Studies on Radionuclide Imaging and Contrast Ultrasound for Sentinel Node Diagnostics in Breast Cancer and Melanoma

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THE FOUR ORIGINAL PAPERS ARE:

1. Nielsen KR, Oturai PS, Friis E, Hesse U, Chakera AH, Callesen T, Nielsen MB, Hesse B. Sentinel node identification in breast cancer patients: Radioactivity at surgery rather than scintigraphy is important. Submitted. (study I)
2. Nielsen KR, Hesse B, Chakera AC, Scolyer R, Stretch J, Nielsen MB, Thompson J, Uren R, Oturai PS. Sentinel Node Imaging in Melanoma Patients: Is Delayed, Planar Scintigraphy Sufficient? Submitted (study II)
3. Nielsen KR, Grossjohann HS, Hansen CP, Nielsen MB. The use of contrast enhanced ultrasound imaging to detect the first draining lymph node in a swine model: Correlation of imaging findings with distance from injection site to the first draining lymph node. Journal of Ultrasound in Medicine, 2008, 27:1203-1209. (study III)
4. Nielsen KR, Charkera AH, Hesse B, Nielsen MB. Sentinel node detection in melanomas using contrast enhanced ultrasound. Acta Radiologica, 2009, 50(4): 412-7. (study IV)

BACKGROUND

The sentinel node

Breast cancer, melanoma and several other solid malignant tumours are known to have a high risk of primary lymphatic spread, initially to the regional lymph nodes and then to more central lymph node basins. Previously, a conventional regional lymphadenectomy was recommended in these cancers for staging and in an attempt to diminish the spread. This procedure is associated with a significant morbidity with complications such as lymphoedema, paraesthesia, reduced mobility and pain [1]. Since the majority of the patients have no regional metastases, conventional regional lymphadenectomy are unnecessary in most patients.

As an alternative and with fewer complications, the sentinel node biopsy (SNB) has been implemented as a standard procedure for patients without known spread of the disease for the regional lymph node staging of breast cancer and melanoma. However, it is generally agreed that if dissemination to regional lymph nodes is found a conventional regional lymphadenectomy should be done in an attempt to eliminate or postpone further spread. A sentinel node (SN) is the first node in the lymphatic system to receive lymphatic drainage directly from a malignant tumour area; therefore it is the first place to look for lymphatic dissemination of the disease. If the surgically removed SNs after histological evaluation are without malignancy, it is widely recommended that further lymph node dissection should not take place [2-9].

In 1992 Morton and his co-workers introduced the SNB technique using dye in melanoma [10], and in 1994 Guiliano et al described this method in breast cancer [11]. Prior to that, Morton et al [12], had used cutaneous lymphoscintigraphy to identify the lymphatic draining pattern in melanoma since 1977 and Lock-Andersen et al since the 1980's. In 1993 Krag and co-workers introduced injection of a radiolabelled colloid for SN detection using preoperative lymfoscintigraphy and a gamma probe in melanoma and breast cancer [13,14].

Today the fundamental principles for SN identification are similar to the technique introduced in the beginning of the 1990's. A combination of two methods is most often used: The radionuclide technique using gamma probe guided detection of SNs with or without preoperative imaging and a blue dye technique.

Standard techniques for sentinel node identification

The radionuclide method

The technique is based on preoperative injection of a radiolabelled tracer close to or directly into the tumour with subsequent gamma camera imaging and gamma probe identification. The tracer is supposed to follow the lymphatic drainage to the regional nodes, where it is caught and retained in the SN(s). However, some of the tracer can pass on to second tier lymph nodes. The most radioactive (hottest) lymph node(s) are visualised preoperatively with a gamma camera, and during surgery they are

found using a handheld gamma probe. Generally they are assumed to represent the SN(s). In Europe the most frequently used tracer is a 99mTc-labelled nanocolloid (Nanocoll, GE Healthcare, Amersham Place, UK), whereas 99mTc-sulpide colloid and 99mTc-antimony trisulphide are preferred in USA and Australia [15]. The size of the colloid for Nanocoll is approximately 5-80 nm [15,16]. Depending on the imaging protocols, the activity injected may vary with the timing of the procedure. The procedure can be a same-day (injection, imaging and surgery on the same day) or a two-day (injection the day before surgery, and imaging either that day or on the day of surgery) protocol. The activity injected and the injection site may also vary according to the type of malignant tumour.
In patients with breast cancer recommendations about injection site include: Subcutaneous periareolar injection, intradermal or subcutaneous injection above the tumour, and peri- or intratumoural injection. Despite many studies and debate in the literature there is still no consensus about which injection site or depth is optimal.
In melanoma the general consensus is to use intradermal injections around the tumour or scar.

Gamma camera imaging of SNs in breast cancer and melanoma
The SNs can be visualised preoperatively with early dynamic imaging and with delayed static imaging. SPECT and/or SPECT/CT of certain regions may be added. In dynamic imaging the radionuclide tracer is followed from the injection site via the lymph channels to the SNs immediately after the injection. The static images of SN(s) in breast cancer and in melanoma are obtained from one to several hours after injection. In breast cancer an anterior and lateral projection is most often used; in melanoma the projection depends on the localisation of the primary tumour. The use of SPECT and/or SPECT/CT is highly variable between centres. In breast cancer there are wide variations, in some centres they use static scintigraphy and SPECT/CT, while others have completely skipped imaging for SNs. In melanoma all institutions include static imaging, and some add dynamic scintigraphy and SPECT or SPECT/CT imaging, maybe in all patients, maybe in certain localizations of the tumour.
The most radioactive extra-tumoural spots are typically marked on the skin as guidance for the surgeon. During surgery the SNs are located using the skin markers, the images, if available, a handheld gamma probe, and most often also visual guidance from the blue dye technique.

The blue dye technique
For the blue dye identification of SNs, vital blue dye e.g. 2.5% Patent blue V (Guerbert, Roissy, France) is injected subcutaneously around the tumour site (or scar) during surgery. This enables direct visualisation of the lymph vessels passing to the SN(s), so that the surgeon can dissect blue stained lymph channels ascending towards the also blue stained SN(s).

Ultrasound and sentinel node
Preoperative identification of SNs has been an important step forward compared to the old days' conventional dissection of all the regional lymph nodes. However, preoperative SN identification still gives no information about the presence of malignant SNs. This would be an important improvement of the preoperative SN diagnostics.

In melanoma and breast cancer patients, the use of high-resolution ultrasound (US) has been introduced for detection of metastases in superficial SN(s). In combination with fine needle aspiration biopsy (FNAB) this can potentially save some patients from the traditional SNB procedure by leading the patients directly to a primary regional node dissection [17]. The US examination is used as a supplement to preoperative gamma camera imaging.
Recently, the use of US contrast agent during US examinations has been suggested for identification of SNs. Contrast enhanced lympho-ultrasonography (CELUS) has been tested successfully for SN detection in different animals, including rabbits, dogs and swine [5,6,8,18-22]. With this technique the US contrast agent is injected either subcutaneously, intradermally or around the tumour and then followed by low mechanical index (MI) US imaging in the lymph channels to the first lymph node(s) visualized – the SN(s). Different contrast agents have been used successfully to detect SN by CELUS in animal studies. The contrast agents most frequently used consist of gas filled microbubbles, including Sonazoid (GE Healthcare, Oslo, Norway), Luminity (Definity) (Bristol-Myers Squibb Medical Imaging, Billerica, MA), and SonoVue (Bracco, Milan, Italy). The microbubbles are lipid coated with a mean diameter of approximately 2-2.5 micrometer [19,23-25], they have a high reflectivity, giving them a hyperechoic appearance on the US images.
An US based technique, which also included SN identification would have the advantages of no ionising radiation and easy access to FNAB. It would be applicable also to tumours with difficult transcutaneous accesses, as the contrast agent could be injected guided by US into or around the tumour. The latter would be potentially valuable if SNB should be used in some abdominal and pelvine tumours. Finally, US can be performed as a bedside examination or in the operation theatre.
The US contrast agent (SonoVue) used in this thesis is a blood pool contrast agent approved for intravenous use. It is well known and used for several examinations including liver and kidney examinations. The contrast agent is not registered for subcutaneous injection.

Histological evaluation of sentinel nodes in breast cancer and melanoma
In breast cancer patients, but not in melanoma patients, the SN(s) removed surgically are usually examined immediately by frozen sections. Next, the SNs removed are examined by a more detailed histological examination usually including immunohistochemistry in both melanoma and breast cancer. The SN technique gives the pathologist the opportunity to focus on one or a few lymph nodes, instead of several lymph nodes following conventional regional lymphadenectomy. This enables a more thorough histological evaluation of more sections in the relevant lymph nodes only [26,27].
Historically, the threshold of "clinically significant malignant dissemination" has decreased to lower and lower levels. Several studies have indicated that presence of micro-metastases has a negative prognostic value [28-31]. It has been indicated that a cut-off at 0.2 mm is too high in melanoma since approximately 10% of the patients still get dis
seminated cancer. Based on prognostics, a cut-off at 0.1 mm has been suggested instead [32]. In breast cancer a recent study demonstrated the same 5-years survival for patients with metastases less than 0.2 mm compared to node-negative patients [33].

However, there is no consensus or prognostic evidence on cut-off level for the lower limit of clinically significant, malignant dissemination, neither for melanoma nor breast cancer [34].

The quantitatively most important cancers, where SN diagnostics are used, are breast cancer and melanoma, but SNB has increasingly been used in other cancers including vulvar cancer, penile cancer and head and neck tumours [16,35,36]. Other malignant diseases are also known to spread mainly by the lymphatic system and could therefore potentially benefit from this method, e.g. cervical and endometrial, gastric, prostate and bladder cancer [37-42].

Even though the SN technique has been implemented as a standard procedure in breast cancer and melanoma patients in most centres worldwide, the details on the procedure varies from centre to centre. Some of the important issues discussed and studied in the present thesis include the necessity of performing preoperative imaging and activity amount that should be injected for reliable SN detection in breast cancer, the complexity of SN imaging in melanoma, and the use of US for SN identification.

