all T-stages, CT has a sensitivity of 67% in the detection of primary tumour (27). In the staging of regional lymph nodes, one meta-analysis found pooled sensitivity for CT in detecting lymph node metastasis of 57% and 83% specificity. For abdominal nodes, the sensitivity was still lower at 42%, with 93% specificity(28). The same meta-analysis showed pooled sensitivity of CT in the detection of distant metastases at 52% and 91% specificity. CT may easily overlook low-volume metastatic lesions such as punctate sites of peritoneal carcinomatosis, as well as small superficial liver metastasis.

1.1.6.4 ^{18}Fluorodeoxyglucose-Positron Emission Tomography (FDG-PET/CT)

Positron Emission Tomography (PET) is a nuclear medicine technique by which physiological and patho-physiological processes may be imaged using radiolabelled pharmaceuticals. The combination of PET and computed tomography in a dedicated system allows for simultaneous imaging of both metabolic/biochemical processes in the body by means of PET-imaging, as well as highly detailed anatomical data by CT-imaging. This type of system also allows for hybridisation of PET and CT images, which greatly increases the depth and width of information available for evaluation.

The most common form of PET imaging is performed using a glucose analogue in the form of deoxyglucose linked to a positron-emitting radionuclide, specifically ^{18}Fluoride, thus forming 2-deoxy-2-(^{18}F) fluoro-D-glucose (^{18}F -FDG).

Almost 100 years ago, the Nobel prize laureate-to-be, Otto Heinrich Warburg first described the eponymous "Warburg effect", by which malignant cancer cells exhibit a manifold increase in glycolysis when compared to healthy cells(29). For many years this phenomenon was considered a promoting factor in the development of cancer, and has recently once again attracted attention as a defining characteristic of cancer diseases, albeit as the result of genetic alterations to cellular metabolism rather than a cancer promotor per se.

Acting as an analogue to glucose, ^{18}F -FDG accumulates in tissues with high glucose consumption such as malignant tumours or tissues that are otherwise hyper-metabolic, most commonly as a consequence of inflammation.

The most commonly used PET-parameters in a clinical setting are variations of the Standardised Uptake Value (SUV). Most often either the maximum or median SUV value in a defined region or volume of interest is measured. The SUV-value is calculated based on several parameters such as patient body mass and current glucose levels in the blood. The parameter is therefore not easily reproducible. When using SUV-values to for instance measure treatment response in a patient, there are several factors that may affect SUV quantification mainly type of reconstruction algorithm, patient specific parameters (e.g. plasma glucose level, weight), the time between ^{18}F -FDG administration and scanning and PET-camera performance.

Hence in our evaluation, we introduced the SUR as the ratio SUV tumor to blood. By instead using a Standardised Uptake Ratio (SUR) parameter which is based on the ratio of the SUV-value in the tissue of interest and the SUV-value of the so-called background uptake, such as

in the large vessels in the mediastinum it is possible to reduce the effect of variations of PET-parameters in variables affecting the availability and uptake of FDG. In a clinical setting, the test-retest variability in SUR-value has been demonstrated to be lower for SUR-values compared to SUV values(30).

The limitations of ^{18}F FDG-PET/CT are similar to those of stand-alone CT due to the inability of limited spatial resolution to accurately differentiate T-stage(31). One study found that correct T staging was performed by EUS in 71% and by CT and PET in 43% of cases(32). For this reason, the value of ^{18}F FDG-PET in early-stage oesophageal cancer is very limited. In addition to the limitations mentioned, pathological FDG-uptake in loco-regional lymph nodes may be obscured by avid FDG uptake in the site of the primary tumour(25). The added value of staging with ^{18}F FDG-PET/CT lies within the detection of distant lymph node metastasis and other types of distant metastasis. Sensitivity for detecting nodal metastasis has been shown to be 86% in EUS, 84% in CT, and 82% in PET. Specificity for detecting nodal involvement was 67% in CT and EUS and 60% in PET. With regard to the specificity of PET for nodal metastasis, it is well known that reactive increases of FDG uptake, primarily in mediastinal lymph nodes, are not uncommonly due to airway infection. Sensitivity and specificity rates for detecting distant metastasis were 81% and 82% for CT and 81% and 91% for PET. ^{18}F FDG-PET/CT also has utility in the detection of synchronous cancers, and in the detection of nodal or distant metastasis following neoadjuvant treatment(33).

^{18}F FDG-PET/CT is currently used for repeat staging of patients following neoadjuvant treatment, and as a basis on which to rule out new metastatic lesions and for definitive choice of potential surgical treatment. The potential role for ^{18}F FDG-PET/CT in the prediction of histological response to neoadjuvant treatment is interesting. Several studies have hitherto been conducted to evaluate the potential of PET/CT examinations in predicting histological response. Results from previous studies have shown conflicing results regarding the impact on changes in PET-parameters following neoadjuvant treatment. Weber and associates where first in investigating the concept of using ^{18}F-FDG PET as a potential tool for predicting histological response in patients with oesophageal cancer. In 2001 they published results of the first prospective study of 37 assessable patients with cancer of the oesophagus or GOJ. This study compared changes in SUV_{max} between baseline PET and following nCT(34).Using ROC analysis, a cut-off reduction of SUV_{max} by >35% was defined as metabolic response. This cut-off value was found to correlate with both clinical and histological response. Histological response was defined as few or no remaining cancer cells

(TRG 1 or TRG 2), using a five-point scale classification system suggested by Manard et al(35). Using this cut-off value, metabolic response was found to be a highly accurate predictor of histological response with 93% sensitivity 95% CI [68-100%] and 95% specificity 95% CI [77-100%]. It has been shown that metabolic changes may be observed early on in treatment. One study of 38 patients with SCC of the oesophagus demonstrated significant reduction of SUV_{mean} in patients with SCC of the oesophagus with histological response (TRG 1 and TRG 2) compared to non-responders following an two-week induction cycle of nCT(36).

In light of these results and using this cut-off value, the MUNICON study was later conducted between 2002-2005. 110 consecutive patients with AC of the oesophagus or GOJ were included. Metabolic response as defined by Weber was used to determine further treatment following a two-week induction of nCT. Metabolic responders completed full cycles of nCT and non-responders discontinued nCT and proceeded to resection. The authors found that 58% of metabolic responders were histologic responders, while none of the metabolic non-responders showed evidence of histological response(37). However, there were no significant differences in metabolic response when comparing pCR with non-pCR.

One retrospective study involving 187 participants with stage II-III cancer of the oesophagus undergoing nCRT found no significant differences in SUV_{max} at baseline between four TRG grades (TRG 0-3). However, follow-up PET/CT showed significant differences in SUV_{max} across the TRG grades, with higher values with lessening degree of histological response. The same study no significant difference in absolute decrease of SUV_{max} between baseline and follow-up in correlation with TRG. However a significant difference in the rate of reduction in SUV_{max} ((follow-up SUV_{max} - baseline SUV_{max}) / baseline SUV_{max}) was observed(38).

1.1.6.5 Histopathological staging

Post-operative pathological staging of the tumour specimen may be achieved by means of semi-quantitative Tumour Regression Grading (TRG) of remaining cancer cells in the specimen. There exist several classification systems for this purpose. Staging may be conducted using TRG grading detailing the ratio of tumour cells to fibrotic cells as described by Manard et al.(35) and later modified by Cheirac et al.(39). TRG as described by Cheirac is graded as follows: TRG 1= no remaining tumour cells, TRG 2 = 1-10% tumour cells, TRG 3 = 11-50% tumour cells and TRG 4 = ≥50% tumour cells. TRG 1 is synonymous with a complete histological response.

1.2 BREAST CANCER

1.2.1 Epidemiology

Breast cancer is the most common cause of cancer death in women worldwide. Rates are significantly higher in the developed countries but rising in developing countries.(40) The cumulative incidence for breast cancer in European and North American women are approximately 2.7% by age 55, about 5.0% by age 65, and about 7.7% by age 75(41).The most well-established risk factors are mainly associated with hormonal exposure and include early menarche, menopause and obesity in post-menopause, as well as high levels of endogenous oestradiol. The use of oral contraceptives and hormone replacement therapy in menopause and, to a lesser extent, alcohol use, increase the risk. There are several known familiar genetic mutations associated with a greatly increased risk of breast cancer, such as mutations of BRCA1 and BRCA2, however the vast majority of breast cancers occur sporadically(42). Protective factors include childbearing, with first birth at a young age greatly reducing the risk. Breastfeeding and physical activity are probable protective factors(40).

1.2.2 Histopathology

Breast cancer comprises a heterogeneous group of neoplasias, with adenocarcinomas of the breast being by far the most common, representing around 95% of all breast cancers(43). For both morphological and clinical reasons, carcinomas of the breast are further divided into invasive and non-invasive forms. As defined by the WHO classification of breast cancer, non-invasive, so-called Ductal Carcinoma in Situ (DCIS) is the proliferation of histologically atypical epithelial cells confined to the epithelial layer of ducts or lobules. It has the potential to become malignant and subsequently transform into invasive cancer. The transformation of DCIS to invasive carcinoma is not obligate, and when it does occur it may be a year-long or even decade-long process(43). The most common type of invasive carcinoma is Invasive Ductal Carcinoma (IDC), which is defined as the proliferation of malignant cells from the breast ducts with stromal invasion in or without the presence of DCIS(43). IDC in turn may be further categorised into several cytoarchitectural subgroups on the basis of distinguishing features. After IDC, the second most common form of invasive carcinoma is the Invasive Lobar Carcinoma (ILD) which arises from cells in the lobuli and which accounts for about 5-15% of all invasive carcinomas(41). Invasive carcinomas have the potential not only for stromal invasion, but therefore also for metastatic spread, mainly by means of lymphatic dissemination. Invasive carcinomas may furthermore be classified by gene-expression of

oncogenic proteins. The clinical utility for this classification rests on prognostic as well as treatment-guiding factors. The expression of Oestrogen Receptor (OR), Progesterone Receptor (PgR), Human epidermal growth factor receptor 2 (HER2) and Ki-67 are the basis of the clinic-pathological, so-called intrinsic subtyping of breast cancer(44).

1.2.3 Clinical features of breast cancer

Breast cancer may present clinically as a palpable mass lesion with or without local pain and discomfort. Unilateral or blood-tinged discharge is not an uncommon symptom of breast cancer. Today around than 50% of all breast cancers in Sweden are detected through the national screening program for breast cancer. The staging of breast cancer is based on the principle of so-called “triple diagnostics”, which constitutes physical examination, radiological imaging examination and cytology and/or tissue biopsy. The clinically most widely used methods for radiological diagnosis are mammography, ultrasound and less often MRI. Tissue samples for pathological diagnosis are routinely obtained by means of ultrasound-guided fine needle aspiration, and when there is remaining concern of malignancy, a core needle biopsy will be performed for more comprehensive pathological staging and in order to facilitate assessment of OR/PgR-receptor expression or HER2 status, as well as Ki-67 expression. Clinically or radiological suspect axillary lymph nodes are routinely biopsied by means of fine needle aspiration for the detection of metastatic cells. The result of this biopsy will influence further surgical management.

1.2.4 Prognostic factors

Currently the 5-year survival following a breast cancer diagnosis is about 90 per cent. The most important prognostic factor in early breast cancer is the presence of axillary lymph node metastasis. The risk of distant metastasis also increases with the number of involved axillary lymph nodes(45). The 5-year survival for patients following ALND ranges from 95 per cent in cases with a low proportion tumour positive lymph nodes to 71.4 per cent I patients with a high proportion positive nodes(46). Increasing tumour size and younger age at time of diagnosis are unfavourable prognostic factors. Other independent factors are expression of HER2, OR/PgR and Ki-67. As in several other malignancies, a complete histological response in the excised tissue is associated with significantly better disease-free progression and long-term survival (HR 0.48 (95% CI 0.33–0.69))(47). Following neoadjuvant therapy, pCR may be observed in 13-22% of cases(48).

1.2.5 Surgical treatment

Most unifocal stage 1-3 breast cancers are treated with breast-conserving partial mastectomy. When properly selected, this procedure is considered a safe alternative to radical mastectomy in terms of survival(49,50). Radical mastectomy is most often used in the setting of multifocal cancer, large-stage T4 cancers and in local recurrence. Historically, radical mastectomy in combination with resection of the underlying chest muscle was the mainstay in breast cancer surgery. This procedure is associated with considerable morbidity. The introduction of radiotherapy led to the current standard of breast conserving surgery. For many decades, axillary lymph node dissection (ALND) was always conducted in the same session in order to provide accurate pathologically-staged metastatic spread and to prevent further distant metastasis. In early breast cancer this procedure has since been replaced by initial Sentinel Lymph Node Biopsy (SLNB), a method that is described in detail below.

1.2.6 Adjuvant and neoadjuvant treatment

In selected cases the addition of adjuvant therapy in the form of radiotherapy, cytotoxic drugs, drugs targeting endocrine processes and anti-HER-2 drugs have improved the prognosis of patients with breast cancer(51).

Patients with primarily operable stage 2 or stage 3 breast cancer (tumour size > 2 cm, clinically and radiologically node-negative) are sometimes treated with neoadjuvant chemotherapy. This has been shown to reduce the need for mastectomy (risk ratio 0.71 (95% CI 0.67 – 0.75)). However there are no significant differences in disease-free progression and long-term survival when compared to patients treated with post-operative adjuvant chemotherapy(47). Routinely an initial regimen is given of Fluorouracil/Epirubicin followed by Docetaxel, and subsequently a longer regimen of Docetaxel, Doxorubicin and Cyclophosphamide (TAC). The same regimen may be used in cases where nCT is opted out as adjuvant treatment following surgery. The addition of neoadjuvant Trastuzumab in the treatment of HER2-positive tumours has been found not only to significantly improve the rate of complete histological response, but has also to improve disease-free progression from 56% to 71% three years following treatment, compared to chemotherapy only (52). OR-positive tumours may also be treated with the addition of anti-hormonal drugs e.g. Tamoxifen.

1.3 THE SENTINEL LYMPH NODE

1.3.1 Definition and concept

The Sentinel Lymph Node (SLN) as defined by Donald Morton in the late 1980s: "*A sentinel node is the initial lymph node upon which the primary tumour drains*"(53). Initially conceived in the clinical setting of staging cutaneous melanoma, the SLN is the hypothetical first lymph node or first echelon of lymph nodes to receive lymphatic fluid drained from the primary tumour. It is defined not necessarily by anatomical proximity to the site of the tumour, but rather by the anatomic and physiological drainage by means of lymphatic vessels from the same(54). Based on lymphatic drainage patterns and the distribution of lymph nodes in relation to the lymphatic vessels, sometimes two or, in rare cases, three or more lymph nodes will in fact be the first to receive lymphatic drainage and are therefore to be considered SLNs. The concept further implies that in cancers with a primarily lymphatic mode of metastasis, if the SLN is free from metastasis, so also will second, third and further echelons of lymph nodes be free from metastasis.

1.3.2 Clinical applications – Sentinel Lymph Node Biopsy

The sentinel lymph node concept may be used in clinical cancer staging by means of pre-operative and intra-operative detection of SLNs. A Sentinel Lymph Node Biopsy (SLNB) may be performed in case of locally advanced melanoma of the skin, and intra-operative Sentinel Lymph Node Biopsy in case of breast cancer. The methods for detection of SLNs are described in detail in the following sections. The Sentinel Lymph Node Biopsy method has revolutionised the staging of melanoma and breast cancer. Unnecessary, extensive loco-regional lymph node dissection for the prevention of potential lymphatic dissemination of cancer may be avoided if the SLNB shows no sign of metastasis. This means that not uncommon and sometimes debilitating complications such as extremity lymphoedema may be avoided. The SLNB concept has been evaluated for feasibility in several types of gastrointestinal cancers(55).

1.3.3 Detection of Sentinel Lymph Nodes

1.3.3.1 Intraoperative detection

In the case of breast cancer staging, Sentinel Lymph Nodes may be detected by the injection of ^{99m}Technetium-labelled colloids and the simultaneous injection of blue dye in the site of the site of the primary tumour shortly prior to primary breast cancer surgery. The surgeon

will detect the radioactive "hot nodes" using a hand-held gamma detector. SLNs will be visually identified by the coloration from the previously-injected blue dye. A novel technique using injection of fluorescent indocyanine green instead of blue dye has also been evaluated. SLNs will be excised and an intra-operative pathological freeze section will be made. If there is evidence of metastasis, ALND will be performed. A more detailed pathological examination of SLNs will subsequently be made by means of microscopic examination of chemically fixed and stained tissue sections in order to examine the presence of micro-metastasis. The same principle is applied to the detection of SLNs in oesophageal cancer, where there is endoscopically-guided intra/peritumoural injection of ^{99m}Technetium-labelled colloids.

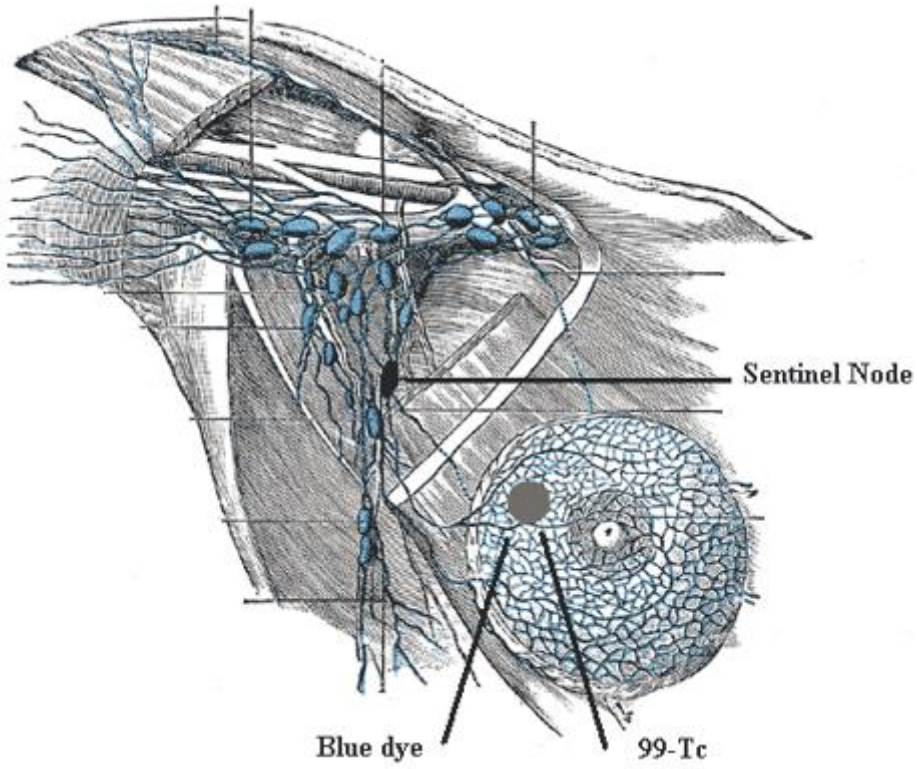

Figure 1. An illustration of techniques used in Sentinel Lymph Node detection. Blue dye and/or ^{99m}Technetium-labelled colloids are injected into an intra/peritumoural location. Sentinel Lymph Nodes are detected either visually or using gamma detection techniques. This image is derived from an illustration by Henry Vandyke Carter published in the textbook "Atlas of the Human body (1918)". This work is in the public domain.

