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PROGNOSTIC FACTORS OF pT1 BREAST CANCER

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To my family – Lauri, Lennart and Julius

”SIC ITUR AD ASTRA”

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I. Liikanen J, Leidenius M, Joensuu H, Vironen J, Heikkilä P, Meretoja T. Breast cancer prognosis and isolated tumor cell findings in axillary lymph nodes after core needle biopsy and fine needle aspiration cytology. European Journal of Surgical Oncology, 2016;42(1):64–70.

II. Liikanen JS, Leidenius MH, Joensuu H, Vironen JH, Meretoja TJ. Prognostic value of isolated tumour cells in sentinel lymph nodes in early-stage breast cancer: a prospective study. British Journal of Cancer, 2018;118(11):1529–1535.

III. Liikanen JS, Leidenius M, Joensuu H, Meretoja TJ. Long-term survival outcomes of patients with small (≤ 1 cm) node-negative HER2-positive breast cancer not treated with adjuvant anti-HER2-targeted therapy: a 10-year follow-up study. Breast Care, 2022;17:279–287.

The publications are referred to in the text by their roman numerals.

LIST OF ABBREVIATIONS

AI: aromatase inhibitor
ADH: atypical ductal hyperplasia
AJCC: American Joint Committee on Cancer
ALH: atypical lobular hyperplasia
ALND: axillary lymph node dissection
ASCO/CAP: American Society of Clinical Oncology/College of American Pathologists
BCS: breast conserving surgery
CISH: chromogen *in situ* hybridization
CNB: core needle biopsy
CT: computed tomography
DCIS: ductal carcinoma in situ
EBCTCG: Early Breast Cancer Trialists' Collaborative Group
ER: oestrogen receptor
ESMO: European Society for Medical Oncology
FISH: fluorescence *in situ* hybridization
FNAC: fine needle aspiration cytology
HER2: Human epidermal growth factor receptor 2
H&E: haematoxylin-eosin
HUS: Helsinki University Hospital
IHC: immunohistochemistry
ITC: isolated tumour cells
Ki-67: nuclear protein (antigen) expressing cell proliferation
LCIS: lobular carcinoma in situ
MGR: mammography
MIB-1: monoclonal antibody against Ki-67 antigen
MRI: magnetic resonance imaging
NCCN: National Comprehensive Cancer Network
PgR: progesterone receptor
RT: radiotherapy
pN0i-/ i+: isolated tumour cell negative/positive lymph node
OFS: ovarian function suppressive
SN: sentinel node
SNB: sentinel node biopsy
US: ultrasound
VAB: vacuum assisted biopsy
WHO: World Health Organization

ABSTRACT

Aim Breast cancer is the most common cancer in women worldwide. In Finland, like in other high-income countries breast cancer is most often detected in an early stage, due to effective screening programme and breast cancer awareness. Nevertheless, the prognostic factors of small, node-negative breast cancers are still somewhat controversial. The aim of this thesis is to investigate the prognostic factors and breast cancer outcome of node-negative pT1 (≤ 2 cm in diameter) breast cancer in a population-based cohort with a long almost 10-years follow-up time.

Study I investigated whether pT1 (tumour size ≤ 2cm) breast cancer patients undergoing preoperative core needle biopsy (CNB) have higher incidence of isolated tumour cell findings (ITC; pN0i+, ≤ 0.2mm or < 200 cancer cells in a cluster) in their sentinel lymph nodes (SN) compared to those undergoing fine needle aspiration cytology (FNAC). The other aim was to study the influence of the type of preoperative needle biopsy on breast cancer outcome. The objective of study II was to investigate the long-term prognostic significance of ITCs in SN in pT1N0M0 breast cancer. The study III investigated the long-term prognostic importance of human epidermal growth factor receptor 2 (HER2) expression in small node-negative (≤ 1 cm, stage pT1a-bN0M0) breast cancer.

Methods The study cohort is based on consecutive 1,865 patients with unilateral invasive pT1 breast cancer (≤ 2 cm in diameter), operated at the Breast Surgery Unit of the Helsinki University Hospital (HUS) between March 2000 and April 2006. Studies I and II are prospective, observational studies and study III is a retrospective study based on this prospectively collected patient data. The patients in all the studies were followed up for almost 10-years.

In study I, 1,525 patients with pT1 breast cancer were categorised into CNB and FNAC groups according to the type of preoperative needle biopsy performed. The incidence of ITCs was compared between the groups and survival outcomes were analysed. In study II, the survival of 936 pT1N0M0 breast cancer patients were analysed according to SN status. Patients with (pN0i+) and without (pN0i-) ITCs in their SNs were compared. Study III included 334 patients with pT1a-bN0M0 cancer, not treated with adjuvant anti-HER2-targeted therapy. The patients were divided according to HER2-status and hormone receptor status and survival outcomes were compared.

Results In study I, FNAC was performed in 868 patients while 657 patients underwent CNB. Among patients with pN0 stage, 70 patients (4.6%) had ITCs (pN0i+) including 37 in the FNAC group and 33 in the CNB group ($p = 0.798$). The biopsy method did not influence breast cancer-specific survival ($p = 0.461$) nor local recurrence-free survival ($p = 0.814$) in univariable survival analyses. Overall, survival favoured the CNB group in a univariable analysis, but no statistically significant difference in survival was seen in multivariable analysis ($p = 0.718$).

In study II, 861 patients had ITC-negative (pN0i-) and 75 ITC-positive (pN0i+) breast cancer. Patients with pN0i+ cancer underwent more extensive surgery and received more often systemic adjuvant therapy than those with pN0i- cancer. Ten-year distant disease-free survival was 95.3% in the pN0i- group and 88.8% in the pN0i+ group ($p = 0.013$). ITC findings were a statistically significant prognostic factor in Cox regression model (HR=2.34, 95% CI 1.09–5.04; $p = 0.029$) for worse distant disease-free survival, together with a high tumour Ki-67 proliferation index and large tumour size. In addition, ITCs were associated with unfavourable overall survival ($p = 0.005$) and breast cancer-specific survival ($p = 0.001$).

In study III, 44 patients with pT1a-bN0M0, HER2+ cancer, not treated with adjuvant anti-HER2-targeted therapy (the HER2+ group) and 291 pT1a-bN0M0, hormone receptor positive, HER2-negative cancers (the ER+/HER2- group) were identified. Ten-year distant disease-free survival was 84.0% in the HER2+ group and 98.2% in the ER+/HER2- group ($p < 0.001$). Ten-year overall survival was 78.5% in the HER2+ group, but 91.7% in the ER+/HER2- group ($p = 0.09$).

Conclusions Preoperative percutaneous biopsy method is associated neither with a higher ITC incidence nor survival outcome. The current findings suggest that SN ITCs are associated with an increased risk for distant metastases and breast cancer death. Breast cancer HER2 expression is an important factor for unfavourable prognosis also in patients with subcentimetre node-negative (pT1a-bN0M0) cancer.

Further studies, however, are needed.

TIIVISTELMÄ

Tavoitteet Rintasyöpä on maailmanlaajuisesti yleisin syöpä naisilla. Rintasyöpä diagnosoidaan kuitenkin yhä useammin varhaisessa ei-levinneessä vaiheessa. Suomessa ja muissa korkean tulotason maissa tämä on suurelta osin rintasyöpäseulonnan ja yleisen rintasyöpä tietoisuuden ansiota.

Tästä huolimatta pienikokoisen rintasyövän ennusteelliset tekijät ovat edelleen keskustelun ja tutkimuksen alla. Tämän väitöskirjatutkimuksen tavoitteena on tutkia pT1 (kasvaimen koko ≤ 2 cm) rintasyövän ennusteellisia tekijöitä väestöpohjaisella aineistolla lähes 10 vuoden seuranta-ajalla. Ensimmäinen osatyö selvitti vaikuttaako leikkausta edeltävä biopsiamenetelmä ennusteeseen sekä vartijaimusolmukkeista löydettävien yksittäisten kasvainsolujen (ITC; pN0i+, ≤ 0.2 mm tai < 200 syöpäsolun joukko) esiintyvyyteen ja täten pienikokoisen rintasyövän (pT1N0M0; kasvaimen koko ≤ 2 cm) ennusteeseen. Toisessa osatyössä keskityttiin selvittämään vartijaimusolmukkeiden ITC-löydösten merkitystä rintasyövän ennusteelle. Kolmas osatyö tutki onko HER2 (epidermaalisen kasvutekijän reseptori 2; human epidermal growth factor receptor 2) positiivisuus merkityksellinen ennusteelle myös pienikokoisessa imusolmukenegatiivisessa rintasyövässä potilailla, joilla on alle 1 cm kasvain (pT1a-bN0M0).