STUDY AIMS

1. To evaluate the possible relationship between the number of SNs detected and frequency of patients identified with malignant SNs in relation to
A) the activity remaining in the patient at the time of surgery,
B) performing or not performing preoperative gamma camera images before axillary SNB in breast cancer patients.
2. To compare the results of the interpretation of only a delayed static scintigraphy for SN visualization with the combination of early dynamic and delayed static and, most often, also SPECT/CT and ultrasound imaging.
3. To investigate whether CELUS could be used for SN detection
A) in swine

B) in melanoma patients
Additionally, to determine whether the distance from the injection site of the contrast agent to the SN affects the ability of the contrast agent to reach the SN, and thereby the sonographic detection of contrast enhanced lymph nodes (table 1).

Table 1

Overview of purposes, methods and materials of the studies.
Abbreviations: BC: breast cancer; CELUS: contrast enhanced lympho-ultrasonography; CT: computed tomography; MM: melanoma; ptts: patients; s.c: subcutaneously; SNs: sentinel nodes; SPECT: single photon emission tomography; US: ultrasound

Study no.	Clinical questions	Material	Procedures performed	Gold standard
I	SN diagnostics in BC : 1) Is imaging necessary? 2) Influence of radioacitivity amount	858 ptts with BC	Static scintigraphy, gamma probe, blue dye, Histology	SNs removed, histology ↓
II	SN diagnostics in MM: Is dynamic imaging important?	307 ptts with MM	Dynamic, static scintigraphy, SPECT/CT, US, blue dye, histology	SNs removed, histology ↔
III	Can SN be imaged by CELUS in a swine model?	13 pigs	CELUS, blue dye	Blue dye
IV	Acute toxicity in mice by US contrast agent, injected s.c.?	10 mice	Injection of SonoVue, injection of saline	Histology
	Can SN be imaged by CELUS in human subjects?	10 ptts with MM and 1 healthy volunteer	CELUS, static scintigraphy, gamma probe, blue dye, histology	Static scintigraphy, gamma probe

STUDY I: SENTINEL NODE IDENTIFICATION IN BREAST CANCER PATIENTS: RADIOACTIVITY AT SURGERY RATHER THAN SCINTIGRAPHY IS IMPORTANT (SUBMITTED)

Aim
To evaluate in BC patients referred to SNB the possible relationship between the number of SNs detected and frequency of patients identified with a malignant SN in relation to: 1) the activity in the patient at surgery (Actrem), and 2) presence or absence of scintigraphy.

Material and methods
We analysed data from 882 consecutively enrolled and prospectively registered breast cancer patients referred to SNB over a three-year period. Twenty-four patients were excluded because of missing demographic information, resulting in a final population of 858 patients. The inclusion criteria were women with unilateral BC, a tumour diameter ≤5 cm, referred to SNB.
During the first two years the standard SN procedure included preoperative gamma camera imaging, and intraoperative use of blue dye and handheld gamma probe, with occasional omission of imaging for logistic reasons. During the third year scintigraphy was no longer performed, supported by the opinion among surgeons that the images were not necessary. All patients were preoperatively injected subcutaneously around the areola with 99mTc-labelled nanocolloid (Nanocoll, GE Healthcare, Amersham Place, UK). In case of same-day procedures 50 (±10%) MBq were injected and in 2-day procedures 110 (±10%) MBq. We calculated the activity remaining in the patient at the time of surgery (Actrem) as the activity injected corrected for physical decay of 99mTc according to the time interval from injection to surgery and assuming no biological elimination of the radiotracer.
The 858 patients included were divided into three groups according to the Actrem and furthermore all patients were divided in two groups regarding whether imaging were performed or not - as shown in table 2

Table 2

Patients were divided into 3 groups according to the activity calculated to remain in the patient at time of surgery, and into 2 groups according to the presence or absence of scintigraphy.

Patient groups	Number of patients (%)	Definition of group
Gr<10	479 (56%)	< 10 MBq in the patient at surgery
Gr10-20	201 (23%)	10-20 MBq in the patient at surgery
Gr>20	178 (21%)	> 20 MBq in the patient at surgery
Total	858 (100%)	
Gr-imaging	419 (49%)	Patients undergoing scintigraphy
Gr-no-imaging	439 (51%)	Patients not undergoing scintigraphy
Total	858 (100%)	

Image acquisition (only in Grimaging): Anterior and lateral images were obtained using a gamma camera and the hottest extra-tumoral spots were marked on the skin.
Surgery: In all patients the blue dye technique was performed according to standard technique. The SNs were also located using a handheld gamma probe, with or without support of the scintigraphic images. All hot and/or blue lymph nodes and also enlarged nodes or nodes with other signs suggesting malignancy were considered SNs and removed surgically. Focal activity accumulations with less than 10% of the most radioactive SN were considered as second tier nodes.
A conventional axillary lymphadenectomy was performed during the primary operation if 1) no SN could be located, 2) 6 or more SN were removed, 3) in case of a "hot axilla" (all LN and surrounding tissue appearing hot), or 4) if metastasis was detected in the frozen section. If the frozen section was without metastasis and the later histological examination of the paraffin sections showed metastasis, a conventional axillary lymphadenectomy was performed two weeks after primary surgery.
Pathology: All SNs removed were examined first at a frozen section stained with haematoxylin-eosin (HE) and subsequently fixed in formaldehyde, paraffin embedded and examined by histological analysis with HE staining followed by immunohistochemical analysis. The pathology procedure was unchanged in the study period.

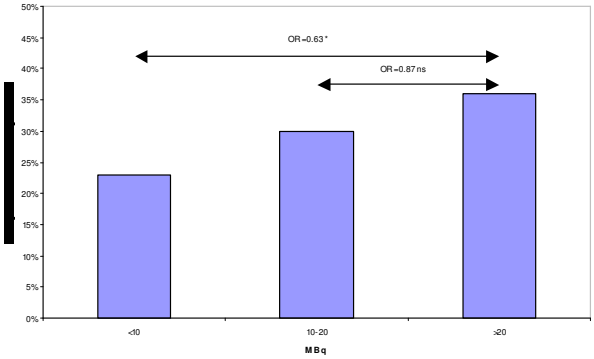

Figure 1
Percentage of patients identified with malignant SNs (y-axis) in relation to different activities at the time of surgery (x-axis). Patients with malignant SN, n= 233. OR = odds ratio. * =Significant (95% confidence limits 0.42-0.93), ns = statistically non-significant (95% confidence limits 0.55-1.36)

Results

The analysis of demographic data between the patient groups with vs. without imaging (Grimaging vs, Grno-imaging) and among the three Actrem patient groups (< 10, 10-20, >20 MBq) revealed no significant differences except for two differences: (1) the time of entry into the study, (2) time interval from injection to surgery (due to enrolment of the patients in either same-day or a two-day protocol). All 680 patients enrolled in a two-day procedure had an Actrem less than 20 MBq with 2/3 <10 MBq whereas the 178 patients undergoing a same-day protocol all had >20 MBq.

Table 3a+b

The influence of: 1) the amount of activity at time of surgery (Actrem) and scintigraphy on an increasing number of sentinel nodes removed at surgery (B1) and 2) the Actrem, scintigraphy and the number of sentinel nodes removed at surgery on the number of patients with malignant sentinel nodes (B2). P-values calculated according to multiple logistic regression analysis. OR - odds ratio, CI – 95% confidents interval, * Significant, NS – non significant

Table 3a: Relation to increasing number of SNs per patient				
↓		OR	(CI)	p-value
Amount of activity at surgery	< 10 MBq	0.474	(0.323-0.698)	0.0003*
	10-20 MBq	0.541	(0.369-0.793)	
	>20 MBq	1		
Scintigraphy	Yes	1		
	No	0.616	(0.447-0.850)	0.0031*
Table 3b: Relation to number of patients with at least one malignant SN				
		OR	(CI)	p-value
Number of SNs removed	1 SN removed	1		0.0025*
	2 SN removed	1.532	(1.052-2.230)	
	3 or more removed	1.981	(1.317-2.981)	
Amount of activity at surgery	< 10 MBq	0.627	(0.422-0.931)	0.0344*
	10-20 MBq	0.865	(0.550-1.360)	
	>20 MBq	1		
Scintigraphy				0.4799 NS

Table 4

Comparison of numbers of "true SN" and "clinical SN"(true SN and possible SN pooled), respectively, described by RH and SMU. Statistical significance was calculated with Fisher's exact test (*) and by analysis of variance (**). CI: 95% confidence interval; NS: not significant difference.

		SMU	RH	p*	Difference SMU/RH (95% CI)
Total number of patient studies		307			
No of patients with at least 1 SN detected		307 (100%)	306 (99.7%)	NS*	
Mean no. of SNs/patient (95% CI)	True	2.31 (2.18-2.44)	2.26 (2.12-2.39)	NS**	0.05 (–0.16 - 0.05)
	Clinical	2.59 (2.43-2.74)	2.71 (2.56-2.86)	<0.05**	0.12 (0.02 - 0.23)
No. of patients with a malignant SN described		38 (17%)	35 (16%)	NS*	

In 97% of all patients at least one SN was removed and 27% (n=233) of the patients had a malignant SN identifyed. Although the numbers of SNs removed per patient did not differ significantly between Grimaging and Grno-imaging, the multiple logistic regression analysis showed that imaging had a significant influence on the number of SNs removed (Table 3a) due to the influence of the activity, cf. below.
In a multiple logistic regression analysis the number of SN(s) removed at surgery and Actrem both had significant influences on the number of patients with malignant SN detected (Table 3b): the probability of finding at least one malignant SN in a patient increased significantly both with the number of SN(s) removed at surgery and with higher Actrem. (Fig 1, below)
Imaging had no significant influence on the probability of identifying malignant SN (Table 3b). It can be argued, however, that Actrem having stronger influence than imaging in the multiple logistic regression analysis, a possible influence of imaging may be concealed by the higher Actrem in patients with preoperative scintigraphy compared to those not undergoing imaging.

In 3% of the patients no SN was found. The most important factor related to non-detection appeared to be low Actrem. Twenty of the 25 patients with non-detection had very low activity (<10 MBq) at the time of surgery. All 25 patients underwent a conventional axillary lymphadenectomy, in 28% of them malignancy was observed in SNs, equally shared between Grimaging and Grno-imaging patients.