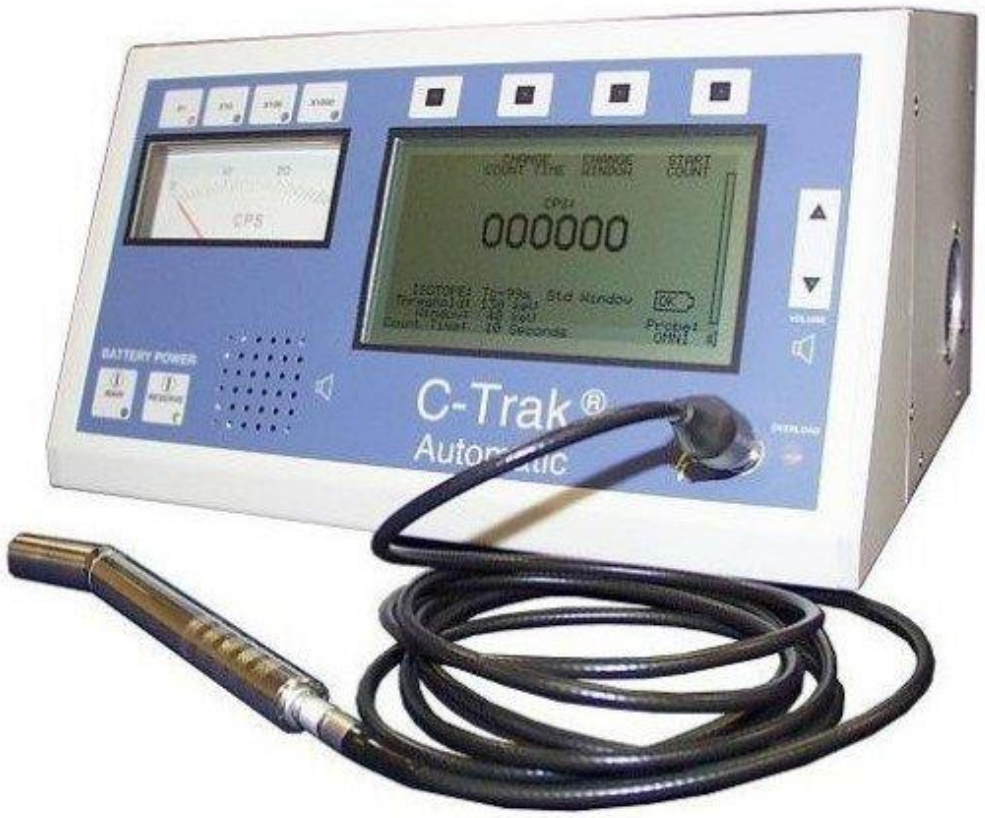

Figure 2. A photograph of a hand-held gamma scintillation detection device and probe used for the intra-operative detection of Sentinel Lymph Nodes. Gamma probes and their use in tumour detection in colorectal cancer ©, *International Seminars in Surgical Oncology 5(1):25*. (Courtesy of Care Wise Medical Products Corp., CA, US), with credit also to the original authors. This image is reproduced in an unaltered state under the terms of the Creative Commons Attribution 2.0 International License.

1.3.3.2 Planar Lymphoscintigraphy

Planar lymphoscintigraphy is a well-established method both in research and in many clinical situations for the pre-operative detection of SLNs. This imaging method can visualise SLNs in as many as 89-91 per cent of breast cancer patients, though it is limited when it comes to the precise anatomical localisation of SLNs (axillary, intrathoracic, parasternal), mainly due to the inherently low spatial resolution and absence of anatomical landmarks(56,57). Two studies have been conducted utilising planar lymphoscintigraphy to study lymphatic drainage patterns prior to and following a surgical biopsy. One study reported a discrepancy in lymphatic drainage in 17 of 25 patients(58). However, a more recent study showed a one hundred per cent reproducibility in 16 out of 18 patients (59). When detecting SLNs in

oesophageal cancer, planar lymphoscintigraphy alone has shown widely disparate detection rates of 40-94% (60,61). One prospective study of 112 patients with clinical T1, N0 oesophageal cancer undergoing pre-operative planar lymphoscintigraphy for SLN detection showed varying detection rates depending of anatomical localisation of SLNs(61). SLNs were detected in 40 per cent of all patients using lymphoscintigraphy.

In this study, 80% of patients with cervical SLNs and 73% of patients with abdominal SLNs were correctly detected. However, the clear majority of SLNs were located in the mediastinum, and of these patients only 39% of patients were accurately assessed. The reason for this inability to accurately detect mediastinal lymph nodes is almost certainly due to so called "shine through", where radiocolloid uptake in SLNs is obscured by the close anatomical proximity to residual radiotracer at the site of injection in the primary tumour.

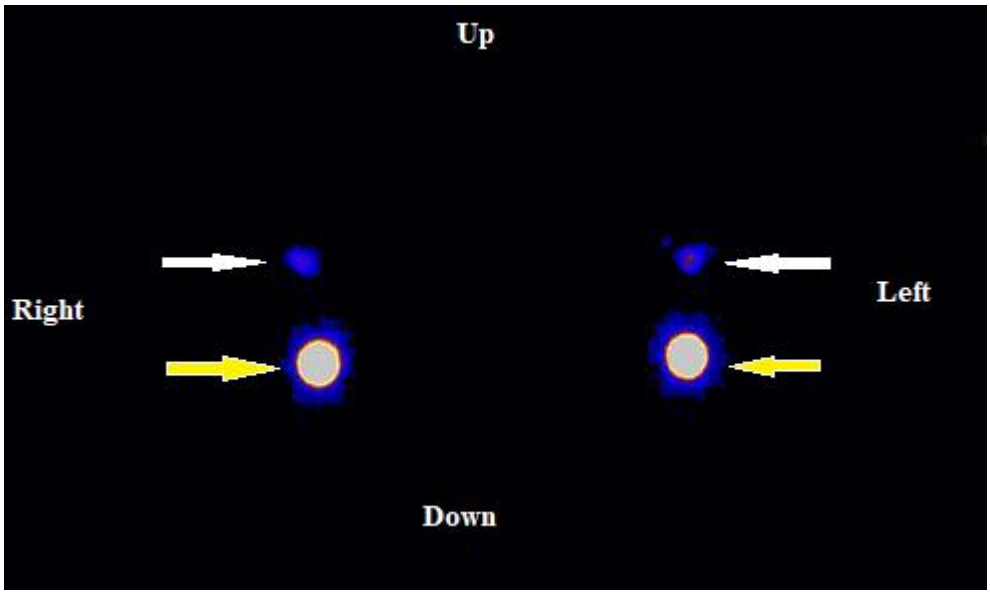

Figure 3. Planar lymphoscintigraphy in anterior-posterior orientation of a patient following peri-areolar injection of ^{99m}Technetium-labelled colloids in both breasts. White arrows indicate radiocolloid uptake in Sentinel Lymph Nodes roughly located in the axillae. Yellow arrows indicate injection sites.

1.3.3.3 Single Photon Emission Computed Tomography (SPECT) Lymphoscintigraphy

Lymphoscintigraphy using Single Photon Emission Tomography (SPECT), with or without simultaneous Computer Tomography, and hybrid technique which combine nuclear medicine imaging using gamma camera technology with radiological imaging by Computed Tomography (SPECT/CT) may overcome many of the limitations inherent to planar lymphoscintigraphy. Compared to planar scintigraphy, SPECT technology offers more

detailed visualisation of lymphatic drainage and lymph node deposits of radiotracer, thereby identifying the SLN. Integrated CT imaging will be used for attenuation correction, giving a truer representation of actual radiocolloid uptake in lymph nodes, as well as offering more precise anatomical localisation of the lymph node/s. The impact of "shine through" obscuring radiocolloid uptake in SLNs located close to sites of injection will hypothetically be reduced, allowing for more accurate detection rates.

Hybrid imaging using SPECT/CT in the detection of SLNs in breast cancer patients has been shown to be valid in several studies. Detection rates of SLNs using SPECT/CT hybrid imaging are somewhat better than with planar scintigraphy. The method is particularly useful in difficult cases, in particular when patients can be assumed to have unusual lymphatic drainage patterns (i.e. intrathoracic, parasternal, supraclavicular), in cases where planar image findings are inconclusive and in cases of non-visualisation of SLNs on planar images(62–65). It has also been shown to improve detection of SLNs in overweight or obese patients. In this patient group, planar lymphoscintigraphy may result in non-visualisation of SLNs, whereas SPECT/CT by means of CT attenuation correction is technically superior(66). In a recent larger prospective study, Uren and associates were able to demonstrate a detection rate of 97.8 per cent using SPECT/CT in patients with primary breast cancer(67). One prospective study of one hundred and eighty-seven consecutive patients undergoing surgery for breast cancer showed a false negative rate of 5.7% in the detection of metastatic SLNs when using SPECT/CT lymphoscintigraphy(68). The method has been used successfully for more precise anatomical localisation of SLNs when comparing lymphatic drainage patterns with different radiotracer injection techniques (subareolar or intra-tumoural)(69,70).

With regard to the added value of preoperative SPECT/CT in the detection of SLNs in oesophageal cancer, one pilot study found that it was feasible to accurately detect SLNs preoperatively in patients undergoing surgery for T2-T3 oesophageal cancer/cancer of the GOJ(71). Addressing the phenomenon of skip lesions, involving lymph drainage to unexpected anatomical locales, SPECT/CT has the potential to precisely visualise SLNs in multiple anatomical locations (neck, thorax, abdomen).

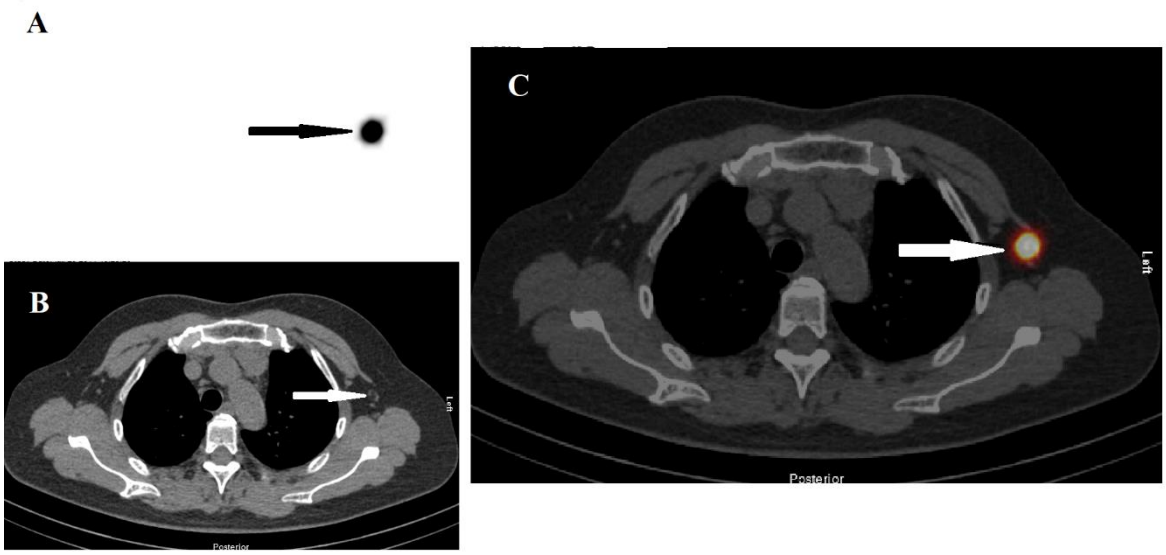

Figure 4. SPECT/CT lymphoscintigraphy of patient undergoing pre-operative Sentinel Lymph Node evaluation of the left breast. **A)** transversal SPECT images **B)** transversal CT images **C)** transversal hybrid SPECT/CT images. Arrows indicate Sentinel Lymph Node uptake in SPECT images and the anatomical location of the SLN in CT images.

1.3.4 Sentinel Lymph Node Biopsy in breast cancer

ALND has been shown to have very high sensitivity in the detection of lymph node metastasis. The sentinel lymph node biopsy (SLNB) method has largely replaced axillary lymph node dissection (ALND) and has been extensively validated as a safe method in the staging of the axilla in patients with early-stage breast cancer with significantly less morbidity compared to ALND(72–75). The SLNB method has been validated in several national and international studies demonstrating detection rates of more than 95 per cent and false negative rates of 0.9 per cent(76–78). Furthermore, a well-established of breast cancer screening programme and likewise an increase in awareness have resulted in earlier detection of small lesions. Patients with morphologically small breast cancers benefit most from SLNB, as roughly 80 % of these women will be node-negative(79,80) About 10 per cent of patients can be expected to undergo a diagnostic surgical operation/excision before a diagnosis of breast cancer can be made; the pre-operative diagnostics of small breast lesions when using ultrasound-guided technique or by means of stereotactic biopsy do not always result in a definitive diagnosis prior to surgery. In these cases, a second operation is needed in order to properly stage the axilla. The feasibility of SLNB after a prior diagnostic operation is debated. One of the arguments is that the method may be invalidated due to iatrogenic transection of the lymphatic vessels(81,82). Other studies have suggested that a SLNB procedure can be performed accurately following excisional biopsies(83–85), while others have shown a clear reduction in the SLN detection rates(57) and also increased false negative

rates for this patient group(81). It is also of utmost importance to correct stage the axilla in order to adequately plan for possible adjuvant therapy. This matter is also impacted due to the exclusion of ALND in certain selected SLN-positive patients(86,87). However, a second SLNB procedure in patients with a history of previous breast surgery remains a matter of debate. Also changes in lymphatic drainage patterns in patients following breast surgery have not been well studied.

1.3.5 Sentinel Lymph Node Biopsy in cancer of the oesophagus or gastro-oesophageal junction

Lymphatic dissemination of cancer of the oesophagus or GOJ to loco-regional lymph nodes is one of the most important prognostic factors. So-called two-field lymph node dissection is routinely performed in stage ≥T2, any N-stage non-cervical oesophageal cancers. This includes the dissection and extirpation of all thoracic mediastinal lymph nodes located below the tracheal carina, as well as abdominal lymph nodes located in proximity to the GOJ, along the minor curvature of the ventricle together with the surrounding coeliac truncus and the proximal branches of the same artery(88). This is an extensive procedure associated with a considerable risk of post-operative complications. There is therefore some appeal in evaluating the SLNB method in this patient group. The method could potentially be used to avoid unnecessary lymph node dissection. A reliable method of predicting lymph node spread could potentially lead to a situation where organ sparing surgery such as ESD could be offered to patients with limited disease (i.e. T1b cancers). Several studies have been conducted in order to investigate the utility of SLNB in cancer of the oesophagus and GOJ. The methods used have included the endoscopic submucosal intra/peritumoural injection of dyes, charcoal for intraoperative visual detection or radiotracers for scintigraphic detection. Where radiotracer has been used, patients have undergone pre-operative lymphoscintigraphy in order to visualise SLNs, and SLNs have been identified intraoperatively using hand-held gamma detector probes(55,89,90). A recent meta-analysis including studies of patients with SCC or AC of the oesophagus or GOJ undergoing SLNB found overall detection rates of 0.93 (95% CI: 0.894- 0.950), with sensitivity at 0.87 (95% CI: 0.811-0.908). The negative predictive value was 0.77 (95% CI: 0.568-0.890) and accuracy was 0.88 (95% CI: 0.817-0.921). In the adenocarcinoma cohort, the detection rate was 0.98 (95% CI: 0.923-0.992), sensitivity was 0.84 (95% CI: 0.743-0.911) and accuracy was 0.87(95% CI: 0.796-0.913). In the squamous cell carcinoma group, the detection rate was 0.89 (95% CI: 00.792-0.943), sensitivity was 0.91 (95% CI: 0.754-0.972) and accuracy was 0.84 (95% CI: 0.732-0.914)(91). However, the validity of the method is not uncontroversial, mainly regarding the

characteristics of lymphatic flow in the oesophagus. It has been suggested that the partly longitudinal orientation of lymphatic vessels is the cause for so called “skip lesions”, where lymph node metastasis may be seen in unexpected locales such as cervical lymph nodes when the primary tumour is located in the middle or distal oesophagus(92). The impact of nCRT on the SLNB method in oesophageal cancer has been the subject of few studies and results have conflicted with detection rates of 54%[29.1–77%] and sensitivity rates of 25% [1–81%](93) in patients undergoing SLNB following cCRT or nCT.

The few studies that have investigated the validity of the SLNB method in oesophageal cancer have included patients with a previous history of neoadjuvant treatment. Most studies have included a mixed population of patients with a significant minority of participants having been exposed to neoadjuvant treatment prior to surgery and SLNB. Another unanswered question is what particle size constitutes an optimal radiotracer in both pre-operative scintigraphic detection and intraoperative detection of SLNs. Most studies were conducted in Japan, mainly using 99mTc-tin colloid (100 nm in size) which allows for lymphoscintigraphy up to 24 h before surgical resection(90). In other parts of the world, radiocolloids such as ^{99m}Tc-antimony trisulphide colloid (3-30 nm in size) have much shorter transit periods in the sentinel nodes but arguably may be better suited to penetrate metastatic lymph nodes due to the smaller particle size.

