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MINIMALLY-INVASIVE BREAST INTERVENTIONS:

METHODS FOR HIGH YIELD, LOW RISK, PRECISION BIOPSY AND CURATIVE THERMAL ABLATION

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Für Marte, Greta und Jakob

ABSTRACT

Advances in medical imaging and the introduction of population-based screening programs have increased the detection rate and overall proportion of small breast tumors. In addition, progress in technology and medical science, in combination with efforts to minimize morbidity, have resulted in the emergence of minimally invasive image-guided interventional procedures for both diagnosis and treatment of breast cancer.

The **aim** of this thesis was to develop and validate new technologies for minimally-invasive diagnosis and treatment of breast cancer.

Specifically, to develop and validate a new biopsy system incorporating novel mechanisms for needle insertion and tissue acquisition designed for accurate lesion targeting and high yield tissue sampling; to clinically validate a biopsy enhancement technology using radiofrequency (RF) pulses to counteract dissemination of tumor cells; and to improve and validate radiofrequency ablation (RFA) for the treatment of small carcinoma and demonstrate feasibility in non-operable elderly patients.

During the course of this work a new biopsy device has been developed which incorporates a pneumatic insertion mechanism combined with a novel needle design. **Paper I** presented the device, compared sampling performance to a standard core needle biopsy (CNB) device in three representative bench models, measured needle dynamics on a specially designed needle trajectory test and evaluated *ex vivo* sample quality. Mean weight of samples were 3.5, 4.6, and 4.3 times higher ($p < 0.01$) than standard CNB device in turkey breast, calf thymus and swine pancreas. The method of tissue acquisition had no negative impact on the histopathologic quality of samples obtained from resected specimens. Maximum measured needle velocity was 21.2 ±2.5 m/s on a stroke length of 2.5 mm.

Paper II investigated whether a technology incorporating the application of RF pulses to the biopsy needle could counteract dissemination of tumor cells. In this proof-of-principle setting the technology was adapted to fine needle aspiration (FNA) and prospectively used in 31 patients. Eighty-eight patients underwent routine FNA. Blood emerging from the skin orifice was analyzed for the presence of tumor cells. Viable tumor cells were found in 74% (65/88) of cases for routine FNA and in 0% (0/31) of cases ($p < 0.001$) when RF pulses where applied. It was observed that application of RF pulses had a hemostatic effect, did not degrade the cytological sample inside the needle and caused no additional pain compared with standard FNA.

In **Papers III**, **IV** & **V**, the technology, method and protocol for RFA in breast cancer were successively developed and evaluated in a total of 55 patients.

Specifically, in **Paper III** the feasibility of a newly developed RF device for ablation of unifocal breast carcinoma <16 mm immediately prior to partial mastectomy was assessed. In 84% (26/31) of cases complete ablation was achieved as assessed by Hematoxylin and Eosin (H&E) staining. Non-complete ablation was associated with incorrect electrode positioning within the lesion and underestimation of lesion extent due to inaccurate preoperative imaging.

In **Paper IV**, tumors ≤20 mm were included and the feasibility under local anesthesia three weeks prior to planned resection using improved technology and protocol was assessed. Magnetic resonance imaging (MRI) was utilized for patient selection. Exclusion criteria included multifocality, diffuse growth patterns, >25% intraductal components and lobular histology. Magnetic resonance imaging, H&E staining and cytokeratine 8 (CK8) immunostaining were used to determine complete ablation. A pneumatic–mechanical insertion mechanism was developed to improve electrode insertion and positioning. Pain was assessed using the Visual Analogue Scale (VAS). In 100% (18/18) of cases MRI showed no residual tumor growth and devitalization of the entire tumor was shown by at least one histologic method. Pain was reported to be a median of 2 and 2.5 for injection of anesthetics and during ablation, respectively, and the difference was not significant ($p = 0.512$).

In **Paper V** the feasibility of RFA as an alternative to surgical resection in elderly breast cancer patients with severe comorbidities that were unfit for or refused surgery was assessed. Six patients aged ≥85 years were included. In all cases, complete ablation was confirmed using MRI and contrast enhanced ultrasound (CEUS) at 1 month as well as staining assays for H&E and CK8 in tissue samples at 6 months. The procedure was well tolerated with mild to moderate pain during the ablation procedure. Follow-up was a median (range) of 54 months (11 to 94 months). Three patients died of non-cancer related causes. Three patients remained alive at 74, 86 and 94 months of which one experienced a loco-regional recurrence at 59 months.

In **conclusion**, this thesis demonstrates that the newly developed biopsy system enables for a novel method of precision needle insertion and achieves high yield tissue sampling. Furthermore, this thesis demonstrates that the presented biopsy enhancement technology can prevent dissemination of tumor cells. Finally, it demonstrates that RF ablation of small breast carcinoma has a high rate of complete ablation, can be performed under local anesthesia with mild to moderate pain, and is feasible as an individualized treatment option in elderly patients with severe co-morbidity who are refusing, or are unfit for surgery.

LIST OF SCIENTIFIC PAPERS

I. High velocity pulse biopsy device enables controllable and precise needle insertion and high yield tissue acquisition.
Schässburger KU, Paepke S, Saracco A, Azavedo E, Ekström C, Wiksell H.

Physica Medica. 2018;46:25-31.

II. Prevention of tumour cell dissemination in diagnostic needle procedures.
Wiksell H, Schässburger KU, Janicijevic M, Leifland K, Löfgren L, Rotstein S, Sandberg PO, Wadström C, Auer G.

British Journal of Cancer. 2010;103:1706 9.

III. Feasibility study on the treatment of small breast carcinoma using percutaneous US-guided preferential radiofrequency ablation (PRFA).
Wiksell H, Löfgren L, Schässburger KU, Grundström H, Janicijevic M, Lagerstedt U, Leifland K, Nybom R, Rotstein S, Saracco A, Schultz I, Thorneman K, Wadström C, Westman L, Wigzell H, Wilczek B, Auer G, Sandstedt B.

The Breast. 2010;19:219-25.

IV. Minimally-invasive treatment of early stage breast cancer: a feasibility study using radiofrequency ablation under local anesthesia.
Schässburger KU, Löfgren L, Lagerstedt U, Leifland K, Thorneman K, Sandstedt B, Auer G, Wiksell H.

The Breast. 2014;23:152 8.

V. Radiofrequency ablation: A tool for individualized treatment of early stage breast cancer in elderly patients?
Schässburger KU, Löfgren L, Fredriksson I, Thorneman K, Auer G, Wadström C, Ekström C, Wiksell H.

Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

ADH	Atypical ductal hyperplasia
ALN	Axillary lymph node
ALND	Axillary lymph node dissection
CEUS	Contrast enhanced ultrasound
CI	Confidence interval
CK8	Cytokeratine 8
CNB	Core needle biopsy
DCIS	Ductal carcinoma in situ
EBCTCG	Early Breast Cancer Trialists' Collaborative Group
ER	Estrogen receptor
FNA	Fine needle aspiration
H&E	Hematoxylin & Eosin
HER2	Human epidermal growth factor receptor 2
IHC	Immunohistochemistry
MRI	Magnetic resonance imaging
NACT	Neoadjuvant chemotherapy
pCR	Pathologic complete response
PgR	Progesterone receptor
RF	Radiofrequency
RFA	Radiofrequency ablation
RR	Risk ratio
SLN	Sentinel lymph node
SLNB	Sentinel lymph node biopsy
US	Ultrasound
VAB	Vacuum assisted biopsy

1 GENERAL INTRODUCTION

The earliest record of breast cancer can be found in the Edwin Smith papyrus dating back to Egyptian times around 1600 BC and describes breast tumors removed by cauterization with a tool called fire drill; arguably the first documented application of medical technology in breast cancer care.

Fast forward a couple of thousand years and modern imaging technologies detect breast tumors long before they become palpable lumps. Public awareness and the implementation of population-based screening programs have resulted in the vast majority of breast cancers being detected when the tumor is small and before the disease has spread to other organs. Minimally-invasive image-guided interventional procedures have taken center stage in diagnosis and are being explored as a treatment alternative to surgery.

To distinguish between a benign and cancerous tumor, and in the latter case, to obtain crucial information for optimal treatment planning, tissue from a suspicious lump in the breast is needed. Surgical biopsies, invasive procedures associated with significant side effects, were routinely performed for this purpose up until the late 1980s. At that time biopsy devices and procedures were developed which enabled acquisition of tissue samples by means of a needle inserted under image guidance using modalities such as mammography, ultrasound (US) and magnetic resonance imaging (MRI). These methods achieved higher diagnostic accuracy than surgical biopsies with vastly fewer side effects. Ultrasound-guided needle biopsies are today the standard-of-care due to high diagnostic precision, low cost and high patient comfort. New treatment paradigms and the emerging era of precision medicine are likely to result in expanding indications for image-guided biopsies and more challenging procedures, as well as increasing demand on both tissue quantity and quality. Current biopsy methodologies lack appropriate targeting and tissue acquisition mechanisms needed to meet these new challenges and while they are less invasive than surgery, general side effects of needle biopsies include risk of infections, dissemination of tumor cells and bleeding.

Removal of a cancerous tumor remains a cornerstone of breast cancer therapy. Before the 1960s, a curative operation of early breast cancer entailed removal of the whole breast, pectoral muscles, and axillary lymph nodes (ALNs) leading to significant short- and long-term side effects. In an effort to decrease side effects, advancements in radiotherapy and progress in understanding the mechanisms of metastasis have led to significant improvements in surgical treatment. Today a more selective approach is the standard of care, wherein only the tumor itself is removed followed by radiotherapy. In the axilla only the lymph nodes draining the breast, the so-called sentinel lymph nodes (SLNs), are routinely surgically removed and assessed. It is only when they show metastases that removal of additional nodes is required.

The next frontier lies in the development and clinical validation of image-guided interventions as non-surgical treatment alternatives. Most of these interventions involve the insertion of an electrode or applicator through the skin into the tumor under image guidance. Different approaches are used to raise or lower temperature of the tumor to induce cell death. These new interventions aim to achieve improved cosmesis, treatment in an office-based

setting, lower costs compared to a surgical procedure, and the possibility to offer a curative option to patients unfit for surgery. Radiofrequency ablation (RFA) of tumors is routinely used in other organs such as the liver and kidney and is considered a promising technique for the breast. However, the technique is in its infancy and numerous technical issues need to be addressed before it can be widely adopted.

Breast cancer is the most frequent cause of cancer death in women. Each year, around 1.7 million women are diagnosed with breast cancer and around 0.5 million die of the disease. Continuing to improve methods and techniques to decrease side effects, patient discomfort and cost while preserving or improving oncologic outcome is paramount.

The aim of the work presented herein was to develop and clinically validate new technologies and procedures for minimally invasive diagnosis and treatment of breast cancer and thereby drive improvement of cancer care.

2 BREAST CANCER

2.1 INCIDENCE, MORTALITY AND SURVIVAL

Worldwide, around 1.7 million women were diagnosed with breast cancer in 2016, see Figure 1. With around 22% of incident cases it is the most frequently diagnosed cancer amongst women and accounts for 10% of all incident cancer cases worldwide [1]. Incidence rates vary greatly across regions, being generally high at about >80 per 100,000 women in more developed regions and low at <40 per 100,000 in less developed regions [2].

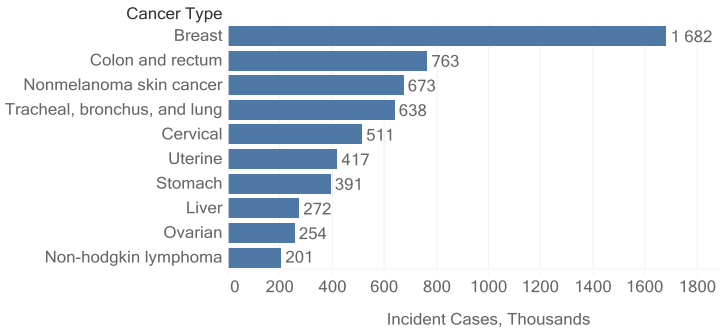

Figure 1. Global incident cases of the 10 most common cancers in women, 2016 data. Source: [1]

The incidence of breast cancer has generally been increasing in developed regions due to an increasing prevalence of known risk factors. A brief decrease after the turn of this century can be attributed to a drop in the use of hormone replacement therapy [3] and possibly saturation in mammography screening [4]. Emerging economies have seen a rapid rise in incidence rates owing to a number of factors such as adoption of a westernized lifestyle and improved diagnostics [2], see Figure 2. From 2008 to 2012, the number of incident cases increased by 21% in developing regions compared with 14% in developed regions [2]. While historically viewed as a disease of the developed world, the number of incident cases is now higher in developing regions than developed regions, accounting for around 51% of cases worldwide [2].

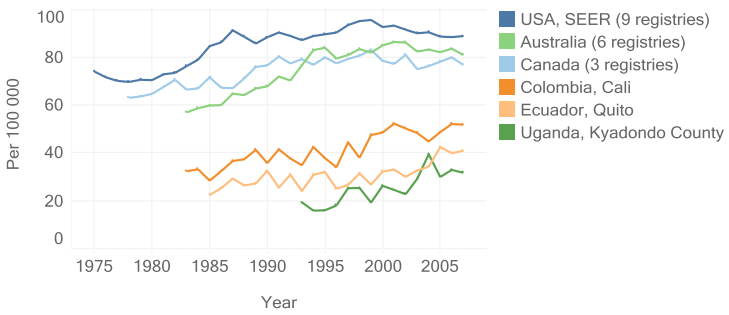

Figure 2. Time trends of breast cancer incidence in selected countries of both the developed and developing world. Age standardized rate using the World Standard Population. Source: [5]

With around 0.5 million cases of breast cancer resulting in death annually worldwide, breast cancer is the most common cause of cancer death in women and the fifth most common cause of cancer death overall [2], see Figure 3. A relatively low mortality rate in developed regions results from higher survival rates compared with developing regions [6].

Better survival can be attributed to the general availability of healthcare, an increase in self-examination and breast cancer awareness that began in the middle of the last century [7], as well as screening mammography and advancements in adjuvant therapies, including cytotoxic drugs, adjuvant hormonal therapy, targeted therapies and radiotherapy [7].

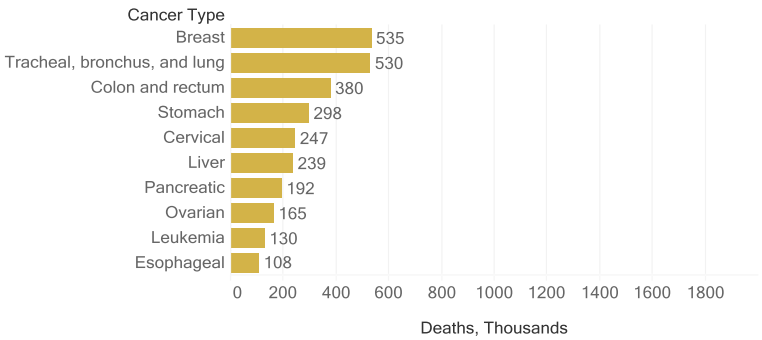

Figure 3. Global number of deaths for the 10 cancers with highest mortality burden in women, 2016 data. Source: [1]

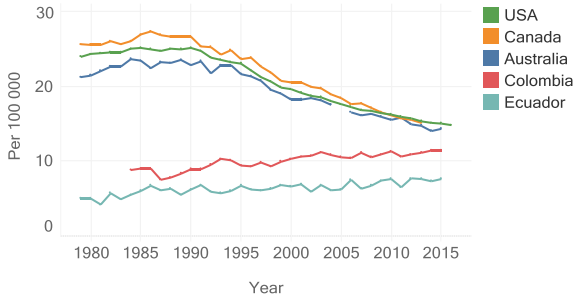

Figure 4. Time trends of breast cancer mortality in selected countries of both the developed and developing world. Age-standardized death rate using the World Standard Population. Source: [8]

In the European Union and the United States of America (USA), mortality rates have decreased by 15% and 18%, respectively from 2002 to 2012 and are expected to decrease further by 12% and 14%, respectively by 2020 [9, 10]. In contrast, rising mortality rates in developing countries are associated with rates of increasing incidence and comparably low survival, see Figure 4. Overall, 62% of all breast cancer deaths occur in developing regions [2].

In Sweden, 7558 women were diagnosed with invasive breast cancer in 2016. Incidence increases with 1% per year [11] and more than 1 in 10 women are expected to develop a breast cancer during their lifetime [12]. At least 100,000 women are living with a breast cancer diagnosis in Sweden and in 2016, 1391 women died of the disease. Breast cancer is the second most frequent cause of cancer death in Swedish women accounting for 26% of cases, second only to lung cancer [12]. Time trends for incidence and mortality are in line with other developed regions, see Figure 5.

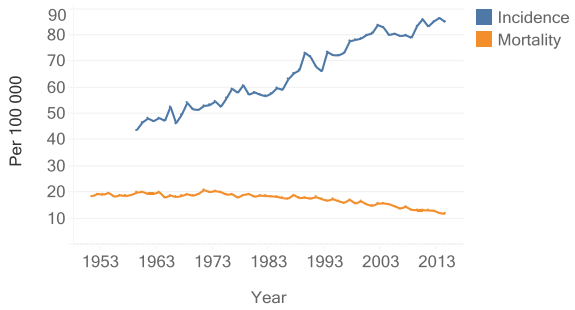

Figure 5. Time trends of breast cancer incidence and mortality in Sweden. Age-standardized rates using the World Standard Population. Source: [11]

In Sweden, the care for patients with breast cancer, is based on a multidisciplinary approach guided by national guidelines issued by the Swedish Breast Cancer Group since 2000 [13]. There are well established health care registries, a national screening mammography program since 1997 and an arguably high level of public awareness. A cancer registry was first established in 1958, a national quality register for breast cancer was established in 2008, a registry for breast reconstruction was established in 2011 and a register for screening mammography is under development [14]. Based on a proposed National Cancer Strategy for the Future [15], regional cancer centers for improved healthcare quality and results alongside more efficient use of health and medical care resources are currently being established.

Due to the disease being routinely detected at an early stage, in 2017 only 14.2% of incident cases in Sweden presented with spread to the lymph nodes and only 2.5% had developed distant metastasis [16]. In 2012 the median size of invasive breast carcinoma that underwent primary surgery was reported to be 17 mm [17]. Consistent with other developed countries, breast cancer survival in Sweden has steadily increased during the last decades, see Figure 6.

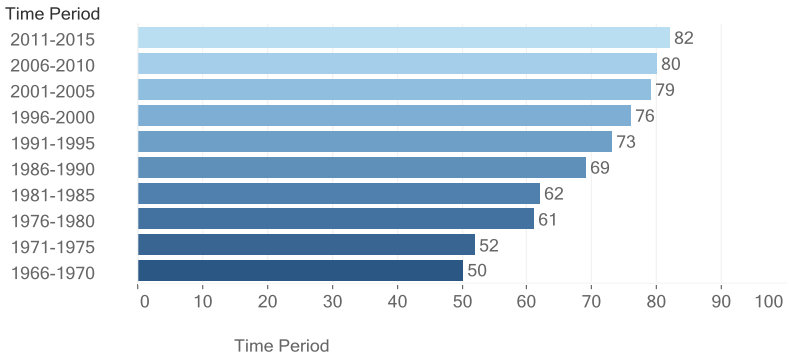

Figure 6. Time trends of breast cancer survival in Swedish women. Age-standardized 10-year relative survival. Source: [11]

2.2 RISK FACTORS

Numerous factors have been associated with increased or decreased risk of breast cancer incidence. Some (e.g. socio-economic status, country of origin and level of education) do not impact biological mechanisms themselves but are thought to be indicators of underlying factors such as life style and reproductive patterns.

Risk factors can either be modifiable (e.g. obesity, parity, or breast feeding) or non-modifiable (e.g. age, age at first menarche, or germline mutations). With breast cancer incidence being very low in men and cancer rates increasing rapidly between the ages of 30 and 70, both age and female gender are two obvious and strong risk factors. Table 1 shows details on a comprehensive list of risk factors well described in the literature.