In conclusion, sufficient activity remaining at the time of surgery appears to be crucial for correct identification of SNs. The activity level in the patient at surgery should at least exceed 10 MBq. The influence of preoperative imaging is less evident from this study, it appears that imaging did not have a great impact on SN findings, but a minor influence cannot be excluded.

STUDY II: SENTINEL NODE IMAGING IN MELANOMA PATIENTS: IS DELAYED, PLANAR SCINTIGRAPHY SUFFICIENT? (SUBMITTED)

Aim

To compare the results of the interpretation of only a delayed static scintigraphy for SN visualization with the combination of early dynamic, delayed static and, most often, also SPECT/CT and ultrasound imaging.

Material and Method

Patients: Three hundred and seven Australian patients were consecutively enrolled in a prospective way. All had histologically verified melanoma. The study is a joined project between Sydney Melanoma Unit (SMU), Australia and Copenhagen University Hospital - Rigshospitalet (RH), Denmark.
Preoperative imaging: Prior to surgery all patients had lymphoscintigraphy including early dynamic and delayed static imaging. Most patients, 98%, also had SPECT/CT of the SN field. The location of the SNs was marked on the skin using a single headed gamma camera. In 97% of the patients a targeted ultrasound examination of the SN and its node field were also performed and a FNAB of the SN(s) was performed when malignancy in the SN was suspected ultra

sonically. All these data were used by the SMU nuclear medicine physician to generate his report.
Surgery: During surgery, the surgeon used: 1) The description of the scintigraphy (dynamic+static) and the images, 2) a handheld gamma probe, and 3) the blue dye method to locate SNs. All the nodes described in the scintigraphy report as SNs and possibly additional blue stained lymph nodes were considered SNs and were removed. Also lymph nodes with a malignant appearance observed during surgery, were removed. Furthermore, lymph nodes preoperatively described on the scintigraphy as "possible SNs" or "possibly second tier nodes" but still marked on the skin, were examined using the probe to determine whether they because of focal, high count rate should be considered SNs.
Pathologists in Sydney examined all removed SNs histologically by HE-staining and by immuno-histochemical analysis.

Postoperative imaging analysis: A secondary description of a computer presentation of only the delayed static images was made as consensus readings by two readers at Rigshospitalet (RH). These readers were blinded for the SMU description, surgical data and pathology. The number and locations of SN(s) were recorded and all SN(s) were categorised as: "true SNs"(including interval nodes) or "possible SNs".

SMU-RH comparison of readings: The number and regional locations of true SNs and possible SNs from the RH description were compared with the SMU description. The category "true SN(s)" were both analysed separately and pooled with "possible SN" giving the category: "clinical SN(s)". Images from patients with a malignant SN were compared twice to

Table 5

Overview of the results from inter/intra observer and consensus examinations in 50 randomly selected patients. Analysis of variance (one-way ANOVA) and Weighted Kappa was used. RH – Rigshospitalet, A and B – referrers to the two interpreters at Rigshospitalet. * significant, level of significance < 0.05.

Consensus: RH 1th reading><RH 2nd reading		**RH1**	**RH2**	**P**	**Weighted kappa**
No. of SN/patient, mean (conf.interval)	True SN	2.4 (2.1-2.7)	2.1(1.8-2.4)	0.032*	0.77
	Clinical SN	2.9 (2.5-3.3)	2.7(2.3-3.1)	0.020*	0.86
Intra observer: B1th reading><B 2nd reading		**B1**	**B2**		
No. of SN/patient, mean (conf.interval)	True SN	2.3 (1.97-2.55)	2.2 (1.91-2.49)	0.083	0.97
	Clinical SN	2.8 (2.42-3.14)	2.7(2.34-3.06)	0.290	0.92
Inter observer: A><B2nd reading		**A**	**B2**		
No. of SN/patient, mean (conf.interval)	True SN	2.1 (1.81-2.35)	2.2 (1.93-2.47)	0.180	0.78
	Clinical SN	2.5(2.19-2.89)	2.7 (2.35-3.05)	0.130	0.87

determine whether RH described the identical localisation of the malignant SN.

Results

Patients: The 307 patients were divided in a subgroup of 220 (72%) patients in whom surgical and pathological information of performed SNB was obtained. In the remaining 87(28%) patients, data of SNB was not available as different surgeons and pathologists located throughout the whole Sydney region were used, which gave some logistic complications when collecting the descriptions. These dropouts of patients were random.
All 307 patients had at least one SN identified by SMU and RH, except for one patient in whom RH did not identify any SN from the delayed image. Thirty-eight (17%) of the 220 patients with SNB had at least one malignant SN removed.

SNs identified by preoperative imaging: RH described slightly, but significantly more "clinical SN(s)" per patient compared to SMU, as SMU described 2.59 and RH 2.71 SN(s) per patient giving a difference of 0.12 (confidence interval 95%, 0.02-0.23). There were no significant differences between the mean numbers of "true SN" (Table 4).
A kappa analysis of the interpretations between the RH and SMU readers gave values of 0.55 and 0.56 for the number of "true SN" and "clinical SN", respectively. The SMU and RH descriptions were identical regarding the numbers of "true SN" and "clinical SN" in 58% and 52% of the patients, respectively. In 3% of "true SN" and in 2% of "clinical SN" differences up to 3-4 SNs were observed between the two readers (Table 4).
Identified and removed malignant SNs: Of the 38 patients with malignant SN, RH identified at least one malignant SN in 35 (96%) of those patients. In one of the remaining three patients it was not possible from the surgical and pathological report to determine if the malignant SN was one of the SN described by RH. In the two other patients the malignant SN were not identified by RH. The risk of over-looking a malignant SN by only delayed static imaging was maximally 1.4% (3/220) compared to the complex of dynamic-static (-tomographic-CT-ultrasound) imaging.

These results are based on an evaluation of the numbers of SNs identified and do not schow whether the SNs identified by SMU and RH were identical since this evaluation were not possible in all patients. However, in the 38 patients with a malignant SN this comparison was possible except in one patient.
Lymph node regions: In 97% of the patients the identified SN regions were identical between the two readers, but in 3% of the patients the regions marked by SMU and RH differed in such a way that it would have a clinical importance.
SPECT/CT and ultrasound: From the SPESC/CT images no additional SN(s) were located but obviously a more precise location of the SN(s) could be registered using this modality. Ultrasound was performed in 97% of all patients. In 11 of the 220 patients a FNAB were performed on the suspicion of malignancy based on the ultrasound examination. In eight of these patients the SN were malignant.

Conclusion: The risk of overlooking a melanoma patients with a malignant SN was around 1% when using delayed static imaging alone compared to using both dynamic and static images and mostly also in combination with

SPECT/CT and ultrasound imaging. The mean number of SNs identified was significantly higher when only delayed imaging was performed, with some discrepancies in the individually patients. However, this difference may not have

a great clinical impact, as only 0.12 SN more per patient were identified.

Inter-/intra-observer and consensus evaluation

These data are not included in appendix form since these evaluations are performed parallel to study II on 50 randomly selected patients from study II. For a consensus evaluation of the interpretations by RH, the delayed static images of these 50 patients were re-evaluated by RH in regards to the numbers of "true SNs" and "clinical SNs". Two month later the two interpreters from Rigshospitalet (A and B) performed individual interpretations of the 50 images. These descriptions were used in an inter observer examination between A and B. Finally, for the intra observer evaluation of interpreter B, an additional second reading was performed by B.

The results are given in Table 5. In a comparison of the two interpretations by Rigshospitalet, there was a significant difference in the number of "true SNs" and "clinical SNs" of 0.24 SNs in both categories. This disagreement could most likely be related to a learning curve for interpreting the static images. There were no significant differences in the intra- and inter observer evaluation.

STUDY III: THE USE OF CONTRAST ENHANCED ULTRASOUND IMAGING TO DETECT THE FIRST DRAINING LYMPH NODE IN A SWINE MODEL: CORRELATION OF IMAGING FINDINGS WITH DISTANCE FROM INJECTION SITE TO THE FIRST DRAINING LYMPH NODE.

Nielsen KR, Grossjohann HS, Hansen CP, Nielsen MB. The use of contrast enhanced ultrasound imaging to detect the first draining lymph node in a swine model: Correlation of imaging findings with distance from injection site to the first draining lymph node. Journal of Ultrasound in Medicine, 2008, 27:1203-1209.

Aim

To evaluate the use of contrast enhanced lympho-ultrasonography (CELUS) for sentinel node imaging in a swine model and to determine whether the distance from the contrast injection site to the SN is important.

Material and Method

Testing hypothesis number III.

Thirteen healthy anaesthesized swine, with a weight between 25-32 kg, were examinated in this study.

For the examinations a GE LOGIQ 9 ultrasound scanner and a 7 MHz linear transducer working at the transmission frequency of 7MHz (Type 7L 2-7 MHz, GE Healthcare, Chalfont St. Giles, UK) were used. CELUS was performed using the manufacturer's preset for contrast imaging and a low mechanical index (MI) of 0.13.

The inguinal lymph nodes in the groin of the swine were located and studied on both sides before contrast injection and images were stored. In every swine, 1 ml of ultrasound contrast agent, Sonovue (Bracco, Milan, Italy) was bilaterally injected subcutaneously below a mamilla. The distances between the injection site and the first draining lymph nodes varied from 6-36 cm. Two examinations were performed on each swine resulting in 26 examinations. The first draining lymph node represents the SN, which is used in the following. Sonovue, is registered as a blood pool contrast agent and consists of the inactive gas, sulphur hexafluoride in phospholipidic coated micro-bubbles. The mean size of the bubbles is approximately 2.5 microns (1-10 micron).

To accelerate the uptake into the lymph channels, the injection site was gently massaged for 2 minutes after the injection of the contrast agent. Contrast enhanced lymphatic channels were visualized and followed to contrast enhanced SNs using low MI CELUS. To ensure that hyperechoic areas within the lymph nodes represented uptake of contrast agent and not background structures e.g. the hilum, the area was scanned with a high MI to destroy the contrast agent. The lymph node was then re-studied to see if the contrast enhancement of the lymph node reappeared

In case no lymphatic channels were visualized, the area of the lymph nodes was examined using ultrasound imaging and if no contrast enhancement was seen, the lymph node was examined every 5 minutes up to 30 minutes until contrast enhancement was detected. Images were saved continuously during the examination.