2 AIMS

2.1 GENERAL AIMS

The aim of this doctoral thesis is to investigate the utility of hybrid imaging using SPECT/CT and PET/CT in the staging of cancer patients. The focus lies on the Sentinel Lymph Node Biopsy method in patients with oesophageal cancer and patients with breast cancer.

2.2 PAPER I

The aim of this paper was to evaluate changes in lymphatic drainage patterns using sequential hybrid SPECT/CT lymphoscintigraphy in patients with presumed benign breast lesions undergoing an excisional operation.

2.3 PAPER II

The aim of this paper was to evaluate the added benefit of pre-operative hybrid imaging with SPECT/CT lymphoscintigraphy in patients with cancer of the oesophagus or gastro-oesophageal junction undergoing an SLNB procedure.

2.4 PAPER III

The aim of this paper was to evaluate changes in lymphatic drainage patterns in patients with cancer of the oesophagus or gastro-oesophageal junction using sequential SPECT/CT lymphoscintigraphy prior to and following neoadjuvant chemo-radiotherapy.

2.5 PAPER IV

The aim of this paper was to evaluate changes in PET parameters in the primary tumour in relation to degree of histological response using sequential hybrid ^{18}F FDG-PET/CT in patients with cancer of the oesophagus or gastro-oesophageal junction.

3 MATERIALS AND METHODS

3.1 PAPER I

3.1.1 Patient population

The patients in this study were prospectively included. The criteria for inclusion were patients planned for unilateral excisional breast surgery for a presumed benign breast lesion (e.g. fibroadenoma or papilloma). Exclusion criteria were patients planned for bilateral breast surgery, significant co-morbidity, pregnancy or communication issues. Patients underwent surgery at Bröstcentrum, Stockholm South County Hospital between the years 2010-2014.

3.1.2 SPECT/CT Lymphoscintigraphy

Patients underwent lymphoscintigraphy the week prior to surgery. Patients were examined first with planar lymphoscintigraphy and immediacy afterwards with hybrid SPECT/CT lymphoscintigraphy using the same Siemens Symbia T16 SPECT/CT system (Erlangen, Germany) with low-energy, high-resolution collimators. A total activity amount of 30 MBq ^{99m}Technetium-labelled Nanocoll® (GE Healthcare, Stockholm Sweden) in a 0.4 ml dilution was injected subcutaneously/subareolarly in each breast. One hour after injection, planar lymphoscintigraphy was conducted in anteroposterior (AP) position using a 256*256 matrix. The acquisition time was 5 minutes. The images were reviewed by the attending Nuclear Medicine physician for radiotracer uptake representing SLNs in both axillae. If no uptake was evident on either side, planar lymphoscintigraphy was repeated after another hour.

SPECT/CT was then performed in the same anatomical region. SPECT acquisition was performed using a 128*128 matrix, 40 seconds per projection and 64 projections over an angle of 360º. The CT acquisition was performed at 110 kV (tube voltage), 75 mAs, (tube current) at a pitch 1.3 and 0.6 s rotation time.

SPECT data were then reconstructed using Hybrid Recon (Hermes Medical Solutions) with OSEM (4 iterations, 8 subsets, Gaussian post-filtration with 0.75 cm FWHM) using resolution recovery, attenuation and scatter correction. CT images were reconstructed optimised for soft tissues with a B60 kernel and 5 mm slice thickness. SPECT/CT images were fused and reviewed in HERMES Hybrid Viewer (Hermes Medical Solutions, Stockholm, Sweden).

The same imaging was then repeated six weeks after surgery. This interval was chosen to represent the time lapse between primary operation and reoperation for axillary staging purposes in cases where malignant findings occur in a previous diagnostic operation.

3.1.3 SPECT/CT Evaluation

Images were reviewed by the co-main authors of the paper. The reviewers were blinded to the side on which the patients had been operated. All conspicuous uptakes in SPECT images corresponding to a lymph node in CT images following image fusion were considered SLNs. Images (pre- and post-operative) were compared for the reproducibility of SLN detection. Patients served as their own controls as both operated and non-operated breast sides were examined and reviewed in comparison to ipsilateral sides. Three levels of reproducibility were defined;

1) Total concordance: The same number and locations of SLNs pre- and post-operativley. Additional SLNs in the post-surgery examination were also included in this group.
2) Partial concordance: At least one of the SLNs detected pre-operativly was detected post-operativly. However the total number of SLNs was lower post-operativly.
3) Discordance: None of the SLNs detected pre-operativly were detectable post-operativly. Patients in whom no SLN uptake was evident either pre-operativly or post-operativly were also included.

Examinations of either breast/axillae where no SLN uptake was evident both pre- and post-operatively were excluded from concordance analysis.

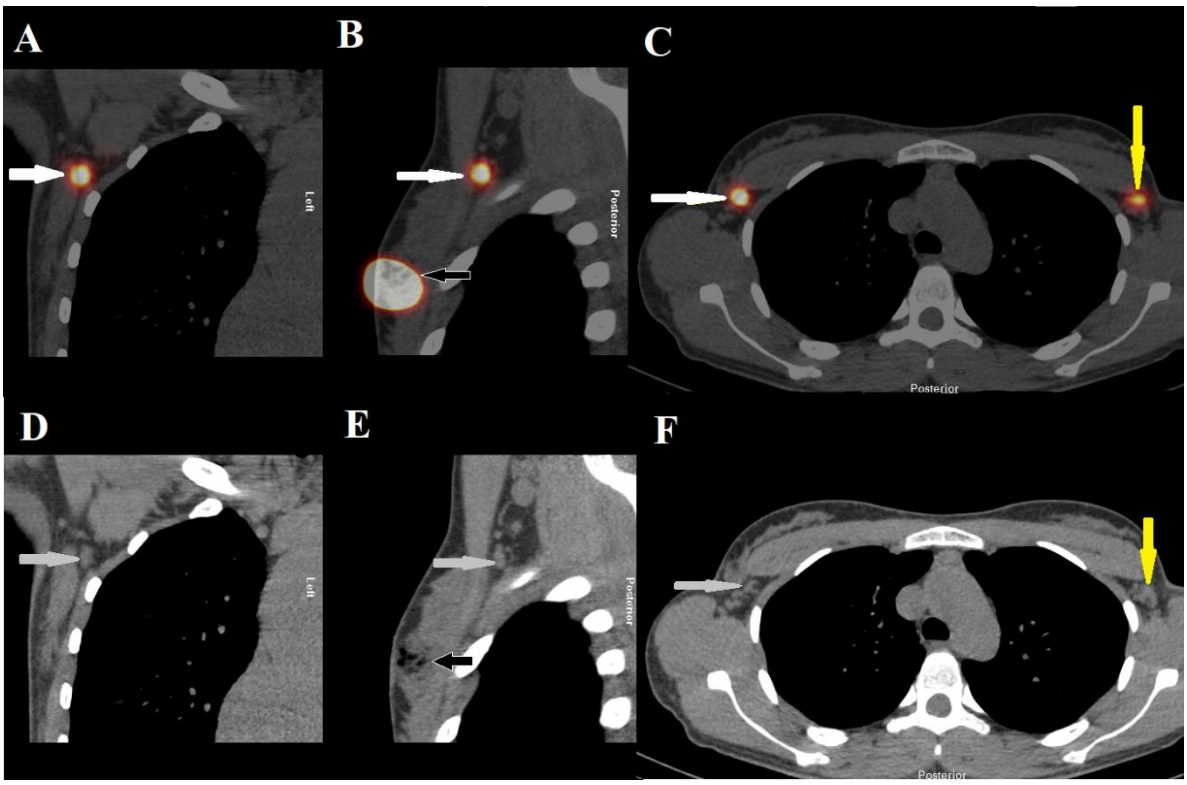

Figure 5. SPECT/CT images from a 45-year old woman undergoing bilateral pre-operative lymphoscintigraphy. **Images A-C** consist of fusion SPECT/CT images in coronal, sagittal and transversal orientation demonstrating SLN uptakes. Images **D-F** depict CT images in coronal, sagittal and transversal orientation. White arrows indicate SLN uptake in the right axilla. Grey arrows indicate corresponding anatomical location of the SLN in the right axilla. Yellow arrows indicate SLN uptake, as well as anatomical location of in the contralateral axilla. Black arrows indicate sites of injection with corresponding radiocolloid deposits and air bubbles.

3.2 PAPER II AND PAPER III

3.2.1 Patient population

The patients in these studies were prospectively and consecutive included between the years 2011-2016. Inclusion criteria in paper II were patients with histologically verified stage T1-T3, any N-stage, M0 cancer of the oesophagus or gastro-oesophageal junction planned either for direct oesophagectomy with curative intent or for oesophagectomy following neoadjuvant chemotherapy or neoadjuvant chemo-radiotherapy. Inclusion criteria in paper III were histologically verified, stage T2-T3, any N-stage, M0 cancer of the oesophagus or GOJ planned for oesophagectomy following neoadjuvant chemo-radiotherapy. Exclusion criteria in both studies were patient age $\geq$ 75 years, considered unfit for oesophagectomy and with a performance status, renal and haematological status not permitting chemotherapy. All patients were staged using endoscopically-guided biopsies, contrast-enhanced Computed Tomography and ^{18}F-FDG Positron Emission Tomography/Computed Tomography

(PET/CT). All patients were scheduled for surgical treatment at the Centre for Digestive Diseases, Karolinska University Hospital, Huddinge.

3.2.2 Sentinel Lymph Node mapping

In paper II, on the afternoon of the day before surgery or the morning of surgery, the patients were given endoscopic submucosal radiocolloid injections of 4 X 0.5 mL in total of 60 MBq ^{99m}Tc-nanocoll (GE Healthcare Srl., Milan, Italy) peri- and intratumourally. For logistical reasons, patients undergoing surgery on a Monday would undergo Sentinel Node evaluation the week prior to surgery. These patients underwent a second endoscopic procedure as described above on the morning of surgery for intra-operative SLN detection. In paper III patients underwent the same procedure the week before commencement of nCRT, and once again following the conclusion of nCRT, within one week preceding oesophagectomy.

3.2.2.1 SPECT/CT lymphoscintigraphy

A Siemens Symbia T16 system with a low-energy, high-resolution collimator was used for all imaging. Whole-body gamma camera imaging was performed 1 hour after radiocolloid injection in order to generally localise SLNs. This information was used to centre the SPECT/CT examination. If no SLN uptake was evident, the same procedure was repeated after another hour. SPECT imaging was performed using a 128 x 128 matrix, with 64 projections over 360° and 40 s per projection. CT scans of the same anatomical region were performed in the same session (110 kV, 75mAs and pitch 1.3.) SPECT data were then reconstructed using Hybrid Recon with OSEM (4 iterations, 8 subsets, Gaussian post-filter with 0.75 cm FWHM) using resolution recovery, attenuation and scatter correction. All image reconstructions and image evaluation were performed using Hermes viewing software. Transverse SPECT images were fused with transverse CT images (0.75 mm/ 0.7 mm recon increment, B31 medium smooth). There was multiplanar reconstruction of all image data. In SPECT images, injection sites were masked using hand-drawn VOIs to accentuate the uptake in SLNs. Any discernible radiocolloid uptake/uptakes in SPECT images corresponding to lymph nodes on fused CT images were considered an SLN.

3.2.2.2 Intra-operative SLN detection

In paper II, the location(s) of SLNs were demonstrated to the operating surgeons immediately prior to surgery. SLN stations were classified according to the 11th edition of the Japanese classification of oesophageal cancer(94). Prior to lymph node dissection, the operating field was scanned using a hand-held gamma scintillation device (gamma probe) (Neoprobe,

Cincinnati, OH, USA) Patients underwent two-field lymphadenectomy (D2), which included abdominal and mediastinal lymph node stations. Following en bloc lymphadenectomy, the dissected lymph nodes were again examined “back table” using the gamma probe. Any lymph node with gamma radiation exceeding 10 times the background level was considered a Sentinel Lymph Node. All identified SLNs were sent for separate pathological evaluation.

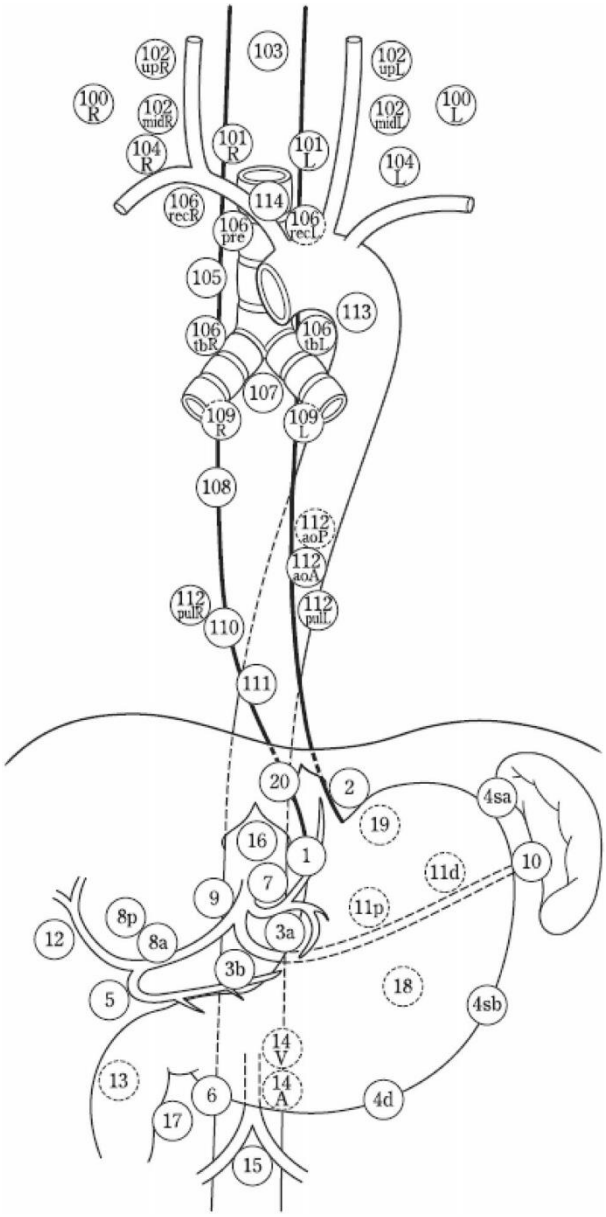

Figure 6. Lymph node stations as per the Japanese Classification of Oesophageal Cancer, 11th Edition: part I. ©, with credit also to the original authors. This image is reproduced in an unaltered state under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/).

3.3 PAPER IV

3.3.1 Patient population

The patients in this study were prospectively included within the randomised controlled Neoadjuvant Chemotherapy versus Chemo-radiotherapy in Resectable Cancer of the Oesophagus and Gastric Cardia Trial (NeoRes). Inclusion criteria were histologically confirmed Tumour stage T1–T3, any nodal stage and non-distant metastatic SCC or AC of the oesophagus or GOJ where there was intent of curative resection. A further inclusion criterion was patient age ≤ 75 years. Patients considered unfit for oesophagectomy and with performance status, renal and haematological status not permitting chemotherapy were excluded. The NeoRes trial was conducted at the Centre for Digestive Diseases, Karolinska University Hospital, Huddinge, and at eight other hospitals in Sweden and Norway, between the years 2006-2013. Patients were randomised to either nCRT consisting of three cycles of Cisplatin/oxaliplatin-5-FU + 40 Gy given in fractions, or to nCT (three cycles of Cisplatin/oxaliplatin-5-FU). Patients in both treatment arms were scheduled for oesophagectomy 4–6 weeks following conclusion of neoadjuvant treatment.

3.3.2 PET/CT examinations

Patients underwent ^{18}F FDG-PET/CT examinations at the Department of Nuclear Medicine Karolinska University Hospital, Solna, using a Biograph 64 True Point V PET/CT system (Siemens Medical Solutions, Erlangen, Germany). A total of 4Mbq/kg bodyweight was administered intravenously 60 min before PET/CT scans. Scans were conducted for 4 min/bed position. Diagnostic non-contrast-enhanced CT scans were obtained in the same session and CT data was also used for attenuation correction. OSEM PET reconstructions were made (four iterations and eight subsets, 168x168 matrix size). Baseline examinations were also used as part of routine clinical staging. Follow-up PET/CT scans were obtained using the same parameters following completion of the neoadjuvant treatment and before surgery. Follow-up examinations were also used for clinical evaluation of the neoadjuvant treatment. Interim PET/CT scans were obtained after the initial induction chemotherapy cycle in both treatment arms.

3.3.3 Image evaluation

Hybrid PET/CT images were reviewed in both separate and fused stacks using the HERMES viewing software to improve definition of the lesion borders. The primary tumour site was delineated using visually-assessed hand-drawn ROIs in the multiplanar orientations. Within the resulting volume of interest (VOI), the primary tumour was

delineated from surrounding tissues using a standardised uptake value (SUV) threshold value of 2.0. SUV_{max} was defined as the SUV of the voxel with the highest value within the VOI.

In our work, the same standard reconstruction algorithm (and associated parameters) were used, additionally, quality controls are periodically performed on the PET camera, ensuring that camera performance is consistent. Further we used a standard investigation acquisition protocol were patient glucose level was monitored before FDG administration, ensuring normal blood glucose levels. However, the time between FDG administration and scanning may vary slightly between examinations.

Hence in our evaluation, we introduced the SUR as the ratio SUV tumor to blood.
To avoid confounders in SUV quantification (i.e. differences in blood glucose levels, availability of FDG, time between FDG injection and scan, etc.), the tissue-to-blood standardised uptake ratio, SUR, was scored. SUR has previously been shown to correlate well with the net uptake of FDG in tissues at late time points(95).
SUR values were calculated as the ratio between SUV_{max} of the lesion and SUV_{mean} of a $1cm^3$ VOI placed within the mediastinal blood pool. For quantification purposes, a linear model of SUR variation with the time elapsed between baseline and follow-up examination was created for each patient. From the model, the SUR rate of change in units per day SUR (days–1) was used as a predictor of decreases in tumour metabolism (for negative rates) or increases in tumour metabolism (for positive rates).