Risk factor	Relative risk (95% CI)		Comment
Hormone related			
- Age at menarche [18]	1.050	(1.044–1.057)	For every year younger at menarche
- Age at menopause [18]	1.029	(1.025–1.032)	For every year older at menopause
- Age at first birth [19]	1.40	(1.15–1.70)	First birth at age ≥35 vs. age <20
- Parity [19]	0.69	(0.61–0,79)	Parity ≥5 vs. 1–2
- Nullparity [19]	1.29	(1.18–1.40)	Nullparity vs. parous
- Breast feeding [20]	0.96	(0.97–0.94)	For every 12 months of breastfeeding
- Free estradiol [21])	2.58	(1.76–3.78)	Highest vs. lowest quintile concentration
- Hormone replacement therapy [22]	1.076	(1.070–1.082)	For current users and every year of use of estrogen-progestin therapy. Risk dissipates 2–5 years after cessation
- Oral contraceptives [23]	1.24	(1.15–1.33)	For current users, decreasing risk with no residual risk left 10 after cessation
Genetic predisposition and family history			
- BRCA1 mutation carrier [24]	36	(25–52)	Age group 30–39 years, decreases in subsequent age groups
- BRCA2 mutation carrier [24]	16	(9.3–29)	
- One first degree relative [25]	1.9	(1.7–2.0)	
- Two first degree relatives [25]	3.6	(2.5–5.0)	
Other			
- Obesity, postmenopausal [26]	1.12	(1.08–1.16)	For every BMI increase of 5kg/mm
- Alcohol intake [27]	1.32	(1.19–1.45)	For 35–44 g of alcohol each day (3–4 alcoholic drinks) vs. no alcohol intake
- Breast density [28]	4.64	(3.64–5.91)	Percentage density ≥75% vs. <5%
- Radiotherapy for Hodgkin's lymphoma [29]	4.70	(3.28–6.75)	

Table 1. Comprehensive list of established risk factors. BMI = Body mass index.

2.3 DISEASE CLASSIFICATION

2.3.1 Histopathological type

Breast cancers are classified into those that have penetrated the limiting basement membrane (invasive) and those that have not (non-invasive, *in situ*).

The most common type of invasive carcinoma, accounting for around two thirds, has historically been referred to as ductal carcinoma. In 2012 the World Health Organization (WHO) changed the classification to "not of special type", since the term better reflects a heterogeneous group of tumors not exhibiting sufficient characteristics to be classified as a single specialized histologic type [30]. The remaining third of invasive cancers can be morphologically classified into a specialized type. These include lobular carcinoma (the most frequent type) as well as tubular, mucinous, and medullary which each account for low single digit percentages.

The most common non-invasive carcinoma is ductal carcinoma *in situ* (DCIS). It rarely presents as a palpable or radiologically detectable mass but more frequently as mammographically detected calcifications. The incidence of DCIS has greatly increased since the introduction of population-based screening programs [31]. An overview of distribution by histologic type is given in Table 2.

Histological type	Frequency
Invasive carcinoma	
- ductal /not of special type	59.4%
- lobular	7.6%
- mixed ductal/lobular	7.2%
- tubular	0.4%
- mucinous	1.5%
- medullary	0.2%
- papillary	0.5%
- other	3.2%
In situ carcinoma	
- ductal	16.9%
- lobular	2.3%
- other	0.7%

Table 2. Distribution by tumor histology among 392 011 reported cases in SEER 18 registries between 2011 and 2015, Source: [32]

2.3.2 TNM classification and disease stage

Breast cancer stage is determined based on the TNM system. The system takes into account information on the tumor (T), i.e. largest diameter and whether or not it is invasive, the extent of regional lymph nodes metastasis (N) and the presence of metastases (M). Combining the T, N, and M classifications the patient is assigned one of five stages denoted with roman numerals 0–IV. Breast cancer stage is a powerful prognostic factor, see Figure 7. As recent as 2017, the classification system underwent a major change to include biological factors such as tumor grade, Human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER), progesterone receptor (PgR) and genomic assays in conjunction with classical anatomical factors. Moreover the American Joint Committee on Cancer has noted that the historic update cycle for the TNM system of 6–8 years will likely be shortened to reflect the increased rate of emerging information from medical science and clinical trials [33].

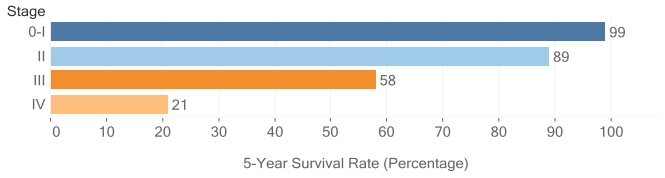

Figure 7. Age-standardized 5-Year Relative Survival in relation to cancer stage of Swedish women diagnosed with breast cancer during 2001–2010. Source: [34]

2.3.3 Histological grade

The concept of histological grade has been widely implemented clinically since the report of Elston et al. [35] in the early 1990s and is based on a scoring system taking into account the

evaluation of tubule formation, nuclear grade and mitotic count. Each category is assigned a value 1–3 depending on pre-defined characteristics. The summation of values result in the assignment of an overall Grade I–III, where a higher value indicates higher aggressiveness, which has shown to have significant prognostic value [35].

2.3.4 Molecular markers

Four biological tumor markers that have proven clinical utility are routinely analyzed during histopathologic examination. Estrogen (ER) and Progesterone (PgR) receptors are analyzed by immunohistochemistry (ICH) methods and the result is given as the percentage of positive receptor cells. Hormone receptor status carries prognostic value and is predictive of response to endocrine therapy using tamoxifen for example. Around 80% of breast cancers are classified hormone receptor positive. The *HER2* gene is overexpressed in around 20% of breast cancer tumors. HER2 status is analyzed using primary IHC and reported as categories 0/1+/2+/3+ according to guidelines [36]. In case of 2+/3+ *in situ* hybridization is performed for confirmation. Human epidermal growth factor 2 status conveys prognostic value and is predictive of response to anti-HER2 treatment such as trastuzumab. Lastly, the cell-cycle specific antigen Ki-67 is analyzed to assess proliferation activity. Immunohistochemistry is used to analyze "hot spots" of Ki-67–positive cell nuclei and the result is given as a percentage. Different cut-off points have been used with the general conclusion that Ki-67 status has prognostic value as well as predictive value with respect to adjuvant chemotherapy.

2.3.5 Intrinsic tumor subtypes

Spurred on by the medical need for improved predictive and prognostic factors with high reproducibility, technological developments in high-throughput gene expression analysis have led to multi-gene assays. In 2000, Perou et al. [37] reported the use of microarrays and hierarchical clustering to identify relevant gene expression patterns in breast tumors. Successive improvements in methodology have resulted in a validated set of 50 genes that identify four distinct intrinsic tumor subtypes with high predictive and prognostic value [38], namely luminal A, luminal B, HER2-enriched and basal-like. Commercial versions (ProSigna, NanoString) are based on cost-effective quantitative polymerase chain reaction and can be performed in both paraffin-embedded or fresh frozen tissue.

There is correlation between the immunohistochemical biomarkers and the identified intrinsic subtypes (see Table 3). Despite not being able to accurately identify subtypes, due to issues of availability and costs, these IHC markers are widely used to classify breast tumors into subtypes.

Molecular subtype	Pathologic surrogate definitions
Luminal A	ER pos, PgR pos, HER2 neg, Ki-67 low
Luminal B	ER pos, HER2 neg and Ki-67 high or PgR low
	ER pos, HER2 pos
Erb-B2 overexpression	ER neg, PgR neg, HER2 pos
Basal-like	ER neg, PgR neg, HER2 neg

Table 3. Condensed table showing surrogate definitions of molecular subtypes according to St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013 [39]

3 DIAGNOSIS

Breast lesions are generally assessed by applying “triple diagnostics”, a methodology which employs three different modalities: Firstly, a clinical examination is performed involving palpation of breast and ALNs. Secondly, imaging diagnostics is performed using primarily mammography, US and MRI. Finally, a biopsy may be indicated using fine needle aspiration (FNA) to obtain tumor cells for cytological tests alternatively core needle biopsy (CNB) or vacuum-assisted biopsy (VAB) to retrieve intact tissues samples for histopathological analysis [13, 40]. Diagnostic surgery is indicated only in exceptional cases.

Around 50% (64% in the screened age group) of breast cancer cases in Swedish women are detected via screening and usually present asymptomatic [13]. The remaining cancers are clinically detected with the patient presenting with a palpable lump or other symptoms such as breast discomfort, redness of the skin, nipple secretion or a lump in the axillary region.

3.1 BREAST IMAGING

3.1.1 Mammography

The principles of modern mammographic technique were conceived in the 1960s when specialized X-ray equipment was used for the detection of breast lesions [41]. In the following decades, mammography was widely adopted with digital mammography largely replacing analog X-ray technology after the turn of the century.

Today, digital mammography is the first-line imaging modality for the assessment of clinical findings in the breast [13]. Sensitivity of mammography in detecting breast cancer is estimated around 85–90% [13]. Mammography alone cannot, however, detect all malignant tumors [42] and sensitivity is known to be low for the detection of diffusely infiltrating tumors such as lobular carcinoma [43, 44]. The major limitation of mammography, however, is represented by women with dense breast parenchyma, especially young women, which limits the detection of cancer in high-risk women, such as those carrying a genetic mutation or with a strong family history of breast cancer [45, 46].

Mammography is the only imaging modality that has proven to be effective for population-based breast cancer screening [30]. In Sweden, screening mammography is offered to women aged 40–74 at intervals of 18–24 months [47]. Sensitivity of screening mammography in the detection of breast cancer ranges between 74% and 95% with specificity between 89% and 99% [48].

Large Swedish clinical studies have consistently reported a decrease in breast cancer mortality as a result of screening mammography [49-51] and there is general consensus that mammographic screening has resulted in a relative reduction of about 20% in breast-cancer mortality [52]. Nevertheless, there is currently a debate regarding population-based mammographic screening [53]. Issues include overdiagnosis resulting in women that suffer psychological distress because of false-positive findings and more importantly women that are treated unnecessarily or [53]. This includes specifically cases of low-grade DCIS, which are treated surgically although they might not have developed any clinically relevant disease.

Another issue is an absence of a significant decline of late-stage breast cancer incidence rates [54] associated with missed so-called interval cancers. Lastly, the overoptimistic perception of women regarding the benefits of mammographic screening is an identified issue [55-59].

3.1.2 Magnetic resonance imaging

Magnetic resonance imaging has emerged as a highly sensitive new imaging modality with sensitivity in the diagnosis of cancer of around 90% whilst showing a low specificity of around 72% [60]. MRI is superior to mammography in detecting lobular cancer [61-63]. Since MRI is costly and its relatively low specificity can lead to extensive additional work-up of indeterminate findings and unnecessary biopsies [13], indications for its use are limited. Breast MRI can be used in high-risk patients with dense breast tissue or hereditary predisposition [13] and for assessment of response to neoadjuvant chemotherapy (NACT) as well as detection of occult primary tumors. Although it can detect multifocal and multicentric disease the cost-effectiveness of its routine preoperative use has not been confirmed [64].

Abbreviated MRI and dynamic contrast enhanced-MRI protocols are being evaluated as possible tools for population-based screening [65].

3.1.3 Ultrasound

Rooted in military equipment, the first clinical use of US was documented in 1952 [66]. Over subsequent decades, US of the breast was mainly used to distinguish between cystic and solid masses.

In the 1990s equipment was vastly improved and allowed to confidently characterize breast lesions as benign or malignant using high-resolution grays-scale US imaging [67]. Breast US showed a sensitivity of 98% in distinguishing benign from malignant masses [67] and was more accurate than both mammography and clinical examination in determining tumor size preoperatively [68].

Advances in US technology have included faster frame rates, power Doppler imaging, higher resolution transducers, harmonic and compound imaging, elastography and three-dimensional US [69]. For diagnostic work-up of breast lesions, wideband linear transducers of 5–12 MHz or 5–18 MHz are most commonly used today [69]. US-contrast agents have been developed which consist of microbubbles containing various gases within a shell and can be used to evaluate the microvascularity of breast lesions in a procedure called contrast-enhanced ultrasound (CEUS).

Breast ultrasound is excellent at demonstrating masses but cannot adequately visualize microcalcifications, the hallmark of early breast cancer. Therefore, it is used to evaluate palpable breast masses, to help characterize non-palpable lesions detected with mammography and MRI [69] and as a complementary breast cancer screening technique in women with dense breasts and negative mammograms [69]. However, recent trials have demonstrated that US used as an adjunct screening modality increases sensitivity but also the rate of false-positives [70].

Ultrasound is also used to examine the axilla and other nodal basins to detect lymph node metastases of breast cancer [13, 71]. It is currently the primary imaging modality for women

aged <30 years and pregnant or lactating women. Due to its unique real-time capability, US is the modality of choice to guide interventional breast procedures [69].

3.2 BREAST BIOPSIES

"... in the era of personalized medicine,... the role of the image-guided percutaneous biopsy is evolving. "[1] *[72]*

The widespread deployment of mammography together with the introduction of population-based breast cancer screening programs has led to increased detection of small and non-palpable lesions which require further diagnostic clarification [73, 74].

A histological diagnosis for non-palpable lesions detected by mammography was initially obtained by preoperative hook wire marking [75] and subsequent surgical excision. However, only 20–30% of these women did in fact have a malignant disease [76] exposing a large numbers of healthy women to invasive surgical procedures with associated risks of morbidity [77-79]. Those patients in which the surgical biopsy did reveal a cancer often had to undergo a second surgical procedure due to narrow margins or required staging of the axilla.

From the beginning of the 1990s percutaneous biopsies [80, 81] started replacing surgical biopsies due to its numerous benefits. Today surgical biopsies in the breast have become virtually obsolete [82].

3.2.1 Tissue sampling techniques

Percutaneous biopsies of the breast were first systematically described in 1930 [83].These biopsies were performed using small diameter needles (20–25G) attached to a syringe creating vacuum. In these procedures known as FNA biopsies, the needle is inserted into the lesion and by moving the needle tip in combination with suction created by the syringe, cells from the surrounding area are drawn into the needle. A cytopathologist subsequently analyzes the cell samples and diagnoses the lesion as benign or malignant. In the late 1960s FNA experienced a breakthrough when Franzen et al. published a comprehensive review of FNA procedures in palpable breast lesions [84].

Fine needle aspiration became widely used in Europe more so than in the USA. However, its popularity soon faded due to a high frequency of insufficient samples [85], incapacity to distinguish invasive from *in situ* lesions, dependence on the experience of the aspirator and cytopathologist [86] and a rather high false-negative rate [87, 88].

In 1978 Elston et al. [87] reported the results of a new biopsy method using so called Tru-Cut biopsy needles for the pre-operative diagnosis of breast carcinoma. The Tru-Cut biopsy method is based on a sharp inner needle with an aperture behind the needle tip enclosed by a hollow outer cannula. The inner needle is manually inserted into the lesion at which tissue

[1] Proceedings from the Society of Interventional Radiology Research Consensus Panel on *Image-guided biopsy in the Era of Personalized Cancer Care* (2016)

enters the aperture. The outer cannula is then manually advanced to cut off the sampled tissue trapped inside the aperture. The needle is subsequently withdrawn and the sample is analyzed by a histopathologist. Diagnostic accuracy was superior to FNA [87] and a general pathologist without special training in cytology was able to interpret the specimen.

Subsequently, Tru-Cut needles were improved with the aim to provide better samples and enabling for a one-handed procedure so that the other hand was free for maneuvering an US transducer. In 1982 Lindgren et al. presented a double spring automatic biopsy device using gun-specific needles [89] and introduced the method now commonly known as core needle biopsy (CNB).

The automatic gun in the CNB method incorporates a spring-trigger system to fire the inner needle and outer cannula. Similar to Tru-Cut, the inner needle has a solid tip and an aperture that functions as a sampling chamber. When the gun is fired, the inner needle advances as far as permitted by design and automatically triggers a system for subsequent advancement of the outer cannula to sever the sampled tissue trapped inside the aperture. The needle assembly is subsequently withdrawn and the tissue sample is removed from the needle for assessment by a pathologist. When obtaining multiple consecutive samples multiple needle channels are created since the needle is fully retracted out of the body after every sampling procedure to retrieve the specimen and to reload the spring-loaded mechanism. Sampling mechanics are shown in Figure 8 and are described in detail elsewhere [90].

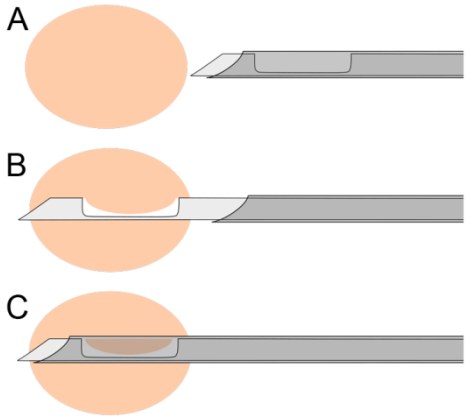

Figure 8. Illustration of CNB sampling methodology. With the aperture closed, i.e. the inner and outer cannula of the CNB are aligned, the needle is inserted towards the suspicious lesion (A). The inner needle incorporates a needle tip of around 5 mm. It is fired approximately 20 mm into the lesion (B). The firing of the inner needle triggers advancement of the outer cannula to sever the tissue sample trapped inside the aperture (C). The biopsy needle is subsequently withdrawn from the breast and the outer cannula is retracted to expose the obtained tissue sample.

Up until the early 1990s image guided percutaneous large core breast biopsy was still considered experimental or potentially fraught with problems. Data published in 1993 [91] is considered pivotal in introducing large gauge needle biopsies as an alternative to the gold standard of surgical biopsies into widespread clinical practice. The analysis of 6152 CNB procedures performed in a total of 20 American institutions concluded that percutaneous CNB was a reproducible and reliable alternative to diagnostic surgical biopsy.

By now comprehensive data from numerous studies has demonstrated that the diagnostic accuracy of CNB is generally high with only around 2% of cancers missed [76]. However,

this technique does underestimate the severity of the disease with relevance in the mammographically guided setting. Atypical ductal hyperplasia (ADH) and DCIS underestimation rates, i.e. ADH and DCIS lesions that are found to be carcinoma or invasive carcinoma at surgery, are 24–36% and 29–43%, respectively [76, 92-94]. These limitations essentially result from the limited amount of material available in a core and uncertainties over whether the sample is representative [95]. See Table 4 for an overview of biopsy performance.

In an effort to overcome the shortcomings associated with CNB, the concept of vacuum assisted biopsy (VAB) was first presented in 1996 by Burbank et al. [96]. Vacuum assisted biopsies are based on CNB methodology, but additionally apply vacuum to prolapse tissue into the aperture. Much like CNB, the VAB method uses the interplay between two cannulas to cut the tissue. The outer cannula consists of a hollow needle with a side aperture and a solid tip. An inner cannula rotates and extends across the aperture to acquire targeted tissue. Throughout this process, vacuum is created inside the device to assist in pulling tissue into the aperture. In combination with larger diameter needles as compared to CNB, this allows for a higher sampling yield and for multiple cores to be taken in a continuous manner with only a single needle insertion. See Figure 9 for details. DCIS and ADH underestimation rates for VAB are reported to be 13% and 24–36% respectively.

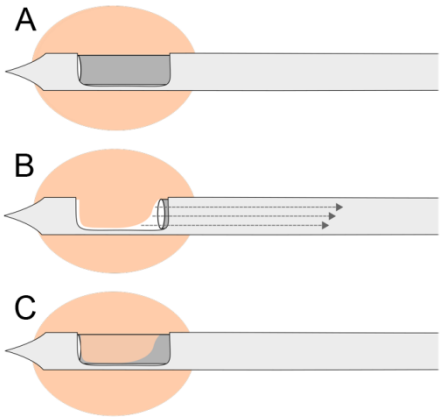

Figure 9. Illustration of insertion and sampling technique of VAB. A VAB needle is inserted to the location of interest with the aperture closed by the inner cutting cannula. The needle is inserted either by manually pushing the needle forward or via an insertion mechanism. The VAB needles incorporate solid tips of around 10 mm length (A). Once at the area if interest, the inner cannula is retracted and vacuum is activated. Surrounding tissue prolapses into the aperture (B). The inner cutting generally rotates and advances, thereby severing the tissue trapped inside the aperture (C). The obtained tissue samples are either transported into a sampling chamber at the proximal end of the cutting cannula or the needle is retracted from the patient and the sample removed after opening the aperture.