After the CELUS examination, blue dye (Blue Patenté V; Guerbet, Roissy, France) was injected at the same locations as SonoVue and dye-guided surgery was performed for localisation the SNs. To confirm that the lymph node detected with the blue dye technique was the same found with CELUS, the lymph node was re-

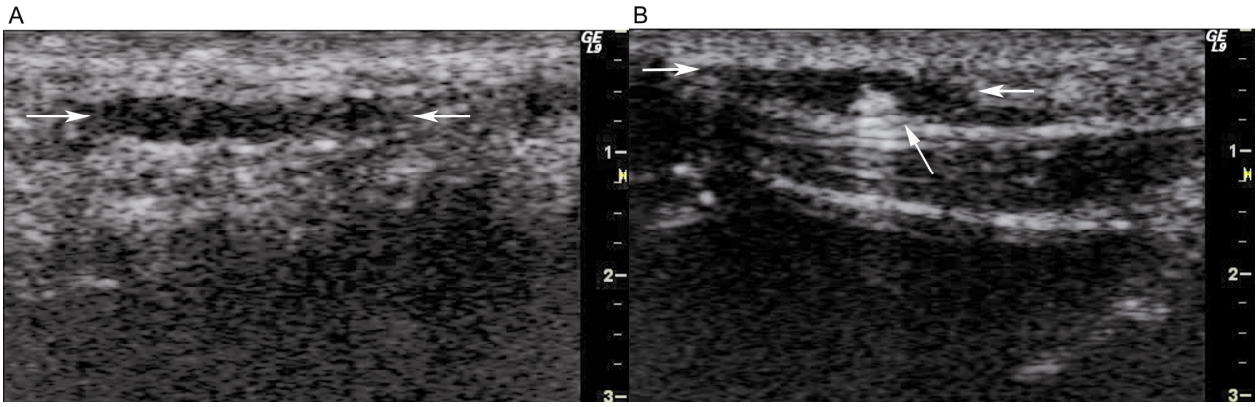

Figure 2A+2B

Confirmation of contrast agent in a SN. One inguinal lymph node illustrated in two sonograms, A (before injection of contrast agent) and B (after injection of contrast agent). The horizontal arrows indicate the lymph node before and after the injection of the contrast agent and the oblique arrow indicate the hyperechoic area, representing contrast agent in the lymph node. The contrast agent only fills a smaller part of the lymph node.

Table 6

Results from detection of contrast enhanced and blue dyed sentinel nodes after bilateral subcutaneous injection of 1 ml Sonovue and ½ ml blue dye in 13 swine

* Lymphatic drainage only towards the lower neck, SNs was not found.

Distances (cm)	**6**		**12**				**18**				**24**								**30**						**36**	
Swine no.	1		2-3				4-5				6-9								10-12						13	
No. of examina-tions	2		4				4				8								6						2	
SN detection by CELUS (+/-)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	- *	- *	- *	- *
SN detection by blue dye (+/-)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	- *	- *	- *	- *

scanned to confirm contrast

enhancement.

Results

Inguinal SNs were detected in 22 of the 26 examinations using both CELUS and blue dye-guided surgery (Fig.2).
These SNs were located at varying distances from the injection sites ranging from 6 cm to 30 cm. Two of the 22 SNs, both in the same swine, demonstrated less contrast en
hancement than the ones seen in the rest of the examinations, however, all SNs were equally filled with dye.
In four of the 26 examinations SNs were not found, neither with CELUS. In these cases (in two swine) the distances between the injection site and the groin were 30 and 36 cm, however, the direction of drainage were only towards the area of the neck. In both animals it was possible to detect the contrast enhanced lymphatic channels by ultrasound extending on both sides towards the neck, but no SN in this area were identified, possibly due to intra-thoracic drainage. Likewise, blue dye was visible in the lymph channels draining towards the neck. Though, dissection for SNs was not performed, as other examinations were carried out in this area complicating the procedure. (Table 6)
The time span for the contrast agent to reach the inguinal SN was less than 5 minutes in all swine except for one with a distance of 30 cm, where the contrast enhancement was not detected until 20 minutes after the injection.

Conclusion: Our results show that it is possible to visualise the SN using CELUS in animals. Furthermore, it indicates that, distances up to 30 cm do not interfere with the ability of the contrast agent to reach the SN, and does not thereby impair the sonographic detection of contrast enhanced SNs.

STUDY IV: SENTINEL NODE DETECTION IN MELANOMAS USING CONTRAST ENHANCED ULTRASOUND.

Nielsen KR, Charkera AH, Hesse B, Nielsen MB. Sentinel node detection in melanomas using contrast enhanced ultrasound. Acta Radiologica, 2009, 50(4): 412-7.

Aim

To investigate the possible use of CELUS to detect SNs in patients.

Material and Method

Mouse study: Since no ultrasound contrast agent is yet approved for subcutaneous administration, a murine study was performed in order to examine the safety of subcutaneous injection of the contrast agent. In ten naked mice 0.1 ml of SonoVue (Bracco, Milan, Italy) was injected subcutaneously on the right side of the flank and, as placebo 0.1 ml isotonic saline was injected subcutaneously on the left side. The injection sites were continuously observed macroscopi

cally and scored for inflammation and necrosis. Eight days after injection the mice were euthanized and the areas
around the injection sites were removed and examined microscopically for signs of inflammation or necrosis. The pathologist was blinded regarding the application of contrast agent or saline.
Patient study: After having excluded any tissue damage from subcutaneous injection of SonoVue the study proceeded in patients. The procedure used in this clinical study was similar to the method used with success in our study in swine.
Ten patients with melanoma on an upper or lower extremity, referred to SN biopsy, were consecutively enrolled in the study.
As US equipment a GE LOGIQ 9 US scanner and a 7 MHz linear transducer working at the transmission frequency of 7MHz (Type 7L 2-7 MHz, GE Healthcare, Chalfont St. Giles, UK) was used.
CELUS was performed using the manufacturer's preset for contrast imaging and a low mechanical index (MI) of 0.13, these settings were not changed during the examinations.
Before injection of the contrast agent the relevant lymph node region was scanned by conventional US, and all visualized lymph nodes were marked on the skin and ultrasonically evaluated regarding possible malignancy. One ml of SonoVue was injected on both sides of the scar from the removed melanoma and the area was gently massaged for two minutes. In eight patients the injected concentration of the contrast agent was 8 µl/ml. In two patients 16 µl/ml was used to study if a higher concentration could improve the contrast enhancement of the lymph nodes. We tried to visualise contrast enhanced lymph channels draining from the injection site towards the regional lymph node basin and contrast enhanced lymph nodes by CELUS examination and stimulated acoustic emission. In case no contrast enhanced lymph nodes were visible the area was examined every 10 minutes for contrast enhancement of lymph nodes. In order to facilitate the lymphatic uptake of the contrast agent different modifications of the procedure were tried in the study: Some patients were asked to sit or walk in between injection and examination, others to remain lying supine on the couch during the examination and some were asked to elevate the examined leg. Also, as mention above the concentration of the contrast agent was doubled in two patients.
After the ultrasound examination, preoperative gamma camera imaging was performed and the patient went to surgery. During surgery the SNs were located using the scintigraphic findings, a gamma probe and blue dye visualization of lymph nodes. All removed SNs underwent histological examination. The nuclear medicine physician interpreting the scintigraphy, the surgeon, and the pathologist were blinded to the results from the US examination.
In addition to the ten melanoma patients, one healthy volunteer was examined only with CELUS. An intradermal injection of

SonoVue, with a concentration of 8 µl/ml, was given on both thighs to compare this route of administration with the experience from subcutaneous injections. The US examination was otherwise identical with that used in the ten patients.

Results

Mouse study: None of the ten mice examined had macro- or microscopic inflammation or necrosis at the injection site of the contrast agent or saline. Neither did they lose weight as a sign of systemic toxicity.

Patient study: In the ten patients, four melanomas were located on the upper extremity and six on the lower extremity. The observations from the CELUS examinations were compared with the scintigraphic and surgical findings. An average of 1.8 SNs (1 - 3) were visualized by scintigraphy, 2.2 SNs (1 – 4) were removed during surgery, and 3.0 LNs (1-5) were observed and subsequently marked on the skin by US imaging. All lymph nodes were located in the inguinal region or in the axilla.

In nine of the ten patients and in the healthy volunteer no contrast enhanced lymph nodes or lymph channels could be visualized using CELUS. In one patient, two nodes were visualized by CELUS and power Doppler were used to create a stimulated acoustic emission representing the contrast bubbles bursting in the contrast enhanced lymph nodes (appears as a colour-flash on the screen) (Fig.3).

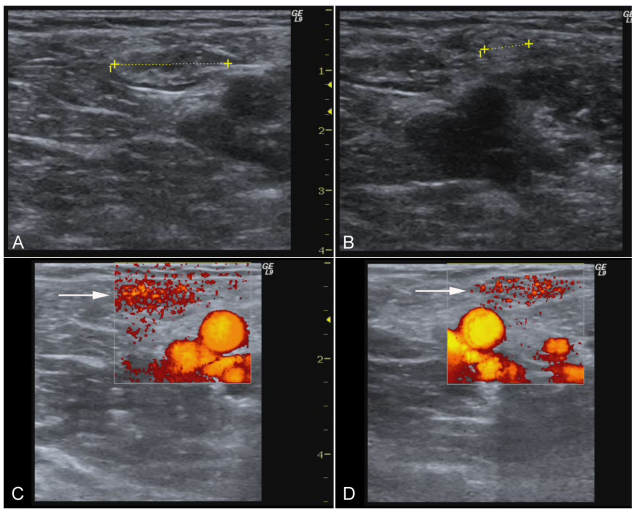

Figure 3

A+B: Sonogram of two inguinal lymph nodes, marked on the scin before the contrast injection.