3.3.4 Pathological evaluation

Postoperative histological grading following oesophagectomy was performed by experienced pathologists in the Department of Pathology, Karolinska University Hospital, Huddinge. Tumours were evaluated using TRG-grading, by which the ratio of tumour cells to fibrotic cells was quantified in a system established by Chirieac et al.(39). TRG was graded as;

1) TRG 1= no remaining tumour cells
2) TRG 2 = 1-10% tumour cells
3) TRG 3 = 11-50% tumour cells
4) TRG 4 = ≥50% tumour cells.

Patients with TRG 1 and TRG 2 were classified as histological responders to neoadjuvant treatment and patients with TRG 3 and TRG 4 were considered as non-responders.

4 RESULTS

4.1 PAPER I

64 patients gave their consent to participation in this study. 37 patients underwent both pre- and post-operative SPECT/CT lymphoscintigraphy and were available for definitive analysis. The details of patients lost to follow-up are given in figure 6.

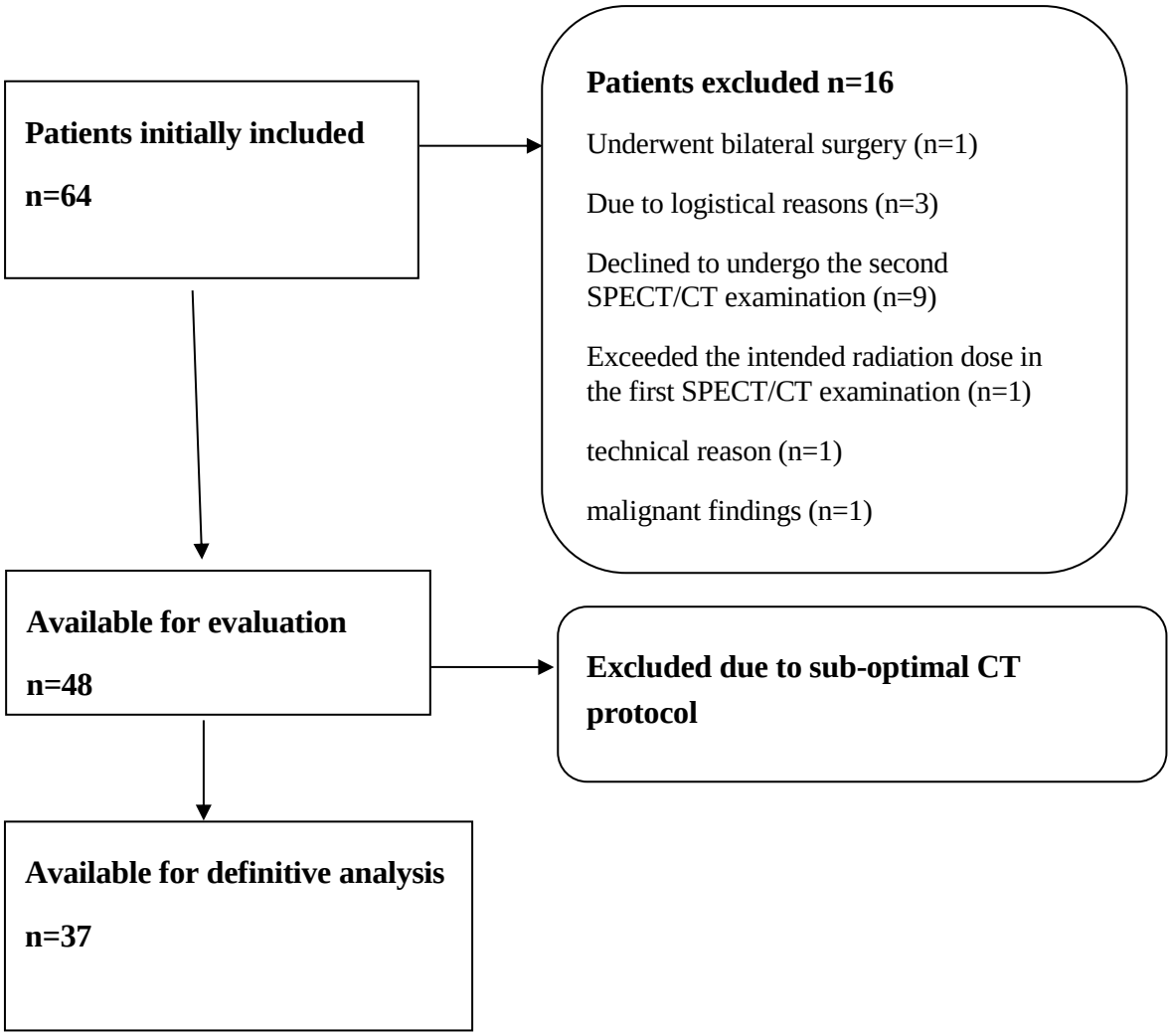

Figure 7. Details of patient inclusion, exclusion and loss to follow-up.

Use of hybrid SPECT/CT lymphoscintigraphy allowed us to identify at least one SLN in 138 of 148 procedures (93.2 per cent). The SLN detection rate was not statistically significantly lower in post-operative examinations on operated sides, being 91.9 per cent (34 of 37 procedures) as compared to the detection rate of 93.7 per cent in pooled non-operated sides pre- and post-operatively and all operated sides pre-operatively (104 of 111 procedures, (P=0.771)). Likewise, there was no statistically significant difference in

reproducibility, with total or partial concordance observed in 85.7 per cent (30 out of 35) of the operated sides, and in 88.9 per cent (32 out of 36) of the non-operated sides, (P=0.735). In the 10 procedures (involving five patients) where we were unable to locate an SLN, the median age was significantly higher than in the patients where SLN detection was successful (70 years; range 61-72, P < 0.000) Likewise the median BMI was significantly higher in patients where we were unable to detect an SLN (BMI=31.7; range 23.4-32.8, P < 0.001).

Three procedures were excluded from concordance evaluation. In these three procedures we were unable to identify an SLN at either pre- or post-operative SPECT/CT examinations. These three procedures occurred in two patients (in one patient on both operated and non-operated breast sides, and in one patient on the non-operated breast side).

Table 1. Concordance rates for SLN detection on operated and non-operated breast sides.

	Total concordance	**Partial concordance**	**Discordance**	**Total**
Operated breast sides	24 (68.6%)	6 (17.1%)	5 (14.3%)	N=35 (100%)*
Non-operated breast sides	28 (77.8%)	4 (11.1%)	4 (11.1%)	N=36 (100%)⁂

* Two procedures were excluded because no SLN was visible in either pre-or post-operative examinations.
⁂ One procedure was excluded because no SLN was visible in either pre-or post-operative examinations.

When analysing only the operated breast sides, we observed no statistically significant impact of the following clinical parameters on concordance (total or partial) or discordance: Patient age, BMI class, Radiological lesion size, length of skin incision at surgery, location of lesion, weight or volume of excised breast tissue.

4.2 PAPER II

Sixty-three patients agreed to participate in this study, and forty-one patients were available for definitive analysis. Using pre-operative SPECT/CT lymphoscintigraphy, at least one SLN was detected in 88% of cases (36/41 patients). The median number of Sentinel Lymph Node stations detected per patient was 1 (range 0–4). In total, 53 lymph node stations were detected in the 41 SPECT/CT examinations.

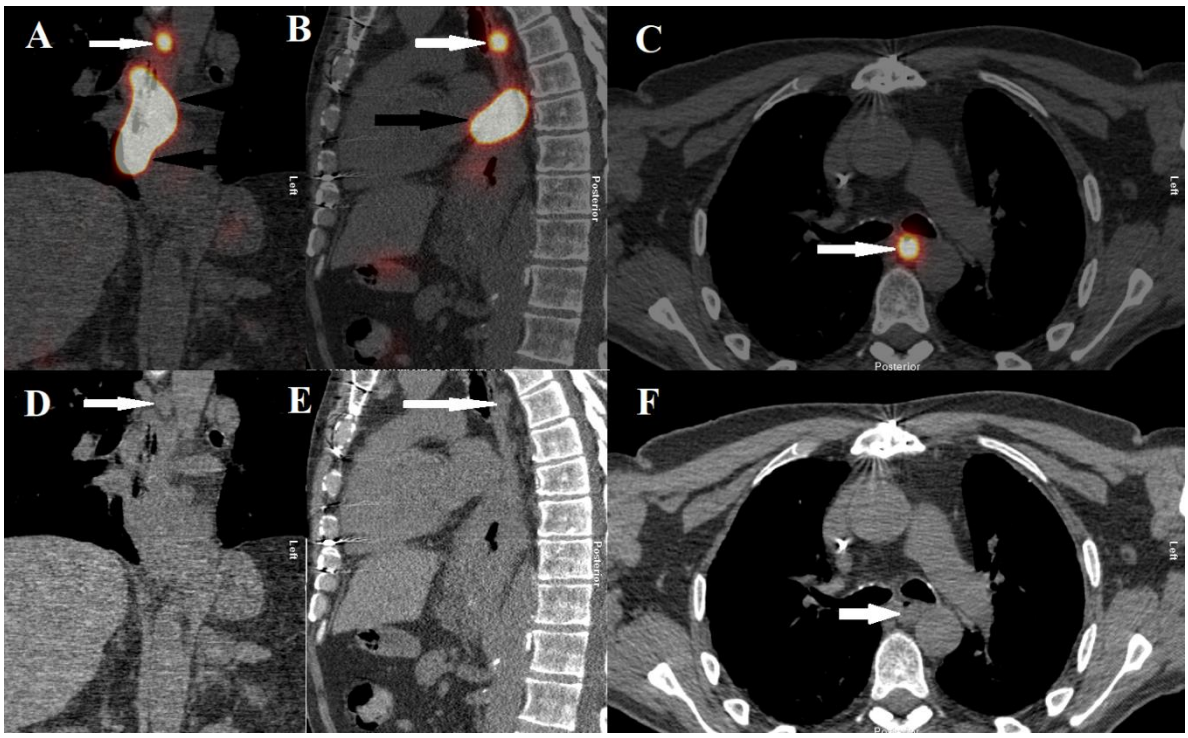

Figure 15. SPECT/CT lymphoscintigraphy images of a 65-year-old male patient with a T3N2M0 adenocarcinoma of the distal oesophagus and GOJ (Siewert 2) undergoing pre-operative SLN mapping. **Images A-C** consist of Hybrid SPECT/CT images in coronal, sagittal and transversal orientations. **Images D-F** are of CT images in an unfused state in the same orientations. White arrows indicate radiocolloid uptake in, and the anatomical location of a Sentinel Lymph Node located in the mediastinum, in the 101L lymph node station (as defined by the Japanese Classification of Oesophageal Cancer, 11th Edition). Black arrows indicate radiocolloid deposit in one of the intra-/peritumoural injection sites.

39/41 patients underwent intraoperative SLNB synchronously with either open or minimally invasive oesophagectomy. A total of 115 Sentinel Lymph Nodes were obtained in these 39 SLNB procedures. The intra-operative SLN detection rates using a gamma-probe were similar to those in the pre-operative imaging, and at least one SLN was detected in 84% of procedures (33/39 patients) (P=0.68). The mean number of lymph nodes per procedure was 2.9 and the median was 2 (range 0–21). At least one suspected Sentinel Lymph Node station was identified in all procedures (39/39). The mean number of identified stations was 1.8 and the median was 1 (range 1–4). In pathological examinations, it was found that in six cases no lymph nodes were evident in the SLN-biopsies. These unsuccessful SLN biopsies contained fat, vessels, nerve tissue etc. Of the six cases where SLNB was unsuccessful, three patients had adenocarcinomas of the distal oesophagus and cardia, and three patients had squamous cell carcinomas of the middle or distal thirds of the oesophagus. Of these same six cases, three patients had undergone neoadjuvant treatment and three had not. A total of 971 lymph nodes were evaluated by pathologists for metastatic occurrence. The mean and median

number of lymph nodes per lymphadenectomy was 24 (range 9–56). Lymph node metastasis was evident in 12/39 patients and in 64/971 lymph nodes. SLNB was a false negative in eight patients where other sites of lymph node metastasis were identified during routine pathological examination. In two of the six patients where the SLNB procedure was unsuccessful, metastatic disease was found in lymph nodes evaluated by routine pathology.

The sensitivity for the 33 successful SLNB procedures was 20%, specificity was 100%, NPV 74%, PPV 100% and accuracy 75%.

4.3 PAPER III

Ten patients were included in this study, and all ten patients underwent SPECT/CT lymphoscintigraphy prior to nCRT, and once again following nCRT. Eight out of ten patients underwent all three cycles of chemotherapy. One patient underwent two cycles due to hyperemesis and one patient underwent only one cycle due to kidney failure. All ten patients underwent the planned radiotherapy.

At baseline examination, the median number of identified SLN stations was 1 (range 0–2). In two patients, no SLN was identified at baseline examinations. At follow-up examination, the median number of identified SLN stations was also 1 (range 0–1). In three patients, no SLN was identified at follow-up examinations.

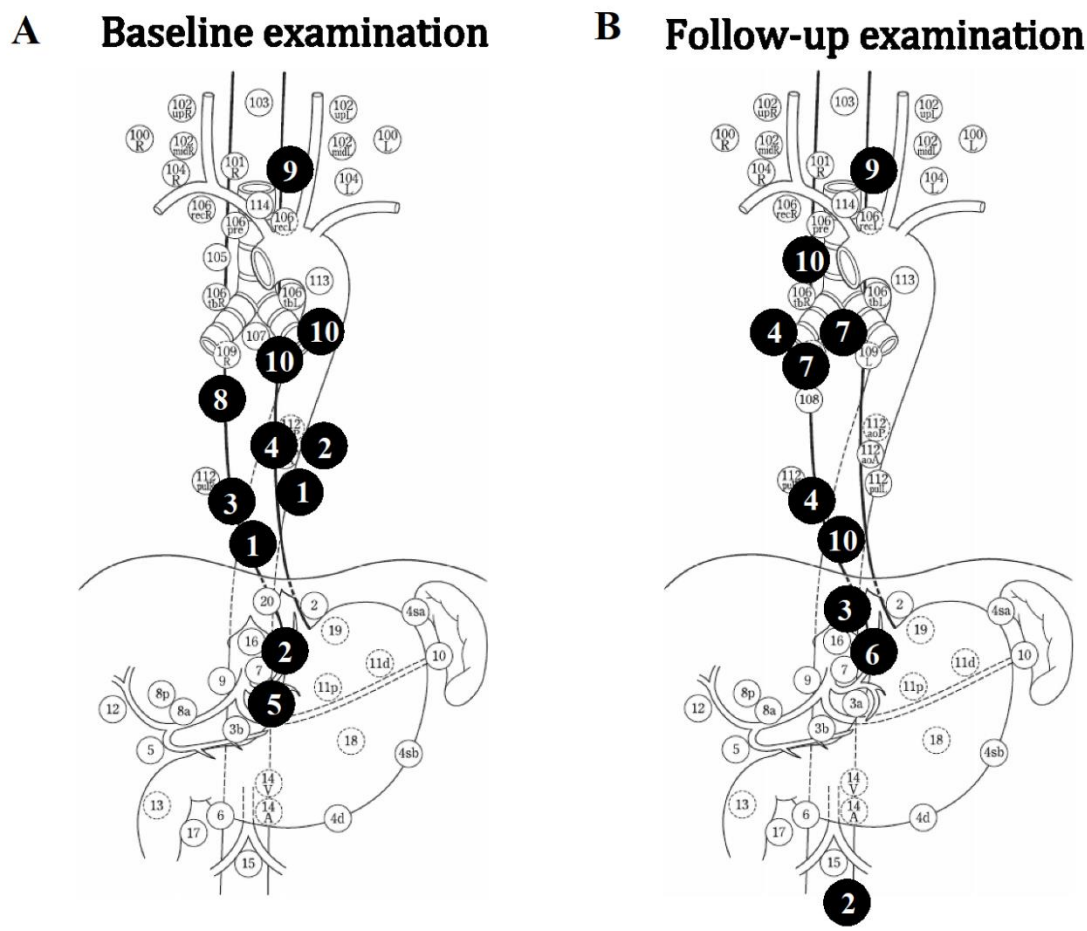

Figure 16. Changes in lymphatic drainage patterns in 10 patients with cancer of the oesophagus/GOJ before and after neoadjuvant chemo-radiotherapy. **A)** Distribution of Sentinel Lymph Node stations as determined by SPECT/CT lymphoscintigraphy at baseline examination. **B)** Distribution of Sentinel Lymph Node stations as determined by SPECT/CT lymphoscintigraphy at follow-up examination. Black circles indicate location(s) of SLNs. The numbers within the circles indicate patient ID. Japanese Classification of Oesophageal Cancer, 11th Edition: part I. ©, with credit also to the original authors. This image is reproduced in a derivative state and modified as indicated in the figure legend under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/).

4.4 PAPER IV

Seventy-nine patients were enrolled, with fifty-one available for analysis. The two treatment groups were well balanced in terms of age, sex and tumour characteristics. The mean rate of SUR change (days-1) was -0.048±0.049 and -0.017±0.041 for pooled nCRT and nCT responders as well as in pooled non-responders, respectively (p=0.02).