Numerous devices have been developed based on the CNB technique. In the breast, features that differentiate devices are needle length (9–11 cm), needle diameter (12–18G), stroke length (9–33 mm) and employed spring characteristics [97].

Some devices feature a semiautomatic firing mode in which the spring-trigger system is able to trigger only one spring to shoot the outer cannula (e.g. BD Achieve, Bard Marquee®). To perform a biopsy, the aperture of the inner needle is placed manually inside the target lesion and the outer cannula is subsequently fired. Some devices are completely disposable (e.g. Hologic Sertera®) while others incorporate a multi-use non-sterile hand piece employing the spring-loading mechanism that can be equipped with single-use needle sets (e.g. Bard

Modality	n studies (n biopsies), for sensitivity and specificity	Sensitivity	Specificity	n studies (n biopsies) for DCIS underestimation	DCIS underestimation probability	n studies (n biopsies) for high risk lesion under-estimation	High risk lesion underestimation probability
CNB, US-guided	27 (16 287)	0.99 (0.98-0.99)	0.97 (0.95-0.99)	14 (307)	0.38 (0.26-0.51)	21 (601)	0.25 (0.16-0.36)
CNB, stereotactic	37 (9 535)	0.97 (0.95-0.98)	0.97 (0.96-0.98)	18 (664)	0.26 (0.19-0.36)	29 (357)	0.47 (0.37-0.58)
VAB, US-guided	12 (1 543)	0.97 (0.92-0.99)	0.98 (0.96-0.99)	5 (48)	0.09 (0.02-0.26)	9 (20)	0.11 (0.02-0.33)
VAB, stereotactic	43 (14 667)	0.99 (0.98-0.99)	0.92 (0.89-0.94)	34 (1 899)	0.11 (0.08-0.14)	40 (1 002)	0.18 (0.13-0.24)

Table 4. Summary estimates of CNB and VAB performance in women with average risk of cancer as assessed by a recent comprehensive mate-analysis [98]. All numbers are median with 95% credible interval. Condensed table from [98].

Magnum®). The most frequently used needle configuration in is a needle with 10-12 cm length, 14G in outer diameter and a 15-22 mm stroke length.

Similarly, several devices based on VAB methodology are employed in the clinic. Features that can differentiate devices are needle length (9-13.6 cm), needle diameter (7-14G), and needle tips varying in length and grinding pattern.

While some lack a placement mechanism (e.g. Mammotome Elite), requiring the operator to manually insert the needle at the desired location, others employ a spring-loaded mechanism (e.g. Bard Finesse®) or electromechanic forward-thrust (e.g. Bard Vacora®). Some VAB devices are able to transport specimen into a collection chamber (e.g. Mammotome elite), thereby omitting the need for multiple needle passes when acquiring multiple samples. Certain VAB devices enable for a flexible aperture, i.e. the option to vary aperture length with regard to the clinical situation (e.g. Bard Encor). Vacuum-assisted biopsy systems can be tethered employing a console (e.g. Bard Encor) or non-tethered as a handheld devices (e.g. Mammotome Elite). While some are indicated for diagnostic procedure only, others are also indicated for the excision of benign lesions.

Complications of CNB and VAB in the breast are rare (<1%) and include bleeding, hematoma formation requiring treatment, infections and vasovagal reactions [76]. VAB may be associated with more severe bleeding than CNB [76].

Today preoperative histological confirmation of a malignant diagnosis is a cornerstone of breast cancer care [99]. Compared with diagnostic surgical biopsies, minimally-invasive biopsies using CNB and VAB imply lower costs, resource requirements, rate of side effects and re-operations combined with improved patient satisfaction and cosmetic result, while maintaining high diagnostic accuracy [76]. Patients with a conclusively benign diagnosis in concordance with imaging and clinical examination are spared an invasive surgical procedure. In patients with a malignant finding, histopathological analysis

can differentiate *in situ* from invasive lesions which facilities surgical planning [100]. Furthermore, preoperative histopathologic analysis of tissues samples enables determination of prognostic (grade, Ki67) and predictive tumor characteristics (ER, PgR, HER2), crucial for adjuvant therapy planning and can help determine the need for preoperative therapy.

3.2.2 Modalities for image-guidance

While it is generally recommended to obtain a biopsy using the guidance modality which best depicts the suspicious lesion, US guidance is preferred due to its numerous advantages [13] including lack of ionizing radiation, lack of contrast injection, use of readily available non-dedicated equipment, accessibility of the entire breast and axilla, accuracy of the procedure under real-time visualization, low cost and high comfort due to no compression and supine patient positioning [91, 101]. See Figure 10 for image of an US room.

For lesions that are only visible under mammography a stereotactic biopsy is performed [102]. For lesions only visible under MRI, dedicated breast biopsy coils are available. Most palpable lesions, as well as lesions detected at mammography or MRI, can be assessed using US-guided biopsies [69]. The number of ultrasound-guided interventional procedures has increased in recent years and US is now the primary biopsy guidance modality in many breast imaging centers [69].

While state-of-the-art US equipment could visualize only around 25% of mammographically detected non-palpable masses in the 1980s [103] this number has increased to 76% only a decade later [104]. Equipment used after the turn of the century was able to detect lesions down to 2 mm in diameter and enabled confident characterization of lesions down to 5 mm [104]. With US quality continuously improving, it is now pushing into the realm of microcalcifications with known mammographic location [105].

Sonographically guided biopsies of small lesions [106], deep lesions [106], lesions adjacent to silicone implants, tumors near the skin and chest wall, calcified lesions and mobile lesions such as small fibroadenoma [106] are described as challenging. In patients with dense breasts or fibrosis, tissue may be difficult to traverse [106]. Core needle biopsy in the axilla is largely described as safe [107, 108]. However, due to the vicinity of the lymph nodes to blood vessels and nerves, it poses challenges and limits the practicability of currently used biopsy devices [108]. Puncture of the wall of a vein or artery could result in heavy bleeding [109] suggesting that a biopsy device with controllable needle progression would be safer to use [110].

All of these are generally cases where the lesion is challenging to reach and to target, cases that result in the lesion being pushed aside by the biopsy needle resulting in empty or non-representative samples and lesions in the vicinity of delicate structure.

Figure 10. Ultrasonography room where US-guided biopsies are performed.

3.2.3 Tumor cell dissemination

"Tumors should never be harpooned, nor should pieces ever be excised from malignant tumors for diagnostic purposes."[2] *[111]*

Historically, seeding of needle tracks with malignant cells and the clinical relevance thereof has been a concern with all diagnostic procedures in the breast, including fine-needle aspiration biopsies and needle-localized excisional biopsies [112-114].

3.2.3.1 Prevalence of epithelial displacement

A plethora of studies is available that use histopathology to demonstrate epithelial cell displacement by CNB procedures. The percentage of needle tracks showing cancer cells ranges from 0 to 69% across reports [115-124]. The wide range may be due to variable methods (surgical specimen assessment versus needle washings), benign and malignant cell displacement, cancer biology and differences in methodology and sampling technique.

Generally, the risk of finding displaced cancerous cells is shown to increase due to the following: Multiple needle passes [120, 125], longer duration of the biopsy procedure [124], a shorter interval between CNB and surgical excision [115], and the use of CNB compared with VAB [115, 124]. Furthermore, risk is increased for carcinoma of higher grade [126], ductal [120, 125, 126] and papillary histology [126].

3.2.3.2 Adverse effects on histopathological assessment and staging

The presence of displaced tumor cells by CNBs is well accepted in the context of potential diagnostic pitfalls in the histologic evaluation of excised specimens [117, 118, 122, 127].

Difficulties may arise in histological assessment of resected specimen particularly in case of DCIS, whereby displaced epithelium can lead to misdiagnosis by resembling stromal invasion [125, 126, 128, 129]. This histologic misinterpretation can erroneously transform a diagnosis of DCIS into a diagnosis of infiltrating ductal carcinoma.

[2] Surgeon William S. Halstedt, pioneer in breast surgery (1898).

Similarly, there has been uncertainty over the significance of displaced malignant cells present within draining lymph nodes. One alleged benefit of sentinel lymph node biopsy (SLNB) is that it allows for a more thorough evaluation of those few lymph nodes with the highest risk of showing metastasis. While lymph nodes obtained during axillary lymph node dissection (ALND) are cut in half and analyzed using Hematoxylin and Eosin (H&E) staining, SLNs can undergo serial sectioning using both H&E and IHC. Pathologists can identify small-volume micrometastasis (< 2mm) and even isolated tumor cells [130]. Authors have reported procedure-related displacement of epithelial cells in lymphovascular spaces [117, 131], ALNs [132] and SLNs [133].

Several large retrospective studies evaluated the association between method of biopsy and metastasis in the SLNs with somewhat conflicting results.

In a retrospective review of 4016 SLNB procedures, multivariate analysis showed that IHC-positive SLN was significantly associated with the method of biopsy. Immunohistochemistry-positive SLNs were present in 1.2%, 3.0%, 3.8%, and 4.6% of patients that had pre-SLNB and had undergone no previous biopsy, FNA biopsy, CNB, or surgical biopsy respectively. This was not the case for H&E–positive SLN [134]. These findings were confirmed in a retrospective analysis of 537 SLNB procedures, whereby IHC-positive SLN was significantly higher in patients undergoing excisional biopsy than CNB (13% vs. 8%). The H&E–positive SLN did not correlate with the method of biopsy [135].

In contrast a retrospective analysis of 1890 patients reported that neither H&E nor IHC positive SLN were associated with pre-operative biopsy [136]. A further retrospective study of 663 patients showed borderline significance for a higher rate of H&E-positive SLN when comparing CNB with excisional biopsy (odds ratio: 1.484; 95% confidence interval [CI] 1.018–2.164) [137].

Some authors have suggested that occult micrometastasis does not represent a step in the natural biology of breast cancer [135, 138] and indeed the clinical relevance of small-volume disease in the axilla has been the subject of considerable debate [139-141] but is beyond the scope of this work.

3.2.3.3 Tumor growth initiated by displaced cells

Epithelial cell displacement does not necessarily transform into malignant tumor growth [125]. The effects of adjuvant treatment, especially radiation therapy and the host immune response likely counteract the formation of clinically relevant tumor development. The clinical significance of these displaced cancerous cells is therefore the subject of considerable debate [115]. The two most recent reviews on this topic [142] [143] could not unequivocally conclude if tumor cell displacement is associated with later recurrence since possible correlation between recurrence and prior biopsy tract remains largely unrecognized.

Isolated cases and small series of clinically relevant tumor growth occurring along the needle tract with strong evidence of association with CNB, can be found in the literature.

Four reports describe a total of six cases [116, 119, 144, 145]. An early report from 1994 describes one case of pathological manifestation of cancer along the needle track [119]. In 2001, two cases are reported which manifested as image-detected local recurrences 12 and 17

month post-biopsy and one case of cancer in the dermal scar 1 month post-biopsy which was apparent on post-resection pathology. All three patients had undergone modified radical mastectomy [116]. In 2011 an image-detected needle tract seeding 3 month post-biopsy on pre-operative MRI was reported [144] and in 2016, a skin recurrence detected at the site of biopsy 5 months prior in a patient undergoing adjuvant chemotherapy [145]. None of the patients had undergone radiotherapy prior to detection of the tumor growth.

Four studies report a total of 15 cases and aimed to assess incidence rates as part of a larger cohort or retrospective analysis [125, 146-148].

In 2006, three cases of recurrences were reported after skin-sparing mastectomy following CNB [146]. None of the patients had received radiotherapy and recurrences were clinically detected at 16, 22 and 23 months after biopsy. These patients constituted 3/11 cases that had undergone skin-sparing mastectomy following CNB over a 4-year period at the reporting institution.

In 2011, two cases of image-detected malignancies were reported along the needle tract detected on routine follow-up at 15 months and 2 months post-biopsy on pre-operative MRI respectively [147]. Both patients had not undergone radiation therapy prior to detection of the recurrence. Retrospectively reviewing 298 malignant results from percutaneous biopsies over a 1-year period, the authors concluded an incident rate of 0.7%.

In 2017, authors from MD Anderson performed a retrospective review of 4010 patients where neoplastic seeding following image-guided needle breast biopsy was found in eight cases (0.2%) [125]. Intriguingly, tumors with aggressive biology, i.e. Grade 3 (three cases) and triple-negative subtype (five cases), were overrepresented. The mean time to detection of seeding was 60.8 days after the biopsy (SD 43.9 days, range 34 to 165 days). Only one of these cases had undergone radiation therapy prior to detection of neoplastic seeding. See Figure 11 for example of image-detected seeding.

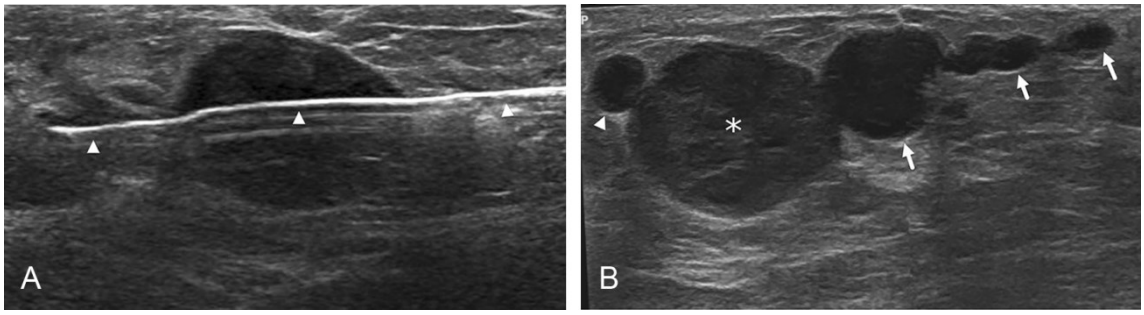

Figure 11. (A) US-image of 14G CNB biopsy of an oval, circumscribed mass confirming invasive papillary carcinoma and DCIS. (B) Follow-up ultrasound showing multiple hypoechoic masses extending laterally (arrows) and medially (arrowhead) from the malignant lesion (asterisk). Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer, Breast Cancer Research and Treatment [125].

These findings prompted the authors to assess the incidence of needle-track seeding in a cohort of triple negative breast cancer patients enrolled in a neoadjuvant therapy trial involving serial biopsies of the index breast lesion. In 1.4% (2/144) of patients, evidence of needle track seeding was detected on follow-up US. The authors concluded a low incidence rate and suggested the impact of serial biopsies on long term outcomes in triple negative patients to be determined in further studies [148].

3.2.3.4 Impact on local recurrence rates and outcome

Recent comprehensive reviews [142, 143] were not able to reliably conclude whether needle tract seeding is associated with increased local recurrence or worse outcome.

Four retrospective studies compared the impact of CNB on recurrence rate with no biopsy [149, 150] and excisional biopsy [151, 152].With a total of 1881 patients and follow-up times of 29–78 months none of the studies could find a significant difference in recurrence rates.

One retrospective study compared survival in Stage I and II patients that had (n =189) and had not (n =530), undergone pre-operative CNB and could not find increased mortality rate associated with this procedure. With a median follow-up of 78 and 71 months in the two groups, respectively, no death occurred in the group that had undergone biopsy, while the mortality rate in the other group that had not undergone biopsy was 4.7% [149].

Within the context of tumor growth in the needle tract, some authors have recommended excision of the needle biopsy exit site [116] or the entire needle tract at the time of definitive surgery to avoid local recurrence [113, 116, 119, 146, 153, 154]. However, this seems neither feasible nor evidently advisable since radiotherapy is generally recommended for both DCIS and invasive ductal carcinoma after breast-conserving surgery [121].

As the ability to visualize lesions is improving, the number of cases where needle tract seeding can be diagnosed preoperatively will likely increase [144]. The developments to limit surgical excision [155] and more limited adjuvant radiation approaches such as accelerated partial breast irradiation [156] or intraoperative radiotherapy [157-159] could impact the frequency of neoplastic seeding occurring [147]. This risk should therefore be kept in mind particularly in patients who have not received adjuvant radiation therapy or have triple-negative breast cancer as detection will impact final management and surgical planning [125, 142].

Percutaneous needle biopsy is frequently performed for diagnosis of other cancers, including, liver, prostate, kidney, head and neck, thyroid, lung, pancreas, brain and melanoma. To varying degrees, the risk and clinical relevance of displaced cancer cells is debated in all of these indications [160].

3.2.4 The evolving role of image-guided biopsies

When the current methods of biopsies were conceived the main goal was to distinguish a malignant from a benign lesion [76]. The role of biopsy has progressed and the standard-of-care today mandates pre-operative tissue samples from the primary tumor with extraction of information such as e.g. histological tumor type, grade, presence of invasion and receptor status. This information is crucial and facilitates pre- and intraoperative planning, improves cosmetic and oncologic outcome and shortens surgery time [161-163].

Several significant and interrelated developments associated with the introduction of population-based screening programs, new treatment paradigms and the emerging era of precision medicine are likely to result in (i) expanding indications for image-guided biopsies and more challenging procedures as well as (ii) increasing demand on tissue quality and quantity.

3.2.4.1 Expanding indications and increasingly challenging procedures

Shift in tumor sizes due to screening programs

The introduction of population-based breast cancer screening programs has seen an increase in the incidence rate and overall frequency of small and non-palpable tumors [31, 164]. Comparing pre- and post-screening periods, the incidence rate of detected tumors <1 cm increased by 53% [31]. The overall proportion of invasive tumor <2 cm increased from 32% to 45% [31].

Routine pre-operative determination of nodal status

Evaluation of the ALNs is an important part of staging and patient selection for adjuvant systemic therapy. Axillary staging for breast cancer has undergone a paradigm shift towards less invasive methods of assessment and surgical management Patients with clinically and radiologically negative axillary nodes proceed to SLNB.

However, if morphologically abnormal lymph nodes are identified during routine sonographic assessment, US-guided lymph node sampling is recommended. Patients with confirmed axillary nodal disease can directly proceed to ALND due to increased likelihood of high nodal disease burden, or may proceed to NACT. Ultrasound-guided CNB has been shown to have higher diagnostic accuracy compared to FNA for axillary staging in sonographically suspicious ALNs [165].

Sequential biopsies to guide treatment and monitor response

Pre-operative treatment with endocrine therapy or chemotherapy allows *in vivo* observation of tumor treatment response [166, 167]. It permits obtaining serial biopsies of the cancer taken at different points in time during the course of pre-operative treatment, allowing for the assessment of biomarker modulations [168, 169]. This approach can be used to identify prognostic and predictive markers [170] and guide treatment decisions [171] depending on response.

Confirmation of pCR after neoadjuvant treatment

A tumor is said to have undergone a pathologic complete response (pCR) if no residual tumor cells remain as assessed by pathological examination following surgery. The pCR has been shown to be a strong predictor of long-term survival [172]. It has garnered increased interest since it was recognized by regulatory authorities as a standard efficacy endpoint to evaluate drugs given in the neoadjuvant setting in early breast cancer clinical trials [173, 174]. The extent of breast cancer surgery after NACT is recommended to include only the radiological residual area of the primary tumor [175]. Extrapolating to patients with a pCR, the need for surgery in these patients is being questioned altogether. Surgery could be safely omitted if a pCR could be identified pre-operatively. Numerous feasibility studies [176-178] have shown promising results and large scale trials [179, 180] are currently evaluating the ability of image-guided biopsies to document pCR after neoadjuvant treatment. In the neoadjuvant setting, needle biopsies as a tool to evaluate pCR could thus substitute surgical excisions, a paradigm shift in therapeutic management [181].