C+D:Sonogram of the same two lymph nodes shown in A and B after injection of contrast agent. US imaging using power Doppler, gives a bubble-bursting-flash (stimulated acoustic emission) representing areas containing contrast agent. In this case the sentinel node (marked with arrows) is visualized. Normal flow signal appears in the femoral vessels.

Both lymph nodes were located in the inguinal region and had a normal appearance at the ultrasound imaging. During preoperative gamma camera imaging two SNs were visualized in the same lymph node region, and two additional more proximally located lymph nodes were visualized in the pelvis. The two inguinal SNs were removed during surgery without any sign of malignancy. The location of the two lymph nodes visualized by CELUS closely corresponded to the location of the two inguinal SNs detected by scintigraphic imaging. The pelvic region was not examined by ultrasound imaging.

In conclusion despite the successful application in animals in study III using the same technique and contrast agent, visualization of SNs in patients was unsuccessful in this first human study using a microbubble contrast agent and CELUS. However, the application of CELUS on SN is still not fully explored in humans, and an alternative set up and / or contrast agent might provide better results.

DISCUSSION

New findings summarised

In this thesis we investigated the need for modification of the radionuclide methods used in breast cancer and melanoma, and whether it is possible to use CELUS for SN detection.

The impact on axillary SNB in breast cancer of the activity remaining in the patient at the time of surgery and of preoperative imaging was evaluated in respect to number of SNs identified and number of patients with malignant SNs. The Actrem had significant influence on the both parameters, indicating that an Actrem above at least 10 MBq is critical for an optimal SN detection. Regarding the influence of preoperative imaging on axillary SN identification, the results were less evident. A minor influence could not be excluded.

The impact of performing dynamic and static scintigraphy compared to static imaging alone was evaluated in melanoma in respect to the same two parameters. It showed a minor benefit of also using dynamic scintigraphy. A small risk of overlooking malignant SNs was present, and there was a tendency towards identifying more SN when only delayed imaging was performed.

In the investigation of the possibility of using an ultrasound microbubble contrast agent and CELUS for SN identification, we found that the method, as shown previously, worked well in an animal model, but could unfortunately not be translated in patients. It had not been investigated before.

General considerations

Some cancers like melanoma and breast cancer predominantly disseminate via the lymphatic system, the first metastases go to regional lymph nodes. To exclude or remove local metastases, surgery therefore previously included primary regional lymphadenectomy, both for staging and in an attempt to eliminate further malignant dissemination. But during this procedure all lymph nodes were removed also in the majority of the patients, who had no malignant spread. The conventional regional lymph node dissection is accompanied by a significant risk of postoperative complications, which will be further be aggravated by radiotherapy. On the basis of this risk the SN procedure was introduced and being virtually without complications rapidly widely accepted.

When the SNB technique was introduced, it was examined if the same results were obtained as with conventional lymphadenectomy [11,43-45]. Since then the technique has undergone several modifications. Today the technique is accepted as state-of-the-art and hence rarely compared with conventional regional lym-

phadenectomy. This is now only performed in patients if no SN can be identified or in case of malignant SNs are identified.
But how do we know that the lymph nodes identified by the SNB procedure then represent the true SNs and only true SNs? If some true SNs are missed, the risk of overlooking cancer dissemination is present, with increasing risk of morbidity and mortality related to the cancer disease. Though, in order not to miss the advantage of the SNB procedure, it is also important not to identify and remove (too many) second tier nodes as SNs. The procedure has a more focused strategy permitting significantly better histological examination of one or a few lymph nodes compared to the old days with 10-15 lymph nodes, and a smaller risk of mobidity.
In many centres treating patients with breast cancer and melanoma the SNB procedure has worked well. Since the drainage from the breast is often confined to the axillary region, some centres have even skipped performance of preoperative imaging in breast cancer relying on intraoperative probe detection and blue dye visualization. Melanomas on the other hand have a rather unpredictable lymphatic draining to one or even several lymph node basins. Therefore, a preoperative scintigraphy must be performed in order to localise which lymph node basins are involved.
The preoperative imaging protocol for SNs procedures vary from centre to centre, from no imaging done in breast cancer, and only static imaging in melanomas, to a combination of several imaging techniques including early dynamic and delayed static imaging, SPECT, SPECT/CT, and US with FNAB in cases of a suspected malignant SN. The more complex SN imaging used in the pursuit of identifying the true SNs, the more precise SN identification is thought to be achieved. But is it also clinically relevant and important? The increasing use of different imaging modalities results not only in higher costs, but also in more complicated logistics, more radiation exposure and a higher risk of complications. When deciding on which SN procedure to use, these all have to be considered in relation to the output.
Adverse effects from the radio-pharmaceuticals used for SN imaging are fortunately mild and extremely rare, including very few allergic reactions [15]. Patent blue V and isosulfan blue (an isomer of patent blue V) are associated with some risk of allergic reactions, but anaphylactic reactions are still rare [46,47].
When using radioactive colloids for SN biopsy, radiation safety issues must be considered for the patient, and for the staff in nuclear medicine departments, in the operating room, and in the pathology laboratories. The effective patient dose from the SN procedure in breast cancer and melanoma patients is small compared with other examinations using ionizing radiation. The skin has a low tissue weighting factor of 0.01 and thereby contributes little to the effective dose in melanoma patients, the breast being somewhat higher (0.12). Still, the effective dose for a breast procedure is small (0.0026 mSv/Mbq [48]), in melanoma generally much lower but dependant on the activity injected and the body region of the tumour. The effective dose has been reported to be 0.0019 mSv/MBq in a 'worst-case' calculation in melanomas [15,49]. The dose received by staff members involved in SNB is very small, also for the surgeon. Therefore, it is in general decided that there is no need for the use of dosimetry. Dependant on the body region and kind of CT performed, additional SPECT/CT imaging in melanoma will increase the effective dose of the patient.
The use of ultrasound in the SN detection could be an advantage, of not using ionising radiation.

THE RADIONUCLIDE TECHNIQUE

Sentinel node detection in breast cancer

The influence of the remaining activity at the time of surgery on axillary SN detection
Recommendations on the activity injected in relation to SNB in breast cancer patients are limited and in published studies the activity injected has varied markedly. Also in relation to the expected time interval to surgery variations are seen.
Our study strongly suggests that a level of radioactivity at the time of surgery less than 10 MBq is not sufficient for an optimal SN identification: SNs may not be found during surgery, which may lead to overlooking malignant SNs in a few patients. By analysis with a multiple logistic regression model using backward selection we demonstrated that the risk of detecting a malignant SN in a patient increased significantly with higher Actrem. We also observed that 20 out of 25 patients without a SN identified had an activity level in her body at the operation calculated to be less than 10 MBq. The surgical procedure in these 25 patients changes from SNB to a conventional axillary lymph node dissection. This was unnecessary in 15 patients, as they had no dissemination to the SNs.
The activity of 99mTc-labelled nanocolloid used in our patients was not small compared with several reports in the literature [50-54]. We concluded that the activity injected around 110 MBq used in our routine procedure for a two-day protocol may be too low if the time interval from injection to surgery exceeds three half lifes of technetium. This agrees with previous findings in our group both in breast cancer and in melanoma [55,56].
According to literature activities injected vary a lot for SN diagnostics in breast cancer. From 7.4 to 120 MBq for same-day procedures and 37 to 370 MBq for two-day procedures [50-54]. European guidelines for SN diagnostics in BC patients from 2007 [48] recommend an injection of 5-20 MBq depending on the expected time to surgery. Guidelines for SN in other diseases as e.g. for oropharyngeal cancers and malignant melanomas [15,16] give limited information about injected activity. Yet, in accordance with the present data both guidelines recommend that at least 10 MBq should be present in the patient at the time of surgery. Hardly any studies have discussed the possible influence of overlooking patients with malignant nodes in relation to Actrem. One study in 60 breast cancer patients [51] concluded that the optimal activity to be injected should be in the range of 7.4 and 37 MBq for same-day and two-day protocols, respectively, leaving an Actrem of approximately 5 MBq or less at surgery. The authors reported that in two of the sixteen patients identified with malignant lymph nodes, the metastatic nodes were not SNs.

The influence of preoperative imaging on axillary SN detection
The recently published EANM guidelines for SN diagnostics in breast cancer [48] recommend that scintigraphy is performed in breast cancer patients. None the less, in the last few years there has been a growing trend towards omitting imaging and relying on the identification of SNs by the probe and dye injection during surgery. The literature documenting the safety of no imaging is limited, and it must be kept in mind that the large bulk of studies documenting the benefit and safety of SN biopsy is based on studies including scintigraphy.

In the 858 patients included in our study, a mean number of about 2 SNs were removed in both groups, but significantly more patients in GrImaging underwent a two-day procedure and therefore had a lower Actrem at the time of surgery compared with the patients in GrNo-imaging. According to the logistic model when "compensating" for these differences in Actrem between the two groups, scintigraphy had a significant impact on the total number of SNs removed per patient. We found no significant impact of scintigraphy on the number of patients identified with malignant SNs. But unfortunately the total number of patients with malignant SNs was limited. It cannot be excluded that preoperative scintigraphy would have shown a significant influence if the Actrem had been the same in imaged and non-imaged patients.
A few other studies do not support an influence of preoperative scintigraphy on axillary SNB [49,54,57,58]. In a prospective study McMaster et al [57] found no significant difference in the detection rate of SNs between patients with and without imaging. The same result was obtained in two other studies with less patients [49,58].
In one retrospective study of 636 patients [54], the detection rate of axillary SN did increase with preoperative scintigraphy. Yet the authors concluded that visualization of SNs in preoperative imaging contributed little to the localization during surgery and is therefore unnecessary in SN diagnostics.