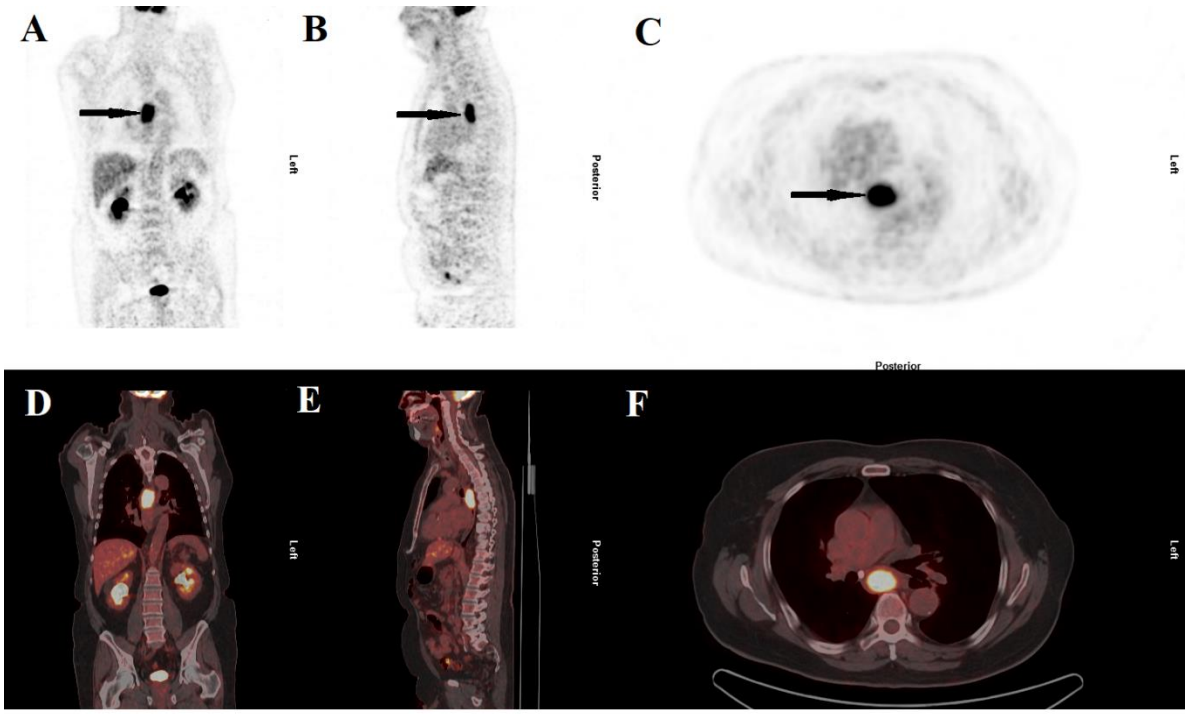

Figure 8. ^{18}F FDG-PET/CT images of a 72-year-old male patient with a T3N1M0 squamous cell carcinoma of the middle oesophagus at baseline examination, preceding neoadjuvant chemo-radiotherapy. **Images A-C** consist of PET images in coronal, sagittal and transversal orientations. Black arrows indicate site of tumour FDG uptake. **Images D-F** are of fusion PET and CT images in the same orientations as above.

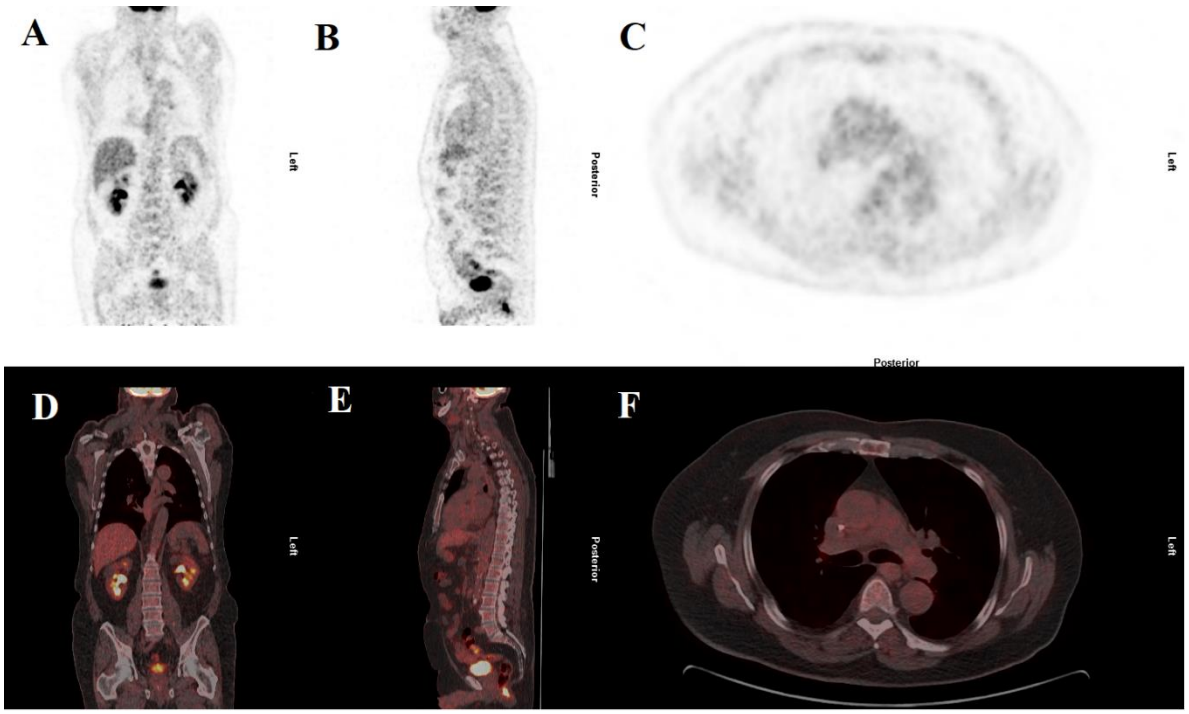

Figure 9. ^{18}F FDG-PET/CT images in unfused and fused states of the same patient as in figure 7, at follow-up examination four weeks following completion of neoadjuvant chemo-radiotherapy. There is no remaining pathological uptake in the tumour.

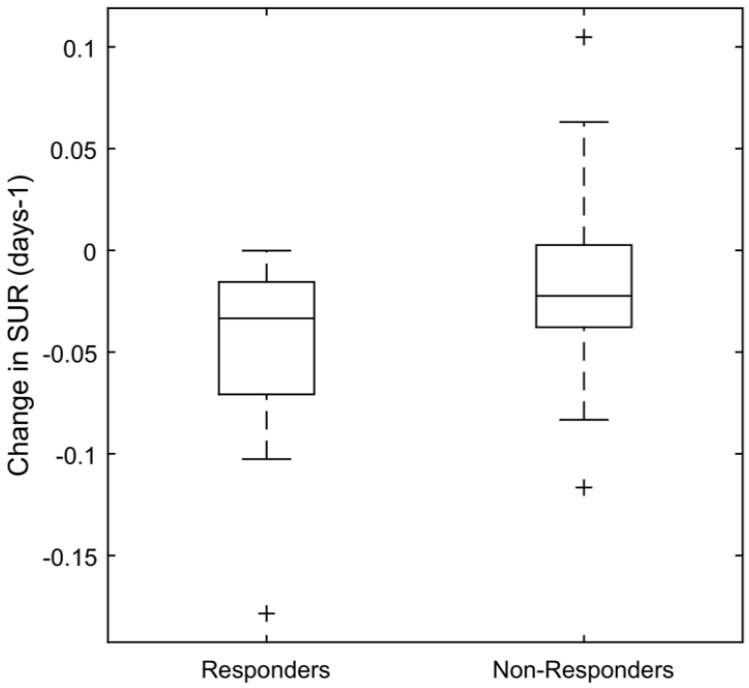

Figure 10. Difference in rate of change of the standard uptake ratio (SUR), from baseline to follow-up after neoadjuvant treatment, and histological response. The data is pooled to include both treatment arms (p=0.02).

The rate of reduction of SUR in histological nCRT responders was statistically significantly higher compared to the rate of reduction in non-responders (P=0.02). The rate of reduction of SUR in nCT responders however was not significantly different from that observed in non-responders (P=0.49). nCRT resulted in a statistically significantly higher rate of reduction in tumour SUR compared to patients treated with nCT (p= 0.04).

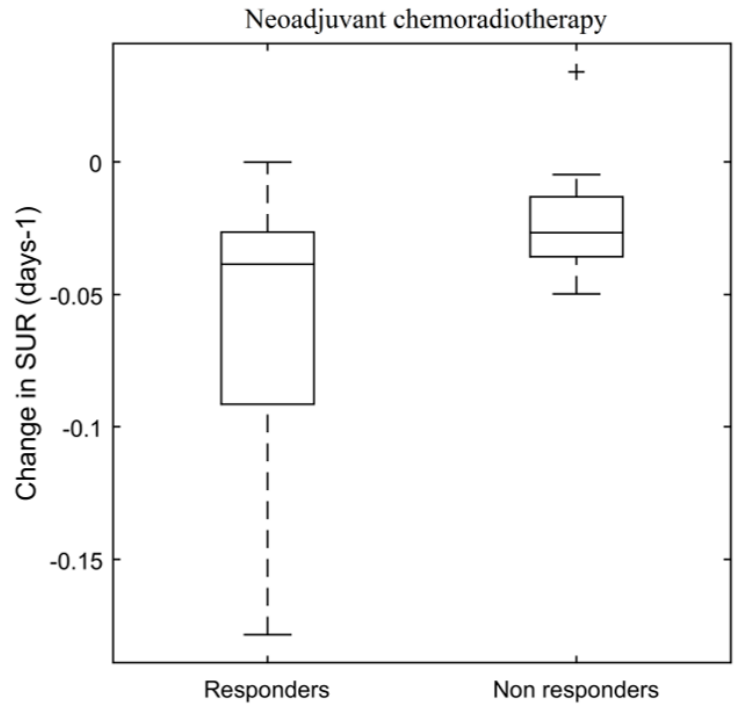

Figure 11. Histological response and differences in rate of change of the standard uptake ratio (SUR), from baseline to follow-up after neoadjuvant chemo-radiotherapy (p=0.023).

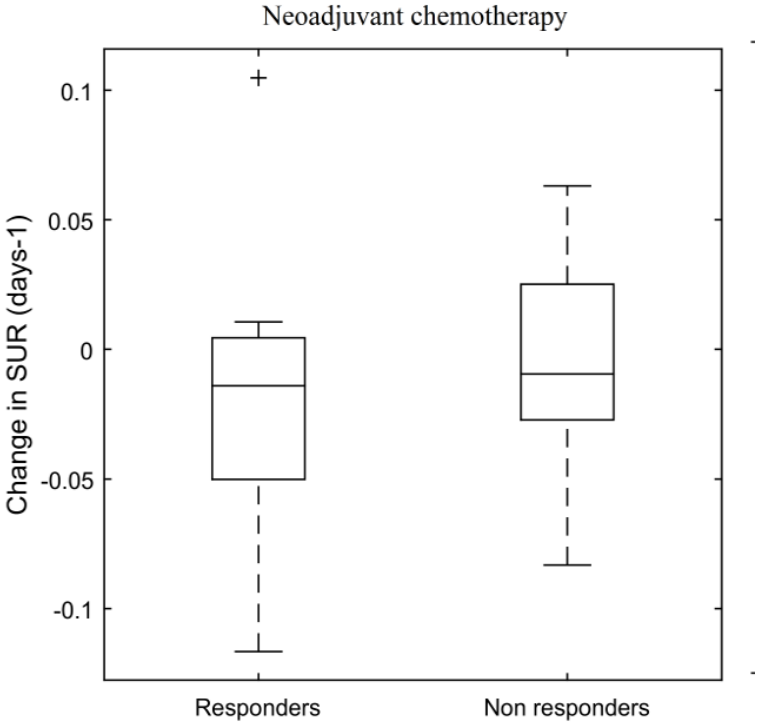

Figure 12. Histological response and differences in rate of change of the standard uptake ratio (SUR), from baseline to follow-up after neoadjuvant chemotherapy (p=0.493).

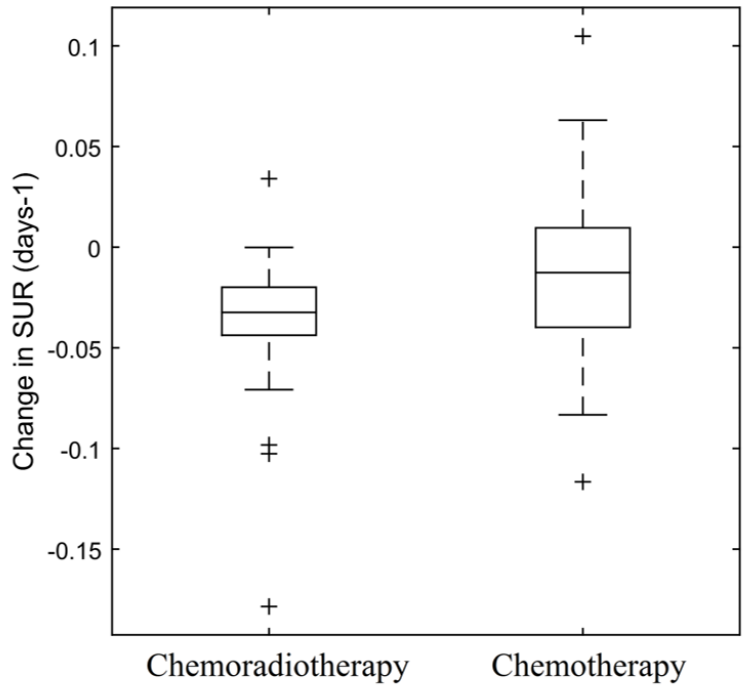

Figure 13. Differences in rate of change in standard uptake ratio (SUR), from baseline to follow-up between treatment arms (nCT or nCRT) (p=0.04).

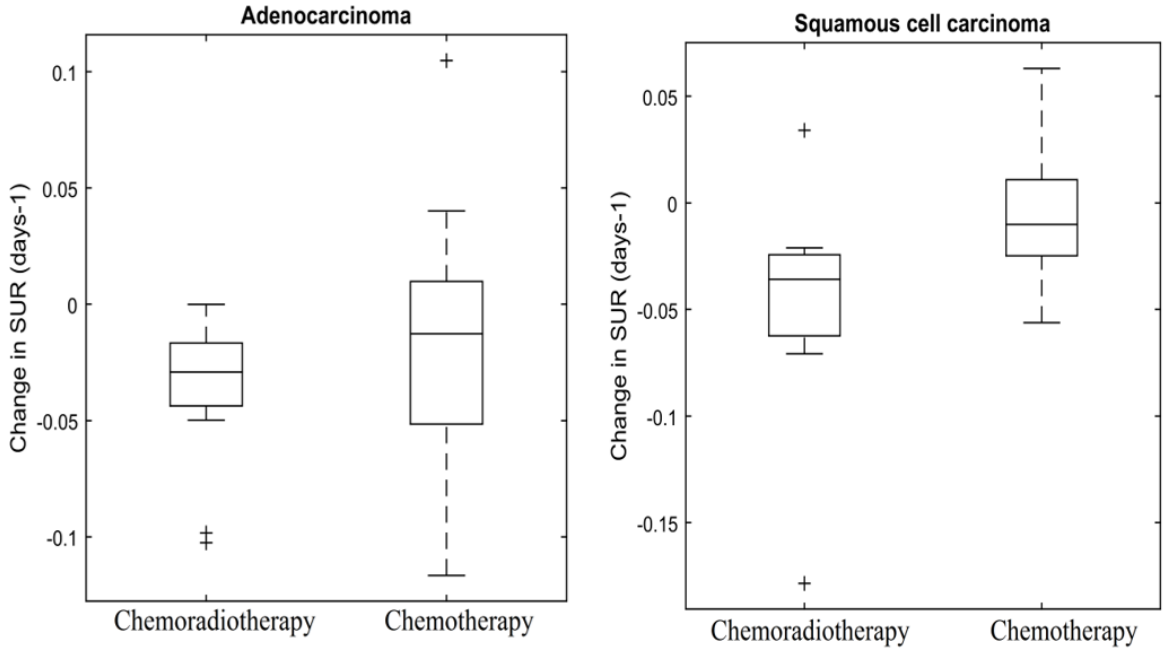

Figure 14. Differences in rate of change in standard uptake ratio (SUR), from baseline to follow-up between treatment arms for histological tumour type (adenocarcinoma or squamous cell carcinoma). (p>0.1 for both histological tumour types).

We observed no statistically significant differences in the rate of SUR changes regarding tumour type (AC or SCC) and in treatment arms (p>0.1 for all combinations). Also, there

were no significant differences in the rate of reduction of SUR when comparing TRG 1 (pCR) to TRG 2–4 in a pooled analysis which included both histological subtypes.

5 DISCUSSION

5.1 PAPER I

In this study, 37 patients underwent sequential hybrid SPECT/CT lymphoscintigraphy before and after a unilateral excisional breast biopsy using sub-areolar radiocolloid injections for evaluating reproducibility in SLN mapping. The patient's own non-operated breast served as a control. Concordance (total or partial) was observed in 85.7 per cent of examinations (30 out of 35) on operated sides and in 88.9 per cent of examinations (32 out of 36) on non-operated sides. The post-operative detection rate of SLNs using hybrid SPECT/CT lymphoscintigraphy was 91.9 per cent (34 out of 37 patient procedures) on operated sides. Our post-operative SLN detection rates were similar to previous studies using pre-operative SPECT/CT lymphoscintigraphy(63,65,68). In the three procedures involving operated breasts where we were unable to either visualise an SLN either pre- or post-operatively, only in one case did this occur only post-operatively. The excisional surgery could therefore only potentially have caused non-visualisation in this one case.

There are some compelling arguments regarding the methodological benefits of using SPECT images compared to conventional planar lymphoscintigraphy using a gamma camera. Apart from the apparent benefit of more detailed anatomical correlation to SLN uptake, there is also evidence that the method produces higher detection rates in this patient group(96,97). One study demonstrated a clear benefit of SLN detection using SPECT compared to planar gamma camera imaging in overweight patients undergoing pre-operative lymphoscintigraphy SLN mapping(66). We found statistically significantly higher BMI and higher age in examinations with non-visualisation. In the previously-mentioned study, it was also established that obesity is associated with lower SLN detection rates. The fact that non-visualisation was associated with higher age is more difficult to interpret and may be due to the rather small patient population.

SLN lymphoscintigraphy appears to be a highly reproducible method. One study using sequential planar lymphoscintigraphy the day before surgery in 25 patients undergoing breast cancer surgery found the same drainage pattern on repeated scintigraphy the following day, immediately prior to surgery in all patients(98).

Regarding the implications for SLNB accuracy in patients with a previous history of diagnostic surgery, earlier studies have been conflicting. One study where the reproducibility of sequential planar lymphoscintigraphy pre-operatively repeated the day following an

excisional biopsy found 100 % reproducibility in the 16/18 patients where an SLN was identified at baseline examination(59).

On the other hand, another study where the same question was addressed by using sequential planar lymphoscintigraphy the day before and again at least two weeks following an excisional biopsy, reproducibility was very poor(58). There were changes in the lymphatic drainage patters in 17 out of 25 patients and the method was reproducible in only 32 per cent of patients (CI 95% 12-52%).
In our study we observed discordance in SLN mapping on the operated sides in 14.3 per cent of patients and on the non-operated sides in 11.1 per cent of patients. These results must be considered an improvement on the previously-mentioned results using planar gamma camera lymphoscintigraphy. The strengths of this study are the use of hybrid SPECT/CT technology with associated improvements to sensitivity and anatomical localisation of SLNs, the weaknesses are the rather small patient population.