Menetelmät Etenevään kohorttitutkimukseen otettiin 1,865 Helsingin yliopistollisen sairaalan rintarauhaskirurgian yksikössä maaliskuun 2000 ja huhtikuun 2006 välillä rintasyövän vuoksi leikattua potilasta. Potilailla oli todettu pienikokoinen pT1N0M0 (kasvaimen halkaisija ≤ 2 cm) rintasyöpä. Osatyöt I ja II ovat prospektiivisia tutkimuksia ja osatyö III retrospektiivinen tutkimus perustuen tähän prospektiivisesti kerättyyn aineistoon. Potilaiden seuranta-aika oli keskimäärin hieman alle 10 vuotta.

Osatyössä I on 1,525 potilasta, joilla oli todettu pT1 kasvain. Potilaat jaoteltiin leikkausta edeltävän biopsiamenetelmän mukaan kahteen ryhmään: CNB (core needle biopsy; paksuneulabiopsia) ja FNAC (fine needle aspiration cytology; ohutneulabiopsia) ryhmä. ITC löydösten insidenssiä sekä potilaiden ennustetta vertailtiin ryhmien välillä. Osatyössä II tutkittiin 936 pT1N0M0 rintasyöpää sairastavan potilaan vartijasolmukkeiden ITC-löydösten vaikutusta ennusteeseen. Osatyöhön III otettiin mukaan 334 potilasta, joilla oli pT1a-bN0M0 syöpä ja joita ei ollut hoidettu anti-HER2 lääkityksellä. Potilaat jaoteltiin ryhmiin HER2 ja hormonireseptoristatuksen mukaan, ja ennustetta verrattiin näiden ryhmien välillä.

Tulokset Osatyössä I ohutneulabiopsia tehtiin 868 ja paksuneulabiopsia 657 potilaalle. Niiden potilaiden joukosta, joilta ei löydetty kainaloimusolmukkeista etäpesäkkeitä (pN0) todettiin 70 potilaalla ITC-löydöksiä: 37 potilaalla ohutneulabiopsia ryhmässä ja 33 paksuneulabiopsia ryhmässä (p = 0.798). Täten biopsiamenetelmällä ei todettu olevan vaikutusta ITC-löydösten insidenssiin. Lisäksi biopsiamenetelmä ei vaikuttanut rintasyöpäspesifiseen elossaoloaikaan (p = 0.461) eikä paikallisuusiutumiseen (p = 0.814) univariaatti analyysissä. Kokonaiseloonjääminen oli parempi paksuneulabiopsiaryhmässä univariaatti analyysissä mutta monimuuttuja-analyysissä paksuneulabiopsia ei ollut enää tilastollisesti merkitsevä ennustetekijä (p = 0.718).

Osatyössä II 75 potilaalla todettiin ITC-löydöksiä (pN0i+) ja 861 potilaalla niitä ei todettu. Potilaat, joilla todettiin pN0i+ syöpä leikattiin laajemmin ja he saivat enemmän liitännäislääkehoitoja verrattuna pN0i- ryhmään. Etäpesäkevapaa elossaolo 10 vuoden seurannassa oli 95.3 % pN0i- ryhmässä ja 88.8 % pN0i+ ryhmässä (p = 0.013*)*. ITC-löydösten todettiin olevan tilastollisesti merkitsevä ennusteellinen tekijä huonommalle etäpesäkevapaalle elossaololle Coxin regressio mallissa (HR=2.34, 95 % CI 1.09–5.04; p = 0.029)*,* yhdessä Ki-67 proliferaatio indeksin ja kasvaimen koon kanssa. Lisäksi ITC-löydökset olivat yhteydessä epäsuotuisaan kokonaiseloonjäämiseen (p = 0.005*)* ja huonompaan rintasyöpäspesifiseen eloonjäämiseen (p = 0.001*)*. Osatyössä III 44 potilaalla todettiin pT1a-bN0M0 HER2 positiivinen syöpä (HER2+ ryhmä) ja 291 pT1a-bN0M0, hormonireseptori positiivinen, HER2-negatiivinen (ER+/HER2- ryhmä) syöpä. HER2+ ryhmässä 10 vuoden etäpesäkevapaa elossaolo oli 84.0 % ja ER+/HER2- ryhmässä se oli 98.2 % ($p < 0.001$*)*. Kymmenen vuoden kokonaiseloonjääminen oli 78.5 % HER2+ ryhmässä ja 91.7 % ER+/HER2- ryhmässä (p = 0.09*)*.

Johtopäätökset Biopsiamenetelmällä ei ole vaikutusta rintasyövän ennusteelle eikä vartijasolmukkeiden ITC-löydösten esiintymiselle. ITC-löydös vartijaimusolmukkeissa on pienikokoisessa rintasyövässä (pT1N0M0) itsenäinen huonomman ennusteen riskitekijä. HER2-monistuma rintasyövässä on tärkeä ennusteellinen riskitekijä huonommalle eloonjäämisennusteelle myös potilailla, joilla on imusolmukenegatiivinen alle yhden senttimetrin rintasyöpä (pT1a-bN0M0). Kuitenkin lisätutkimuksia aiheesta tarvitaan.

1 INTRODUCTION

Every year, more than 2 million women are diagnosed with breast cancer (1). During the last decade, the incidence of breast cancer in developed countries has plateaued or is only slowly increasing. However, in developing countries the breast cancer incidence is increasing due to increasing life expectancy, less pregnancies and the effect of modern lifestyle causing e.g., reduced physical activity and obesity.

The most important prognostic factors in breast cancer are tumour size, axillary lymph node status, histological grade, Ki-67 proliferation index, oestrogen receptor (ER) status and progesterone receptor (PgR) status, human epidermal growth factor receptor 2 (HER2) status, biological subtype and patient age. These factors guide the clinical management of breast cancer such as surgery and adjuvant therapies together with patient characteristics.

The gold standard in breast cancer diagnosis is the triple diagnosis including clinical examination, breast imaging and percutaneous biopsy of suspicious lesions and histopathological evaluation of these biopsy specimens. Breast tumour biopsy is most often performed using ultrasound (US) guided core needle biopsy (CNB). In the past, fine needle aspiration cytology (FNAC) was also used as a diagnostic biopsy method. However, the sensitivity and specificity of FNAC ranges widely depending on studies, being 35–95% and 48–100% respectively, while in CNB these range from 85–100% and 86–100% respectively (2). Due to its inferior diagnostic value FNAC is not anymore recommended as a primary diagnostic method in breast cancer by the Finnish Breast Cancer Group (3).

CNB yields a cylindrical histological tissue sample of the suspected lesion while FNAC includes only aspirated cells. From CNB sample the pathologist can readily define several previously mentioned prognostic and predictive factors, including tumour histology, ER- and PgR-receptor status, HER2-status and Ki-67 proliferation index.

Axillary lymph node status is an important prognostic factor in breast cancer (4,5). During the last two decades sentinel node biopsy (SNB) has replaced axillary lymph node dissection (ALND) in axillary staging of clinically node-negative breast cancer. SNB has been found to be accurate in axillary staging (6) with less morbidity (7) and without decreased survival when compared to ALND (8,9). Before the SNB era the axillary lymph nodes were examined by haematoxylin and eosin (H&E) staining and in only one or two slides per node. After the introduction of SNB, the sentinel nodes (SN) were examined more meticulously by serial sectioning and after negative H&E staining also with

immunohistochemical (IHC) methods. (10) Consequently, isolated tumour cells (ITC; pN0i+, ≤ 0.2mm or < 200 cancer cells in a cluster) and micrometastases (pN0mi, ≥0.2mm– ≤ 2mm, or more than 200 cancer cells but less than 2mm) are more often found in SNs (10,11).

Since their definition, the prognostic value of ITC findings in SN has been questioned. Some consider ITCs to have true metastatic potential and prognostic importance (12–14) while others consider them as artefacts from benign transportation after tumour manipulation such as CNB (15–18).