The influence on axillary SN detection of some of the other factors not examined in our study
Radiopharmaceuticals: The different radiopharmaceuticals available vary in the size of the radiocolloid, and an optimal particle size of 100-200nm has been suggested. The most widely used radiolabelled tracer in Europe is Nanocoll (Nanocoll, GE Healthcare, Amersham Place, UK), which was also used in this study. Nanocoll have a particle size of approximately 5-80 nm. An impact on the SN detection rate may depend on the size of the colloid compared to the time interval to imaging and surgery, as small particles are transported faster than larger particles in the lymphatic system.
Probe sensitivity: The identification of a "hot" SN in breast cancer patients is also depending on the probe used. If the radiosensitivity is low, the demand for a higher Actrem increases. The recommendations for γ-probes for intraoperative use have been described in several reports [15,16,48,59-61]. The probe used in our study is a commonly used probe and has a medium sensitivity when used with a collimator compared with the general level of the commercially available probes, as recently described [61].
Lymph vessel obstruction: Tumour cells in regionally disseminated cancer may obstruct the lymph channels leading to a malignant SN, so that the SN tracer passes with the lymph to another, non-malignant lymph node. Hence, this may result in a false negative, non-detection of the malignant SN [62,63].
Injection site: Different injection sites have been suggested in the literature, including intra- or peri-tumoural injections, intradermal and subdermal injections into the skin over the tumour or in the periareolar area. The intra- or peri-tumoral injections appear logical since it will reflect the natural lymph drainage. The argument for periareolar injection is based on the general lymph drainage pattern of most parts of the mammary gland going to the areolar region and from there to the axilla. The superficial injection techniques are easier and require less activity injected compared to peritumoural injections leaving less activity in the breast and thereby a minor risk for complication in the SN identification from spill-over to neighbour regions. Several studies describe a better SN identification rate and a smaller rate of non-visualization of lymph nodes from performing either dermal or periareolar injections compared to intra- or peri-tumoral injections [55,64-67]. However, while the superficial injection techniques will demonstrate more axillary SNs, they rarely show drainage to the internal mammary lymph nodes, resulting in non-visualisation of these nodes. Visualisation of extra-axillary SNs is obviously better with peritumoral injection compared to periareolar injections [64,67,68]. A more correct SN identification using both peritumoural and periareolar injection has been suggested [69].

Extra-axillary SNs
By omission of the scintigraphy the detection of possible extra-axillary SNs remains a problem. A few studies suggest that about 5% of the patients may have extra-axillary SNs visualized by preoperative imaging [49,54]. But malignant lymphatic spread is often not limited to this region. Other studies observed much higher values and as mentioned above [68], they claimed that it was related to the injection type of the tracer. The majority of centres do not use intratumoral injection. They do not look for possible internal mammary SNs, and if observed they do not necessarily make biopsies from this region because of the difficulties in the procedure and a risk of complications [54,68]. Furthermore, internal mammary node dissection has not been shown to improve survival [70. But, if metastases are only found in intra-mammarian SNs, staging and postoperative therapy will be affected [71].
Malignant spread to the supra- and infraclavicular nodes are known to be related to a poorer prognosis, but these lymph nodes are fairly easily located by the probe [57,72]. Several studies demonstrate that the risk of malignant SNs in extra-axillary LN basins is small, if the axillary basin is free of metastases [57,68,71,73,74]. None of the studies supported a significant influence of preoperative imaging.
It has been debated if SNB should be performed in patients with a primary breast cancer for the second time in the same breast or with a recurrence, as the lymphatic draining pattern might have been changed by surgery. For the visualisation of a possible alternative lymph drainage pattern to extra axillary SNs, which is more represented in these patients, the need for SNB has been suggested [75].
.

Study limitation
Our study was not a controlled, double-blind, randomised study. It was an analysis of two groups of breast cancer patients, one underwent imaging, the other one did not because of change in procedures at the hospital over a four year period. All patients were prospectively enrolled in a database recording SNB data. All the patients undergoing imaging were included before 2006 and then matched with a similar group of consecutive patients, who did not undergo imaging, included in the same period, but mostly also after 2006. In any other respect including preoperative diagnostic work-up and procedures related to SNB, the two groups were comparable, as regards surgery, pathology, patient demographics, tumour size, and histology.

Scintigraphic sentinel node detection in melanoma – comparison of imaging techniques

Many centres use only delayed static imaging for SNB in melanoma. However, some clinics are changing their procedures to include also dynamic imaging, in some centres for all melanomas, in others only for melanomas located in certain regions such as the head and neck [15]. Furthermore, SPECT/CT and US examination of the SNs gain ground in the evaluation of SNs.

In the present study nearly the same number of SNs and nearly all patients with malignant SNs were identified by the use of static imaging alone compared to dynamic and static imaging in combination with SPECT/CT.

However, there was a tendency towards identifying a slightly higher number of SNs per patient when interpreting only static images. There was a moderate disagreement between the use of static imaging alone to the use of the combined procedure as regards identical numbers of "true SN" and "clinical SN" in the individual patients. Identical number of "true SNs" and "clinical SNs" were described by SMU and RH in 58% and 52% of the patients, respectively. In the majority of the remaining patients the differences were small (≤2SNs in 39% and 46% of all patients, respectively).

To our knowledge this study is the first comparison of static imaging alone vs. dynamic-static imaging in melanoma. A study in breast cancer showed that dynamic imaging only prevented removal of one or two echelon nodes in 5 % of the patients compared with static imaging after 3 hours [76]. This benefit is of the same magnitude as our findings in melanoma.

In our study design it was only possible to evaluate the number of SNs and lymph node basins identified by the two reading sites, as the different SNs identified by SMU were not marked for a comparison of the two nuclear interpretations. Hence, due to doubt about the exact location of a SN in some of the SMU reports, the precise location within the basins of the different SNs could only be identified in part of the patients.

The influence of SPECT/CT

The use of SPECT/CT in SN detection in melanoma patients makes a precise anatomical location of the SN(s) possible and thereby, during surgery, an easier and faster location of the marked SN [27,77-81]. Identification of additional SNs by SPECT/CT has been reported for head and neck tumours with a complex and especially deep draining pattern [77,82]. The same goes for other types of drainage to deeper regions like the pelvis or retroperitoneum [80]. However, the clinical implications are not yet documented. In our study we did not examine the influence on the number of SNs detected by SPECT/CT compared to no SPECT/CT, but the technique undoubtedly contributed to a more precise description of the location of certain SNs.

Number of patients identified with malignant SNs

Of the 220 patients with surgical and pathological information on performed SNB, included in the present study, 38 patients had metastases in SNs. We found a risk of overlooking patients with a malignant SN of 5-8% when only delayed static imaging was performed compared with the combination of early dynamic imaging and delayed static imaging. The surgeon might possibly identify some of these malignant SNs by intraoperative palpation or by the use of blue dye. If the discrepancy is related to the total number of patients examined, including the patients without malignant spread, the combination of early dynamic and delayed static imaging would result in approximately 1% more patients identified with malignant SNs.

Overlooking a malignant SN will have consequences for the patient. A false negative SNB will result in missing conventional regional lymphadenectomy and thereby increase the risk of further dissemination of the disease. SNB followed by immediate regional node dissection after malignant SN histology significantly increases the three- and five-year-survival rates in melanoma patients with regional lymph node metastases [83-85]. This is compared to patients in whom a regional node dissection is delayed until appearance of regional metastases. The three- and five-years-survival rates were 89% and 83% in node negative patients compared to 56% and 53% in node positive patients[84,86]. The number of malignant SN(s) found per patient seems to have an impact on the mortality and on the risk of developing further malignant spread [87,88]. It is therefore crucial for melanoma patients that the malignant SN(s) are located, because removal of these malignant SNs has the potential of either curing or prolonging the time without further dissemination.

The cost, time and logistics

Regarding the costs, the clearly more expensive SN procedure performed by SMU including dynamic and SPECT/CT imaging must be analysed in relation to patient output of identified malignant SN(s). This may have an impact on patient therapy, monitoring, morbidity and survival.

Our study suggests a risk of overlooking a melanoma patient with a malignant SN by using only static imaging in 1-1.5% compared to the more comprehensive and sophisticated imaging procedures used as the gold standard for SN identification in this study. It thereby increases the risk of serious morbidity and mortality in these patients.

The purpose of SNB in melanoma patients is to identify the patients with a malignant SN with as few complications for the patients as possible. The performance of dynamic and static imaging perhaps in combination with SPECT/CT is a more expensive procedure, takes more time and is logistically more demanding. It can be discussed whether the benefits of performing this combined procedure compared to performance of only static imaging justify the need for the combined and complex procedure. The better detection rate should also be set in relation to the basic, false negative rate reported for the SNB procedure in melanoma of about 3-5% [84,86,88]. There is no simple answer to ethical and economical health policy questions about cost-efficiency, but it is necessary to raise the questions on the basis of knowledge about consequences and use of resources [89].

Study limitations

The comparison between the two reading sites were based on an average number of identified SNs and not on a "node-to-node" comparison of the individual lymph nodes described. Some of the SNs identified by the two readers as being located in the same region may not be identical nodes. Furthermore, the readings of the complex dynamic-static-SPECT/CT and US imaging were done in the clinical routine, whereas the readers of only static images analysed the images with no clinical information and on a different work station, only allowing for threshold and colour changes of the images. Hence, the interpretations should be compared

with some caution. It cannot be excluded that if only static images had been interpreted in clinical routine, it might have changed (deteriorated) the interpretation slightly, but that might be compensated by better facilities for handling the original images on the original work station. Anyway, identification of the same patients with malignant SNs is probably the most critical parameter analysed. Out of 38 patients with a malignant SN, described by the combined dynamic-static imaging, three patients were not identified looking only at static images.

CONTRAST ENHANCED LYMPHO-ULTRASONOGRAPHY (CELUS)

The possible use of CELUS for sentinel node detection

Ultrasonography in relation to SN diagnostics

Ultrasound imaging (US) can be used for detection of metastatic lymph nodes and a number of criteria have been suggested (size, shape, loss of central echogenicity etc [17]). Recently, the use of US for detection of metastasis has been suggested in the SNs diagnostic. Although the axial resolution of high-frequency US theoretically is less than 1 mm, it is not possible to detect small clusters of tumour cells within an otherwise normal lymph node, and micro-metastases cannot be detected. Metastatic deposits in SNs of 2-4 mm have been reported as the limit for US detection [90-92]. US in combination with guided biopsy has been described for identification of regional metastatic lymph nodes in both melanoma and breast cancer patients [17,93,94], giving a moderate sensitivity (39%- 65%), and a high specificity (87-99%) [17,92,95-97].