5.2 PAPER II

In this prospective study of 39 patients with cancer of the oesophagus or GOJ undergoing radio-guided SNLB following pre-operative SPECT/CT lymphoscintigraphy SLN mapping, we found an acceptable detection rate for intraoperative gamma detector-guided SLNB. The SLNB procedure was successful in 33/39 patients (82%). These intra-operative detection rates are consistent with results from previous studies.

Unlike most previous studies of SLNB in cancer of the oesophagus or GOJ, most of the patients in our study, 30/39 (77%), had undergone neoadjuvant treatment with either nCT or nCRT. In a previous study of 112 patients with cancer of the oesophagus or GOJ undergoing radio-guided SLNB following pre-operative planar lymphoscintigraphy(61). The total SLN detection rate was comparable to our results and at least one SLN was identified in 120/134 patients (90%). However, in patients who had received nCRT, at least one SLN was identified in only five out of eleven cases (45.5%). It is worth noting that pre-operative SLN detection using planar lymphoscintigraphy was successful only in 45/112 patients (40.2%), compared to 88% of patients in our study.

In another study of 23 patients undergoing SLNB guided by pre-operative whole-body SPECT lymphoscintigraphy, four out of 23 patients had undergone nCRT, and the intra-operative SLN detection rate was 91% (21/23). The two patients in whom SLN detection was unsuccessful had both undergone nCRT. The authors make no mention of the SLN detection rates in the pre-operative SPECT imaging(99).

Conversely, in a similar setting Takeuchi et al. described intra-operative SLN detection rates in 71/75 patients (95%), and a successful SLNB was carried out in all the four patients who had undergone nCT. The authors of this paper likewise did not report on the SLN detection rates with pre-operative planar lymphoscintigraphy(90).

Regarding previous history of neoadjuvant treatment, the study with a population most similar to ours was conducted by Thompson et al. In this study, 31 patients were included and 19/31 patients (61%) had received nCRT in a regimen not unlike that used in our protocol. Patients underwent intra-operative radio-guided SLNB. The SLNB detection rate in this study was 94 per cent (29/31 patients) (60). Using SPECT/CT, we were able to demonstrate a similar SLN detection rate of 90 per cent (27/30 patients) of the cases that had received neoadjuvant therapy. This demonstrates that it is possible to achieve a fairly high frequency of SLN detection in patients who have received neoadjuvant treatment, which is consistent with the findings this previous study.

Considering the low sensitivity and accuracy of SLNB in our study, it is important to compare previous investigations of the SLNB method in the present patient group.

One previous study showed excellent sensitivity and accuracy for SLNB in patients with T1 tumours (sensitivity 91.7%, accuracy 98.2%), and more often false negative cases in T2 tumours (sensitivity 66.7%, accuracy 80.6%) and in T3 tumours (sensitivity 54.2%, accuracy 60.7%). In a small subgroup that had received nCRT, false negative rates were considerably higher and evident in three out of eleven cases (sensitivity 0%, accuracy 40%, n=11)(61). Contrary to these findings, Kim et al. found no false negative cases (0/9) in a cohort with higher T-stages, which was comparable with our study, albeit with only 17 per cent of the patients having previously undergone neoadjuvant CRT.

Takeuchi et al. observed false negative SLNBs in 4/33 cases (sensitivity 88%, accuracy 94%). However, the clear majority of patients in that study were T1 stage (76%) and 2/4 false negative SLNBs were found in T3 tumours. The authors observed no correlation between previous neoadjuvant chemotherapy and accuracy in SLNB, but only 4/75 patients had been treated with neoadjuvant chemotherapy and none with CRT.

The poor sensitivity and accuracy that we found may be explained by the high proportion of patients with advanced ≥T3-stage tumours (82%) and a high proportion with a history of neoadjuvant treatment (73%) in our patient population. Since only very few patients with T1 tumours or patients that had not received neoadjuvant therapy were included in this study, analysis of the influence of these factors was not feasible. Due to technical limitations, it is

also possible that we were unable to detect SLNs located immediately proximal to the tumour and/or radiocolloid injection sites, which may have interfered with the detection of SLNs due to so called “shine through”. One previous study showed an accuracy of 96% (55/57 patients) for SNLB procedures in patients with adenocarcinoma of the oesophagus or GOJ.(89). However, it is difficult to compare our results with these findings, as the authors did not report on T-stages or usage of neoadjuvant treatment.

However, Thompson et al. observed only one false negative SLNB in 29 patients. The sensitivity was 90% and the accuracy was 96% in a population like that used in our study. We were thus unable to repeat these results, with a sensitivity of only 20% and accuracy of 75%. We believe that these different results may be explained in part by differences in T-stage in the patient populations. In our study, 63% of patients were ≥pT2-stage compared to 43% in this previously-conducted study.

5.3 PAPER III

In this pilot study of ten patients with cancer of the oesophagus or GOJ undergoing sequential SLN mapping using hybrid SPECT/CT lymphoscintigraphy, we found that the reproducibility following neoadjuvant radio-chemotherapy appears to be very poor. We were only able to identify the same SLN station in examinations before and after neoadjuvant chemo-radiotherapy in one out of ten patients. In three cases where at least one SLN station was observed at baseline, there was neither any visible uptake in the same stations at follow-up, nor were there any other detectable SLN stations. In two patients where no SLNs could be detected at baseline, at least one SLN station was detected at follow-up examination. Out of the five patients where SLN stations could be detected at both examinations, the SLN stations were not the same at follow-up compared to baseline examination in four of these cases.

The impact and underlying mechanisms of neoadjuvant treatment on lymphatic drainage patterns in oesophageal cancer are currently not well understood. Our study suggests that nCRT may have a significant impact on lymphatic drainage patterns from the oesophagus or GOJ. These potential changes may adversely affect the accuracy of the SLNB method in this patient group and may in part explain the poor accuracy of SLNB in patients who have received nCT in previous studies (61,99).

It is worth noting that the one case where we were able to reproduce the SLN mapping involved the one patient with a lower T-stage (T2) stage tumour; all other patients in this study were staged as T3. It is also interesting that this patient was one of two patients with an

SCC tumour located in the mid-oesophagus, with the SLN station being located in the cervical region (station 109L). Due to the very limited patient population, the significance of these characteristics is unclear. The detection rates in baseline SPECT/CT examinations where we were able to identify at least one SLN station in 8/10 patients (80%) are consistent with previous findings using intra-operative radio-guided technique with detection rates of 77.5% [57.4–89.8%] in T3-T4 stage tumours(93).

As there are no previous studies specifically addressing the effects of chemo-radiotherapy on changes in lymphatic drainage in the oesophagus, the results presented here can instead be compared to other cancers, e.g. breast cancer, where Sentinel Lymph Node imaging has been applied in patients exposed to radiation or chemotherapy. In one study of 22 patients with breast cancer previously treated with mantle radiation due to Hodgkin's Lymphoma, and undergoing SLN mapping with SPECT/CT lymphoscintigraphy and intra-operative radio-guided SLNB, the authors were less often able to identify an SLN in previously treated patients compared to the control group (14% and 3% respectively (P=0.01)).(100).

The same research group later conducted a study on the reliability of SLNB in patients with recurrent breast cancer. In this study, which used pre-operative SPECT/CT lymphoscintigraphy, thirty-six out of 114 (31%) patients included in this study had undergone breast-preserving therapy, including radiotherapy without axillary lymph node dissection(101). The authors found that the detection rates were significantly lower in patients with a history of breast-conserving therapy, compared to the whole study population, with detection rates of 72% compared to 85% for the latter (P=0.01). However, it is impossible to quantify with any certainty how the effect of radiotherapy on the breast, radiotherapy to the axilla and variation in surgical technique would affect the flow of lymphatics from the breast and subsequently the accuracy of an SLNB procedure. The authors conclude that the SLNB method is probably more reliable in a setting where there has been no prior no iatrogenic disturbance of the lymphatics.

One large multi-centre cohort study looked at women with clinically node-negative breast cancer undergoing SLNB prior to chemotherapy(102). In this study the detection rates and accuracy of the SLNB was very good, and at least one SLN was identified in of 1,013/1,022 patients (99.1%). Interestingly, in a subgroup undergoing a second SLNB procedure following nCT, the detection rates were much lower, with successful SLNB in only 213/360 patients (60.8%). Likewise, the false negative rate for SLNB was high in this subgroup, with false negative SLNBs occurring in 33/64 patients (51.6%).

Similarly, in a recent study of the accuracy of SLNB accuracy prior to neoadjuvant chemotherapy in 224 breast cancer patients, the detection rate was one hundred per cent, with at least one SLN identified using radio-guided/blue dye intraoperative SLNB technique(103). Approximately half of the patients in this study (98 patients) underwent a second SLNB procedure following nCT, with a success rate of SNL detection of only 69.4%. The false negative rates in SLNB were also significantly higher in a repeated procedures group (25% compared to 7.4% in the first procedure). The authors of this paper advise against a second SLNB following nCT due to low SLN detection rates and the unacceptably high rate of false negative biopsies. To our knowledge, ours is the first study of the effects of neoadjuvant chemo-radiotherapy on changes in lymphatic drainage patterns in patients with cancer of the oesophagus or GOJ. Due to the very small patient population, our results must be interpreted with caution. Concerning SLN detection rates, our results are in line with some, but not all, previous studies(93). There are no previous studies on the reproducibility of SLN mapping of the oesophagus in healthy subjects, or in patients without a history of neoadjuvant chemo-radiotherapy. In light of this, it is important to consider certain methodological uncertainties in our study. Since there is a several week-long interval between the two SPECT/CT examinations and there may or may not have been any morphological changes to the primary tumour, the radiocolloid injections may have been considerably different in the two procedures. This could conceivably have an impact on the lymphatic drainage from the tumours and result in an alternative SLN in the second procedure. Meanwhile, the SLNB method has a high accuracy in T1-stage tumours, albeit in the absence of specific studies (104). This may imply that the reproducibility of pre-operative SLN mapping using a method such as ours would be better in patients with limited disease and in the absence of neoadjuvant treatment.

5.4 PAPER IV

In this study of 51 patients randomised to either of two well-defined regimens of nCRT nCT, we observed a significantly higher rate of reduction of tumour SUR in patients with histological response when compared to non-responders. We found a significantly higher rate of reduction of tumour SUR in histological responders following nCRT, but not in responders following nCT. A statistically significant higher rate of reduction of SUR was also observed in subsequent nCRT when compared to nCT. We interpret these findings as mirroring the greater impact on tumour metabolism resulting from this more aggressive treatment regimen. It also correlates with the overall lower TRG scores in the nCRT treatment arm. When

stratified, we did not find a statistically significant difference in the rate of change in SUR when comparing treatment arms with respect to tumour type (AC or SCC).

We chose to study changes in SUR rather than changes in SUV_{max} or SUV_{mean} in order to avoid some of the inherently imprecise factors associated with these last-mentioned PET parameters. Some previous studies used ROC analysis levels of reduction of SUV_{max} following neoadjuvant treatment and thereby were able to find cut-off values for the predication of pCR((105,106)). The uncertainty when determining a significant level of change, in this case SUV_{max}, was one of the reasons why there was no ROC analysis in this study of changes in SUR in regard to pCR. It is the authors' belief that the SUR parameter is more reliable compared to the clinically-established SUV parameters in reflecting changes in tumour metabolism following neoadjuvant therapy.

The results from previous studies into changes in PET parameters in the evaluation of neoadjuvant treatment have been contradictory ((34,37,105,107,108)). The fact that we observed a significantly higher rate of reduction of SUR in patients with histological response in comparison with histological non-responders is in line with previous findings. The same can be said regarding the significantly higher rate of SUR reduction in responders following nCRT. However, we could not demonstrate any significant differences in the rate of SUR reduction in responders following nCT. Several previous studies have shown significant reductions of SUV values following nCT ((34,37,109)). One explanation for this may be that only a small number of patients were histological responders to nCT treatment 3/26 (12%). This mirrors the larger population of the NeoRes trial, in which histological response was observed in 12/91 (15%) of patients in the nCT treatment arm. It is nevertheless possible that our rather small patient population in this study could hide a potential difference (i.e. a type 2 statistical error). Furthermore, no correlation was observed with tumour type (SCC or AC) and changes in SUR when comparing responders to non-responders. It is also apparent from these calculations that the small patient population may have affected the lack of significant findings.

It is very important to note that in our study, changes in SUR could not be reliably used to discriminate pCR from subtotal response. However, these findings are the most difficult to interpret when comparing our results to similar studies. In the present study, only 6/51 patients (12%) achieved pCR, which is rather low when compared to current clinical experience. It is possible that a larger patient population may have yielded different results. Nevertheless, pCR was attained in 29/181 (16%) of patients in the total NeoRes population, which is comparable to the findings in this paper. Previous studies have investigated PET-

negative tumours in relation to histological response following neoadjuvant treatment. They were unable to reliably rule out limited residual disease (remaining viable cancer cells) in PET-negative tumours. The false negative rates of PET-negative tumours ranged between 17.9% (13/73) ((38)) to 29.5% (5/17) ((110)). Conversely one large retrospective cohort study of patients with adenocarcinoma of the oesophagus and GOJ came to other conclusions ((108)). In this study, no significant correlation was observed between PET-negative tumours and pCR. Moreover, there were no statistically significant differences in ΔSUV_{max} or SUR at baseline or follow-up, and thus it was not possible to distinguish pCR from subtotal histological response.

The prospective design of this study and the randomisation of patients with histological carcinoma types representative of the population in large to one of two well-defined regimens of neoadjuvant treatment are considered strengths. The main weakness is the comparably small patient population. Another weakness, which is due to epidemiological and clinical factors, is the presence of considerable heterogeneity, primarily in T-stages, in the patient population.

6 CONCLUSIONS

6.1 PAPER I

In patients who have small, breast malignancies that have not been diagnosed pre-operatively and who are planned for an excisional biopsy, it is not as common to find tumour-containing lymph nodes at a secondary axillary procedure compared to patients with pre-operatively diagnosed breast malignancies. In this patient group the tolerance for accepting discordant or partial concordant findings arguably would be lower. We observed discordance on operated breast sides in 14.3 % and on the non-operated sides in 11.1 % of breast sides. These findings are different from the 28 % discordance rate found in the study by Estourgie et al. When comparing these two studies, ours had the larger patient population, which is an advantage. The study participants also underwent bilateral imaging, and by these means the non-operated breasts served as controls for evaluating reproducibility. In addition, hybrid SPECT/CT was used instead of planar lymphoscintigraphy. By using this method, we were able to gather detailed information on the precise anatomical location of the SLNs before and after surgery.

6.2 PAPER II

This study shows that pre-operative SPECT/CT imaging combined with intraoperative radio-guided detection yields a high frequency of SLN detection in patients with cancer of the oesophagus or GOJ. To our knowledge, only one similar previous study using planar imaging technique that has reported on SLN detection rates in pre-operative SLN lymphoscintigraphy has hitherto been conducted (61). Compared to these findings, we observed a much higher detection rate (88 per cent compared to 40.2 per cent). We suspect that the increased detection rates are due to increases in sensitivity when utilising SPECT imaging in combination with CT attenuation correction of SPECT data. We also perceive a clear benefit when correlating SPECT findings to CT images in the process of image fusion.

Most tumours in this study were ≥T3 and the clear majority of patients had received neoadjuvant treatment with either chemotherapy or chemo-radiotherapy. The sensitivity for the SLNB procedure was low, with unacceptably high occurrence of false negative biopsies. SLNB cannot, therefore, be recommended as a staging tool in patients with ≥T3 tumour stages or a previous history of neoadjuvant treatment. Having considered our findings, further investigation of the SLNB method in cancer of the oesophagus or GOJ has been discontinued at our hospital. However, our findings lend support to the added value of pre-operative fusion SPECT/CT lymphoscintigraphy, and use of this method could facilitate the intra-operative

identification of SLNs, which may be useful for T1 stage cancer, where there may still be a potential for SLNB staging of cancer of the oesophagus or GOJ.

6.3 PAPER III

In this small pilot study, we observed very poor reproducibility in SLN detection in patients with oesophageal cancer before and after nCRT. We conclude that nCRT may have a clinically relevant impact on lymphatic drainage in the vast majority of these patients. We suggest that this may negatively influence the accuracy of an SLNB procedure in the same way as demonstrated in other cancer forms.

6.4 PAPER IV

The potential utility of predicting pCR in a clinical scenario would likely be quite valuable. Arguably a surveillance regimen with endoscopy and biopsies and expectance could be used in patients who achieve a complete histological response to neoadjuvant treatment. In this patient group potentially, unnecessary surgery with oesophagectomy possibly could be avoided. The clinical value in the discrimination of differentiation of pCR and subtotal histological response compared to non-responders following neoadjuvant treatment could improve on the prognostic evaluation of the response to treatment. Patients with TRG2 would likely benefit from neoadjuvant treatment. On the other hand, patients with TRG 3 or TRG 4 arguably might be better suited for surgery without neoadjuvant treatment. Perhaps the greatest obstacle to the implementation of ^{18}F-FDG PET/CT in treatment response evaluation of oesophageal lies in identifying the optimal PET parameters.

Another obstacle is the current lack of minimal standards of care in the management of this patient group. Furthermore, the comparably low spatial resolution in images when using current PET-technology also limits the ability to accurately rule out any limited residual disease in patients with cancer of the oesophagus/GOJ following neoadjuvant treatment with either nCRT or nCT.

7 FUTURE ASPECTS

The aim of this thesis was in part to evaluate the impact of a previous limited surgical procedure to the breast on a prospective SLNB procedure. The present study indicates that an SLNB procedure could be considered safe in this patient group. We have no current plans for further investigation into this matter.