Biopsy of metastases to guide treatment

Primary tumors are often used as proxies for systemic disease at the time of recurrence [182]. A biopsy of breast cancer metastases can have several potential benefits. It can provide tissue or cells to confirm metastatic disease, reveal benign disease, other primary tumor and evaluate the concordance of relevant biomarkers such as ER, PgR and HER2 receptors. This constitutes information that can add to optimal management of patients with metastatic breast cancer [183-185]. There has been a reported disagreement regarding hormone receptor status in up to 30 percent of cases and regarding HER2 status in 5–10% of cases. This causes the treatment strategy to change in every sixth case [186-192]. Identifying targetable genomic alterations in metastases could identify subpopulations of patients who will benefit from approved targeted agents [193, 194].

3.2.4.2 Increasing demand on tissue quantity and quality

Pretherapeutic tumor assessment

Neoadjuvant treatment is increasing [16]. In the framework of neoadjuvant treatment the only possibility to perform a comprehensive baseline assessment of primary tumor characteristics is in histologic samples provided by image-guided biopsy [100].

Impact of tumor heterogeneity

Histopathological assessment of mixed-type tumors by image-guided biopsy could be incorrect due to the inability of biopsy needles to obtain samples from all parts of the tumor [76].

Spatial heterogeneity can impact immunohistochemically analysis of ER, PR and HER2 in spatially separated tumor samples sometimes associated with heterogeneity in morphology [13, 195-197]. Proliferation markers such as Ki-67 are likewise subjected to substantial intra-tumor heterogeneity [198] with higher expression in certain hot-spots and in the tumor margins [199]. Intratumor heterogeneity of ER in itself is shown to be a prognostic factor especially in luminal A tumors [200].

Intratumor genetic heterogeneity and clonal evolution have been subject to intense research since the seminal publication by Gerlinger et al. [201] which reported on genetic heterogeneity and branched evolution using next-generation sequencing (NGS) data. Comprehensive data from breast carcinoma shows that biopsies often fail to identify a wide range of subclonal driver alterations [202]. In multifocal tumors significant genetic heterogeneity was shown despite identical clinical characteristics of foci. With the number of approved targeted agents growing, it is suggested inadequate to only characterizing the largest lesion for the optimal management of multifocal breast lesions [203].

A sequencing-based treatment strategy relies on a representative tissue sampling of the tumor. Tumor heterogeneity poses a challenge to personalized medicine since subclones with distinct sensitivities or resistances may exist within a tumor [202]. It is suggested that analysis of multiple samples is necessary to compensate for heterogeneity [204].

Increase of tissue based biomarkers

Development and advances in pathologic techniques, including IHC staining, fluorescence *in situ* hybridization, polymerase chain reaction and gene sequencing contribute to the development of new biomarkers [205]. Testing for biomarkers ER and PgR for predictive endocrine sensitivity and prognosis and HER2 for predicting the response to anti-HER2 therapies are currently used in clinical practice. Assessment of ER, PR and HER2 is recommended to be performed on recurrent lesions [13] if feasible. Ki67 may be used as a clearly established factor determining prognosis.

Recently, several tumor biomarker essays have been proposed for predicting outcome in patients with newly diagnosed primary invasive breast cancer, these include MammaPrint, EndoPredict, Oncotype DX, Breast Cancer Index, urokinase plasminogen activator, plasminogen activator inhibitor-1 and the Prosigna Breast Cancer Prognostic Gene Signature Assay (PAM50 test) [206]. Many of these are currently being validated in prospective clinical trials. Oncotype DX and Mammaprint have already demonstrated clinical utility for determining prognosis and aiding decision-making. These tests can identify patients with low genomic risk which, although indicated by clinical–pathological parameters, do not benefit form additional chemotherapy [207, 208]. Recommendations for use are currently being implemented into clinical guidelines [206].

Image-guided biopsies need to deliver tumor rich specimen adequate for NGS testing. Delivered breast biopsy specimen have been reported to be inadequate for NGS in 43.2% [209] and 38% [193] of cases, the main general reason being low percentage of cancer cells [193]. In addition, emerging immunotherapies targeting immune checkpoint molecules will require the assessment of additional predictive molecular markers in cancer tissue specimen [210].

Research biopsies and tissue biobanks

Given that the Food and Drug Administration requires targeted therapies to be accompanied by diagnostic tests for adequate patient selection [211] the importance of image-guided biopsies in oncologic trials increases [212, 213]. Lack of adequate tissue samples has been reported to be one of the most important hinders to the development and validation of biomarkers [214].

A further drive in requirements on tissue quality and quantity result from increasing interest in storing biopsy tissue for future clinical or research purposes [205, 215] and possibly from establishing biobanks of metastatic biopsy specimens which are important for investigating mechanisms of metastasis [183].

4 TREATMENT OF EARLY-STAGE BREAST CANCER

"Perhaps the day will come in the new millennium when we can offer a woman not only minimally invasive diagnosis but also minimally invasive treatment of her breast cancer."[3] *[216]*

All breast cancer patients should be discussed in a multidisciplinary conference both pre- and postoperatively according to Swedish guidelines [13]. Multidisciplinary conferences were established in Sweden several decades ago and comprise a breast surgeon, breast oncologist, breast radiologist, pathologist and a contact nurse. Based on comprehensive diagnostics as described in the previous chapter, a treatment recommendation is given. Treatment can encompass surgery, radiotherapy, chemotherapy, endocrine treatment and molecular targeted therapies. Treatment decisions consider disease stage, tumor characteristics, comorbidities, and patient preferences.

Advances in treatment during the last decades has contributed greatly to increased patient survival. Personalized therapy avoiding both over and undertreatment has now become a major focus [217] and predictive tests to guide adjuvant treatment decisions are being developed [218].

4.1 SURGERY

In 1882, the first radical mastectomy was performed. The whole breast as well as the underlying pectoralis major and minor muscles together with most of the ipsilateral ALNs were removed [111]. This extensive procedure proved to be successful in reducing rates of local recurrence and was globally adopted as a standard procedure for decades to come. It was, however, associated with immense morbidity such as disfigurement, pain, lymphedema, restricted shoulder mobility and sensory loss [111].

It was only in 1948 that significant progress was reported in decreasing postoperative morbidity and breast disfigurement by preserving the pectoralis major muscle, a procedure known as modified radical mastectomy [219]. Discontent with the morbidity of mastectomy, increased understanding of tumor biology and metastasis, along with the increased detection of smaller localized tumors and development in adjuvant therapy [220] led to the introduction of breast-conserving surgery in the 1960s.

Breast-conserving surgery combined with radiotherapy resulted in higher local recurrence rates but achieved survival rates comparable to mastectomy in randomized multicenter trials with long term follow-up [221, 222]. Breast conserving surgery has been widely adopted. Today in Sweden, 83% of patients with invasive cancer <3 cm undergo a breast-conserving surgery for their primary breast cancer [16].

[3] Laura Liberman, pioneer in minimally-invasive breast interventions, in her Centennial Dissertation for the American Roentgen Ray Society (2000)

From the beginning of the 1990s significant advancements were made in reducing morbidity associated with surgical ALN procedures. Metastatic ALNs have historically been an important prognostic factor.

Complete ALND is performed to stage the patient, to improve regional control and guide adjuvant treatment decisions. Axillary lymph node dissection implies the removal of ipsilateral lymph nodes underneath the pectoralis minor muscle. It is associated with morbidity such as lymphedema, shoulder dysfunction, pain, and paresthesia [223, 224]. Furthermore, women presenting with clinically node-negative disease show nodal metastases in only 20–35% of cases [225-227]. Many patients were thus subject to morbidities without gaining a therapeutic benefit.

In 1994 a less invasive approach for axilla management was introduced with the concept of the SLN. The sentinel node is defined as the first lymph node draining directly from a tumor. In respect to tumor infiltration it is considered representative for the status of the lymph node region. After initial findings on the feasibility and accuracy were published [228], large randomized trials evaluated sentinel lymph node biopsy (SLNB) as a staging procedure for clinically node negative breast cancer. Long-term follow-up confirmed that SLNB is suitable for staging the axilla in patients with clinically node-negative breast cancer and that ALND can be omitted for a negative SLN without significant differences in overall survival [229, 230]. Its implementation reduced the risk of postoperative morbidity and unnecessary axillary dissection in node negative patients [231]. Currently in Sweden, 95% of patients with invasive cancer and clinically negative lymph nodes undergo a SLNB [16].

With improvements in systemic therapy radiotherapy, the clinical utility of ALND in patients with a positive SLN was questioned. The ACOSOG Z0011 trial was initiated to assess if ALND can be safely omitted in patients with a positive SLN. The trial enrolled patients with an invasive cancer <2 cm who underwent breast-conserving therapy and one or two positive SLNs and randomized them to ALND or no further surgery. Initial results showed that ALND could safely be omitted for this patient group [232].

Although the initial results generated controversy [233, 234] partly because the study was underpowered it did have significant impact on clinical management of women with SLN metastasis [235-237]. The results of the trial have been confirmed in long-form follow-up [232], in other trials with similar design [238-240] with results from further trials pending [241].

4.2 RADIOTHERAPY

Postoperative radiotherapy has shown to decrease local recurrence rates as well as marginally improve patient survival in a number of settings. This has been confirmed by a recent meta-analysis by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) for patients with invasive breast cancer treated with breast-conserving surgery [242] and mastectomy [243] respectively.

The Meta-analysis shows that the absolute decline in local recurrence rate and improved survival depends on the extent of the underlying risk of recurrence which in turn depends on

tumor size, tumor type, lymph node status, patient age and type of surgery [244]. It is thus important to balance overall risk and benefit for each individual patient.

Radiotherapy has shown increased cardiac mortality as well as rates of primary lung and esophageal cancer [245, 246]. During radiotherapy acute side effects such as fatigue, radiodermatitis and, swelling of the breast can occur. Post-treatment pneumonitis is seldom but can occur [247] and long-term effects on lung function are documented [248, 249]. In long-term smokers, the benefits of radiotherapy may be outweighed by the absolute risks [245]. Modern techniques in breast cancer radiotherapy reduce lung and heart radiation doses and are expected to improve the side effects profile [245].

A recent comprehensive study showed that axillary radiotherapy provides axillary control comparable to ALND but with significantly less morbidity [250] in clinically node negative T1–2 primary breast cancers with a positive SLN. The results are currently being implemented into clinical practice [13].

Biomarkers predictive of response to radiotherapy are being evaluated [251-253]. Recent data suggests a low risk patient sub-set in which postoperative radiotherapy can be safely omitted [254].

4.3 SYSTEMIC THERAPY

Systemic regimen can be given in an adjuvant or neoadjuvant setting [255]. Adjuvant therapy is given in addition to primary surgery with curative intent. Generally patients with an estimated 10-year relapse risk of >10% are seen as candidates for neoadjuvant or adjuvant chemotherapy [217]. Triple negative tumors (i.e. ER/PR/Her2-negative) are generally indicated for chemotherapy. Patients who are HER2-positive are generally indicated for chemotherapy together with HER2-targeted monoclonal antibody trastuzumab. Hormone-positive, HER2-negative tumors are indicated for endocrine therapy and depending on criteria such as proliferation, tumor grade, age or lymph node involvement also for chemotherapy. Multigene assays can be used to for risk assessment and prediction of chemotherapy response.

4.3.1 Chemotherapy

Chemotherapy has been in use since the 1960s, initially only in patients with breast cancer recurrence [256]. Recent comprehensive individual-patient data meta-analysis by the EBCTCG have given good insights into the long-term efficacy of various cytotoxic regimes [257, 258].

Standard CMF (the combination of three chemotherapy drugs: cyclophosphamide, methotrexate and 5-fluorouracil) has shown to decrease 10-year breast cancer mortality compared with no cytotoxic treatment (risk ratio [RR] 0.76)[257]. Anthracyclines based regimes are more efficient than CMF in decreasing 10-year mortality (RR 0.80)[257]. The addition of taxanes into anthracycline-based regimes further improves efficacy and resulted in a 5-year breast cancer mortality decrease (RR 0.88)[257].

Modern regimes can reduce 10–year risk of breast cancer mortality by about a third [257]. Proportional reduction of breast-cancer mortality is essentially independent of nodal status,

age, tumor diameter, tumor differentiation or ER-status. A low absolute risk implies a low absolute benefit.

Initially NACT was only used in patients with large, locally advanced or inflammatory breast cancer to reduce the tumor load in order to enable or minimize surgical procedure. In a meta-analysis by the EBCTCG that assessed data of 4756 women with early breast cancer from ten randomized trials [258], NACT has shown to achieve equal 15-year breast cancer mortality compared with adjuvant chemotherapy, but resulted in an increased frequency of breast–conserving surgery (rate ration 1.28).

A partial or complete clinical response was seen in more than two–thirds of women allocated to NACT [258]. A pCR after NACT, defined as a lack of residual invasive tumor in the breast and axilla, has been associated with improved disease–free and overall survival [172].

In 2015, 19% of Swedish patients that received chemotherapy did so in a neoadjuvant setting [16]. In Germany this number was on average 15% in breast centers in 2014 [259], but was reported to be as high as 65% in highly specialized breast cancer centers [259].

4.3.2 Endocrine therapies

Approximately 80% of breast cancer patients have ER-positive tumors, the growth of which is stimulated by hormones. Adjuvant endocrine therapy either blocks or lowers the circulating endogenous hormone levels, thereby reducing both local and distant recurrences.

A meta-analysis of the EBCTCG showed that 5 years of tamoxifen reduces recurrence rates by about half and breast cancer-specific mortality by one third during the first 15 years after starting treatment [244]. Aromatase inhibitors have recently shown to further decrease recurrence rates and 10-year mortality compared with tamoxifen in postmenopausal women [260] and are now standard in this patient group. It has been shown that recurrences continue to occur 15 years after the course of 5 years adjuvant endocrine therapy [261]. Longer and combination endocrine treatment regimens are being evaluated in clinical trials [262].

4.3.3 Molecular targeted therapies

Trastuzumab is a monoclonal antibody that binds to the HER2 receptor and impairs HER2 signaling, thereby reducing proliferation. Since 2006 it is available for the treatment of primary breast cancer. Adding trastuzumab to chemotherapy has been shown to lead to a 34% relative improvement in overall survival in HER2-positive breast cancer patients [263]. Another HER2–antagonist currently studied for use in combination with trastuzumab is pertuzumab.

4.4 ASPECTS OF TREATMENT IN ELDERLY PATIENTS

In 2016 women aged ≥80 years accounted for around 13% of new breast cancer cases in Sweden and this proportion is most likely to rise due to an aging population [264].

Primary breast cancer diagnosed in elderly women more often belong to the group of biologically less aggressive tumors reflected by an increased percentage of patients with tumors that are ER–positive [265-267] genomically stable [268], show a lower grade [265] and less vascular invasion [265, 266]. However, this does not lead to a better outcome. The

risk of dying of breast cancer increases with age and the lowest relative survival is seen in the eldest age group [269]. A recent systematic review showed that breast cancer-specific mortality for women ≥80 years was higher at 5 years (25.8% vs 17.2%) as well as 10 years (32.7% vs 26.6%) [265].

Reasons for this are manifold. Breast cancer in elderly patients is generally diagnosed at a more advanced stage [266] due to non-inclusion in screening programs, possibly less self–examination and a general reluctance to act upon health–related issues due to older age.

However, older patients experience worse breast cancer outcomes irrespective of disease stage or subtype [270]. It is suggested that this patient group generally receives suboptimal treatment [253, 266, 271, 272] due to limited available evidence from clinical trials [273], a high prevalence of co-morbidity [269] ignorance of life expectancy data as well as lack of data concerning treatment preferences. At least 50% of patients in this age group present with co-morbidities, with the number and severity of co-morbidities rising with age [274].

The management of these patients is complex and requires careful balancing between risk of breast cancer death and the competing risks of death due to comorbidity and treatment–related toxicity [275] as well as taking into account factors such as logistics (e.g., transportation, finances) and psychosocial characteristics [276].

Surgery is the recommended first–line of treatment of breast cancer independent of age. Older women not undergoing surgery for their breast cancer have a higher risk of breast cancer-related death than those receiving surgery, and this risk exceeds the risk of dying from other causes [276]. A significant proportion of elderly women do not undergo surgery with the likelihood of them actually receiving surgery decreasing with age [277]. Uncertainty on optimal treatment strategies has resulted in significant international differences in the proportions of elderly breast cancer patients receiving surgery. The percentage of patients aged 85–89 that do not receive surgery is reported to be >50% in Ireland, around 25% in Switzerland, Belgium and the Netherlands and less than 5% in USA and Germany [277]. In the United Kingdom, around 60% of women aged ≥80 years do not have surgery for their breast cancer. Hence, increased mortality from breast cancer could be the result of different treatment patterns [278, 279].

Breast surgery is considered low risk and age itself is not associated with a higher risk of complication or death [280]. However, presence and severity of other diseases affects perioperative risk [281]. Older patients are at a greater risk of postoperative infections, they can often carry multi-resistant bacteria and treatment is more often costly treatment due to co-morbidity. Although co-morbidity is associated with a reduced surgery rates, it does not explain the underuse of surgery in this patient group [282].

Patients may refuse surgery mainly due to concerns on their quality of life and independence [283] or physicians may withhold surgery because of severe comorbidities and the overall health status of the patient [284].

Elderly patients rely largely on primary endocrine therapy [277] with a clear trend demonstrating an increase in the use of endocrine therapy correlating with a decrease of primary surgery in this patient group over the last two decades [285]. However,

comprehensive studies have shown that treatment with tamoxifen only is inferior to surgery in terms of progression-free survival and local control [286, 287] and not all breast cancers in elderly women are hormone sensitive. About 80% of patients initially responded to primary tamoxifen treatment, but the response remained on average only for 1.5–2 years [286, 287]. Even 80-year-old women have a remaining life expectance of 6.6–11.6 years depending on co–morbidity status [288]. Secondary surgery to achieve local control is then required at a more advanced stage of disease and at an increased patient age.

The paradigm of individualized treatment strategies taking into account biological tumor properties, co-morbidity, family support, functional status as well as patient preferences, demands new treatment options in this particular patient group.

4.5 NON-SURGICAL ABLATION MODALITIES

Efforts of treatment minization, combined with the increasing proportion of small and non-palpable lesions as well as US as the emerging method of choice for breast interventions have led to the exploration of minimally invasive or non-invasive modalities for the treatment of early-stage breast cancer. These promise better cosmesis, health-economic benefits and additional advantages such as treatment of patients in poor medical condition unfit for surgery. In general, these procedures could contribute to downsize the effect of overdiagnosis and overtreatment. Several modalities are being explored; see Table 5 for an aggregated overview.

Ablative modality	Guidance modality	Advantages	Disadvantages
Cryoablation	US	▪ Analgesic effect, pain well tolerated	▪ Cost, especially with multiple probes Time consuming
Laser ablation	US, CT	▪ Photocoagulation effect decreases bleeding ▪ Time efficient	▪ Treatment zone not well visualized without MRI
Microwave ablation	US	▪ High thermal efficacy ▪ Time efficient	▪ Relatively more complications, i.e. higher risk of thermal injury on skin / pectoralis muscle
High-intensity focused US	US, MRI	▪ Noninvasive	▪ Long treatment time ▪ Variable success rate ▪ Very high equipment cost
Radiofrequency ablation	US, MRI	▪ Tumor is preferentially heated rather than normal tissue ▪ Time efficient	▪ Ultrasound cannot measure adequately ablation progress during procedure

Table 5. Comparison of image-guided percutaneous ablation techniques. Aggregated table from [289], [290] and [291]. US = Ultrasound, CT = Computer tomography, MRI = Magnetic resonance imaging.

Cryoablation creates low temperatures inside the tumor by circulating liquid nitrogen or decompression of argon gas via an inserted applicator. A cytotoxic effect results from intracellular and extracellular ice crystal formation during a freeze cycle and vascular stasis during the thaw cycle results in cell death by ischemia. In laser ablation, thin fibres are inserted through a needle into the tumor, light emitted by a laser is absorbed by tissue and converted into heat, increasing temperature in the tumor and causing cell death. For

microwave ablation, a microwave applicator is inserted into the lesion, electromagnetic microwaves produce friction and heat resulting in celullar death by coagulation necrosis. For high intensity focused US, piezoelectric transducers create ultrasonic energy and focuse it within a small focal volume of tissue, both hear and/or mechanical effects (e.g. cavitation, micro streaming) can result. Certain phenomenon can screen or even reflect focal volume, a common problem. Friction effects result in a rapid rise of temperature leading to coagulative necrosis.