Human epidermal growth factor receptor 2 (HER2) expression is considered an independent factor for unfavourable prognosis in early breast cancer when patients are not treated with anti-HER2-therapy such as trastuzumab (19–22). However, the prognostic importance of HER2 expression in small (≤1 cm, stage pT1a-b), node-negative breast cancer is still incompletely known and under debate, since subcentimeter, node-negative tumours were excluded in trials investigating the treatment effect of trastuzumab.

Several studies have concluded that perhaps the afore-mentioned prognostic factors are not completely valid in small node-negative breast cancers where the prognosis is thought to be excellent. Therefore, in this thesis, the prognostic factors of small node-negative breast cancer (pT1N0M0, tumour size ≤ 2cm) in a large patient cohort and during a long-term, almost 10-year follow-up were investigated. Firstly, it was investigated whether the preoperative method of biopsy influences the ITC incidence and prognosis in pT1 breast cancer. Secondly, the aim of the study was to investigate the prognostic importance of ITC findings in SN in pT1 node-negative breast cancer. Thirdly, it is of interest, whether HER2 positivity is an independent prognostic factor in pT1a-bN0M0 breast cancer. The joint aim is to produce novel information on prognostic factors of small node-negative breast cancer.

2 REVIEW OF LITERATURE

2.1 BREAST CANCER EPIDEMIOLOGY

Invasive breast cancer is the most common cancer in women worldwide in developed and developing countries. Worldwide, 2.26 million breast cancers were diagnosed in 2020. Half of the breast cancer cases are currently detected in developing countries. Breast cancer is also the leading cause of cancer death in women with ca. 685,000 deaths worldwide in 2020. (23) Due to differences and inequality in treatment admission, diagnostic and treatment possibilities the 5-year survival rate ranges between developed and developing countries, being ca. 91% in Finland and ca. 40% in South Africa (24).

2.1.1 BREAST CANCER INCIDENCE AND SURVIVAL IN FINLAND

In 2019, breast cancer was the second most common cancer in women after basal cell carcinoma with an incidence of 170 per 100,000 inhabitants (age standardised). In Finland, 5,136 women were diagnosed with breast cancer in 2019 and 892 women died of breast cancer. (25) Breast cancer incidence in Finland is one of the highest in Europe (23). During the last decades the breast cancer incidence in Finland has increased but seems to have plateaued during the last five years. This increase in incidence during past decades was mostly due to the increased life expectancy in women. It is estimated that every 8th Finnish woman will be diagnosed with breast cancer during her lifetime. (25)

Access to early diagnosis and optimal treatment are considered as key factors in international comparisons in cancer survival (26). In Finland, like in other high-income countries, breast cancer is more often diagnosed in an early and localised stage due to effective screening program and breast cancer awareness. Also access to treatment in Finnish health care system is well and equally established to all citizens. Consequently, the 5-year age standardised breast cancer-specific survival in Finland was as high as 91% in 2019 (25) compared to worldwide survival of early breast cancer being 80–90% (27).

2.1.2 RISK FACTORS OF BREAST CANCER

Risk factors of breast cancer include female sex, higher age (>45 years), family history of cancer, race, early menarche, and late menopause, nulliparity or pregnancy at higher age, exogenous oestrogen exposure (e.g., hormone replacement therapy), alcohol and tobacco use, obesity, and low physical activity. There are also several gene mutations that cause increased breast cancer risk such as pathogenic mutations in *BRCA1*- and *BRCA2*-genes. (28)

Also, other patient related factors such dense breast tissue detected in mammography is associated with increased breast cancer risk (29,30). Women with proliferative breast lesions without atypia such as cysts, fibrosis and simple fibroadenomas have a slightly increased risk of breast cancer (31). However, atypical ductal hyperplasia (ADH), atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS) are high-risk lesions and the risk of invasive breast cancer is 4–7-fold in the two first mentioned and 8–10-fold in the latter (27,31–34).

2.2 DIAGNOSIS OF BREAST CANCER

2.2.1 TRIPLE DIAGNOSIS OF BREAST CANCER

The gold standard in the diagnosis of breast cancer is the triple diagnosis, including clinical examination, breast imaging and percutaneous biopsy of the suspicious lesion and histopathological evaluation of the biopsy specimen. Clinical examination includes patient and family history as well as inspection and palpation of the breasts and lymph node regions.

The most common symptom and sign of breast cancer is a palpable lump in the breast tissue or in the axilla and/or in the supraclavicular fossa. Other symptoms include for example change in the shape, size or appearance of the breast, retraction of the mamilla or the breast skin as well as an eczema in the nipple-areola complex. Inflammatory cancer is a rare and aggressive form of breast cancer and manifest as redness, and oedema of the breast. Sometimes the first symptoms of breast cancer are due to metastatic disease with the symptoms depending on the metastatic site and are such as musculoskeletal pain in bone metastases or headache and dizziness in brain metastases.

Breast imaging in a symptomatic patient includes high-quality mammography and US of the breast and the axilla. In women under 35 years, and in those who are breast feeding or pregnant, US can be used as the primary method of imaging. If suspicious findings are seen in imaging, percutaneous CNB is performed. (35)

If on any step of the triple diagnosis a suspicion of malignancy is aroused, the CNB can be renewed, or the suspicious lesion can be surgically removed. Vacuum assisted biopsy (VAB) is also an option for biopsy or even for surgical removal of small suspicious or high-risk breast lesions. (36)

2.2.2 BREAST IMAGING

The standard first line diagnostic imaging modality of patients with breast cancer symptoms is high quality mammography (MGR) together with US of the breast and the axilla. High quality MGR is also the method for breast cancer screening. In asymptomatic women, the sensitivity of MGR alone is ca. 77% and for US it is ca. 75%. Nevertheless, MGR and US together has ca. 94–97% sensitivity. US is more sensitive in dense breasts when compared to MGR. (37,38) Nevertheless, the specificity of MGR has been reported to be as high as 98.8% (37).

Magnetic resonance imaging (MRI) is the most sensitive of the current breast imaging modalities. The sensitivity of MRI in the diagnosis of malignant breast lesions ranges from 75%–100% and is generally over 80%, while the specificity ranges from 83% to 98.4%. (39) Due to its sensitivity to diagnose invasive cancer, MRI screening is recommended in women with high lifetime risk of breast cancer such as carriers of high-risk breast cancer gene mutations or in women who have received radiotherapy to thorax region at the age between 10–30 years. In addition, MRI is more sensitive in detecting breast cancer and evaluating its extent in younger women with very dense breast tissue (40,41). Pre-operative MRI may also slightly reduce the rate of re-excisions in patients with invasive lobular carcinoma (41,42). MRI is also recommended to patients with axillary metastasis, but without a primary breast tumour in MGR and US. It is also used to estimate the treatment response in patients with neoadjuvant therapy. The disadvantages of MRI include the false positive findings, high cost and limited availability. Therefore, MRI is not routinely used in breast cancer diagnostics. Moreover, MRI does not provide survival benefit in patients with average risk of breast cancer. (39,41,42)

Computed tomography (CT), isotope bone scintigraphy and positron emission tomography (PET) are used as staging methods in patients with a considerable risk of distant metastases (3,43).

2.2.2.1 Breast cancer screening

All women aged 50–69 in Finland are invited to participate in a biannual mammography screening programme. In screening programme asymptomatic women are investigated with the goal to detect breast cancer early thus aiming to improve prognosis. (44) Over 80% of Finnish women participate in the screening programme and two thirds of the breast cancers of screening aged women are diagnosed within the screening programme. Remaining breast cancers are diagnosed in women who are not in screening age, or do not participate in the screenings or are diagnosed in between the screening rounds. (25)

However, the participants are also exposed to the risk of false-positive findings causing 8–21% of women being referred to further studies which turn out as unnecessary. On the other hand, false-negative findings may cause delays in breast cancer diagnosis. (45) Women participating in screening programmes have been reported to have an estimated 15–28% reduced relative risk of dying in breast cancer compared to nonparticipating women in approximately 13 to 20 years of follow-up. (46,47) In a Finnish study, the relative risk of Finnish women dying in breast cancer was reduced by 33% in women who participate in the screening programme compared to nonparticipants (48). Albeit, the absolute risk reduction is reported to vary depending on studies from 0.05% to 0.56% (46,47).