Concerning the applicability, and the possible impact of, nCRT on an SLNB method in patients with cancer of the oesophagus/GOJ, it is important to consider epidemiological factors. Some researchers suggest using the SLNB method in the staging of early oesophageal cancer patients where the evidence points towards the method being more reliable(111). For instance, intra-operative SLNB could be performed in selected cases of cT1N0 stage cancers. If the SLN was found to be in the abdomen, the surgeons could proceed with a laparoscopic minimally-invasive oesophagectomy and only perform a limited abdominal lymphadenectomy, completely omitting the currently-used mediastinal lymphadenectomy. Conversely, if there is evidence of metastatic spread to the SLN, the surgeons would proceed to extended thoracoscopic lymphadenectomy. A pilot study was recently conducted in which patients with cT1N0M0 adenocarcinoma of the oesophagus underwent intra-operative thoraco-/laparoscopically radio-guided SLNB without oesophagectomy. Instead, an endoscopic resection of the tumour was conducted in the same procedure(112). This method was found to be feasible, though more research into the area is needed. However, at my academic centre, these low-grade tumours represent a minority of cases. In part due to the discouraging results in the articles included in this thesis, further investigation into the applicability of the SLNB method has been discontinued for now.

The prospects are more hopeful in respect of the utility of ^{18}F-FDG PET/CT in evaluating response to neoadjuvant treatment in patients with cancer of the oesophagus and GOJ.

We are currently involved in the ongoing NeoRes II trial, in which patients with resectable cancer of the oesophagus/GOJ are randomised to two time-frames between end of neoadjuvant treatment and surgery. Similarly, to the protocol used in the NeoRes I trial, patients undergo ^{18}F-FDG PET/CT examinations at baseline, and again at follow-up. Hopefully evaluation of PET parameters such as SUR values will prove useful as a predictive or prognostic factor in this context.

The ongoing Systematic Surgery Versus Surveillance and Rescue Surgery in Operable Cancer of the Oesophagus with a Complete Clinical Response to Radio-chemotherapy

(ESOSTRATE) multi-centre study will use evaluation by means of ^{18}F-FDG PET/CT to identify patients with a complete clinical response to neoadjuvant chemo-radiotherapy. Patients with a complete response will be randomised to either direct oesophagectomy or to monitoring and rescue oesophagectomy. Advances in PET-imaging technology entailing new techniques such as fully-digital PET systems may prove to offer higher sensitivity compared to those currently used. A significant increase in sensitivity could perhaps result in the possibility of ruling out residual limited disease, and to accurately discriminate a complete response to neoadjuvant treatment.

Hopefully the availability of PET-hardware capable of dynamic PET-studies will increase the role of PET in the response evaluation of cancer patients. The added value of being able not only to measure the steady-state properties of a tumour but also the rate of influx of FDG to the same will likely prove valuable in the evaluation of changes in tumour metabolism.

In addition, ^{18}F-Fluorothymidine (^{18}F-FLT) is a PET-tracer considered to be more specific to cellular proliferation, rather than cellular metabolism in the case of ^{18}F-FDG. This difference in pharmaco-physiological properties makes it a compelling tracer for investigation in this patient group. One advantage would be the fact that residual inflammatory reaction to radiotherapy would not have a significant impact on FLT uptake in the affected tissues. The uptake of FLT in tumours would rather reflect proliferation in residual cancer cells. We are currently conducting a study in our research group of the utility of ^{18}F-FLT PET/CT in evaluating the response to neoadjuvant treatment in patients with potentially curable cancer of the oesophagus or gastro-oesophageal junction.

8 ACKNOWLEGEMENTS

First and foremost, I would like to express my heartfelt thanks to my supervisor, teacher and friend, Professor **Rimma Axelsson**, who first introduced me to the field of scientific research and who, through her expert guidance and enthusiastic support, has made these years a joy. Thank you for inspiring me to always look forward in the face of set-backs and also to take pride in my accomplishments.

To my co-supervisor, Associate Professor **Jon Tsai**, for introducing me to the research area of disease-staging in oesophageal cancer, many thanks for your extensive and invaluable help in all aspects of our collaboration.

To my co-supervisor, Associate Professor **Alejandro Sanchez-Crespo**, for all your guidance and for your dynamic and insightful demeanour.

To my co-supervisor, Dr. **Fuat Celebioglu**, for introducing me to the research area of Sentinel Lymph Node Biopsies and for kindly sharing your expert knowledge in this area.

To my collaborators in the department of upper abdominal surgery, Professor **Magnus Nilsson**, Dr. **Fredrik Klevebro**, Dr. **Ioannis Rouvelas** and Associate Professor **Mats Lindblad**, for your tireless efforts in finding study participants and for conducting the endoscopically-guided radiocolloid injections and SLNB procedures. Many thanks also for sharing your research material and extensive knowledge of clinical research.

To my collaborators in the departments of breast surgery, Dr. **Linda Zetterlund** of Södersjukhuset and Associate Professor **Jana de Boniface** and Professor **Jan Frisell** of Karolinska University Hospital for your efforts.

To **Berit Sunde** for your outstanding help in the planning of examinations and keeping track of dates for surgery.

To my collaborator, **Annie Bjärebäck**, for introducing me to the technical aspects of SPECT/CT imaging and for all your efforts in planning and maintaining study protocols.

To the staff at the Department of Nuclear Medicine, Karolinska University Hospital, for all the hard work that has gone into planning and producing examinations of the highest quality and for taking care of our patients.

To my dear friends and colleagues: Dr. **Artur Tomson**, Dr. **Carl Samuelsson**, Dr. CPT. **Henrik Lehmann**, Dr. **Marcus Danell** and Dr. **Niklas Berg** for all your encouragement and all the good times, and with hopes for many more!

To my friend and colleague, Dr. **Irina Savitcheva**, for teaching me so much in the field of Nuclear Medicine, for all your support and for always showing such kindness and understanding to me and to my family.

To the director of studies at the Radiology department, Dr. **Marie-Louise Wretling**, who encouraged me to pursue my PhD studies.

To Dr. **Kamelia Kostova** for encouraging me to choose a career in medical radiology.

To the faculty and board of the National postgraduate school in Clinical Cancer Research (NatiOn) for all their hard work and guidance.

To my fellow students in NatiOn (2014-2017) for sharing your experiences in other areas of expertise and life experiences.

To my loving and supportive parents, **Annelie and Henrik Gabrielson**, without whose efforts in all things practical and ethereal this work would not have been possible.

To my dear brother, Dr. **Carl-Henrik Fermhede**, and my sister, **Madeleine Gabrielson**, for always encouraging my efforts and for bringing joy to my life.

To my grandmother, Dr. **Malina Staneva**, for always showing interest in my studies and for being such a warm and supportive granny.

To my late grandfather, Dr. **Stanislav Stanev**, for inspiring me to follow in his footsteps and become a physician.

To my parents in law, **Christina and Göran Fjellman**, for your encouragement and for your outstanding generosity and hospitality during our visits.

To my old friend and mentor, Dr. **Sipan Khalil**, for all the memories and for our companionship ever since our first adventures in Gothenburg.

To my amazing partner, Dr. **Karin Fjellman**, for all your love and support and for giving me the greatest gift imaginable – our sons Erik and Arvid.

9 REFERENCES

1. Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, MacIntyre MF, et al. The Global Burden of Cancer 2013. JAMA Oncol. 2015 Jul 1;1(4):505.

2. Edgren G, Adami H-O, Weiderpass E, Nyrén O. A global assessment of the oesophageal adenocarcinoma epidemic. Gut. 2013 Oct;62(10):1406–14.

3. Arnal MJD. Esophageal cancer: Risk factors, screening and endoscopic treatment in Western and Eastern countries. World J Gastroenterol. 2015;21(26):7933.

4. Arnold M, Soerjomataram I, Ferlay J, Forman D. Global incidence of oesophageal cancer by histological subtype in 2012. Gut. 2015 Mar;64(3):381–7.

5. Abnet CC, Arnold M, Wei W-Q. Epidemiology of Esophageal Squamous Cell Carcinoma. Gastroenterology. 2018 Jan;154(2):360–73.

6. Lagergren J, Smyth E, Cunningham D, Lagergren P. Oesophageal cancer. The Lancet [Internet]. 2017 Jun [cited 2017 Jul 6]; Available from: http://linkinghub.elsevier.com/retrieve/pii/S0140673617314629

7. Shaheen NJ, Richter JE. Barrett's oesophagus. The Lancet. 2009 Mar;373(9666):850–61.

8. Hao W, Shen Y, Feng M, Wang H, Lin M, Fang Y, et al. Aspirin acts in esophageal cancer: a brief review. J Thorac Dis. 2018 Apr;10(4):2490–7.

9. Kim J, Bowlby R, Mungall AJ, Robertson AG, Odze RD, Cherniack AD, et al. Integrated genomic characterization of oesophageal carcinoma. Nature. 2017 Jan 4;541(7636):169–75.

10. Enzinger PC, Mayer RJ. Esophageal Cancer. N Engl J Med. 2003 Dec 4;349(23):2241–52.

11. Herszényi L, Tulassay Z. Epidemiology of gastrointestinal and liver tumors. Eur Rev Med Pharmacol Sci. 2010 Apr;14(4):249–58.

12. Lambert R, Hainaut P. Epidemiology of oesophagogastric cancer. Best Pract Res Clin Gastroenterol. 2007 Dec;21(6):921–45.

13. Eloubeidi MA, Desmond R, Arguedas MR, Reed CE, Wilcox CM. Prognostic factors for the survival of patients with esophageal carcinoma in the U.S.: The importance of tumor length and lymph node status. Cancer. 2002 Oct 1;95(7):1434–43.

14. Akutsu Y, Matsubara H. The significance of lymph node status as a prognostic factor for esophageal cancer. Surg Today. 2011 Sep;41(9):1190–5.

15. Miyata H, Sugimura K, Yamasaki M, Makino T, Tanaka K, Morii E, et al. Clinical Impact of the Location of Lymph Node Metastases After Neoadjuvant Chemotherapy for Middle and Lower Thoracic Esophageal Cancer. Ann Surg Oncol [Internet]. 2018 Oct 29 [cited 2018 Nov 29]; Available from: http://link.springer.com/10.1245/s10434-018-6946-z

16. van Hagen P, Hulshof MCCM, van Lanschot JJB, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BPL, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med. 2012 May 31;366(22):2074–84.

17. Ychou M, Boige V, Pignon J-P, Conroy T, Bouché O, Lebreton G, et al. Perioperative Chemotherapy Compared With Surgery Alone for Resectable Gastroesophageal Adenocarcinoma: An FNCLCC and FFCD Multicenter Phase III Trial. J Clin Oncol. 2011 May;29(13):1715–21.

18. Sjoquist KM, Burmeister BH, Smithers BM, Zalcberg JR, Simes RJ, Barbour A, et al. Survival after neoadjuvant chemotherapy or chemoradiotherapy for resectable oesophageal carcinoma: an updated meta-analysis. Lancet Oncol. 2011 Jul;12(7):681–92.

19. Chirieac LR, Swisher SG, Ajani JA, Komaki RR, Correa AM, Morris JS, et al. Posttherapy pathologic stage predicts survival in patients with esophageal carcinoma receiving preoperative chemoradiation. Cancer. 2005 Feb 17;103(7):1347–55.

20. Klevebro F, Alexandersson von Döbeln G, Wang N, Johnsen G, Jacobsen A-B, Friesland S, et al. A randomized clinical trial of neoadjuvant chemotherapy versus neoadjuvant chemoradiotherapy for cancer of the oesophagus or gastro-oesophageal junction. Ann Oncol. 2016 Apr;27(4):660–7.

21. von Döbeln GA, Klevebro F, Jacobsen A-B, Johannessen H-O, Nielsen NH, Johnsen G, et al. Neoadjuvant chemotherapy versus neoadjuvant chemoradiotherapy for cancer of the esophagus or gastroesophageal junction: long-term results of a randomized clinical trial. Dis Esophagus [Internet]. 2018 Aug 22 [cited 2018 Nov 29]; Available from: https://academic.oup.com/dote/advance-article/doi/10.1093/dote/doy078/5078143

22. Bollschweiler E, Hölscher AH, Metzger R. Histologic tumor type and the rate of complete response after neoadjuvant therapy for esophageal cancer. Future Oncol. 2010 Jan;6(1):25–35.

23. Taniyama Y, Sakurai T, Heishi T, Okamoto H, Sato C, Maruyama S, et al. Different strategy of salvage esophagectomy between residual and recurrent esophageal cancer after definitive chemoradiotherapy. J Thorac Dis. 2018 Mar;10(3):1554–62.

24. Naveed M, Kubiliun N. Endoscopic Treatment of Early-Stage Esophageal Cancer. Curr Oncol Rep [Internet]. 2018 Sep [cited 2018 Aug 31];20(9). Available from: http://link.springer.com/10.1007/s11912-018-0713-y

25. Old OJ, Isabelle M, Barr H. Staging Early Esophageal Cancer. In: Jansen M, Wright NA, editors. Stem Cells, Pre-neoplasia, and Early Cancer of the Upper Gastrointestinal Tract [Internet]. Cham: Springer International Publishing; 2016 [cited 2017 Jul 7]. p. 161–81. Available from: http://link.springer.com/10.1007/978-3-319-41388-4_9

26. Bunting D, Bracey T, Fox B, Berrisford R, Wheatley T, Sanders G. Loco-regional staging accuracy in oesophageal cancer—How good are we in the modern era? Eur J Radiol. 2017 Dec;97:71–5.

27. Räsänen JV, Sihvo EIT, Knuuti MJ, Minn HRI, Luostarinen MES, Laippala P, et al. Prospective Analysis of Accuracy of Positron Emission Tomography, Computed Tomography, and Endoscopic Ultrasonography in Staging of Adenocarcinoma of the Esophagus and the Esophagogastric Junction. Ann Surg Oncol. 2003 Jan;10(8):954–60.

28. van Vliet EPM, Heijenbrok-Kal MH, Hunink MGM, Kuipers EJ, Siersema PD. Staging investigations for oesophageal cancer: a meta-analysis. Br J Cancer. 2008 Feb 12;98(3):547–57.

29. Warburg O. On the origin of cancer cells. Science. 1956 Feb 24;123(3191):309–14.

30. Hofheinz F, Apostolova I, Oehme L, Kotzerke J, van den Hoff J. Test–Retest Variability in Lesion SUV and Lesion SUR in ^{18} F-FDG PET: An Analysis of Data from Two Prospective Multicenter Trials. J Nucl Med. 2017 Nov;58(11):1770–5.

31. Erasmus JJ, Munden RF. The Role of Integrated Computed Tomography Positron-Emission Tomography in Esophageal Cancer: Staging and Assessment of Therapeutic Response. Semin Radiat Oncol. 2007 Jan;17(1):29–37.

32. Lowe VJ, Booya F, Fletcher JG, Nathan M, Jensen E, Mullan B, et al. Comparison of Positron Emission Tomography, Computed Tomography, and Endoscopic Ultrasound in the Initial Staging of Patients with Esophageal Cancer. Mol Imaging Biol. 2005 Nov;7(6):422–30.

33. Schmidt T, Lordick F, Herrmann K, Ott K. Value of Functional Imaging by PET in Esophageal Cancer. J Natl Compr Canc Netw. 2015 Feb 1;13(2):239–47.

34. Weber WA, Ott K, Becker K, Dittler HJ, Helmberger H, Avril NE, et al. Prediction of response to preoperative chemotherapy in adenocarcinomas of the esophagogastric junction by metabolic imaging. J Clin Oncol Off J Am Soc Clin Oncol. 2001 Jun 15;19(12):3058–65.

35. Mandard AM, Dalibard F, Mandard JC, Marnay J, Henry-Amar M, Petiot JF, et al. Pathologic assessment of tumor regression after preoperative chemoradiotherapy of esophageal carcinoma. Clinicopathologic correlations. Cancer. 1994 Jun 1;73(11):2680–6.

36. Wieder HA, Brücher BLDM, Zimmermann F, Becker K, Lordick F, Beer A, et al. Time Course of Tumor Metabolic Activity During Chemoradiotherapy of Esophageal Squamous Cell Carcinoma and Response to Treatment. J Clin Oncol. 2004 Mar;22(5):900–8.

37. Lordick F, Ott K, Krause B-J, Weber WA, Becker K, Stein HJ, et al. PET to assess early metabolic response and to guide treatment of adenocarcinoma of the oesophagogastric junction: the MUNICON phase II trial. Lancet Oncol. 2007 Sep;8(9):797–805.

38. Swisher SG, Erasmus J, Maish M, Correa AM, Macapinlac H, Ajani JA, et al. 2-Fluoro-2-deoxy-D-glucose positron emission tomography imaging is predictive of pathologic response and survival after preoperative chemoradiation in patients with esophageal carcinoma. Cancer. 2004 Oct 15;101(8):1776–85.

39. Chirieac LR, Swisher SG, Ajani JA, Komaki RR, Correa AM, Morris JS, et al. Posttherapy pathologic stage predicts survival in patients with esophageal carcinoma receiving preoperative chemoradiation. Cancer. 2005 Feb 17;103(7):1347–55.

40. Key TJ, Verkasalo PK, Banks E. Epidemiology of breast cancer. Lancet Oncol. 2001 Mar;2(3):133–40.

41. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52 705 women with breast cancer and 108 411 women without breast cancer. The Lancet. 1997 Oct;350(9084):1047–59.

42. Foulkes W. *BRCA1* and *BRCA2* - update and implications on the genetics of breast cancer: a clinical perspective: Commentary. Clin Genet. 2014 Jan;85(1):1–4.

43. Makki. Diversity of Breast Carcinoma: Histological Subtypes and Clinical Relevance. Clin Med Insights Pathol. 2015 Dec;23.

44. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol. 2013 Sep;24(9):2206–23.

45. Cianfrocca M. Prognostic and Predictive Factors in Early-Stage Breast Cancer. The Oncologist. 2004 Nov 1;9(6):606–16.

46. Yang J, Long Q, Li H, Lv Q, Tan Q, Yang X. The value of positive lymph nodes ratio combined with negative lymph node count in prediction of breast cancer survival. J Thorac Dis. 2017 Jun;9(6):1531–7.

47. van der Hage JH, van de Velde CC, Mieog SJ. Preoperative chemotherapy for women with operable breast cancer. In: The Cochrane Collaboration, editor. Cochrane Database of Systematic Reviews [Internet]. Chichester, UK: John Wiley & Sons, Ltd; 2007 [cited 2017 Aug 17]. Available from: http://doi.wiley.com/10.1002/14651858.CD005002.pub2

48. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. The Lancet. 2014 Jul;384(9938):164–72.