For an RFA treatment procedure, a pad electrode is often attached to the patient and a needle electrode is inserted into the tumor under image-guidance. Radiofreqency ablation in solid tumors generally employs medium-frequency current paths with a frequency of 460–1500 kHz. When the radiofrequency (RF) current is passed between electrodes the molecular ions induce vibration resulting in frictional heating. The heating is highest in close proximity to the needle electrode where current density is highest. The generated heat results in the destruction of cells by induction of protein coagulation [292].

Radiofrequency ablation is generally considered be the most promising modality since it has shown high success rates in several studies, low complication rates and the procedure only takes a short time [293].

The modality does have advantages related to the difference in tissue properties between a tumor and surrounding tissue with high fat content. High fat content is known to interfere with heating during RFA [294, 295]. Difference in impedance is well studied [296-299] and is known to result in a preferential heating of the tumor compared with surrounding tissue with varying degrees of fat. Lower thermal conductivity of fatty surrounding tissue significantly further increases temperatures within a defined ablation target and some authors have coined the term “oven effect” [300, 301].

There is ample experience in the clinical use of RFA in the treatment of cancer. It is used in a curative setting for patients with unresectable hepatocellular carcinoma and is an emerging treatment option even in resectable cases [302]. Radiofrequency ablation is an available clinical tool for the treatment if inoperable and high-risk operable patents with lung cancer [303] as well as high-risk surgical patients with small renal carcinoma [304]. The superficial location of the breast in combination with no adjacent organs renders it an ideal case for the application of RFA [305].

However, the technique is still in the early stage of development and several issues need to be adressed before it can expect widespread acceptance as a curative modality in early breast cancer. Issues pertain to patient selection, adequate assessment of ablation completeness, improvement of technical efficacy and determination of feasiblity in an outpatient setting.

The confirmation of complete ablation using histological methods remains difficult. Standard H&E staining is reported to be unreliable [306], generally underestimating tissue damage when performed shortly after treatment [307, 308]. Both mammography and US have been reported unsuitable to radiologically assess treatment outcome [309, 310]. Evidence of using MRI for assessment is limited [311, 312].

The force of the electrode during insertion can easily deform soft breast tissue which may lead to tumor dislocation [313, 314]. It has been found that even a slight error in positioning the electrode would result in significant mismatch in the shape of ablation volume produced during RFA application [315] and outcome is thus highly dependent on precise placement of the electrode into the geometric center of the tumor [315]. Some authors have reported issues with electrode insertion [316], difficulty to gain visibility of all electrodes during US-guidance [317], and deployment near the skin [312].

Finally, most reported studies were performed under general anesthesia resulting in a lack of evidence on RFA in breast cancer in an outpatient setting under local anesthesia with some authors questioning feasibility altogether [318].

5 AIMS OF THE THESIS

The aim of this thesis was to develop and validate new technologies for minimally invasive diagnosis and treatment of breast cancer. Specific aims of respective paper were as follows:

Paper I

- Develop a biopsy system providing improved needle control during insertion and tissue sampling efficiency
- Pre-clinically validate newly developed biopsy system

Paper II

- Clinically validate application of RF pulses to a biopsy needle to counteract dissemination of tumor cells

Paper III–V

- Clinically validate RFA for the treatment of small breast carcinoma
- Successively develop technology, protocol for patient selection and assessment of complete tumor ablation
- Demonstrate feasibility of RFA in non-operable elderly breast cancer patients in an outpatient setting

6 MATERIAL AND METHODS

6.1 PAPER I

6.1.1 Biopsy system

During the course of this thesis a new biopsy system for US-guided biopsy procedures was developed which incorporates a new mechanism for needle insertion as well as tissue sample acquisition.

Figure 12. Developed biopsy system consisting of sterile single-use biopsy device and stationary base unit (A).

The handheld biopsy device consists of a hand piece that incorporates the insertion mechanism and a 14G sampling needle. A base unit supplies the biopsy device with power and controls its operation. Two oil-free piston compressors are incorporated into the base unit. One compressor creates pressurized air for the pneumatic driver and the other provides suction to aid tissue acquisition. The system is shown in Figure 12.

Applying principles of fracture mechanics [319] the means of needle insertion applied herein is based on a high acceleration leading to high velocities at short stroke length. During needle insertion into tissue, this leads to instantaneous tissue fracture and minimized tissue displacement. When this insertion mechanism is integrated into a biopsy device the movement of the needle created by the insertion mechanism in combination with manual advancement of the biopsy hand piece generates stepwise needle advancement when penetrating tissue. This is especially meaningful for procedures under US-guidance, where real time visualization of the needle is possible. The pneumatic-mechanical insertion mechanism employed in the device is detailed in Figure 13.

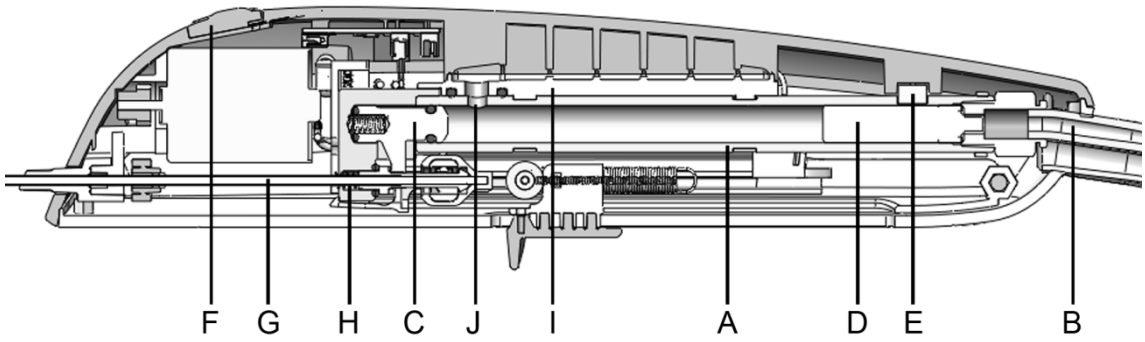

Figure 13. Cross-sectional view of the developed biopsy device. The central functional unit of the insertion mechanism is composed of a 7 cm stainless steel tube (A) connected to a flexible PVC tube (B) at the proximal end and incorporating a piston arrangement (C) at the distal end. Inside the tube, a 12 g stainless steel weight (D) is held in place in the proximal position by magnets (E). The 3 m PVC tube is connected to compressor via a high speed response solenoid valve and pressure regulator delivering pressure pulses at 4.5 bar gauge pressure. When a button at the top of the device is activated (F), a valve in the base unit opens for 50 ms and a pressure pulse traverses the tube. Pressure builds at the proximal end of the steel weight which starts accelerating as soon as magnetic forces are overcome. At the distal end of the tube the energy of the projectile is transferred to the biopsy needle (F) via the piston (C). The needle, in turn, is propelled forward. Movement is restricted to 2.5 mm before the needle is returned via a coil spring (H). As the projectile travels forward, pressure increases in the air compartment (I) that is connected to the tube via an opening (J). This pressured returns the projectile weight back to its starting point. Air compartment and magnets ensure both return to and stable positioning of the projectile at its initial position independent of device angle.

To maximize sampling yield with minimal tissue trauma a distal-tip sampling needle design is employed, see Figure 14. A distal-tip sampling needle consists of a cannula with an open needle tip. The entire inner needle volume can thus be used as a sampling cavity without the need for a solid and sharp needle tip. The axis of the steel tube and the sampling needle are offset to each other. This enables for a solid trocar to be inserted and withdrawn from the sampling needle via its proximal end, thereby blocking healthy tissue from entering the sampling needle during insertion towards the lesion.

Challenges concerning open-tip sampling needle pertain to sufficient filling of the needle with target tissue and robust separation of obtained samples from surrounding tissue [320, 321]. For the developed device, filling of the needle with target tissue during advancement is supported by rapid insertion, needle grind and applied negative pressure to the sampling needle. Tissue separation is based on a rotational needle movement and specialized needle tip design, wherein a slit increases the momentum applied during rotation. The tissue acquisition mechanism employed in the device is detailed in Figure 14.

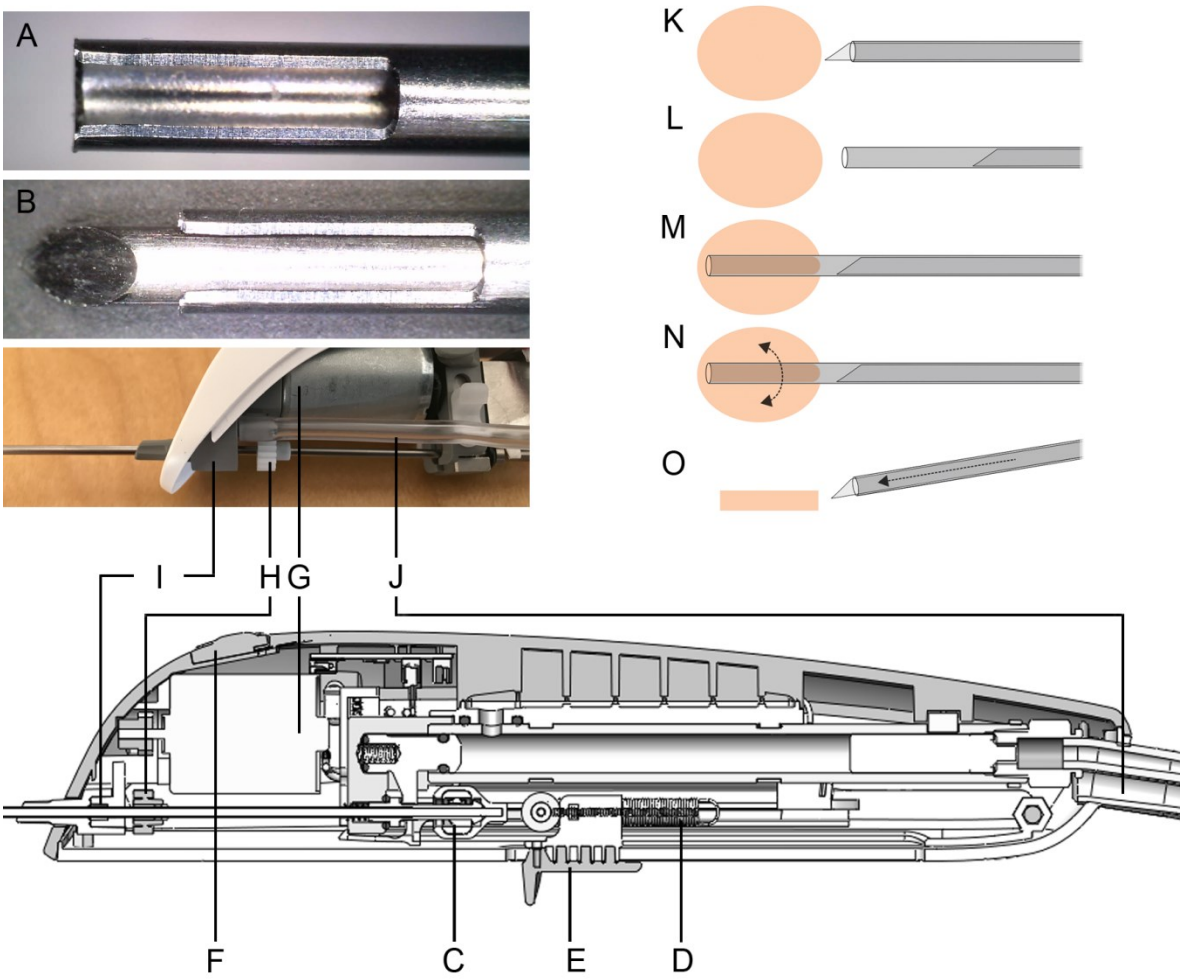

Figure 14. Details on needle design, mechanics of sample acquisition and illustration of procedure. Magnified picture of the sampling needle tip (A). Inward grind and incorporated slit can be seen. Biopsy needle tip with the trocar in its extended position (B). Trocar and sampling needle are detachably connected via a mechanism (C). Upon activation via a button on the side of the device (not shown) the trocar is released from the sampling needle and retracted 6 cm via a coil spring (D). Using a lever (E) the trocar can be pushed forward and re-attaches to the sampling needle. When a button is activated (F), an Electrical motor (G) rotates the needle via cogwheels (H) to separate the sample from surrounding tissue. Cogwheels permit for longitudinal needle movement during application of pneumatic pulses. The suction connector (I) is connected via a PCB tube (J) to a compressor generating negative pressure. Suction is transferred to the needle via a number of small holes in wall of the sampling needle. The suction connector allows for longitudinal and rotational needle movement. Illustration of sampling procedure is given by (K)–(O). The needle is advanced towards the lesion through healthy tissue with the trocar in extended position (K). Once the needle tip has reached the lesion, the trocar is retracted and the open-tip sampling needle is exposed (L). Vacuum suction is activated and a negative pressure of 60 kPa applied to the inside the sampling needle within 5 seconds. Pulses are applied to stepwise insert the needle into the tumor and during that process fill the open-tip sampling needle with tumor tissue (M). Theoretically, the incorporated needle design enables arbitrary sample length depending on insertion length. By activating a button at the top of the device (F) a rotational pattern is applied to the needle (50 ms clockwise followed by 50 ms pause and subsequent 50 ms counter clockwise turn) to separate the tissue inside the sampling from surrounding tissue (N). The biopsy needle is withdrawn from the breast. The trocar is subsequently extended via a lever to gradually eject the tissue sample from the sampling needle (O).

6.1.2 Performance assessment

To evaluate needle dynamics a test bed was developed consisting of a fixture for the biopsy hand-piece and a sensor unit (see Figure 15). The sensor unit incorporates two light emitting diodes and complementary metal-oxide semiconductor (CMOS) sensor (S11105, Hamamatsu Photonics). The setup enables readout with a framerate of 83.3 kHz at a spatial resolution of 12.5 µm. Ten measurements were performed for respective setting. 1.5, 2.5, 3.5, and 4.5 bar gauge pressure.

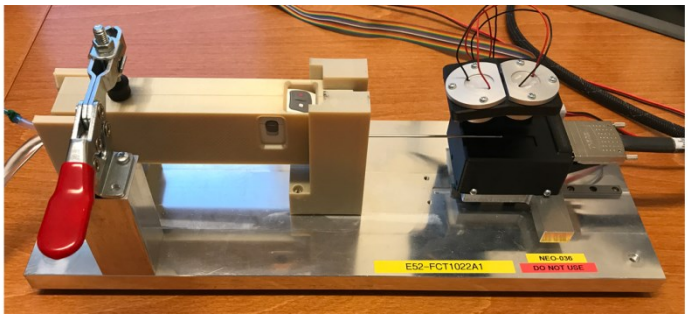

Figure 15. Developed test bed.

Sample performance testing was performed in turkey breast, swine pancreas and calf thymus which reflect a wide range of tissue properties. The tissue models were placed in a transparent test box with in the wall to allow for sampling through the side wall. A 280 g lid was placed in the tissue model for stability. To compare performance with a well-documented and routinely used device a Bard Magnum biopsy device equipped with 14G needles was used. The device was configured for a 22 mm stroke length. The developed biopsy device was inserted 22 mm to facilitate comparison.

Tissue samples obtained with current CNB and VAB methodologies can contain crush artifacts [127], be fragmented [322] and occasionally completely inadequate for histological assessment [322]. The tissue sample obtained by the new device is subject to distinctly different forces when the needle is inserted into the tumor, the sampling needle is rotated and the sample subsequently ejected. Therefore, to rule out any systematic sample quality issues, samples were obtained from resected breast specimens and histopathologically analyzed by an experienced pathologist.

Approval of the Regional Ethics Committee was obtained. Under ethical permit 2013/705-31/2 and amendment 2014/824-32 resected breast specimens were obtained from patients with histologically confirmed breast cancer ≥15 mm in size. Postoperatively, specimens were immediately transported the pathology department. Specimens were cut in the plane of the tumor. Under the supervision of a pathologist the device was used to obtain samples from the exposed tumor in a total of 11 specimens. After routine histopathological preparation tissue samples were analyzed by an experienced pathologist.

6.1.3 Statistical analysis

For comparing the sampling yield between the two biopsy devices an independent samples t-test was performed using SPSS statistics Version 24 (IBM, USA).

6.2 PAPER II

6.2.1 Patients

Approval of the Regional Ethics Committee was obtained. The study was covered by ethical permit 03-416 and amendments 2007/1372-32, 2008/1026-32. All study-related procedures were performed in agreement with the ethical standards of the World Medical Association (Declaration of Helsinki). If a patient fulfilled all inclusion criteria she was given detailed information on the study. If the patient agreed to study inclusion, a written consent was obtained.

Patients presented with a suspicious lesion and were indicated for a FNA biopsy procedure. All patients were diagnosed according to standard hospital protocols. Patients were excluded if they had undergone a prior CNB procedure or NACT. Patients with confirmed invasive carcinoma as determined by post-surgical histopathology were included in analysis.

We included 57 patients at a local breast center (set 1). Subsequently, 31 patients were included at a second breast center (set 2) and underwent both routine FNA and subsequent FNA with applied RF pulses.

Patient characteristics are provided in Table 6.

		Overall		**Set 1 FNA**		**Set 2 FNA / FNA+RF**	
Number of patients		88		57		31	
Tumor size [mm], average ± SD		21.3 ± 11.9		23.7 ± 13.1		17.0 ± 7.8	
Guidance modality	Freehand	65%	(57)	100%	(57)	0%	(0)
	US	35%	(31)	0%	(0)	100%	(31)
Histological Type, frequency (number)	Ductal	78%	(69)	75%	(43)	84%	(26)
	Lobular	18%	(16)	21%	(12)	13%	(4)
	Tubular	2%	(2)	2%	(1)	3%	(1)
	Papillary	1%	(1)	2%	(1)	0%	(0)
Grade, frequency (number)	I	9%	(8)	4%	(2)	19%	(6)
	II	64%	(56)	61%	(35)	68%	(21)
	III	27%	(24)	35%	(20)	13%	(4)

Table 6. Patient, tumor and procedure characteristics.

6.2.2 Biopsy instrumentation

For patient set 1, biopsies were performed using 22G fine needles mounted on a standard syringe hand-piece.

For patient set 2, biopsies were performed using 22G fine needles. The physician held the needle at its hub and by means of flexible tubing the needles were connected to a syringe operated by a technician.

For biopsies incorporating RF pulses, biopsies were performed using specially designed biopsy instrumentation. Treatment instrumentation was reported to the Swedish Medical Products Agency (Uppsala, Sweden) under permit 461:2010/517417 for use according to

clinical study protocols. Instrumentation was tested according to internal hospital protocol prior to use. Instrumentation met the insulation demands of cardiac floating with a frequency weighted patient leakage current less than 10 µA.

Instrumentation incorporated an RF generator, control console and a specially designed 22G FNA needle as well as a dispersive pad-electrode, see Figure 16. The needle incorporates an electrically insulated shaft with a bare tip of around 6 mm length. Radiofrequency pulses were triggered when the needle was retracted. Radiofrequency pulses had a duration of 0.2 s and delivered around 6 J energy. Consecutive pulses were separated by a minimum time interval of around 0.5 s. Pulses were not applied at low needle velocities and in proximity to the skin.