To accurately identify which lymph node is the sentinel node, recent animal studies have suggested the use of subcutaneous or intradermal injected US contrast agent for identifying the lymphatic tracts and the SN. The use of this technique in combination with FNAB in relation to SN diagnostics is of clinical interest, since this method may by able to detect the SNs and identify the malignant SNs prior to surgery. As a supplement to already existing methods this method may hopefully reduce the false negative rate of the SNB procedure.

Identification of SNs using CELUS in animals

The possibility of using an ultrasound contrast agent to depict the lymphatic tract and identify the SN has been suggested in the literature in 2002 and it was confirmed by our study in swine (study III) as well as others in dogs, rabbits and swine [5,6,8,18-22,98,99]. Overall a number of different scanning methods and presets have been used which make direct comparison of the studies difficult, however, all were successful in identifying the SN (Table 7, below).

Other studies have not discussed how or if the distance from the injection site to the SN could influence the CELUS method. We know from intravenous injections that the contrast agent only persists in blood vessels for 5-10 minutes,
which could be too short a time for the contrast agent to pass in the lymphatic tracts. Although we cannot give data for the maximum time the contrast agent remains in the lymphatic system (and thereby indirectly suggest a maximum distance between injection site and lymph node) we were able to detect contrast at the injection site even 20
minutes after injection. Because of the swine anatomy it was impossible to obtain a distance longer than 30 cm from an injection site at a mamilla to the SN.

Table 7

Overview of different studies investigating the possibility of using CELUS for SN identification. 1 Alliance pharmaceutical corp., San Diego, USA, 2 GE Healthcare, Oslo, Norway,3 Bristol-Myers Squibb Medical imaging, Billericia, MA, USA,4 Bracco, Milan, Italy. MM – melanoma, BC – breast cancer, SAE – Stimulated Acoustic Emission

	Subjects	Contrast agent + type of injection	US imaging for CELUS	Trans-ducer frequency	CELUS Result	Gold standard
Animal studies						
Wisner et al[21] 2002	14 dogs, 2 inj./dog in 13 of the dogs	Non-commercial contrast agent. Subcutaneous	Continuous power Doppler mode MI=1.3	4-7 MHz	SN identified in 11/14 dogs	-
Mattrey et al[8] 2002	15 rabbits	Imavist1	Pulse inverted harmonic US	7.2 MHz		x-ray lympho-graphy
Omoto et al[20] 2002	9 pigs 2 inj./pig	5% (5 pigs) or 25%(4 pigs) albu-min solution Subcutaneous	Grey scale US	5-8 MHz	0 SN by 5% alb.solution 8 SN by 25% alb.solution	-
Wisner et al[22] 2003	11 dogs	4 non-commercial contrast agents	Continuous power Doppler mode MI=1.3, PRF=1-12 kHz	4-7 MHz	Identified 34/40 SN	Preoperative Scintigraphy
Goldberg et al[5] 2004	6 pigs with 17 MM	Sonazoid2 Intradermal	Pulse inverted harmonic US (MI=0.2-0.5) + colour Doppler mode for SAE (MI>0.9)	7.5 MHz	28/31 SN	Preoperative scintigraphy (31 SN) Blue dye (27 SN)
Goldberg et al[6] 2005	8 pigs 4 rabbits 7 dogs 1 monkey	Sonasoid2 36 inj. Subcutane-ous, 14 inj. submu-cocal and 8 inj. parenchymal	Pulse inverted harmonic US (MI=0.2-0.5) + colour Doppler mode for SAE (MI>1) 3D US	7.5 MHz	SN was identi-fied after all injections	Blue dye in 6 of the animals
Lurie et al[19] 2006	10 dogs	Definity (Lumin-ity)3 Subcutaneous	Continuous power Doppler mode MI=1.3, PRF=12 kHz	4-7 MHz	SN was identi-fied in 8/10 dogs	Preoperative Scintigraphy (1-2 SN in all 10 dogs
Nielsen et al 2009	13 pigs 2 inj./pig	SonoVue4	Pulse inverted harmonic US (MI=0.13)	7 MHz	SN was identi-fied in 22/26 injections	Blue dye (22 SN)
Wang et al[99] 2009	12 rabbits	SonoVue4	Pulse inverted harmonic US (MI=0.32) + colour Doppler mode for SAE (MI=1.9)	7 MHz	17/19 SN 1-2 SN/dog	Blue dye (19 SN)
Wang et al[98] 2009	5 dogs 4 inj./dog	SonoVue4	Pulse inverted harmonic US (MI=0.32) + colour Doppler mode for SAE (MI=1.9)	7 MHz	21/23 SN + US identified 18 of 20 SN-Basins	Blue dye (23 SN)
Patient study						
Omoto et al[100] 2006	23 BC patients	25% albumin solution Subcutaneously over the tu-moursite	Grey scale US	7.5-10 MHz	SN was identi-fied in 12/23 patients	Axillary lymph node dissection
Nielsen et al 2009	10 MM patients	SonoVue4	Pulse inverted harmonic US (MI=0.13) + colour Doppler mode for SAE	7 MHz	SN was identi-fied in 1/10 patients	Preoperative scintigraphy + probe Blue dye
Omoto et al[101] 2009	20 BC patients	Sonazoid2 Subcutaneously	Pulse inverted harmonic US MI=0.15-0.19		SN was identi-fied in 14/20 patients	Preoperative scintigraphy + probe (20 SN) Blue dye (15 SN)
Sever et al[102] 2009	54 BC patients	SonoVue4 Intradermal	Pulse inverted harmonic US MI=0.2-0.4	14 MHz	SN was identi-fied in 48/54 patients	Preoperative scintigraphy + probe Blue dye

The use of different ultrasound contrast agents

Also, different ultrasound contrast agents have been used in previous animal studies: Sonazoid (GE Healthcare, Oslo, Norway) was used by Goldberg et al in different animals [5,6], Lumin-ity/Definity (Bristol-Myers Squibb Medical Imag

ing, Billerica, MA) used by Lurie et al in dogs [19] and SonoVue, used in our study in swine and by Wang et al in rabbits and dogs [98,99]. Other contrast agents like Imavist [8] and albumin suspension [20] have also been suggested.
The three US contrast agents SonoVue, Sonazoid and Luminity (Definity) all consist of lipid coated micro-bubbles with a similar mean bubble size (2 - 2.5 microns), but are composed of different substances [19,24,25,103]. It is possible that this could influence the CELUS examination. In the swine model used by Goldberg et al [5] Sonazoid seems to fill the lymph nodes better than SonoVue did in our study and in the study by Wang [98]. This higher accumulation may give a higher contrast to the surroundings. There are no published studies comparing the different contrast agents for CELUS, and it was not possible for us to obtain Sonazoid.

Identification of SNs using CELUS in patients
When we started part study IV in 2006 there were no similar studies of CELUS using a micro-bubble contrast agent in humans. Obviously, we had expected that it performed the same way in human as it had in animal studies, but it did not. Afterwards three studies have described ways to identify the sentinel node, all in breast cancer patients [100-102].
One study by Omoto [100] in 2006 used subcutaneous injections of 5 ml of a 25% albumin suspension as a "negative" ultrasound contrast agent. The contrast agent was visualised as hypoechoic areas within the lymph node. The technique identified at least one lymph node in the axilla in all 23 patients included. However, the lymphatic tracts were not visualized, and there was no gold standard for comparison.
In a second study by Omoto from 2009 Sonazoid was injected subareolarly in 20 breast cancer patients [101]. Three methods were performed on the same day as the operation for breast cancer: the CELUS-guided, dye-guided and gamma-probe-guided methods. SNs were identified in 14 of the total of 20 cases by the CELUS-guided method, in 15 of the 20 by the dye-guided method and in all 20 by the radionuclide method.
Sever [102] in 2009 also demonstrated the possibility of using CELUS for SN detection in 54 breast cancer patients using SonoVue. In the 54 patients examined using CELUS 48 had SN(s) identified, giving a detection rate of 89%. As gold standard the combination of gamma camera imaging, probe detection and the dye method was used.
The set-up in these three studies was in many ways similar to ours. A clear explanation on the difference in the success rate is not obvious, except that our examinations were done on extremities vs. examinations in breasts and also two of three other studies used a different contrast agent. One explanation could be a difference in choice of equipment inclusive the transducer, transducer frequency or mechanical index. However, if this is the case, it still puzzles us why the technique worked in our animal study, but not in our patients study.

These three recent studies show more promising results for the method; however, the application of CELUS for SN detection is still not fully explored in humans. Unfortunately, for the time being, Sonazoid is only approved for human use in Japan, and only for liver imaging [104] which sets a limit to the use of this contrast agent.

Other possible explanations for non-visualisation of SN using CELUS
Bubble size
The size of the micro-bubbles in the contrast agent is larger than the radionuclear tracer normally used for SN detection, with a mean size of approximately 2500 nm in diameter vs. 2.5 – 1000 nm [23,105,106]. This could theoretically result in a slower drainage of the ultrasound micro-bubbles to the SN. However, in our and other studies the contrast enhanced lymph nodes were seen within few minutes [5,19,22] and for several minutes after the injection, indicating that some of the bubbles must have been small enough to enter the lymphatic system and be retained in the macrophages.
Differences in lymph node architecture
There is an anatomical difference in the architecture of lymph nodes in swine and humans. The lymph node of the swine shows a reverse entrance of the lymphatic vessels into the node compared to other animal and human lymph nodes/vessels [107]. The afferent vessels of the swine lymph nodes enter together in the hilum region and the efferent vessels have dispersed origins from the cortical region. The germinal centres are located centrally opposed to the cortical position in other animals and humans. However, the structure of the lymph node in other animals used for SN detection using CELUS are fairly similar [107] to the human one. Another possible explanation relates to the echogenecety of the hilum, which in human lymph nodes is more hyperechoic than in swine. This makes it more difficult to distinguish whether the hyperechogenecety is normal or due to the contrast agent.
Differences in the US equipment
In daily work with US contrast agents most would have observed that there is a difference between US machines, transducers, and software versions, all matters that may influence the success rate when testing a new application like CELUS. Also, all presets would be optimised for intravenous injections, but not for CELUS. The choice of transducer frequency would also influence the US image and in Table 8 there is a wide range of frequencies from 4 to 14 MHz.