On the other hand, screening also causes overdiagnosis. That is breast cancers which would not cause any symptoms in person's lifetime. The risk of overdiagnosis in European data has been reported to vary from 1–10% and in recent data from United States 15.4% (49,50). In a Finnish study overdiagnosis was seen in less than 10% of women participating breast cancer screening (51). However, the benefit of the screening is still regarded higher than the risks of overdiagnosis and possible overtreatment (52). Nevertheless, in the future the screening philosophy is evolving towards more personalised detection and screening aiming to find those at higher risk of breast cancer, and thus reducing overdiagnosis of low-risk breast cancers (53).

2.2.3 BIOPSY METHODS OF THE BREAST AND THE AXILLA

Biopsy of suspicious breast lesions are most often performed using percutaneous US guided CNB. If the lesion is not visible in US, then a stereotactic or even MRI guided CNB is an option.

Also vacuum assisted biopsy (VAB) can be used. The advantage of VAB is that it provides larger specimen than CNB and therefore better accuracy also in the histopathological diagnosis (54). VAB can be used e.g., if CNB

provides insufficient or inconclusive histopathological findings, or in patients with diffuse microcalcifications or otherwise small or diffuse breast lesions (36).

CNB has replaced FNAC as a diagnostic procedure in breast cancer, as CNB is more accurate and further biopsies are needed less often when compared with FNAC (36,55). Although, FNAC has been proven to be quick, easy, and inexpensive method of biopsy, its disadvantage lies in the inferior accuracy as well as its inability to distinguish between invasive and in situ cancer. The sensitivity and specificity of FNAC varies between 35–95% and 48–100%, while in CNB it ranges between 85–100% and 86–100%, respectively. (2)

The advantage of CNB is also that it gives a cylindrical tissue sample from which a pathologist can also readily evaluate several prognostic and predictive factors, including tumour grade and histology, steroid hormone receptor status, HER2-status, and Ki-67 proliferation index. CNB is recommended in breast cancer diagnosis by the Finnish Breast Cancer Group due to its superior diagnostic value (3). According to Finnish Breast Cancer Group FNAC should only be used in cyst diagnostics and in the rare situations where CNB is not technically possible such as in patients with poor co-operation or in those with bleeding disorders. When the axillary US reveals suspicious lymph nodes, FNAC or CNB is taken from the node, of which CNB is more accurate (56).

Since the introduction of CNB, several studies have concluded CNB to cause cancer cell dissemination along the needle track due to the mechanical trauma of the larger needle (57–60). However, whether it is just seeding with no prognostic significance (61–66), or includes an increased risk of local and/or distant recurrence has been under debate (67). During the early SNB era, concern about breast cancer cell seeding through lymph vessels to SN after CNB was raised. Some studies suggested that tumour manipulation detached breast cancer cells causing ITC findings in SN (68,69), while others were stating that biopsy method has no effect on ITC findings (62,65,70–72) .

2.3 HISTOPATHOLOGY AND BREAST CANCER STAGING

2.3.1 HISTOLOGICAL SUBTYPES

Mammary glands are formed of 15–25 lobes, which are connected with ducts and surrounded by fibrous and fat tissue. A lobe consists of ductal-lobular units lined by epithelial cells. Breast carcinomas arise from these epithelial cells of the breast tissue. There are several histological types of breast cancer.

Ductal carcinoma in situ (DCIS) is a pre-invasive form of invasive carcinoma. Carcinoma cells have not yet invaded through the basement membrane but are growing inside the mammary ducts. DCIS do not have the potential to

metastasize but has significant potential to develop into invasive carcinoma, if not treated and is therefore effectively treated with surgery. Paget's disease of the nipple is its own entity, but concomitant DCIS or invasive carcinoma is often diagnosed.

World Health Organization (WHO) classification divides invasive breast carcinomas into invasive carcinoma of no special type (NST), formerly known as invasive ductal carcinoma and here on also called as invasive ductal carcinoma, invasive lobular carcinoma, tubular carcinoma, cribriform carcinoma, mucinous carcinoma, mucinous cystadenocarcinoma, invasive micropapillary carcinoma, carcinoma with apocrine differentiation and metaplastic carcinoma. In addition, there is a group of rare and salivary gland-type tumours and neuroendocrine neoplasms. (73)

In Finland, approximately 70–80% of invasive carcinomas of the breast are invasive ductal carcinomas, while 15–20% are invasive lobular carcinomas (3). Invasive ductal carcinomas are a very heterogenous group of cancers from grade 1 indolent tumours to aggressive triple negative cancers, whereas invasive lobular carcinomas are more often grade 1–2 with a low Ki-67 proliferation index. Nonetheless, pleomorphic lobular carcinoma is seen to be more aggressive in nature. (73,74)

2.3.2 BREAST CANCER STAGING

Breast cancer stage is an important prognostic factor. The clinical and pathological disease stages are determined according to the tumour size (T), the presence of lymph node metastases (N), and possible distant metastases (M). Clinical staging is based on the physical examination and imaging while the pathological stage is determined after surgery. In breast cancer staging the most often used staging system is TNM classification of The American Joint Committee Of Cancer (AJCC) (75). In 2002, AJCC TNM-classification defined and distinguished ITCs (pN0i+, ≤ 0.2mm or < 200 cells in a cluster) from micrometastases (pN0mi, 0.2– ≤ 2mm, or more than 200 cells but less than 2mm). (75) (Table 1.)

Table 1. Clinical and pathological TNM- classification of breast cancer according to the American Joint Committee on Cancer 8th Edition (p= proved by pathology, c=clinical, T=primary tumour, N= regional lymph nodes, M=distant metastases, LN=lymph node)

TNM-class	Criteria
pTX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis (DCIS)	Ductal carcinoma in situ
Tis (Paget)	Paget disease of the nipple not associated with invasive carcinoma and/or DCIS
T1	Tumour size ≤ 20mm
T1mi	Tumour size ≤ 1mm
T1a	Tumour size ≥ 1mm to ≤ 5mm
T1b	Tumour size ≥ 5mm to ≤ 10 mm
T1c	Tumour size ≥ 10mm to≤ 20mm
T2	Tumour size ≥ 20mm to ≤ 50mm
T3	Tumour size ≥ 50mm
T4	Tumour size any but invasion to chest wall and/or to skin
T4a	Extension to chest wall (pectoralis major not included)
T4b	Ulceration of skin, macroscopic satellite nodules, skin oedema (including peau d'orange)
T4c	T4a and T4b both present
T4d	Inflammatory carcinoma
pNX	Regional LN cannot be assessed (removed previously, or not removed for pathological study)
pN0	No regional LN metastasis or ITCs only
pN0(i+)	ITCs only (malignant cell cluster ≤ 0.2mm)
pN0(mol+)	Positive molecular findings by reverse transcriptase polymerase chain reaction (RT-PCR); no ITC detected
pN1	Micrometastases or metastases in 1–3 axillary LN
pN1mi	Micrometastases, metastatic cell cluster larger than 0.2mm bur smaller than 2mm
pN1a	Metastases in 1–3 axillary LN, at least one larger than 0.2mm
pN1b	Metastases in ipsilateral internal mammary SN, excluding ITCs
pN1c	pN1a and pN1b both present
pN2	Metastases in 4–9 axillary LN, or positive ipsilateral internal mammary LN by imagining in the absence of axillary LN metastases
pN2a	Metastases in 4–9 axillary LN and on > 2.0mm
pN2b	Metastases in clinically detected internal mammary LN with or without microscopic confirmation and pathologically negative axillary LN
pN3	Metastases in 10 or more axillary LN OR in infraclavicular LN OR positive ipsilateral internal mammary LN by imaging in the presence of one or more positive Level I or II nodes OR in more than three axillary LN and micrometastases or macrometastases by SNB in clinically ipsilateral internal mammary LN
pN3a	Metastases in 10 or more axillary LN (one > 2.0mm) OR metastases to the infraclavicular nodes (Level III)
pN3b	pN1a and pN1b in the presence of cN2b (positive internal mammary nodes in imaging)
pN3c	Metastases in ipsilateral supraclavicular LN

M0	No clinical or radiographic evidence of distant metastases (imaging studies are not required to assign the cM0 category)
cM0(i+)	No clinical or radiographic evidence of distant metastases in the presence of tumour cells or deposits larger than 0.2mm detected microscopically or by molecular techniques in circulating blood, bone marrow, or other non-regional nodal tissue in a patient without symptoms and sings of metastases
cM1	Distant metastases detected by clinical and radiographic means
pM1	Any histologically proven metastasis in distant organs or if in non-regional node metastases greater than 0.2mm

2.4 PROGNOSTIC AND PREDICTIVE FACTORS OF BREAST CANCER

Prognostic factors are used to estimate the risk of cancer recurrence and cancer death. Tumour size, axillary lymph node status, histological grade, Ki-67 proliferation index, ER-status and PgR-status, HER2-status, biological subtype and patient age are known prognostic factors of breast cancer. Some of these prognostic factors are also predictive factors. Predictive factors are used to estimate response to cancer treatments and the most important ones include tumour grade, hormone receptor status, HER2-status, Ki-67 proliferation index as well as the biological cancer subtype.