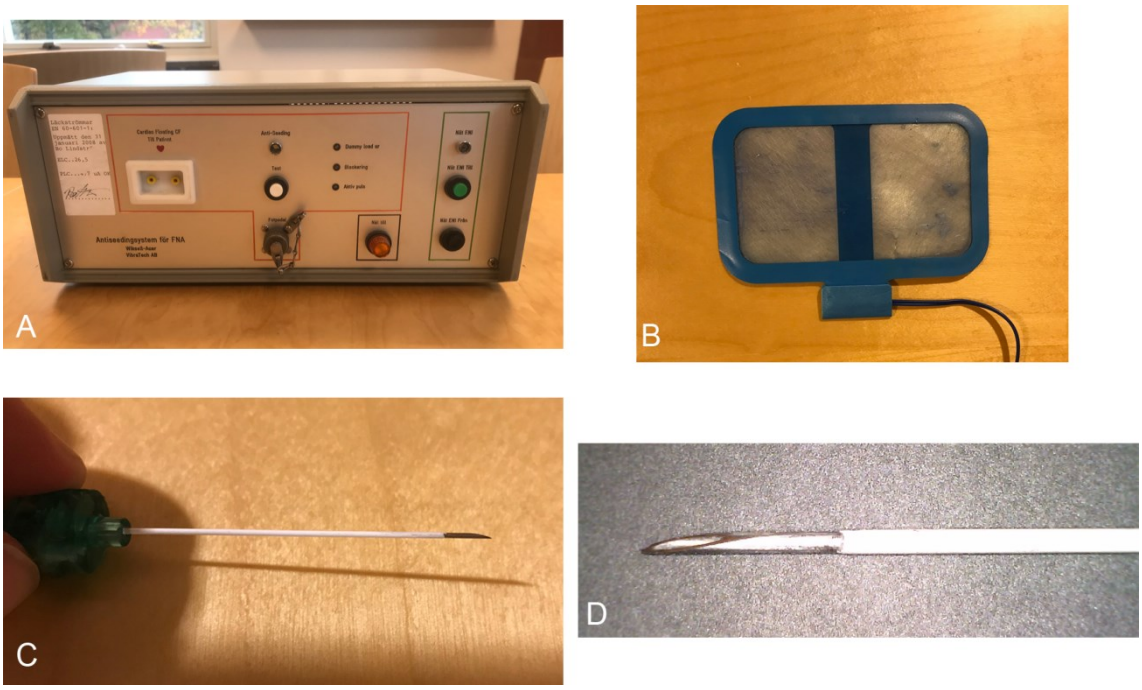

Figure 16. Images of specially design biopsy instrumentation. Controle console (A), dispersive pad electrode (B) and biopsy needle which held at the hub (C). The bare tip as well as the insulated shaft can be seen (D, 20x magnification).

6.2.3 Biopsy protocol and cytological assessment

For patient set 1, biopsies were performed by two experienced cytopathologists with freehand guidance. For patient set 2, biopsies were performed by an experienced radiologist under ultrasound-guidance. Different insertion sites were used for biopsies procedures with and without RF pulses. Biopsies were performed without local anesthesia und the needle was maneuvered back and forth inside the lesion 10–15 times.

After withdrawal of the biopsy needle from the breast, blood and/or secretion droplets emerging from the incision site were sampled on glass slides. Samples were air-dried, stained using May-Grünwald-Giemsa and analyzed by two experienced cytologists for the presence of tumor cells.

6.2.4 Statistical analysis

To compare the occurrence of tumor cell dissemination with and without applied RF pulses a two-sided McNemars' test was performed using SPSS statistics (IBM, USA).

6.3 PAPER III-V

6.3.1 Patients

Approval of the Regional Ethics Committee was obtained. The study reported in Paper III was covered by ethical permit 2005/1280-31/1 and amendments 2007/685-32, 2007/1512-32, 2008/342-32. The study reported in Paper IV was covered by ethical permit 2008/1018-31/3 and amendment 2010/963-32. Finally, the study in Paper V was additionally covered by amendment 2011/216-32. All study-related procedures were performed in agreement with the ethical standards of the World Medical Association (Declaration of Helsinki). If a patient fulfilled all inclusion criteria she was given detailed information on the study. If the patient agreed to study inclusion, a written consent was obtained.

Patients reported in Paper III had to present with a unifocal, clearly distinguishable lesion under US. Patients where the tumor was located close to the axilla were excluded due to possible interference with the SLNB procedure. Two successive patient sets were accrued. The initial five patients had a lesion <10 mm and subsequent patients had a lesion size of <16 mm.

Patients reported in Paper IV presented with a clearly distinguishable, unifocal lesion with a largest diameter of ≤16 mm under mammography, US and MRI. Exclusion criteria were among others multifocality, cancer of diffuse growth as well as surrounding DCIS, lobular and aggressive lesions, i.e. Elston Grade 3, hormone receptor negative, HER2-positive.

In accordance with ongoing trials on minimally-invasive breast cancer treatment at the time inclusion criteria were subsequently altered as per amendment to include tumors that were ≤2 cm, HR-negative, HER2-positive, Elston grade 3 and showed ≤25% of intraductal components.

Patients reported in Paper V had to comply with the amended criteria as described above and additionally be unfit for surgery due to older age, anesthetic risk (American Society of Anesthesiologists [ASA] III–IV) or refuse to receive a surgical breast procedure.

Patient characteristics in Paper III–V are provided in Table 7.

		Paper III (set 1)	Paper III (set 2)	Paper IV	Paper V
Number of patients		5	26	18	6
Age [years], median (range)		62 (46–83)	63 (49–80)	67 (46–84)	87 (85–92)
Tumor size [mm], median (range)	US	9 (7–10)	12 (6–15)	10 (6–15)	18 (8–20)
	Mammo	10 (9–10)	12 (7–18)	10 (6–15)	15 (8–20)
	MRI	n/a	n/a	11 (5–20)	13 (9–20)
Grade, frequency (number)	I	40% (2)	27% (7)	28% (5)	17% (1)
	II	60% (3)	58% (15)	72% (13)	67% (4)
	III	0% (0)	12% (3)	0% (0)	17% (1)
	n/a	0% (0)	4% (1)	0% (0)	0% (0)
Histological Type, frequency (number)	Ductal	100% (5)	73% (19)	83% (15)	83% (5)
	Lobular	0% (0)	4% (1)	0% (0)	17% (1)
	Duct./ Tub.	0% (0)	8% (2)	6% (1)	0% (0)
	Mucinous	0% (0)	4% (1)	0% (0)	0% (0)
	Tubular	0% (0)	12% (3)	11% (2)	0% (0)
ER, frequency (number)	Positive	100% (5)	96% (25)	100% (18)	67% (4)
	Negative	0% (0)	4% (1)	0% (0)	33% (2)
PgR, frequency (number)	Positive	60% (3)	85% (22)	72% (15)	50% (3)
	Negative	40% (2)	15% (4)	28% (3)	50% (3)
HER2, frequency (number)	0	80% (4)	73% (19)	83% (15)	67% (4)
	1+	0% (0)	0% (0)	0% (0)	0% (0)
	2+	0% (0)	8% (2)	17% (3)	17% (1)
	n/a	20% (1)	19% (5)	0% (0)	17% (1)
Ki67, frequency (number)	<10%	20% (1)	54% (14)	55% (10)	50% (3)
	10–20%	40% (2)	46% (12)	33% (6)	33% (2)
	20–30%	40% (2)	0% (0)	6% (1)	0% (0)
	30–40%	0% (0)	0% (0)	6% (1)	17% (1)
	>40%	0% (0)	0% (0)	0% (0)	0% (0)

Table 7. Characteristics of patients that were treated as part of Paper III, set 1 (n=5), Paper III, set 2 (n=26), Paper IV (n=18) and Paper V (n=6). Patients were classified as ER/PR positive if receptors were expressed in >10% of cells. For tumor size the largest diameter was used. Due to rounding, not all total percentages equal 100%.

6.3.2 Radiofrequency ablation instrumentation

Treatment instrumentation was reported to the Swedish Medical Products Agency (Uppsala, Sweden) under permit 461:2010/503820 for use according to clinical study protocols. Instrumentation was tested according to internal hospital protocol prior to use.

The RF instrumentation consisted of a specially designed RF generator with floating low impedance output (0–950 W, 1.5 MHz) and a dedicated treatment electrode with integrated temperature monitoring and internal water cooling system. The needle is composed of coaxial cannula design with diameters sufficient to permit a predetermined flow. Cooling flow was approx. 1 ml/s with a temperature of around 20°C. External cannulas were 1.8 mm and

1.5 mm in diameter with insulated surface except for 20 mm at the distal end. A teflon insulated constantan wire was laser welded to the outer cannula. Thermocouples to measure temperature were chosen because they can be easily miniaturized to ensure minimal cooling water flow obstruction in the thin design plus an adequate short time constant. The very low signal level obtained from the thermocouples (53 μV/°C) suffers interference from the applied RF power field, approximately 100 $V_{RMS.}$ This demands that the low-pass filtering before the amplification has very high damping. The RF signal was damped approximately 70 dB with a tuned low-pass filter. The RF power feeding and low-level feed-back circuits must further comply with a frequency weighted patient leakage current less than 10 μA. The whole temperature circuit has been built using a Faraday cage design, static screen between primary and secondary galvanic insulating windings and anode grounded system configuration. Large creeping distances and very low leakage current (conform to better than IEC 60-601-1 body floating).

Electrical impedance was measured to ensure that no self-insulating phenomenon occurred [295]. Temperature at the tip is measured directly by momentarily stopping energy and cooling flow. When the insulation phenomenon occurs during treatment the impedance increases due to local decrease of conductivity near the electrode cause by temperature dependent gas bubble formation. This increases the energy absorption in the near region even further, increasing the temperature and bubble formation in an avalanche-like manner ultimately leading to electrical insulation of the electrode.

In order to avoid this phenomenon the power level must be kept on a moderate level which in turn limits the ablation size. The coagulation zone with the monopolar regime is therefore be limited to approximately 13 mm in diameter during *in vivo* protocols in muscle tissue [323]. Several approaches have been proposed to increase lesion size by preventing insulation phenomenon. There are multiprobe electrodes [324], saline injected electrode [325] and internally cooled electrodes [317]. We have chosen an internally cooled electrode design which has been shown to generate more homogenous lesions [326, 327].

A handheld unit for pneumatically-assisted insertion of the treatment electrode was developed. The electrode was designed incorporating robust tip on the back of the electrode. The driver encorporated a bowl tip design which could be coupled to the tip at the back of the treatment electrode. The driver incorporated a steel tube of 10 cm housing a plunger weighing approx 5.5 g. The plunger was accelerated by a working pressure of 4.5 bar. The driver unit aids the operator by delivering triggered mechanical pulses advancing the electrode forward over a controlled distance. Power-assistance was used for the last two patients of Paper IV and last four patients part of Paper V. An overview of the instrumentation and procedure is presented in Figure 17.

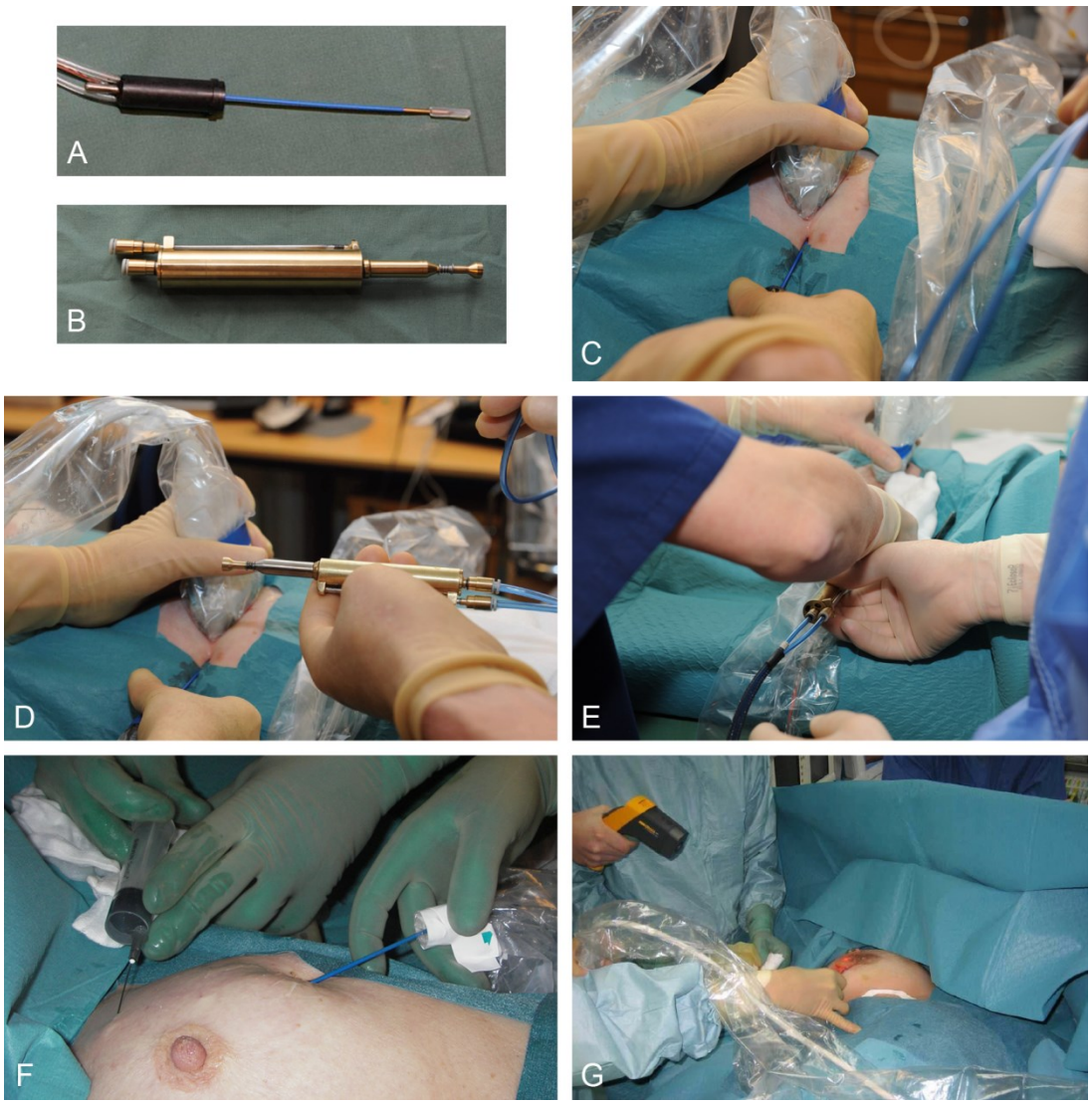

Figure 17. Images of electrodes and procedure. Treatment electrode incorporating solid tip at proximal end (A). Pneumatic-mechanical insertion device with bowl tip at distal end (B). Insertion of the treatment electrode under US-guidance (C). Aiding treatment electrode insertion by applying mechanical pulses (D,E). Injection of local anesthetics to separate tumor from skin (F). Infrared (IR) thermometer used for temperature control (G).

6.3.3 Imaging modalities

Sonographic procedures were performed using an iU22 US device (Philips, Netherlands) with transducer L17–5 (5–17 MHz frequency range), see Figure 18. Magnetic resonance instrumentation was performed using a 1.5 T Signa system (GE Healthcare, USA) with an intravenous gadolinium contrast agent ProHance (Bracco Diagnostics, Italy). Contrast enhanced ultrasound was performed incorporating an intravenous administration of Sonovue (Bracco Diagnostics, Italy), a second generation US-contrast agent that consist of sulfur hexafluoride (SF_6) within a phospholipid shell. Microbubbles have a size of 1–10 µm. A L9–3 (3–9 MHz frequency range) transducer was used.

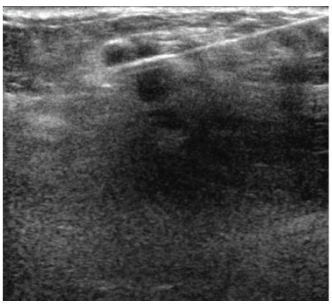

Figure 18. Placement of treatment electrode inside the lesion under US guidance.

6.3.4 Treatment protocol

In all included patients, CNB was performed prior to all RFA procedures.

For the RFA procedure, a dispersive pad electrode was attached to the back of the patient. Treatment electrode was inserted through a small incision. Images were taken in different projections to verify correct electrode placement. Tissue temperature was maintained at around 85°C for 10 minutes. The electrode was active upon retraction to counteract dissemination of tumor cells. For minimizing complication rate several measures were taken. The skin temperature was monitored with an infrared (IR) thermometer, ice-pads were used for temperature control, injecting boli of local anesthetics was performed to separate tumor from skin or pectoral muscle and sutures were attached to the skin to increase distance between tumor and skin.

Procedures reported in Paper III were performed in the operating theatre under general anesthesia. Sentinel lymph node biopsy was performed using pre-operative injection of radioisotope and blue dye labeling.

Procedures reported in Paper IV were performed under local anesthesia. The initial four patients underwent RF ablation in the operating theater, subsequent patients in the US suite of the breast center. Time between RFA and surgical resection and SLNB was a median of 14.5 days (range 6 to 22 days).

Procedures reported in Paper V were performed under local in the US suite of the breast center. No subsequent surgical resection was planned.

6.3.5 Pain assessment

In Papers IV & V, RFA was performed under local anesthesia. The patient was asked to specify pain pre-procedure, during administration of local anesthetics, during RFA treatment and postprocedure on the visual analogue scale (VAS, from 0 to 10; 0 =no pain, 10 =unbearable pain).

6.3.6 Radiological assessment

In Papers IV & V, both MRI and CEUS was performed pre- and post-RF treatment. Magnetic resonance imaging was performed using a 1.5 T Signa system (GE Healthcare, USA) with intravenous gadolinium contrast agent ProHance (Bracco Diagnostics, Italy). In a subset of patients, CEUS was performed incorporating an intravenous administration of Sonovue

(Bracco Diagnostics, Italy), i.e. sulfur hexafluoride microbubbles with a size of 1-10 μm. A L9–3 (9–3 MHz frequency range) transducer was used.

6.3.7 Histological assessment

In Papers III & IV resected specimens were sent to the pathology department for histopathological assessment. Tissue was fixed in 4% buffered formalin solution and subsequently cut into 3–4 mm slices parallel to the electrode channel. For histopathological evaluation using H&E, the sections were stained following standard hospital procedures. In Paper III Gieson-elastin stain was additionally performed. In Paper IV additional immunohistochemical staining was performed with a monoclonal antibody to cytokeratine 8 (CK8; 35betaH11, Ventana Medical Systems, Inc, USA).

In Paper V, CNB obtaining multiple samples was performed at 6 months post-RFA. Samples were assessed according to protocol described in Paper IV.

Histopathological assessment was carried out by an experienced histopathologist. A second experienced physician was consulted in case of uncertainty.

6.3.8 Technical efficacy assessment

In Paper III, complete ablation was assessed by H&E staining on the resected specimen. In Paper IV, complete ablation was assessed using MRI and a combination of histological assays for H&E and CK8. In Paper V, complete ablation was assessed using MRI and CEUS at 1 month and assays for H&E and CK8 on US-guided CNB samples were taken from several areas of the ablated region at 6 months.

6.3.9 Follow-up protocol

In Paper III patients were followed-up according to hospital protocol. Women in the study described in Paper IV underwent an additional MRI and US/CEUS evaluating post-RFA and prior to surgical resection. In Paper V, follow-up with MRI and CEUS was performed 1, 6 and 12 months after treatment. Subsequently the patients were called for clinical follow-up at intervals of 6 or 12 months depending on patient status.

6.3.10 Statistical analysis

No formal sample size calculation has been performed due to the explorative nature of the reported studies. In general, data is summarized by means of summary statistics.

In Paper IV, a Wilcoxon-Signed-Rank test (alpha =0.05) was used to compare pre- vs. postprocedure pain as well as pain during administration of local anesthetics vs. pain during ablation procedure. Statistical evaluation of pain measurements was aided by substituting missing values with median values from each measurement stage due to low sample size.

7 RESULTS AND DISCUSSION

7.1 PAPER I

The developed biopsy system showed characteristics distinctly different from currently used CNB and VAB devices.

Maximum velocity reached by the newly developed device was higher than compared to commonly used CNB devices [97, 328] and was reached over a significantly shorter stroke length. Table 8 shows biopsy needle dynamics in comparison to other needles described in the literature. Acceleration and delivered power to the sampling needle is significantly higher.

Device	Quick-Core	Magnum	New biopsy system
Placement mechanism	Mechanical spring	Mechanical spring	Pneumatic driver
Control	Manual	Manual	Automatic
Stroke length [mm]	20	22	2.5
Stroke frequency	Single-shot	Single-shot	Multiple
Needle mass [g]	3	11	3.4
Max. velocity [m/s]	15.59	8.19	21.2

Table 8. Comparison of needle dynamics. Measurement performed in air. Pneumatic driver at 4.5 bar gauge pressure.