Toxicity and side effects
Because the ultrasound contrast agents are approved only for intravenous use, the potential toxicity using the contrast agent for subcutaneous injection should be considered. We therefore performed a safety study in mice before initiating the human study and found no tissue damage. Allergic reaction from SonoVue after intravenous injections has been seen, but are rare [23]. In clinical trials the most commonly reported side effect were headache (2.3%), injection site reaction (bruising, burning, paraesthesia) (1.7%), and pain at the injection site (1.4%) [23]. Permanent tissue damage, like necrosis has to our knowledge never been reported as an adverse effect of erroneous subcutaneous injection in humans and no adverse reactions were recorded in the mice, the eleven patients or in the swine examined in our studies.

Study limitations
In our patient study it is a limitation that we had to change the procedure during the study period, since this would compromise the reproducibility. However, it was a pilot study where we tried to test and create a method working in patients, and we were frustrated by our poor results. The melanoma patients were

supposed to be an "easy human model" for SN identification by CELUS. We still do not know the explanation of our failure in human subjects. We wish to do further, systematic animal studies including the testing of other contrast agents before new human applications.
Melanomas have a rather unpredictable lymphatic draining pattern and often drains to one or even several lymph node basins. In our patient study the US examination was performed prior to the preoperative scintigraphy giving the physician performing the US examination no information on which lymph node region to examine. However, the melanomas examined were on an extremity giving a more predictable drainage, and this was also the reason for the study design. In retrospect all melanomas in study IV drained to the predicted region. US imaging with CELUS is at best a supplement to the traditional SN procedure, and in a normal clinical routine the data from the gamma camera images would be available.

GOLD STANDARD

The purpose of performing a SNB is to identify the patients with malignant SNs. However, the overall goal of the SNB procedure is to improve survival or at least postpone further spread of the disease. Therefore, when evaluating SN procedures the ideal gold standard would be patient survival and/or recurrence of lymph node metastasis examined in a controlled, randomised study, blinding obviously not possible. These outputs need long study periods of well-matched, often quite large patient groups. The survival and recurrence rates of the patients enrolled in our breast cancer study were not analysed, and we do not believe that it could disclose any statistically significant difference within a feasible time period. It was irrelevant in the melanoma study of comparing static vs dynamic-static image interpretation, since all the patients were operated following the combined image interpretation. Consequently we wanted to use other, less "hard" gold standards. We decided, as often done in the literature, to use the number of SNs as a surrogate parameter for an endpoint in both our studies. The parameter "number of SNs" does not tell whether the SNs identified were true SNs or false positive lymph nodes. Our results in the two studies point in different directions in regard to this parameter: The breast cancer study suggests the risk to be a failure of detecting all (true positive) SNs when the activity is too low and possibly when omitting imaging. In our melanoma patients the risk might rather be related to identifying too many lymph nodes and possibly not the correct ones.
In our opinion another surrogate parameter is therefore stronger, though not often used, i.e. the number of patients identified with malignant SNs. We used this parameter as an important endpoint for both clinical studies. The identification and removal of malignant SNs will obviously have an impact both as predictor for prognosis and possibly also a direct impact on survival. The effect of the SN procedure has previously been well evaluated in comparison with the traditional regional lymphadenectomy. It is generally agreed that the SN procedure is an effective and reliable procedure for staging most breast cancers and melanomas without clinical sign of dissemination. When testing new SN procedures, the traditional SN procedures therefore seem acceptable as kind of gold standard for a new technique. Accordingly, the use of CELUS for SN detection was compared to the two standard SN procedures for the SN identification rate, preoperative scintigraphy and the blue dye method.

CONCLUSIONS

Even though the overall concept of the SNB is the same within different cancers, there are several differences in the SN procedures. As described in this thesis the procedure for preoperative SN imaging is going into different directions in patients with breast cancer and melanoma. There is a tendency towards performing no imaging in breast cancer patients and more imaging in patients with melanoma.
In regard to the performance of preoperative gamma camera images in the SNB, our results suggest that the influence of preoperative imaging on the clinical outcome for patients with breast cancer is not great regarding axillary SN findings. Preoperative scintigraphy had a significant influence on the number of SNs identified per patient, but we found no significant influence on the number of patients with malignant SNs. Yet, a minor influence on the final outcome cannot be excluded; only a randomised trial will give the answer.
The mean numbers of SNs identified by the two reading sites were rather similar, but in a patient-to-patient comparison of the numbers of SNs described from dynamic-static vs static images in patients with melanoma, only a moderate agreement was shown. However, this did not lead to a big difference in the detection of malignant SNs: In 16% of the patients a SN containing metastasis was described from delayed static images vs. 17% by the use of combined dynamic and static images.
Finally our results indicate that it is crucial for correct identification of all axillary SNs including the malignant nodes in breast cancer patients that a sufficient radioactivity amount is still present at the time of surgery. At surgery it should exceed at least 10 MBq.
In the investigation of the possibility of using an ultrasound micro-bubble contrast agent and CELUS for SN identification, we found that the method worked well in our animal model, but unfortunately it could not be confirmed in the patients. However, the application of CELUS on SN is still not fully explored in humans, and an alternative set up and / or contrast agent might provide better results.

PERSPECTIVES

Possible modifications within few areas of optimal preoperative SN imaging have been clarified in this thesis while many other matters still have to be discussed as mentioned above. One important issue would be to make clinical follow up studies of patient outcome in relation to imaging modifications, such as a follow-up study of the impact of skipping preoperative imaging for axillary SNB in relation to the 5 years survival and recurrence rate in our breast cancer patients. A prospective randomised study would provide even stronger data, but has a significantly longer time perspective. However, it is important - and unfortunately rarely done - to undertake controlled, diagnostic trials using hard endpoints, when new techniques or modifications of existing methods are introduced.
Our CELUS studies demonstrated the theoretical potential for the technique to detect SNs. Although our patient study was negative, three recent human study [100-102] on CELUS imaging for SN detection confirmed the promising animal data for the clinical future. CELUS is a non-invasive method that does not involve ionising radiation and may be especially attractive because of the possibility of injecting the contrast agent around deeper, parenchymal tumours [42]. Further studies on larger groups of patients

are needed, as well as solving the technical problem we experienced.
Recently preliminary studies have suggested the use of contrast enhanced MRI and CT for visualisation of regional lymph nodes as an analogy to the lymphoscintigraphy. Gadolinium-based contrast agents can be used for intradermal and subcutaneous injections. Interstitial injection of a gadolinium chelate contrast agent have shown nodal contrast enhancement on MRI in different animals and humans [108-110]. The contrast enhancement of the lymph nodes was seen within 15-30 min. MRI has also been suggested for preoperative SN staging using an ultrasmall superparamagnetic ironoxid (USPIO) contrast agent [111], the results were promising, but the study group only comprised 10 breast cancer patients. Furthermore, Wisner et al. have repeatedly shown the possibility of using contrast enhanced CT imaging for the performance of lymphosvintigraphy in various animals [112-115]. Injections were given in several locations, as subcutaneous injection, into the rectum, the stomach, the cervix and into the colonic submucosa using a 15%wt/vol iodinated nanoparticle suspension. Contrast enhanced lymph nodes were visualized within a maximum of 24 hours. This procedure has not yet been tested in patients.
The beauty of the MRI and CT methods would be the possibility of also differentiating benign from malignant SNs. The obvious drawback will be the lack of sensitivity compared to pathology combined with the lack of using the method for surgical identification of the nodes detected by imaging. Therefore a combination, the SPECT/CT, offers a great advantage by potentially disclosing some of the malignant SNs and tracing the other SNs during surgery. The same will be true for technique involving US, as discussed above. Finally PET/CT might theoretically offer a very attractive solution with FDG as a (malignant) tumour marker including systemic dissemination, a tracer for the surgeon, and CT for perfect localization of the lymph nodes. But in practice the method has not demonstrated sufficient sensitivity [116,117], and the nuclear medicine tracer is not useful as a SN tracer.
The SNB procedure is a multidisciplinary field where all areas may be important, and at the precent the accuracy of some areas may need more focus than others depending on the type of cancer. It is well documented that the SNB as currently performed is a good standard procedure for breast cancer, melanoma, and a few other cancers, but further investigations will hopefully make the procedure even better for the treatment of those and other malignant diseases as well.

SUMMARY

Malignant involvement of the regional lymph nodes in breast cancer, melanoma and other cancers is considered an important prognostic factor and determines the further treatment of the patient. Currently two methods are most often combined for SN detection, intra-operative blue dye injection around the tumour site and the radionuclide technique.
The aims of this thesis were to evaluate the possibility of optimising the radionuclide SN procedures in patients with breast cancer and melanoma, and to examine the possibility of using contrast enhanced lympho-ultrasonography (CELUS) for SN detection. The radionuclide method was evaluated in patients with breast cancer (study I) and in melanoma patients (study II). CELUS was tested in animals (pigs and mice, study III and IV) and in melanoma patients (study IV).

I. We investigated the influence on axillary SN biopsy in breast cancer patients of: a) Preoperative scintigraphy, used by some, but omitted by other centres, b) The variable activity remaining in the patient at surgery, due to differences in activity administered and to time to surgery.

II. This study compared the interpretation of delayed static imaging alone with the interpretation of early dynamic and delayed static imaging in combination with SPECT/CT in the SN diagnostics in melanoma.

III. This study describes the possibility of using CELUS to detect SNs in a pig model. The method worked well for SN detection in this model, in agreement with previous studies in pigs and other animals.

IV. In this study we examined the possibility of using CELUS with micro bubbles to detect SN in melanoma patients.

Conclusions: In breast cancer patients it is essential for SN detection that the injected activity is high enough for optimal SN detection, preoperative scintigraphy may be of some clinical value.
A combination of the three imaging modalities works only slightly better for SN detection than a simple static gamma camera imaging in patients with melanoma, the combined procedure used as gold standard identifies 1% more patients with malignant SNs.
CELUS as performed in our study worked well for SN detection in a pig model, but could not be used to detect SN in patients.

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