2.4.1 TUMOUR SIZE

Tumour size is an important prognostic factor. The larger the tumour the worse the prognosis is (5,76). Larger tumours are also more often node-positive and biologically more aggressive compared to smaller tumours (77).

2.4.2 AXILLARY LYMPH NODE STATUS

Lymph node status is an even more important prognostic factor than tumour size. The survival probability decreases when the number of metastatic axillary lymph nodes increases (4,5). In studies with a long follow-up the overall survival of N0 patients has been seen to vary between 93.5–93.9% compared to 74.3–81.4% in N1 patients, in patient populations treated with systemic adjuvant therapies according to modern guidelines (18,78) . In addition, patients with three or more metastatic nodes have reported to have over five-fold relative risk of death compared to those with negative nodes (79).

Furthermore, the size of lymph node metastasis plays a role in survival. Reduced disease-free survival has been reported in patients with micrometastases in axillary lymph nodes compared to node-negative patients (14,80–82), while some argue that micrometastases or smaller occult metastases has no role on breast cancer outcome (15,83). Nevertheless, macrometastases are associated with a worse breast cancer outcome compared to micrometastases (80).

2.4.2.1 Isolated tumour cells

Since the definition of ITCs their clinical importance has been debated. Some are convinced that ITCs represent true metastatic potential (12,72,84), while others consider them as artefacts from benign transportation after preoperative tumour manipulation such as CNB (66,68,70), or otherwise of only little prognostic importance (15). Especially, the influence of ITCs on long-term prognosis and their value in tailoring adjuvant therapy is still under debate. According to ACOSOG Z0010 trial, immunohistochemically detected metastases are not of clinical importance and not associated with decreased survival (16). Nevertheless, in ACOSOG trial 78% of patients were treated with systemic adjuvant therapy. Accordingly, the National Comprehensive Cancer Network (NCCN) guideline (85) do not recommend routine IHC in defining nodal involvement and treatment choices should only be based on routine H&E staining, which seldom can detect isolated tumour cells (86).

Nonetheless, others have addressed that micrometastases and even ITCs are associated with unfavourable prognosis and should be actively detected by IHC methods (12–14,87). The MIRROR study even concluded that adjuvant systemic therapy improves survival in patients with ITC findings and micrometastases (14). In HUS, ITCs as such are not considered as an indication to chemotherapy, but as an additional risk factor to be evaluated case by case.

2.4.3 HISTOLOGICAL GRADE

Breast cancer is graded according to its morphological characteristics. Three morphological features are included in the tumour grade: degree of tubule or gland formation, nuclear pleomorphism and mitotic count (88). Grades from these are counted together and the final grade is determined according to Scarff-Bloom-Richardson scale: Grade I tumours are well differentiated, grade II moderately differentiated, and grade III poorly differentiated. Higher grade tumours are associated with an early recurrence and worse survival,

while low grade tumours have shown a very good survival rate but can cause late recurrences (89–91). High grade tumours (III) have also a high Ki-67 proliferation index and are often either HER2-positive or triple negative (91). Tumour grade is not used as a predictive factor alone in breast cancer but together with other predictive factors it guides the treatment choices.

2.4.4 KI-67

Ki-67 nuclear protein (antigen) is only expressed in the cell nucleus in cells which are in proliferative phase and is therefore used as proliferative marker. More precisely, particularly Ki-67 antigen is expressed in G1, S, G2 and M (mitosis) phases, in which Ki-67 antigen is relocated on the surface of chromosomes. (92) With immunohistochemical methods the cell nuclei expressing the Ki-67 antigen can be stained with the MIB-1 monoclonal antibody in paraffin fixed sections. Also, polyclonal anti-Ki-67 can be used to recognise the Ki-67 antigen in fixed material giving almost equivalent result with MIB-1 antibody, both are in use in Finland (93,94). The Ki-67 proliferation index tells the percentage of the tumour cell nuclei which has Ki-67 antigen among the total number of cell nuclei seen and is a marker of cell proliferation rate (88). The prognostic and predictive value of Ki-67 proliferation index has been under debate due to discrepancy between various laboratory methods and different cut-off values used in different pathology laboratories when reporting Ki-67 proliferation rate (95,96). The values also range in Finland between laboratories but being close to the those agreed in the 2013 St. Gallen consensus meeting: low Ki-67 proliferation index ≤ 14% and high ≥ 14% (97). International Ki-67 In Breast Cancer Working Group (IKWG) was established in 2011 to standardize technical procedure and scoring of Ki-67 assessment. IKWG concluded that Ki-67 has significance as prognostic marker and clinical utility but with limitations and it is only suggestive in nature. They however reported that “low” Ki-67 is ≤ 5% and high ≥ 30%. (98) High Ki-67 levels are associated with higher grade tumours (95,99). Nevertheless, AJCC does not recognise Ki-67 proliferation index as a single prognostic factor and as “reliable factor in clinical practise” (75).

Furthermore, high Ki-67 index is seen as a predictive factor for better response to neoadjuvant chemotherapy and it is associated with a higher complete pathological response rate (98,100,101).

2.4.5 OESTROGEN AND PROGESTERONE RECEPTORS

Tumour ER- and PgR-status are assessed with IHC methods. Results are reported as percentages of the stained ER- or PgR-positive cancer cell nuclei from 0–100%. Over 1% is seen as positive (43,88). Patients with ER-positive (HER2-negative) breast cancer have good to excellent prognosis compared to ER-negative breast cancer. Especially the 5-year disease free survival of ER-negative cancer is worse compared to ER-positive (HER2-negative) cancer, since most of the recurrences occur during the first three years after diagnosis. (79,102). However, ER-positive cancer can recur after a long period of time. Over 50% of the recurrences in ER-positive cancer occur after first five years but recurrences after ten years is not uncommon (103,104). Recurrence risk is higher in younger premenopausal women who tend to have lower ER levels, higher tumour grade and have higher stage at diagnosis compared to post-menopausal women. (105,106) Hence, ER-receptor status is an important prognostic factor in breast cancer.

ER-positivity has also an important predictive value: the treatment of ER-positive breast cancer with 5 years of tamoxifen reduces the 10-year risk of death and recurrence about 50% and breast cancer mortality is reduced ca. 30% throughout the first 15 years (relative risk) (107,108).

Approximately 80–85% of cancers are ER-positive and of these ca. 64% are also PgR-positive, since PgR is ER-regulated (109,110). Only about 1–4% of breast cancers are ER-negative and PgR-positive (110,111). Positive PgR-status has been found to be an independent predictor for better endocrine therapy response and better disease-free and overall survival compared to a low or a negative PgR (112,113), even in metastatic breast cancer (114). However, the role of PgR as a prognostic factor is not well established and is seen modest (112,115).

There is controversy about the cut-off value for ER-positive result (116). Nowadays, 1% or higher ER-status is regarded as an indication to adjuvant endocrine therapy in many guidelines (43,97). At the time of our study, and currently, the cut-off value for hormone receptor positivity is 10% in the Finnish guidelines (3). According to some studies tumours with values from 1–10% may act like hormone receptor negative tumours and may not be endocrine therapy sensitive (117). ASCO/CAP guideline recommends that tumours with 1–10% should be called “ER-low positive” (118). Over 50% of stained nuclei is considered to predict good response to endocrine therapy (88).