Testing in tissue models and ex vivo specimen was performed at 4.5 bar gauge pressure. This specific pressure was chosen following empirical testing in different tissue models that confirmed a pressure level necessary to enable inertia stabilization of surrounding tissue, i.e. the sampling needle pulses forward with minimal movement of surrounding tissue. The newly developed biopsy system delivered samples with significantly higher weight in all tissue models compared to a routinely used CNB device (Bard Magnum), see Table 9. The tissue type with softest texture, calf thymus, showed both largest differences over the standard device and highest spread of values. This is likely related to the negative pressure applied to the sampling needle. Turkey breast is the most widely used tissue model for biopsy device benchmarking [329-331]. In turkey breast, results for the Magnum device were consistent with other published reports [329]. Samples obtained using the developed device were heavier than reported for 14G CNB devices and comparable to samples obtained by 14G VAB devices. Comparison with VAB devices however is problematic. Vacuum assisted biopsy devices generally employ non-circular needle assemblies. The marketed needle size often corresponds to the dimensions of the inner cutting cannula, while the overall needle diameter is significantly larger [331].

	Tissue sample weight, median ± SD [mg]				p
Tissue model	Newly developed biopsy system		Magnum		
Turkey breast	49.6 ± 8.0	n=29	14.3 ± 3.6	n=59	<0.01
Swine pancreas	45.8 ± 15.0	n=29	10.1 ± 3.8	n=90	<0.01
Calf thymus	66.5 ± 14.3	n=29	14.3 ± 4.0	n=60	<0.01

Table 9. Evaluation of tissue sampling performance in different bench models.

Thirty-eight samples were obtained from 11 specimens using the developed device (Figure 19). The pathologist judged samples to contain no significant artifacts or levels of fragmentation as to hinder histopathologic evaluation. No adverse effect of the sampling mechanism compared to current methodologies was observed.

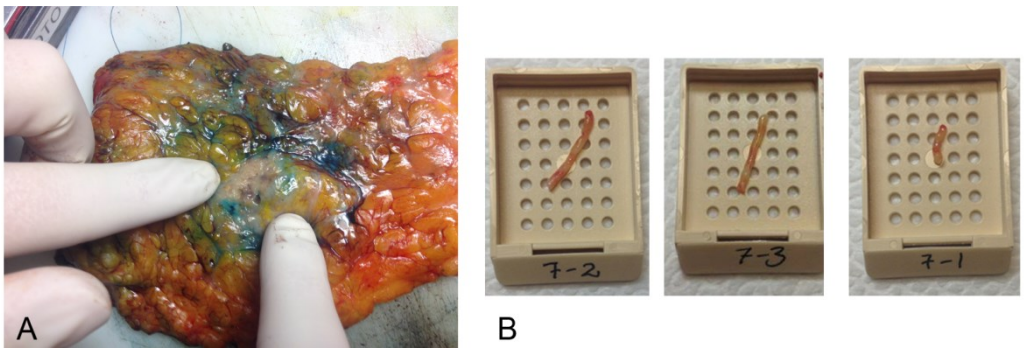

Figure 19. Resected breast specimen cut in the plane of the tumor center (A). Tumor was exposed and could be targeted without problems. Examples of obtained tissue samples (B).

Strength of this work lies in robust and conclusive pre-clinical results, namely insertion characteristic that are distinctly different form standard devices and good sample acquisition ability demonstrated in representative tissue models. The specific characteristics could enable new tissue sampling paradigms. In principle, the method could be used in all biopsies of solid tumors where currently large-diameter biopsy needle are used.

While the work demonstrated robust pre-clinical performance, only limited conclusions can be drawn about performance in a clinical setting. While bench models represent a wide range of tissue properties they can never be fully representative of human tissue. Furthermore, while we have established the absence of any systematic adverse effect on the histopathological quality of the tissue sample we have no evidence of the samples being of better quality. Neither was the ability of the device validated to obtain tissue samples of arbitrary length depending on insertion length. The pneumatic-mechanical insertion mechanism does enable for a novel way of needle insertion over currently used devices applying manual insertion or spring-loaded firing action. However, the performance in insertion precision has not been quantified. Lastly, no clinical benefits can be claimed from the presented data.

During the last decade several new biopsy technologies for US-guided histologic tissue sampling have been developed that employ novel methods of both needle insertion and tissue acquisitions. These include devices incorporating a helix-cutting mechanism (Spirotome/Coramate, Cook Medical, USA), cryo-assisted stick freeze methodology (Cassi II, Scion Medical Technologies, USA), modalities for RF-assisted excision of complete lesion (Intact, Medtronic Surgical Technologies, USA) and robot-assisted ultrasound-guided breast biopsy systems [314, 332]. Methods differ widely as does their intended clinical benefits as well as the body of available clinical documentation.

The helix cutting mechanism intends to access the lesion in a direct and frontal way and decrease patient pain. Clinically, it has merely been shown that the devices delivers good diagnostic accuracy [333, 334]. The cryo-assisted stick freeze methodology immobilizes the lesion by freezing it. The device is designed to provides highly accurate, targeted tissue sampling while delivering more biopsy tissue in fewer passes. However, there is a paucity of clinical data. Radiofrequency-assisted excision of complete lesions was developed with the goal of decreasing upstaging rates of high risk lesions. Low rates of upstaging using the device have been well documented in large clinical studies [333]. Robot-assisted methods

aim at improving targeting and decreasing needle placement error but is still in a very early stage with no clinical data available.

The data indicates that the pneumatic insertion mechanism enables for a novel way of needle insertion into the breast and axilla. Clinical studies will need to demonstrate to what extent and in which specific lesions these characteristics can transform into clinically relevant benefits such as shorter procedure time, higher diagnostic accuracy or less patient trauma when compared to existing devices.

7.2 PAPER II

After standard FNA, blood emerging from the needle incision site was easily sampled and showed varying levels of tumor cell concentration. Reduced bleeding was observed for biopsies with applied RF pulses and light mechanical pressure was applied to the breast to extract secretion. Overview of tumor cell detection is given in Table 10.

		Overall, FNA		Set 1, FNA		Set 2, FNA		Set 2, FNA/RF	
Number of procedure			88		57		31		31
Tumor cells present	Yes	74%	(65)	77%	(44)	68%	(21)	3%	(1)
	No	26%	(23)	23%	(13)	32%	(10)	97%	(30)

Table 10. Detection of tumor cells in blood/secretion emerging from the biopsy incision site.

For standard FNA, tumor cells were detected in a total of 75% (65/88) of cases overall. In patient set 2, disseminated tumor cells were detected in 68% (21/31) cases for standard FNA, and in only 3% (1/31) of cases with applied RF pulses ($p < 0.001$). Notably, the solitary case of tumor cell presence showed cells that were denatured, see Figure 20.

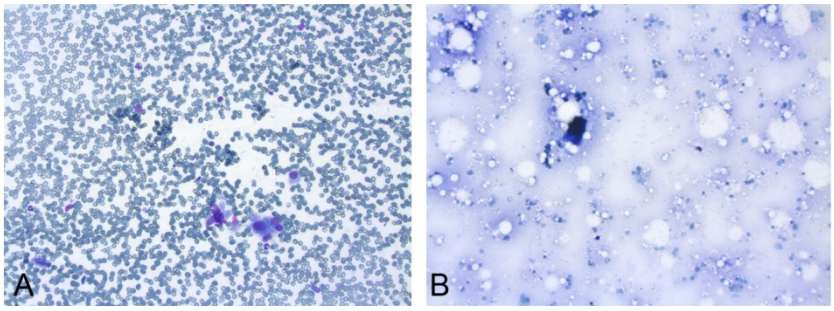

Figure 20. Images of May-Grünwald-Giemsa stained smear of blood/secretion droplets. Following standard FNA, well preserved cancer cells surrounded by erythrocytes can be observed (A). Following FNA with applied RF pulses, reduced number of erythrocytes and a denatured tumor cell can be observed (B).

To decrease the risk of tumor cell dissemination related to percutaneous interventional procedures several actions have been proposed. Subsequent to percutaneous ablation procedures using RFA or microwave (MW) ablation the electrode can remain active when it is retracted from the respective organ to cauterize the needle tract [335-337]. For biopsies fewer needle passes are associated with decreased tumor cell dissemination [120, 125] and a coaxial approach using an introducer sheath can be used which remains in position during multiple cutting needle sampling [338]. In clinical practice this can merely be seen as a guideline. Placement of gelatin sticks loaded with chemotherapeutics in the needle tract post-biopsy has been developed and tested in animal models [339].

Tumor cell displacement and occurrence of bleeding in biopsies naturally interrelated, since mechanical forces of biopsy procedures both directly displace tumor cells and cause bleeding that can further disseminate cells. Especially for liver biopsies where hemorrhage is the most common severe complication of percutaneous needle biopsy [340], new approaches have been reported in the literature to decrease bleeding. These include injection of fibrin sealant [341], gelatin sponge injection [342], placement of steel coils [343] as well as RF-cauterization of the needle tract.

Using several different technical approaches, RF cauterization of the biopsy needle tract has been shown to decrease bleeding in experimental studies in various animal models [340, 344-347]. Advantages of RF ablation of the needle tract over alternatives are a lack of risk for vascular embolization, avoidance of implant placement, no thickened introducer sheath and no prolonged procedure time. While some of these studies insinuated a possibly decrease in tumor cell dissemination [345, 347] non demonstrated proof to that claim. While we did not quantify bleeding in our study, we did notice reduced bleeding, corroborating these reports.

To our knowledge, this is the first data presented on a biopsy technology demonstrating a decrease in tumor cell dissemination in a human *in vivo* setting.

Application of RF pulses did not adversely affect the quality of the cytological samples. Samples were indistinguishable from samples obtained with standard FNA. The aspirate inside the needle is protected within the Faraday's cage realized by the needle cavity and thus RF-induced heating. Movement of the needle provides cooling of the sample and counteracts conductive heating from surrounding tissue. Thermal injury of biopsy specimen due to excessive heating has previously been reported [340]. No additional pain or other adverse events were observed in our study.

The presented data draws strength from the simplicity in methodology and pronounced differences in outcomes with and without applied RF pulses, with the patient acting as its own control. The efficacy of the method to denature tumor cells was corroborated by cells that were found and analyzed. The method itself is elegant in that, as other have stated concerning the reduction of bleeding, omits the need of implant placement, does not add needle thickness, needs no additional items such as an introducer sheath and does not prolong procedure time.

While the method of tumor cell assessment in the emerging blood droplet might be straightforward, it does have limitations. Tumor cells could in theory remain in the needle tract occult to detection. Neither bleeding nor pain perceived by the patient was quantified. The effect of heating on post-surgical histopathological assessment, repeat biopsies as well as imaging assessment will have to be assessed and the method to be adapted to the now more widely used CNB.

Ultimately, randomized clinical trials with long-term follow-up are needed to compare biopsy procedures with and without application of RF pulses. This would be able to confirm both that presented methodology works and that tumor cell dissemination ultimately has a detrimental effect on patient outcome.

7.3 PAPER III-V

Over the course of studies presented in Papers III–V, 55 breast cancer patients underwent RFA treatment in different settings. Inclusion criteria, technology, treatment method as well as histological and radiological modalities were successively developed.

Radiofrequency ablation treatment of small breast carcinoma showed an overall high rate of complete ablation. Table 11 gives an overview over complete ablation rate in the respective setting. Across studies, complete ablation was achieved in 91% (50/55) cases as assessed by respective histologic and radiologic protocol. Two factors were associated with incomplete ablation as identified in Paper III. Firstly, inaccurate pre-operative imaging, i.e. a tumor extent that was larger than pre-operatively described, was the cause for incomplete ablation in two cases. Secondly, incorrect electrode placement was likely associated with incomplete ablation in three cases. In subsequent procedures presented in Papers IV and V patient inclusion criteria were changed, MRI was added for pre- and post-operative imaging and histological assays for CK8 were added. Furthermore in a subset of patients CEUS was used pre– and post–operatively and a pneumatic insertion mechanism was used for improved electrode insertion and positioning.

Paper	n	Efficacy		Setting	Tumor size	Efficacy assessment
I (set 1)	5	100%	(5/5)	General anesth.	< 10 mm	H&E
I (set 2)	26	81%	(21/26)	General anesth.	< 16 mm	H&E
II	18	100%	(18/18)	Local anesth.	≤ 20 mm	MRI 1 month, (CEUS 1 month) H&E, CK8
III	6	100%	(6/6)	Local anesth.	≤ 20 mm	MRI & CEUS 1 month, H&E & CK8 of CNB samples at 6 months

Table 11. Number of patients (n), setting, included tumor sizes, assessment protocol and success rates.

A meta-analysis published in 2017 found technical efficacy, i.e. rate of complete ablation, of RFA in breast cancer to be 82% (95% CI 74–88%) on the basis of 23 published studies, (including Paper III and IV) reporting on a total of 576 patients [348]. Success rates presented in this thesis as well as in the meta-analysis have to be seen in the context of a technique that is successively developed and improved. Many of the studies evaluated in the meta-analysis were initial studies performed to assess safety and technical feasibility in a research setting. Protocols varied with regards to inclusion criteria, surgical setting, radiologic and histologic assessment.

Across the studies presented here, treatment showed a low rate of complications, see Table 12. Defining major complications as skin burns Grade 2 and 3, necrosis of the skin and pneumothorax, the rate across the studies was 7% (4/55). The reported ipsilateral pneumothorax in Paper III was diagnosed on the third postoperative day. The pleura was not punctured by the electrode but was possibly damaged by the ablation procedure. The incident was reasonably found to be associated with the study procedure. Rate of side effects is well within the reported rates of the meta-analysis which found major complication rates to occur in 6% (95% CI 4–9%) of cases [348]. Long term minor side effects concerning cosmesis can occur and include skin and nipple retraction [349], see example in Figure 21.

Paper	n	Adverse events
I (set 1)	5	-
I (set 2)	26	1x skin burn
		1x thermal damage chest muscle
		1x thermal damage chest muscle, pneumothorax
II	18	-
III	6	1x skin burn

Table 12. Overview of adverse events across studies.

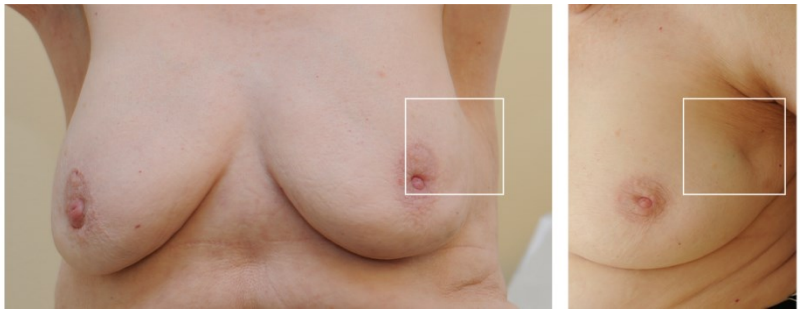

Figure 21. Patient number 1 in Paper III at the 12–month follow-up visit. The palpable lesions after RFA had decreased in size to 15 mm after measuring 25 mm and 30 mm at the one and 6–month follow-up respectively. Symmetry was judged as very good (left) with a slight retraction of skin (right).

In Paper III, MRI was proposed as a tool for improved patient selection with regards to tumor size and presence of extensive intraductal carcinoma as well as to assess complete ablation. In Papers IV & V MRI was added to the inclusion and post-RFA assessment protocol. None of the patients showed extensive intraductal carcinoma upon histological analysis. In all cases the contrast enhancement had disappeared post-RFA. An ablation region surrounded by a peripheral ring could be observed (see Figure 22). Complete ablation was achieved in all cases. Pre-RFA MRI resulted in an additional suspect finding and related diagnostic interventions in two patients, one case ipsilateral and one contralateral. Biopsies confirmed findings to be benign. Magnetic resonance imaging appears to deliver good results in patient selection and post-ablation assessment. However, the implications of additional findings and associated diagnostic procedures should not be underestimated with regards to costs and patient discomfort. This is especially relevant in the context of the ongoing debate on the overtreatment of breast cancer patients. Several studies report on the use of MRI for post-ablation assessment [309, 350-352] and generally conclude that it reliably predicts histologic findings. Two studies [309, 351] specifically report larger tumor sizes for MRI compared with US pre–ablation.

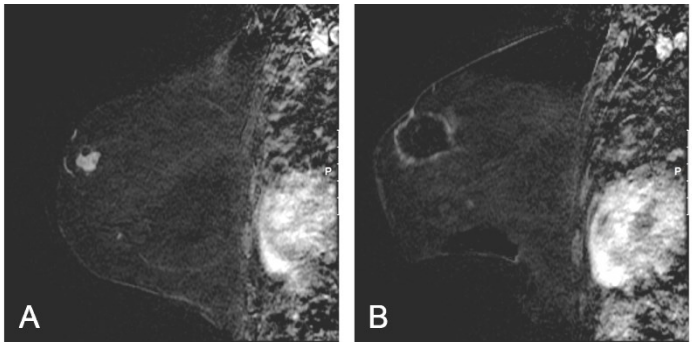

Figure 22. MR images of patient number 3 in Paper V Pre-RFA (A) and 1 month post-ablation (B).

Across the studies CEUS was used in 12 patients and ablation margins could be depicted post–treatment. Pre–RFA CEUS images were heterogeneous and reflect vascularization

patterns and tissue properties of the tumor and surrounding tissue. Post–RFA, images showed a distinguishable ablation margin (see Figure 23). Contrast enhanced ultrasound is also a recognized tool for assessing treatment efficacy of RFA in the liver [353-355]. In the breast CEUS has so far shown promise for diagnosis, differential diagnosis and prognosis of invasive breast tumors [356, 357] and for identifying SLN [358] but no studies have previously reported on the use of CEUS in RFA of the breast. Implementation of CEUS has several advantages such as low cost, patient comfort, no waiting time and seamless integration into clinical flow. It leverages the advantages of RFA as a method for local treatment with regards to low cost and high availability.

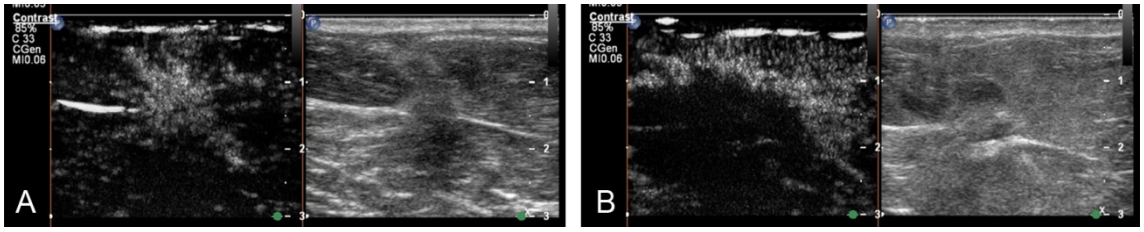

Figure 23. CEUS images in patient number 12 reported in Paper II. CEUS images are on the left, standard US images are on the right. These images are taken pre-RFA (A) and post-RFA (B). CEUS overestimates tumor size pre-RF, most likely due to vascularization around the tumor mass. There is a marked visible ablation margin following treatment.

Adding CK8 immunostaining to the assessment protocol proved to be valuable. In Paper IV it was used on the resected specimen, in Paper V on the CNB samples obtained 6 months post-treatment. It facilitated assessment of complete tumor ablation and was a suitable adjunct to H&E, see Figure 24.

A drawback of H&E staining is the lack of protocol for assessing thermally ablated tissue [359] and the reported underestimation of cell devitalization less than 6 months after ablation [308]. Several studies have reported the use of enzyme histochemical analysis of cell viability using nicotinamide adenine dinucleotide (NADH)-diaphorase analysis [293]. However, this technique requires frozen tissue which poses several problems: it does not work well for fatty tissues like breast as fat impedes the cutting of frozen sections, it requires costly infrastructure and it does not preserve morphological details as well as paraffin sections [308, 360].