49. Veronesi U, Cascinelli N, Mariani L, Greco M, Saccozzi R, Luini A, et al. Twenty-Year Follow-up of a Randomized Study Comparing Breast-Conserving Surgery with Radical Mastectomy for Early Breast Cancer. N Engl J Med. 2002 Oct 17;347(16):1227–32.

50. Fisher B, Anderson S, Bryant J, Margolese RG, Deutsch M, Fisher ER, et al. Twenty-Year Follow-up of a Randomized Trial Comparing Total Mastectomy, Lumpectomy, and Lumpectomy plus Irradiation for the Treatment of Invasive Breast Cancer. N Engl J Med. 2002 Oct 17;347(16):1233–41.

51. Pondé NF, Zardavas D, Piccart M. Progress in adjuvant systemic therapy for breast cancer. Nat Rev Clin Oncol [Internet]. 2018 Sep 11 [cited 2018 Nov 29]; Available from: http://www.nature.com/articles/s41571-018-0089-9

52. Gianni L, Eiermann W, Semiglazov V, Manikhas A, Lluch A, Tjulandin S, et al. Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. The Lancet. 2010 Jan;375(9712):377–84.

53. Morton DL, Wen DR, Wong JH, Economou JS, Cagle LA, Storm FK, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. Arch Surg Chic Ill 1960. 1992 Apr;127(4):392–9.

54. Nieweg OE, Tanis PJ, Kroon BB. The definition of a sentinel node. Ann Surg Oncol. 2001 Jul;8(6):538–41.

55. Kitagawa Y, Fujii H, Mukai M, Kubota T, Ando N, Watanabe M, et al. The role of the sentinel lymph node in gastrointestinal cancer. Surg Clin North Am. 2000 Dec;80(6):1799–809.

56. Brenot-Rossi I, Houvenaeghel G, Jacquemier J, Bardou V-J, Martino M, Hassan-Sebbag N, et al. Nonvisualization of axillary sentinel node during lymphoscintigraphy: is there a pathologic significance in breast cancer? J Nucl Med Off Publ Soc Nucl Med. 2003 Aug;44(8):1232–7.

57. Borgstein MD PJ, Pijpers MD R, Comans MD, PhD EF, van Diest MD, PhD PJ, Boom MD, PhD RP, Meijer MD, PhD S. Sentinel Lymph Node Biopsy in Breast Cancer: Guidelines and Pitfalls of Lymphoscintigraphy and Gamma Probe Detection. J Am Coll Surg. 1998 Mar;186(3):275–83.

58. Estourgie SH, Valdés Olmos RA, Nieweg OE, Hoefnagel CA, Rutgers EJT, Kroon BBR. Excision biopsy of breast lesions changes the pattern of lymphatic drainage. Br J Surg. 2007 Sep;94(9):1088–91.

59. Asadi M, Shobeiri H, Aliakbarian M, Jangjoo A, Dabbagh Kakhki VR, Sadeghi R, et al. Reproducibility of lymphoscintigraphy before and after excisional biopsy of primary breast lesions: A study using superficial peri-areolar injection of the radiotracer. Rev Esp Med Nucl E Imagen Mol. 2013 May;32(3):152–5.

60. Thompson SK, Bartholomeusz D, Jamieson GG. Sentinel lymph node biopsy in esophageal cancer: should it be standard of care? J Gastrointest Surg Off J Soc Surg Aliment Tract. 2011 Oct;15(10):1762–8.

61. Uenosono Y, Arigami T, Yanagita S, Kozono T, Arima H, Hirata M, et al. Sentinel Node Navigation Surgery is Acceptable for Clinical T1 and N0 Esophageal Cancer. Ann Surg Oncol. 2011 Jul;18(7):2003–9.

62. van der Ploeg IMC, Valdés Olmos RA, Nieweg OE, Rutgers EJT, Kroon BBR, Hoefnagel CA. The additional value of SPECT/CT in lymphatic mapping in breast cancer and melanoma. J Nucl Med Off Publ Soc Nucl Med. 2007 Nov;48(11):1756–60.

63. Vercellino L, Ohnona J, Groheux D, Slama A, Colletti PM, Chondrogiannis S, et al. Role of SPECT/CT in Sentinel Lymph Node Detection in Patients With Breast Cancer. Clin Nucl Med. 2013 Jul 19;

64. Husarik DB, Steinert HC. Single-photon emission computed tomography/computed tomographyfor sentinel node mapping in breast cancer. Semin Nucl Med. 2007 Jan;37(1):29–33.

65. Lerman H, Metser U, Lievshitz G, Sperber F, Shneebaum S, Even-Sapir E. Lymphoscintigraphic sentinel node identification in patients with breast cancer: the role of SPECT-CT. Eur J Nucl Med Mol Imaging. 2006 Mar;33(3):329–37.

66. Lerman H, Lievshitz G, Zak O, Metser U, Schneebaum S, Even-Sapir E. Improved sentinel node identification by SPECT/CT in overweight patients with breast cancer. J Nucl Med Off Publ Soc Nucl Med. 2007 Feb;48(2):201–6.

67. Uren RF, Howman-Giles R, Chung DKV, Spillane AJ, Noushi F, Gillett D, et al. SPECT/CT scans allow precise anatomical location of sentinel lymph nodes in breast cancer and redefine lymphatic drainage from the breast to the axilla. Breast Edinb Scotl. 2012 Aug;21(4):480–6.

68. Coffey JP, Hill JC. Breast sentinel node imaging with low-dose SPECT/CT. Nucl Med Commun. 2010 Feb;31(2):107–11.

69. Noushi F, Spillane AJ, Uren RF, Cooper R, Allwright S, Snook KL, et al. High discordance rates between sub-areolar and peri-tumoural breast lymphoscintigraphy. Eur J Surg Oncol EJSO. 2013 Oct;39(10):1053–60.

70. Brouwer OR, Vermeeren L, van der Ploeg IMC, Valdés Olmos RA, Loo CE, Pereira-Bouda LM, et al. Lymphoscintigraphy and SPECT/CT in multicentric and multifocal breast cancer: does each tumour have a separate drainage pattern? Results of a Dutch multicentre study (MULTISENT). Eur J Nucl Med Mol Imaging. 2012 Jul;39(7):1137–43.

71. Tsai JA, Celebioglu F, Lindblad M, Lörinc E, Nilsson M, Olsson A, et al. Hybrid SPECT/CT imaging of sentinel nodes in esophageal cancer: first results. Acta Radiol Stockh Swed 1987. 2013 Mar 18;

72. Ashikaga T, Krag DN, Land SR, Julian TB, Anderson SJ, Brown AM, et al. Morbidity results from the NSABP B-32 trial comparing sentinel lymph node dissection versus axillary dissection. J Surg Oncol. 2010 Jul 14;102(2):111–8.

73. Mansel RE, Fallowfield L, Kissin M, Goyal A, Newcombe RG, Dixon JM, et al. Randomized Multicenter Trial of Sentinel Node Biopsy Versus Standard Axillary Treatment in Operable Breast Cancer: The ALMANAC Trial. JNCI J Natl Cancer Inst. 2006 May 3;98(9):599–609.

74. Krag DN, Anderson SJ, Julian TB, Brown AM, Harlow SP, Costantino JP, et al. Sentinel-lymph-node resection compared with conventional axillary-lymph-node dissection in clinically node-negative patients with breast cancer: overall survival findings from the NSABP B-32 randomised phase 3 trial. Lancet Oncol. 2010 Oct;11(10):927–33.

75. van der Ploeg IMC, Nieweg OE, van Rijk MC, Valdés Olmos RA, Kroon BBR. Axillary recurrence after a tumour-negative sentinel node biopsy in breast cancer patients: A systematic review and meta-analysis of the literature. Eur J Surg Oncol J Eur Soc Surg Oncol Br Assoc Surg Oncol. 2008 Dec;34(12):1277–84.

76. Bergkvist L, Frisell J, Swedish Breast Cancer Group, Swedish Society of Breast Surgeons. Multicentre validation study of sentinel node biopsy for staging in breast cancer. Br J Surg. 2005 Oct;92(10):1221–4.

77. Veronesi U, Paganelli G, Viale G, Luini A, Zurrida S, Galimberti V, et al. A randomized comparison of sentinel-node biopsy with routine axillary dissection in breast cancer. N Engl J Med. 2003 Aug 7;349(6):546–53.

78. Krag D, Weaver D, Ashikaga T, Moffat F, Klimberg VS, Shriver C, et al. The sentinel node in breast cancer--a multicenter validation study. N Engl J Med. 1998 Oct 1;339(14):941–6.

79. Radford DM, Cromack DT, Troop BR, Keller SM, Lopez MJ. Pathology and treatment of impalpable breast lesions. Am J Surg. 1992 Nov;164(5):427–32.

80. Walls J, Boggis CR, Wilson M, Asbury DL, Roberts JV, Bundred NJ, et al. Treatment of the axilla in patients with screen-detected breast cancer. Br J Surg. 1993 Apr;80(4):436–8.

81. Krag DN, Anderson SJ, Julian TB, Brown AM, Harlow SP, Ashikaga T, et al. Technical outcomes of sentinel-lymph-node resection and conventional axillary-lymph-node dissection in patients with clinically node-negative breast cancer: results from the NSABP B-32 randomised phase III trial. Lancet Oncol. 2007 Oct;8(10):881–8.

82. Feldman SM, Krag DN, McNally RK, Moor BB, Weaver DL, Klein P. Limitation in gamma probe localization of the sentinel node in breast cancer patients with large excisional biopsy. J Am Coll Surg. 1999 Mar;188(3):248–54.

83. Celebioglu F, Frisell J, Danielsson R, Bergkvist L. Sentinel node biopsy in non-palpable breast cancer and in patients with a previous diagnostic excision. Eur J Surg Oncol EJSO. 2007 Apr;33(3):276–80.

84. Wong SL, Edwards MJ, Chao C, Tuttle TM, Noyes RD, Carlson DJ, et al. The effect of prior breast biopsy method and concurrent definitive breast procedure on success and accuracy of sentinel lymph node biopsy. Ann Surg Oncol. 2002 Apr;9(3):272–7.

85. Javan H, Gholami H, Assadi M, Pakdel AF, Sadeghi R, Keshtgar M. The accuracy of sentinel node biopsy in breast cancer patients with the history of previous surgical biopsy of the primary lesion: systematic review and meta-analysis of the literature. Eur J Surg Oncol J Eur Soc Surg Oncol Br Assoc Surg Oncol. 2012 Feb;38(2):95–109.

86. Galimberti V, Cole BF, Zurrida S, Viale G, Luini A, Veronesi P, et al. Axillary dissection versus no axillary dissection in patients with sentinel-node micrometastases (IBCSG 23–01): a phase 3 randomised controlled trial. Lancet Oncol. 2013 Apr;14(4):297–305.

87. Giuliano AE, McCall L, Beitsch P, Whitworth PW, Blumencranz P, Leitch AM, et al. Locoregional recurrence after sentinel lymph node dissection with or without axillary dissection in patients with sentinel lymph node metastases: the American College of Surgeons Oncology Group Z0011 randomized trial. Ann Surg. 2010 Sep;252(3):426–32; discussion 432-433.

88. Matsuda S, Takeuchi H, Kawakubo H, Kitagawa Y. Three-field lymph node dissection in esophageal cancer surgery. J Thorac Dis. 2017 Jul;9(S8):S731–40.

89. Lamb PJ, Griffin SM, Burt AD, Lloyd J, Karat D, Hayes N. Sentinel node biopsy to evaluate the metastatic dissemination of oesophageal adenocarcinoma. Br J Surg. 2005 Jan;92(1):60–7.

90. Takeuchi H, Fujii H, Ando N, Ozawa S, Saikawa Y, Suda K, et al. Validation study of radio-guided sentinel lymph node navigation in esophageal cancer. Ann Surg. 2009 May;249(5):757–63.

91. Nagaraja V, Eslick GD, Cox MR. Sentinel lymph node in oesophageal cancer-a systematic review and meta-analysis. J Gastrointest Oncol. 2014 Apr;5(2):127–41.

92. Kuge K, Murakami G, Mizobuchi S, Hata Y, Aikou T, Sasaguri S. Submucosal territory of the direct lymphatic drainage system to the thoracic duct in the human esophagus. J Thorac Cardiovasc Surg. 2003 Jun;125(6):1343–9.

93. Dabbagh Kakhki VR, Bagheri R, Tehranian S, Shojaei P, Gholami H, Sadeghi R, et al. Accuracy of sentinel node biopsy in esophageal carcinoma: a systematic review and meta-analysis of the pertinent literature. Surg Today. 2014 Apr;44(4):607–19.

94. Japan Esophageal Society. Japanese Classification of Esophageal Cancer, 11th Edition: part I. Esophagus. 2017 Jan;14(1):1–36.

95. van den Hoff J, Oehme L, Schramm G, Maus J, Lougovski A, Petr J, et al. The PET-derived tumor-to-blood standard uptake ratio (SUR) is superior to tumor SUV as a surrogate parameter of the metabolic rate of FDG. EJNMMI Res. 2013;3(1):77.

96. van der Ploeg IMC, Olmos RAV, Kroon BBR, Rutgers EJT, Nieweg OE. The hidden sentinel node and SPECT/CT in breast cancer patients. Eur J Nucl Med Mol Imaging. 2009 Jan;36(1):6–11.

97. Mucientes Rasilla J, Farge Balbín L, Cardona Arboniés J, Moreno Elola-Olaso A, Delgado-Bolton R, Izarduy Pereyra L, et al. [SPECT-CT: a new tool for localisation of sentinel lymph nodes in breast cancer patients]. Rev Esp Med Nucl. 2008 Jun;27(3):183–90.

98. Tanis PJ, Valdés Olmos RA, Muller SH, Nieweg OE. Lymphatic mapping in patients with breast carcinoma: reproducibility of lymphoscintigraphic results. Radiology. 2003 Aug;228(2):546–51.

99. Kim HK, Kim S, Park JJ, Jeong JM, Mok YJ, Choi YH. Sentinel Node Identification Using Technetium-99m Neomannosyl Human Serum Albumin in Esophageal Cancer. Ann Thorac Surg. 2011 May;91(5):1517–22.

100. van der Ploeg IMC, Russell NS, Nieweg OE, Oldenburg HSA, Kroon BBR, Olmos RAV, et al. Lymphatic Drainage Patterns in Breast Cancer Patients Who Previously Underwent Mantle Field Radiation. Ann Surg Oncol. 2009 Aug;16(8):2295–9.

101. van der Ploeg IMC, Oldenburg HSA, Rutgers EJT, Baas-Vrancken Peeters M-JTFD, Kroon BBR, Valdés Olmos RA, et al. Lymphatic drainage patterns from the treated breast. Ann Surg Oncol. 2010 Apr;17(4):1069–75.

102. Kuehn T, Bauerfeind I, Fehm T, Fleige B, Hausschild M, Helms G, et al. Sentinel-lymph-node biopsy in patients with breast cancer before and after neoadjuvant chemotherapy (SENTINA): a prospective, multicentre cohort study. Lancet Oncol. 2013 Jun;14(7):609–18.

103. Zetterlund L, Celebioglu F, Axelsson R, de Boniface J, Frisell J. Swedish prospective multicenter trial on the accuracy and clinical relevance of sentinel lymph node biopsy before neoadjuvant systemic therapy in breast cancer. Breast Cancer Res Treat. 2017 May;163(1):93–101.

104. Takeuchi H, Kawakubo H, Nakamura R, Fukuda K, Takahashi T, Wada N, et al. Clinical Significance of Sentinel Node Positivity in Patients with Superficial Esophageal Cancer. World J Surg. 2015 Dec;39(12):2941–7.

105. Cerfolio RJ, Bryant AS, Talati AA, Cerfolio RM, Winokur TS. Change in maximum standardized uptake value on repeat positron emission tomography after chemoradiotherapy in patients with esophageal cancer identifies complete responders. J Thorac Cardiovasc Surg. 2009 Mar;137(3):605–9.

106. Molena D, Sun HH, Badr AS, Mungo B, Sarkaria IS, Adusumilli PS, et al. Clinical tools do not predict pathological complete response in patients with esophageal squamous cell cancer treated with definitive chemoradiotherapy: Retrospective review at one institution. Dis Esophagus. 2014 May;27(4):355–9.

107. Baksh K, Prithviraj G, Kim Y, Hoffe S, Shridhar R, Coppola D, et al. Correlation Between Standardized Uptake Value in Preneoadjuvant and Postneoadjuvant Chemoradiotherapy and Tumor Regression Grade in Patients With Locally Advanced Esophageal Cancer: Am J Clin Oncol. 2015 Dec;1.

108. Arnett ALH, Merrell KW, Macintosh EM, James SE, Nathan MA, Shen KR, et al. Utility of 18F-FDG PET for Predicting Histopathologic Response in Esophageal Carcinoma following Chemoradiation. J Thorac Oncol. 2017 Jan;12(1):121–8.

109. Kauppi JT, Oksala N, Salo JA, Helin H, Karhumäki L, Kemppainen J, et al. Locally advanced esophageal adenocarcinoma: Response to neoadjuvant chemotherapy and survival predicted by $^{[18F]}$ FDG-PET/CT. Acta Oncol. 2012 May;51(5):636–44.

110. Yen T-J, Chung C-S, Wu Y-W, Yen R-F, Cheng M-F, Lee J-M, et al. Comparative study between endoscopic ultrasonography and positron emission tomography-computed tomography in staging patients with esophageal squamous cell carcinoma: Accuracy of preoperative esophageal cancer staging. Dis Esophagus. 2012 Jan;25(1):40–7.

111. Takeuchi M, Takeuchi H, Kawakubo H, Kitagawa Y. Update on the indications and results of sentinel node mapping in upper GI cancer. Clin Exp Metastasis [Internet]. 2018 Aug 22 [cited 2018 Aug 30]; Available from: http://link.springer.com/10.1007/s10585-018-9934-6

112. Künzli HT, van Berge Henegouwen MI, Gisbertz SS, van Esser S, Meijer SL, Bennink RJ, et al. Pilot-study on the feasibility of sentinel node navigation surgery in combination with thoracolaparoscopic lymphadenectomy without esophagectomy in early esophageal adenocarcinoma patients. Dis Esophagus. 2017 Nov 1;30(11):1–8.