2.4.6 HER2

The gene for HER2 protein is located on chromosome 17 and its amplification has been found to promote tumourigenesis, cell proliferation, differentiation, angiogenesis, invasion and metastasis in several cancers including breast cancer (73). HER2 protein overexpression is assessed by IHC reported on a scale from 0 to +3. Which of 0 and +1 are negative, +2 equivocal and +3 HER2-positive breast cancer. However, +2 and often also +3 result are confirmed as positive using *in situ* hybrization. At the time of this study confirmation was made by chromogen *in situ* hybrization (CISH) and/or fluorescence *in situ* hybridization (FISH) indicating the HER2 gene amplification. These have been replaced by silver *in situ* hybridization (SISH) and now dual SISH is in use in HUS.

HER2 positivity is a known independent prognostic factor for unfavourable recurrence-free survival and breast cancer-specific survival (119,120). HER2-receptor status is also an important predictive factor since anti-HER2-targeted therapy for HER2-positive cancer reduces significantly both recurrences and mortality (19,20). The joint analysis of NSABP-31 and NCCTG N9831 trials showed a 48% decrease in the risk of recurrence and 39% reduction in death in patients who received trastuzumab plus chemotherapy compared to those with chemotherapy only (22).

2.4.7 BIOLOGICAL SUBTYPES

Originally Perou and Sørlie et al identified five intrinsic subtypes of breast cancer according to molecular classification and gene expression profiling: Luminal A, Luminal B, normal breast like, HER2-enriched, and basal-like. (121) However, gene expression profiling is expensive and not readily accessible in every institution. Thus, in clinical setting IHC methods can be used to determine the biological surrogates of molecular subtypes by assessing the ER-receptor status, the PgR-receptor status, the HER2-status and the Ki-67 proliferation index.

Accordingly, invasive breast cancer is divided into four surrogate subtypes: luminal A-like, luminal B-like, HER2-positive and triple-negative. Characteristics of these subtypes according to the Finnish guidelines are as follows: The luminal types are always ER-positive. They are also the most common subtypes. The Ki-67 proliferation index and the PgR-status are used to distinguish between the luminal A-like and the luminal B-like cancers. Luminal A-like subtype is ER-positive and/or PgR-positive, HER2-negative, and has a low Ki-67 proliferation index and the recurrence risk is low. Luminal A-like has the best prognosis of the subtypes. Luminal B-like cancers are ER-positive, PgR is low or negative, HER2-negative or positive and high Ki-67

proliferation index. The recurrence risk of Luminal B-like cancer is higher when compared to luminal A-like subtype. HER2-positive cancers are either ER-positive (Luminal B-like HER2-positive) or negative (HER2-positive) and have a poor prognosis without anti-HER2 therapy. Therefore, as regards to chemotherapy and anti-HER2 targeted therapy, all HER2-positive cancers are considered as HER2-positive subtype. In triple-negative cancer ER-, PgR- and HER2-receptors are all negative. (3) (Table 2)

Triple-negative cancer is associated with the highest risk of recurrence and cancer death. However recently, immunotherapy has been suggested as an addition to treatment to gain better outcome in triple-negative cancer. (122) Thus, immunotherapy can be used in patients with PD-L1 expressing advanced triple-negative cancers as first line treatment and perhaps also as neoadjuvant treatment in the future. (123)

These biological subtypes are used as a tool in evaluating the prognostics differences among these groups and for treatment planning (97,124). The cut-off values for Ki-67 index, ER and PgR must be evaluated in every laboratory through quality assurance programmes. According to ESMO guidelines, which again are adapted from the St. Gallen consensus conference recommendations from 2013, low Ki67-proliferation index is ≤ 14% and high ≥ 14% (43,97).

Table 2. Intrinsic breast cancer subtypes

Intrinsic subtype	**Biological surrogate definition**
Luminal A	**Luminal A-like:** ER- and/or PgR-positive HER2-negative Ki-67 low *
Luminal B	**Luminal B-like:** ER-positive PgR low or negative HER2-negative or positive Ki-67 high**
HER2-enriched	**HER2-positive:** HER2-positive ER- and PgR- negative
Basal-like	**Triple-negative:** ER- and PgR-negative HER2 negative

Abbreviations: ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; Ki-67, Ki-67 proliferation index; PgR, progesterone receptor. * < 14%, ** > 14% (3,43)

2.4.8 PATIENT AGE

Very young age at diagnosis (<40) is a factor for unfavourable prognosis. Breast cancer in young women is often aggressive being often triple-negative and larger in size. Young age (<40) has been also seen as an independent factor for unfavourable prognosis in ER-positive cancer compared to postmenopausal women (106). Age under 35 has been reported as a factor for unfavourable disease-free survival (125). This is partly because hereditary breast cancer, which is often of an aggressive type, is more frequently diagnosed in younger patients. However, also in the older age group (>70) the prognosis is worse, but this is mostly due to suboptimal treatment because of co-morbidities or even undertreatment due to physician neglection (126).

2.5 PROGNOSTIC AND PREDICTIVE FACTORS IN SMALL (≤1CM) NODE-NEGATIVE BREAST CANCER

Due to screening programmes, very small invasive breast cancers (pT1a-b, tumour size ≤ 1cm) are increasingly diagnosed. The prognosis of small, node-negative breast cancer has been regarded as excellent. In patients with pT1a-b tumours, 10-year overall survival as high as 92% has been reported, while in pT1c (1–2cm) tumours overall survival of 75% was observed. (127) Therefore, patients with node-negative subcentimetre tumours have been excluded from many trials evaluating the benefit of systemic adjuvant therapy, especially chemotherapy. Consequently, there has been debate whether there is a certain threshold for tumour size when systemic adjuvant therapies should be considered (128–130). However, some small node-negative breast cancers are biologically aggressive, such as HER2-positive and triple-negative cancers. The prognosis may not always be so excellent even in pT1a-bN0 tumours, so that chemotherapy with or without anti-HER2-therapy can be omitted (131,132). Approximately 6% to 12% of pT1a-bN0M0 breast cancers overexpress HER2 protein (119,120,133). It has been reported that in small tumours (pT1a-c), the HER2-status, ER-status and the grade play a more important role than the tumour size (134,135).

2.6 MULTIDISCIPLINARY DIAGNOSIS AND TREATMENT OF BREAST CANCER

Breast cancer diagnosis and treatment is teamwork of several professionals. The core multidisciplinary team includes radiologists, surgeons, pathologists, medical and radiation oncologists and breast cancer nurses. When the multidisciplinary team works effectively, the patient goes through a well-planned clinical pathway, ensuring high quality treatment.

2.7 BREAST CANCER SURGERY

Due to the MGR screening program and patient awareness in Finland, breast cancer is most often detected at an early stage. Therefore, less radical breast surgery is needed. The aim of surgery is tumour removal with negative resection margins and enabling of pathological staging of the tumour and lymph nodes. Surgery is performed as sparing as possible maintaining good functional and aesthetic outcomes, but without an increased risk of recurrences.

2.7.1 BREAST CONSERVING SURGERY

In breast conserving surgery (BCS), the tumour is removed together with adequate amount of surrounding breast tissue to achieve sufficient resection margins. No ink on tumour in invasive carcinoma and 2mm in DCIS are considered as sufficient margins (136,137). In patient selection, the most important criterion is the tumour size in relation to the breast size. Nowadays, BCS can be an option even in large or multifocal tumours due to oncoplastic surgical methods. With neoadjuvant therapy the downstaging of large tumours is also possible often enabling BCS in patients, who otherwise were candidates for mastectomy.

In BCS, the goal is to achieve good aesthetic results without compromising the oncological outcome. Good aesthetic outcome after BCS is associated with a better quality of life. With BCS and post-operative radiotherapy (RT), an equivalent disease control is achieved as with mastectomy (138).

2.7.2 MASTECTOMY

Mastectomy is recommended if BCS is not possible with sufficient margins and acceptable aesthetic result, or on patient's request. In addition, mastectomy is indicated, if patient has received prior RT to the same region and further RT after BCS is not possible. Mastectomy is also the treatment of choice in inflammatory breast cancer. Also, risk reducing mastectomies are performed to patients with for example *BRCA1* or *2* gene mutations.