Cytokeratins are epithelium-specific intermediary filaments and CK8 is cleaved early in apoptosis so it can be used to assess cell viability. The ability to assess cell viability after RFA in breast cancer using immunostaining for CK8 on paraffin embedded tissue has been reported comparable to NADH-diaphorase staining in frozen tissue [360]. The tumor must be sampled and analyzed for CK8 positivity before ablation since a significant, albeit small, number of breast tumors are CK8 negative [361]. Studies have reported good results on the use of CK8 IHC to determine cell viability after RFA in the breast [351, 360].

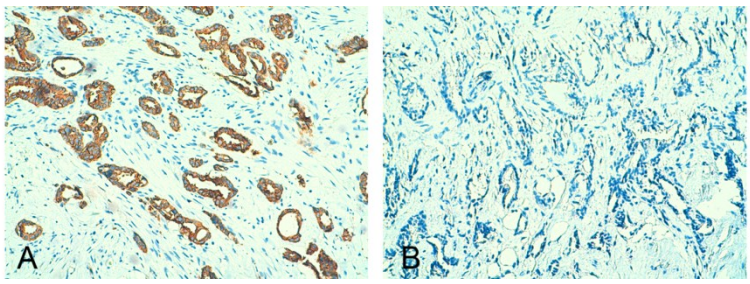

Figure 24. In patient number 18 in Paper II the biopsy shows positivity for CK8 (left, magnification 10x) pre–RFA. Post–RFA, no staining is visible (right, magnification 10x)

In Paper IV RFA treatment was performed under local anesthesia and pain was assessed. In this study, 40–75 ml of mepivacaine (5 mg/ml) and bupivacaine (2.5 mg/ml) combined with 5 µg/ml epinephrine were injected for pain control and the separation of tumor from skin and pectoral muscles. Radiofrequency ablation under local anesthesia was considered mild with a median value on the VAS of 2.5.

Pain perceived during the thermal ablation procedure did not differ significantly from pain perceived during the injection of local anesthetic with median values of 2.5 and 2 respectively (n =18, Z =-0.656, p =0.512). Pain reported immediately after treatment ceased compared with before the procedure, with median values of 0.5 and 0, respectively, with the difference being statistically significant (n =18, Z =-2,032, p =0.042), see Figure 25. Patients could return home around 1 hour after the procedure.

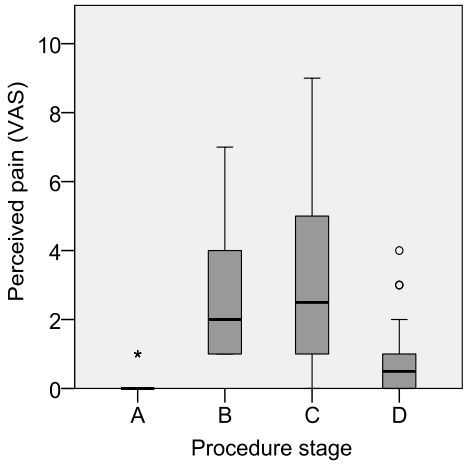

Figure 25. Box-plot–including outliers (°) and extreme outliers (*)–of pain scores reported at different stages, i.e. pre-procedure (A), during administration of local anesthetics (B), during ablation (C) and postprocedure (D) as reported in Paper IV.

While some authors reported RFA under local anesthesia to be unfeasible due to significant pain [291], several reports are available [309, 326, 350, 351, 362-365]. Only two studies quantitatively reported on pain scores. One study reported 10 patients which were treated under local anesthesia using a mean (range) of 42 ml (32 to 55 ml) of 1% lidocaine [351]. On a scale from 0 (no pain) to 5 (worst possible pain) eight patients scored 0, and two patients scored 1 and 3, respectively. Another study used a scale from 1-10 and reported a median value of 4 for a total of 22 treated patients [365]. Pre-treatment, intravenous sedation with titrated doses of midazolam and fentanyl had been administered. Nine patients who also had an intercostal nerve block experienced considerable discomfort and it was reported that most felt that this was the worst part of their experience. Reported pain can be compared to

previously reported pain scores for US-guided CNB and VAB procedures, which were on average 2.3 on a scale from 1 to 10 for a total of 235 procedures [366].

In Paper V six elderly patients (median age 87 years) and with severe co–morbidity profiles were enrolled and treated with RFA in an outpatient setting under local anesthesia. Three patients had refused surgical resection and three were considered to be of high surgical risk. In this study, 40–60 ml of mepivacaine (5 mg/ml) and bupivacaine (2.5 mg/ml) combined with 5 µg/ml epinephrine were injected for pain control and the separation of tumor from skin and pectoral muscles. Electrode insertion was performed without complications and correct placement was verified by US. Median (range) reported pain during local anesthetic injection and thermal ablation was 4 (1 to 8) for both, with the median (range) difference between them 0 (-2 to 2). Patients reported no postprocedure pain and returned home approximately 1 hour after treatment.

Complete ablation was achieved in all cases according to MRI and CEUS at 1 month and histological analysis of two to four CNB samples at 6 months. Radiologically well-delineated, non-contrast loading spheres were observed with the largest diameters of median (range) 30 mm (30 to 35 mm, MRI) and 31 mm (28 to 40 mm, CEUS), respectively. Follow-up, using MRI and CEUS, was performed in six and four patients at 6 and 12 months, respectively. Compared with measurements obtained at 1 month, the largest diameter had decreased by a median (range) of 33% (17 to 57%) and 16% (0 to 31%) at 6 months and 49% (40 to 57%) and 25% (22 to 39%) at 12 months, calculated using MRI and CEUS, respectively.

Clinical follow-up data were available for a median (range) of 25 months (6 to 76 months) and survival data for a median (range) of 54 months (11 to 94 months). Three of six patients died from non-cancer–related causes at 11, 13 and 33 months after treatment. Three patients remained alive 74, 86 and 94 months after treatment, respectively. One of these patients, who received no radiotherapy and only 16 months of endocrine therapy, experienced a loco-regional recurrence at 59 months.

These data support findings of previous reports on the feasibility of RFA under local anesthesia as an alternative to surgery in elderly non-operable patients [326, 362-364]. The number of patients reported in these studies is low but authors report a high rate of complete ablation and agree that RFA is safe, effective and feasible for patients who are refusing and/or are unfit for surgery. The included patient populations differed in age, tumor size, location and biology. An overview is given in Table 13. In comparison, patients in the study presented here, were elderly and exhibited a more severe co–morbidity profile.

Author, Year	Inclusion criteria, disease	Inclusion criteria, patient	Number of patients (tumors)	Age [y]	Setting & anesthetic protocol	Complications	Technical efficacy	Follow-up [M]	Outcome
Marcy, 2007 [326]	<5 cm, HR+, Low grade (SBR), distant to skin, nipple, pectoralis muscle, node negative	>70 years. Patients had to request a minimally invasive treatment because they were afraid of	4 (5)	81, 81, 79-82	Sterile conditions in interventional radiology suite, patients discharged once stable and free of sedative effects; Local anesthesia - subcutaneous lidocaine	25 % (1/4), infection 9 M after RFA - Abscess, aspiration revealed morganella morgani germ. Partial breast resection	80 % (4/5) according to MRI, US, mammo and CNB at 3 M	28, 26, 24-36	25 % (1/4) LR - at 4 M
Susini, 2007 [362]	<2 cm, >1 cm from skin and chest wall	>75 years Inoperability or very high surgical and anesthetic risk	3 (3)	81, 82, 76-86	1 hour hospital stay; Local anesthesia - mixture of lidocaine and naropine	0 %	100 % (3/3) according to US, mammo at 1 M. MRI, CNB at 12 M	18, 18, 18-18	0 % (0/3) LR or death during follow–up
Brkljacic, 2010 [364]	-	Unsuitable for surgery or general anesthesia (ASA III/IV) and/or refused surgery	6 (7)	76, 78, 63-85	Radiology department, overnight stay; Local anesthesia - 20 ml of 2 % lidocaine, 20 ml of 0.5 % bupivacaine	16 % (1/6), infection 4 month after RFA - immunosuppressed patient. Successfully treated with antibiotics	86 % (6/7) according to US, mammo, CNB at 2 M, MRI if possible - One tumor located close to	27, 29, 2-49	33 % (2/6) death non-cancer related - leukemia at 42 M - acute myocaradical infarction at 2 M
Palussière,2012 [363]	<3 cm after 6 M neoadjuvant HT, HR+, >1 cm from skin, nipple and chest wall, monocentric tumor	>70 years Contraindications to surgery (relative contraindications to complete sedation) or patient declined surgery	21 (21)	NR, 79, 70-80	Radiology suite, patients hospitalized at least 24 h; Local anesthesia + sedation (n=15) - 1 % lidocaine, nitrous oxide and propofol Local-regional anesthetic block (n=6); - 5 mL ropivacaine at 7.5 mg/ml in each paravertrebral space 30 minutes before RFA	19 % (4/21) skin burn with necrosis - One led to 21 day hospitalization - Two healed within 2 weeks - One healed within 2 M 14 % (3/21) nipple retraction	95 % (20/21) according to MRI follow-up up to 12 M. CNB for suspicious MRI - One recurrence at 9 M confirmed by CNB	NR, 50, 17-77	19 % (4/21) LR - at 9, 30, 48, and 60 M 10 % (2/21) death breast cancer related - Two patients at 60 M 10 % (2/21) death non-cancer related - cardiac failure at 25 M - natural causes at 54 M

Table 13. Comprehensive overview of studies reporting on RFA in elderly breast cancer patients with contraindications for surgery. Age and follow-up given as mean, median, range. LR=Local recurrence, M=Month, HT=Hormone therapy, NR=Not reported.

The work presented in Paper III–V draws strength from the successive development of protocols and methods implemented into a well-functioning clinical workflow to demonstrate feasibility and clinical validity. Patient groups were well selected and described. Results were coherent and consistent. Specifically, patients selected for Paper V were of older age and exhibited a more complex morbidity profile than patients in other studies, constituting a highly relevant group given that these patients did not have treatment alternatives.

Radiofrequency ablation for the treatment if small breast carcinoma has consistently demonstrated high success rates and low complication rates. Since this work was initiated, several studies with low patient numbers have been reported. With each new study the value of incremental information has decreased. By now the torrent of studies has ebbed and given way to systematic reviews and meta-analysis on minimally invasive procedures in general [290, 348] and on RFA specifically [305, 367], see Table 14.

	RFA	LA	HIFU	CA	MW
No. of scientific publications	23	7	6	6	3
No. of patients	576	227	129	146	78
Age range [years]	22–92	34–84	41–92	34–82	34–78
Pooled technical success rate	96% (93–97)	98% (95–99)	96% (90–98)	95% (90–98)	93% (81–98)
Pooled technical efficacy rate	82% (74–88)	59% (35–79)	49% (26–74)	75% (51–90)	90% (n/a)
Minor compl. rate	7% (4–12)	11% (3–33)	15% (7–28)	8% (1–36)	14% (3–46)
Major compl. rate	6% (4–9)	4% (1–17)	10% (5–20)	2% (1–7)	4% (1–17)

Table 14. Summary of data extracted from 45 scientific publications included in the systematic review by Mauri et al. [348], Table modified from [348]. Data presented as percentage (95% CI). RFA=Radiofrequency ablation. LA=Laser ablation. HIFU=High intensity focused ultrasound. CA=Cryoablation. MW=Microwave ablation.

A recently emerged possible benefit of these ablation modalities is its effect on the immune system. Local ablations using several modalities, among them RFA, have shown systemic immune stimulation in preclinical and clinical studies [368]. This is especially interesting with regards to recent comprehensive investigations which clarified the role of the immune microenvironment surrounding tumor cells in tumor progression, metastasis, and response to treatment. There are numerous immuno–oncological agents currently undergoing clinical trials.

Limitations of the work presented in Paper III–V are the low number of patients included into the trials and the arguably low level of evidence that can be derived from a statistical point of view. Recruitment was specifically slow for the study presented in Paper V. Swedish guidelines for breast cancer management strongly emphasize a treatment strategy independent of physical age and recommends surgical treatment for elderly patients to be offered on the same terms as for a younger patient population. This likely resulted in older age and severe co–morbidity status of the patients reported here. To be enrolled into the study, patients had to fulfill narrow tumor inclusion criteria and consent to additional visits for radiologic assessment and follow-up which further hampered inclusion rate. Another limitation of Paper V is the short follow-up time. Further limitations are the low number of patients where the newly developed insertion device and CEUS was applied.

One inherent limitation of the modality is the inability to assess treatment margins due to the lack of surgical specimen. In breast conserving surgery, the re–incision rate mostly due to positive margins, is reported to be around 8% in Sweden [16]. This limitation has to be overcome by using adequate imaging modalities for example MRI and possibly extensive percutaneous biopsies with histopathological assays for H&A and CK8.

A further limitation is the persistence of an, albeit possibly temporary, residual lump at the tumor location. Presence of a palpable, yet ablated, mass in the breast after local treatment can cause discomfort and anxiety [369, 370]. Data on this issue is sparse. One study with long-term follow-up on elderly patients found disappearance of palpable mass in only 3/21 cases at 1-year follow-up. It is suggested that tumor size, location, breast size and breast density may be associated with the resorption of ablated tumors [371].

Indications for RFA in breast cancer remain to be defined. The focus has been in the treatment of small primary breast cancers in the general breast cancer population as well as specifically in elderly patients whose condition precludes surgery. For the general population, questions remain as to whether to limit it to cancers with low aggressive profile. Other possible indications include patients with local recurrence or patients with tumor residue after neoadjuvant treatment.

To leverage the properties of this treatment modality, the role of ALN interventions is important. Today, SLN is indicated for clinically negative lymph nodes and ALND is warranted for metastasis–positive SLN. Even though SLNB can be performed under local anesthesia, the drawbacks would be significant if RFA of the primary breast tumor would still be accompanied by a surgical procedure in the axilla. The benefit of SLNB for some patient groups, e.g. >70 years with T1–2N0 and HR–positive breast cancer, has been questioned [372]. Ongoing clinical trials assess whether SLNB can be omitted without detrimental long-term effects in selected patient groups, i.e. the SOUND trial [373] and others [374]. The SOUND trial is especially intriguing, in that patients with no evidence of axillary disease on pre–operative assessment (US ±biopsy) are randomized to SLNB versus no axillary surgery. This is a non-inferiority trial that aims to recruit 1560 women (780 in each arm), with the primary endpoint being disease-free survival. Results are expected within the coming years.

The major issue however remains the comparison with standard of care with regards to long–term outcome. To the best of my knowledge no clinical trial is currently ongoing that addresses this question. Two somewhat similar, prospective single-arm trials (150–200 patients, tumors <15 mm, HR-positive, HER2-negative), are currently evaluating a minimally invasive treatment alternative (cryotherapy) in breast carcinoma without surgical resection and are determining long-term outcome. Five-year follow-up data on recurrence rates and survival is awaited for 2021 and 2023, respectively. Patients enrolled in the FROST trial do not undergo SLNB since patients are included on the basis of, amongst other criteria, their genomic profiling score (Oncotype DX) indicating a low risk of recurrence and a safe adjuvant treatment with endocrine medication only.

Parallels in study design considerations can be drawn from ongoing trials in which the gold standard of surgical excision is tested against a less invasive alternative. This is the case in ongoing trials comparing active monitoring to surgical resection in patients with low risk

DCIS, e.g. the LORIS trial [375]. Health–economic assessment shall take into account all costs incurred on the health care system. The substitution of a surgical resection with a less invasive alternative initially likely saves procedural cost. However, long-term monitoring can cause inclusion of additional imaging follow-up programs and patient counseling. The role of imaging for RFA treatment follow-up is especially relevant since MRI, if included, is in itself costly and can entail further interventions due to incidental findings, such as MRI-guided biopsies. Immediate and long-term local side effects, pain, persistence of residual lump, breast cosmetics satisfaction, together with quality of life related aspects (e.g. anxiety) using adequate questionnaires should be assessed.

Care should be taken when powering the trial. Background recurrence rate is generally low in small early stage breast cancer and to reach statistical significance in a non-inferiority trial setting patient numbers of >1000 are required. A trial of this magnitude in combination with long-term follow-up requires significant resources. Furthermore, progress in adjuvant breast cancer treatment leads to progressively better outcome which might render priori statistical calculations inadequate. A recent example is the ACOSOG Z0011 trial which failed to reach statistical significance partly because of improving patient outcomes during the study period. However, an important lesson learned from this trial was that even though statistical significance was not achieved, the results were convincing enough as to significantly change clinical practice pending results from longer-term follow-up and other trials.

8 CONCLUSIONS

Concerning the biopsy system that has been developed and evaluated as part of Paper I:

- The device provides a novel way of biopsy needle insertion for histologic tissue acquisition compared to currently use CNB and VAB devices.

- The sampling mechanism delivers a high tissue yield in a wide range of representative bench models and has no apparent adverse effects on sample quality.

Regarding the technology presented in Paper II:

- Application of RF pulses to the biopsy needle has shown potential to prevent dissemination of viable tumor cells in the needle track as well as decrease bleeding, without negatively affecting the sample inside the needle or causing additional pain.

The work presented in Papers III–V constitutes first Swedish data presented for the use of RFA in breast cancer patients. It has established my group as the European research group, amongst a few others in Italy [324, 350], France [316, 363], Greece [294] and the Netherlands [337], with the largest reported series of breast cancer patients undergoing RFA. The presented data contributes to the growing body of evidence demonstrating that RFA in the breast has a high efficacy rate and low complication rate. Specifically, conclusions are:

- The developed RFA device using a monopolar cool-tip electrode presented herein achieves a high success rate in small unifocal breast carcinoma as determined by histopathological methods for H&E and CK8 staining as well as MRI. A prerequisite for high complete ablation rate is careful patient selection using MRI and correct placement of the treatment electrode.

- The intervention showed a low rate of major and minor complications with given safety measures, i.e. injection of bolus to separate tumor from skin and pectoral muscle, ice-pad cooling, monitoring temperature using IR thermometer and sutures attached to the skin to increase distance between tumor and skin.

- Contrast enhanced ultrasound demonstrated promising properties regarding post-treatment evaluation of treatment success. Further exploration is warranted if this modality has the ability to substitute the more costly and less patient-friendly MRI for depicting the physical boundaries of the ablated region.

- A pneumatic driver for electrode insertion showed promise in counteracting tumor dislocation and improving electrode insertion and placement.

- Performing the intervention in an outpatient setting and under local anesthesia is feasible. Patient experienced mild to moderate pain during ablation, nearly no pain immediately post-ablation and could return home after around 1 hour.

- The intervention is feasible as an individualized treatment option in elderly patients with severe co-morbidity and/or who are refusing or are unfit for surgery.

- A prospective trial to assess long–term outcome, cosmetics, quality of life and health-economic aspects in a well-defined patient group is warranted.

9 FUTURE DEVELOPMENTS

The biopsy system developed and evaluated as part of this thesis has recently become commercially available and has been used in a handful of hospitals in Sweden, UK, Germany, France and Austria. A recent publication reports on a small patient series in whom the device was used in technically difficult cases of axillary lymph node biopsies where prior FNA or CNB had yielded non-diagnostic results. The device yielded conclusive results in all cases which had implications on management in half of these [376]. A current multi-centre study in Germany is systematically evaluting the performance and use of the device in clinically positive axillary lymph nodes. In general, only prospective clinical studies can show if the novel mechanisms of needle insertion and sampling acquisiton translate into clinical benefits such as shorter procedure time, less patient trauma or increased diagnostic accuracy. In the long term, the device could be adapted and used in other organs such as the liver, prostate, thyroid or kidney.

The method of applying RF pulses to the biopsy needle will need to be adapted to the now commonly used CNB or VAB. Randomized clinical trials with long-term follow-up are needed to compare biopsy procedures with and without application of RF pulses. This would be able to demonstrate both that presented methodology works and that tumor cell dissemination ultimately has a detrimental effect on patient outcome.

Regarding the teatment of early-stage breast cancer the general trend towards less invasive treatment regimes providing comparable oncological outcome remains strong. As has been the case in other organs, RFA in the breast will most likely be initially used in patients with high surgical risk. Meanwhile, large prospective trial are needed to assess long-term outcome, cosmesis, quality of life and health-economic aspects in comparison with standard of care.

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