In radical mastectomy, the whole breast is removed as en-block, including over-lying skin, pectoralis major and minor muscles and level I–III axillary nodes. It is nowadays rarely needed. Instead, modified radical, simple, skin sparing or nipple- areola complex sparing mastectomies are used. When mastectomy is the surgery of choice, immediate breast reconstruction or delayed breast reconstruction should be discussed with the patient in terms

of improving quality of life after breast cancer surgery. Reconstructions can be performed with implants or with autologous tissue such as a deep inferior epigastric perforator (DIEP) flap or a latissimus dorsi (LD) flap. (3,139)

2.7.3 SENTINEL LYMPH NODE BIOPSY

SNB is the standard of care in staging clinically node-negative breast cancer, except inflammatory cancer. SN is considered as the first lymph node in which metastatic cancer cells most likely pass through the lymphatic system from the tumour site. The gold standard in SN localization has been preoperative lymphoscintigraphy and intra-operative SN detection with gamma probe and blue dye. In addition, all macroscopically suspicious nodes must be removed and sent to pathology laboratory to decrease the risk of false negative results (140). Nowadays, magnetic tracer and indocyanine green immunofluorescent dye are also used as options in SN localization. The use of radioactive tracer with preoperative lymphoscintigraphy is the standard in HUS Breast Surgery Department, but blue dye is used only occasionally.

The false negative rate of SNB in clinically node-negative breast cancer has varied from 5 to 10 % and the sensitivity being 90–95% (9,141,142). When comparing SNB to diagnostic ALND, no survival disadvantages is seen. Only 0.5–1% axillary recurrence rates have been reported after negative SNB and omitting ALND during a 5-year follow-up. (9,142,143) After SNB, the risk of both short- and long-term morbidity is significantly lower when compared to ALND (7,8,144).

2.7.3.1 Histopathology of sentinel nodes

Before SNB era, the axillary lymph nodes were examined by H&E staining and only one or two sections per node were examined. The SNs are examined more meticulously by serial sectioning, and after negative H&E staining also with IHC (10). Due to this, isolated tumour cells and micrometastases are more often found.

The incidence of ITC and micrometastases after SNB and using IHC methods is reported to be 6–10% and 5–16%, respectively. (11,145,146)

The methods in pathology laboratories however vary worldwide. In HUS, when metastases in SN are found in routine H&E staining additional IHC staining is performed, but in many institutions IHC is not used, at least not routinely.

2.7.4 AXILLARY LYMPH NODE DISSECTION

Before the SNB era, ALND was the gold standard in nodal staging in breast cancer providing also excellent locoregional control. In ALND, lymph nodes from Berg levels I and II are dissected (lymph nodes lateral to and posterior to the pectoralis minor) and also level III, if overt axillary metastases are detected. Performing ALND provides the knowledge about the number on metastatic lymph nodes. Nonetheless, ALND causes significantly more morbidity compared to SNB. Lymphoedema in the arm and in the breast, limited mobility of the arm, numbness and neuropathic pain due to nerve injuries in the ipsilateral arm are the most common morbidities after axillary surgery. (7) Moreover, the role of ALND has been questioned by the NSABP B-04 trial, already before the SNB era (4).

In the beginning of the SNB era, ALND was the gold standard in the treatment of SN positive patients, even those with micrometastases or ITCs. However, after ALND, non-sentinel node metastases were found in only approximately 40% of the patients (147,148). The most often reported risk factors for non-sentinel node metastases include tumour size ≥ 2cm, lymphovascular invasion, the size and number of the SN metastasis, and the extra-capsular growth of the SN metastasis (149). The role of ALND in the surgical treatment of SN positive patients has been questioned in randomised trials, including ACOSOG Z0011, IBCSG- 23-01, AMAROS and OTOASOR (150–154). None of these studies showed benefit of ALND in terms of regional recurrences or survival when compared to observation or axillary radiotherapy. The morbidity, especially arm lymphoedema, was less common after observation or axillary RT when compared with ALND (150,151,153).

After publication of the 10-year follow-up results of the AMAROS trial (151), the Finnish National guidelines were revised. Accordingly, in most patients with upfront surgery and SN metastases ALND is omitted, but the patients receive RT to the axilla (3).

In Finland, ALND is still recommended in patients who have clinically positive axillary lymph nodes, in patients with SN metastases or even ITC after neoadjuvant treatment, and in patients with SN macrometastases (> 2mm), but not suitable for RT.

2.8 RADIOTHERAPY

RT is the standard of care after BCS. RT has shown to reduce local recurrences by 65–75%, all recurrences by 50% and breast cancer mortality by about one-sixth (relative risk reduction) (107). In elderly, >65–70 year, patients RT after BCS reduces the risk of local recurrences but does not have effect on survival (155,156). Accordingly, in these patients with ER-positive tumours endocrine therapy may be sufficient and RT may be omitted after breast conserving surgery (156). In the postoperative whole-breast radiotherapy the target volume usually includes the lower part of the axilla. A booster dose of 10 to 16 Gy to the tumour bed is considered in premenopausal women (3). The booster dose reduces local recurrence rate, but it does not improve survival and patients with booster dose have poorer cosmetic outcome (157).

According to meta-analysis by Early Breast Cancer Trialists' Collaborative Group (EBCTCG), the addition of RT after mastectomy and ALND reduces the risk of local recurrence and increases overall survival in all patients with node-positive disease (158). In Finland, radiotherapy after mastectomy is recommended in all patients with node-positive, macrometastatic disease and in patients with T3-T4 tumours. In patients with T2 tumours or in those with axillary micrometastases the benefit of postmastectomy RT is evaluated case by case. RT to regional nodes is given in patients with axillary nodal macrometastases. (3)

2.9 SYSTEMIC ADJUVANT THERAPY OF THE BREAST CANCER

In Finland, the protocol for systemic adjuvant therapy is based on national and international recommendations and guidelines such as the Finnish Breast Cancer Group, ESMO, St.Gallen and ASCO. These are again based on data and evidence from clinical trials. First of all, systemic adjuvant treatment is tailored according to the patient and disease characteristics. In general, adjuvant systemic therapy is administered in patients with a moderate-to-high risk of recurrence which again depend on clinical and pathological characteristics of the primary tumour. In Finland, 10% risk of recurrence during next 10 years is regarded as an indication to systemic therapy (159). In treatment choices, also patient age, general health, and co-morbidities must be considered. The aim is to start systemic adjuvant therapy within 4–6 weeks after breast surgery. (3)

2.9.1 ENDOCRINE THERAPY

According to St Gallen 2019 Guidelines, all patients with hormone receptor positive (ER ≥ 1%) breast cancer are recommended to be treated with endocrine therapy (160). In Finland, endocrine therapy is recommended in node-positive cancer and in node-negative cancer larger than 10 mm, when ER is ≥10%. It can be considered also in smaller tumours or those with ER-positivity less than 10%. (3) ER-receptor positive cancers have a significant risk of late recurrence. Endocrine therapy has reported to provide a 30–40% relative risk reduction in breast cancer mortality compared to patients not receiving it (161,162).

Tamoxifen is a selective oestrogen receptor modulator and binds to oestrogen receptor blocking the action of circulating oestrogen which then cannot affect to target tissues. The EBCTCG reported in 2011 that 5 years of tamoxifen treatment reduced the relative risk of breast cancer mortality by about one-third through 15 years and significantly reduced breast cancer recurrence in ER-positive cancers. During the years 0–4 recurrence rate was halved and during years 5–9 it was reduced by one third (relative risk reduction). (115)

Tamoxifen causes menopausal symptoms, increases the risk of thromboembolism and the risk of endometrial cancer but causes less risk to osteoporosis compared to aromatase inhibitors.

Aromatase inhibitors (AI) such as letrozole, anastrozole or exemestane block the synthesis of oestrogen in ovaries of pre-menopausal women and in extra-gonadal organs such as liver and in adipose tissue, which are the most important sites of action of AIs in postmenopausal women. AIs are more effective in postmenopausal women. In post-menopausal women five-year treatment with AI has reported to provide a relative risk reduction of about 30% in breast cancer recurrence rate compared to tamoxifen, but for overall survival the data is somewhat controversial (162,163).

The standard treatment for premenopausal women with ER- or PgR-positive cancer is tamoxifen for a time period of 5–10 years. The prolonged 10-year treatment with tamoxifen is indicated in patients with high-risk of recurrence and reported to decreases recurrence and breast cancer mortality especially after the first 10 years (164). Addition of ovarian function suppressive (OFS) medication has been seen to improve disease-free and overall survival in premenopausal women compared to tamoxifen alone (165). Especially, premenopausal women under 35 years and /or having four or more lymph nodes positive could benefit from inclusion of OFS (160,166,167). Therefore, in premenopausal women with a high-risk of recurrence exemestane with OFS for 5 years is recommended (3).

Postmenopausal women usually receive an aromatase inhibitor or tamoxifen for 5 years. In patients with a high risk of recurrence, such as those with node-positive disease, therapy extension up to 10 years further decreases the recurrence risk (168).

2.9.2 CHEMOTHERAPY

The aim of chemotherapy is to eliminate the microscopic cancer cells and thus cure the patient. Young patients and those with aggressive triple-negative and HER2-positive breast cancers benefit most of chemotherapy. The chemotherapy recommendations were previously based solely on prognostic factors, that is the risk of recurrence. The recommendations were largely based on a EBCTG meta-analysis, indicating that chemotherapy reduces breast cancer mortality by one-third, not depending on nodal status, tumour size, or tumour differentiation, oestrogen receptor status or tamoxifen use (169). However recently, the multigene assays like 70-gene signature test and 21-gene recurrence score have proved out to be not only of prognostic value but also predictive for the benefit of chemotherapy in ER-positive HER2-negative N0-N1 cancer (170–172). Nevertheless, the gene signature tests are expensive and might even cause delay in initiation of the treatment and therefore not yet in routine use in Finland.

According to the guideline of HUS Comprehensive Cancer Center chemotherapy is indicated in triple-negative and HER2-positive breast cancer of stage pT1bN0 or higher and patients with pT1a cancer and axillary metastases. Chemotherapy is also indicated in luminal B type cancers when node positive or pT1c. In luminal A type cancers chemotherapy is recommended in premenopausal patients and in those with pN2-3 axillary nodal stage. The results from IHC staining are not always reliable enough to distinguish between luminal A and B type cancers. In these cases, the genomic risk scores or multiple gene assays can be used to guide the decision making for or against chemotherapy.

Chemotherapy may cause hair loss, fatigue, leuko- and neutropenia, anaemia and nausea and vomiting. In elderly patients and even in younger with serious co-morbidities, the benefits of chemotherapy must be carefully evaluated and compared to the risk of recurrence.

2.9.3 NEOADJUVANT CHEMOTHERAPY

Neoadjuvant chemotherapy is given prior to surgery, and it is considered as effective as adjuvant chemotherapy (173). It was traditionally indicated in patients with locally advanced breast cancer to improve operability. It is currently indicated also in operable cancers, especially in biologically aggressive (triple negative and HER2-positive carcinomas), to avoid delays caused by surgery itself and possible post-operative complications. Also, the aim of neoadjuvant chemotherapy may be to reduce the tumour size to facilitate BCS. The neoadjuvant chemotherapy regimen varies depending on patient and tumour characteristics and usually 6–8 chemotherapy cycles are given before the surgery.

2.9.4 ANTI-HER2 THERAPY

Trastuzumab is a humanised monoclonal antibody against the HER2 extracellular domain and blocks signal transduction and thus proliferation and induces apoptosis. It also decreases vascular endothelial growth factor production and potentiates chemotherapy in HER2-positive breast cancer. From 2005, trastuzumab has been the standard of care in patients with HER2-positive tumours. It provides a relative reduction in relapse of about 50% and it reduces the relative risk of death approximately 30% compared to patients not receiving it. (20,22,174)

The treatment of small node-negative HER2-positive breast cancer has been under debate. According to some studies the pT1a-bN0M0 HER2-positive breast cancers have such a good long-term prognosis that systemic adjuvant treatment is not necessary (131,132,175). On the other hand, others argue that a survival advantage is seen even in pT1a-bN0M0 HER2-positive breast cancer when treated with systemic adjuvant therapy including anti-HER2 treatment (128,129,176–179). According to Finnish national guidelines trastuzumab has been recommended routinely for years in patients with node-negative, HER2-positive pT1b and larger tumours and in all node-positive tumours. St Gallen panel 2019 advised taxane chemotherapy and trastuzumab routinely in pT1b and larger HER2-positive tumours, even when node negative and for pT1a HER2-positive node-negative tumours case by case.

The discussion about the duration of effective trastuzumab treatment is ongoing. The routine duration of trastuzumab is 12 months since a longer duration has not shown any benefits (174). Even a shorter duration of trastuzumab has been effective (20,180), although not considered as the standard of care. In addition, the shorter duration of trastuzumab caused less adverse effects of which left ventricular ejection fraction decrease and following cardiac failure is the most common (20,180).

In addition to trastuzumab, also other anti-HER2 drugs are used. Pertuzumab is also a monoclonal antibody binding to HER2 protein but at different site than trastuzumab. Pertuzumab is used together with trastuzumab in neoadjuvant therapy in node-positive breast cancer and in metastatic breast cancer. (116,181) Trastuzumab emtansine is recommended in patients with residual disease in breast, axilla or in both after neoadjuvant therapy (182).

There are also adverse events in anti-HER2-therapy. The risk of left ventricular dysfunction is the best-known toxicity in trastuzumab treatment and must be discussed with the patient. (183,184)

3 AIMS OF THE STUDY

In general, this doctoral thesis aims to investigate the prognostic factors in small node-negative (pT1N0, ≤ 2cm) breast cancer and their impact on breast cancer survival in this patient group.

The more specific aims are:

I. To investigate does the method of preoperative biopsy affect breast cancer outcome. In addition, whether a preoperative core needle biopsy (CNB) increases the incidence of isolated tumour cells (ITC) in the axillary sentinel lymph nodes and impairs breast cancer outcome when compared to fine needle aspiration cytology (FNAC).

II. To evaluate the prognostic value of sentinel node isolated tumour cells (ITCs) in node-negative pT1 breast cancer.

III. To investigate the prognosis and prognostic factors of pT1a-bN0M0 (≤ 1cm tumour) HER2-positive breast cancer during a long follow-up time.

4 MATERIALS AND METHODS

The study was conducted at the Breast Surgery Unit of Helsinki University Hospital, Finland. Consecutive 1,865 patients with unilateral invasive pT1 (the largest tumour diameter ≤ 2 cm) breast cancer operated between March 2000 and April 2006 were prospectively collected in a database and formed the basis of the study. Patients were not previously treated for invasive breast cancer or other malignancy during the last five years prior to the detection of breast cancer and were not treated with neoadjuvant therapy.

The date of breast cancer recurrence, the cause of death, and the date of death were extracted from the hospital records. In addition, data on cancer survival were obtained from the Finnish Cancer Registry, which has a coverage exceeding 95% in the population.

The research protocol of the study was approved by an Ethics Committee of HUS in 1999.

4.1 PATIENTS

Study I is a prospective observational cohort study of 1,525 patients. Patients were categorised in two groups according to the preoperative biopsy method (FNAC or CNB), which was chosen by the preference of the radiologist who performed the biopsy. All needle biopsies were image-guided and performed by a specialised breast radiologist. Both FNAC and CNB was performed in patients with an inconclusive FNAC result and these patients were included in the CNB group. We investigated whether performing CNB in breast cancer diagnosis increases the incidence of ITC findings in the axillary sentinel lymph nodes compared to FNAC or influences patients' oncological outcome

Study II is a prospective population-based cohort study including 936 patients with unilateral pT1N0 cancer. Patients who underwent ALND without preceding SNB were excluded, since lymph nodes removed at ALND are not as meticulously investigated as the SN, and ITCs are often missed. The survival of patients with (pN0i+, n = 75*)* or without (pN0i-, n = 861) ITC findings in their SN was compared.

The study III is a retrospective analysis based on this prospectively collected database including 414 patients with pT1a-bN0M0 breast cancer. Altogether 335 patients with pT1a-bN0M0 breast cancer were included in the study: 44 patients with HER2+ cancer, not treated with adjuvant anti-HER2-targeted therapy (the HER2+ group) and 291, hormone receptor positive, HER2-

negative cancers (the ER+/HER2- group). Patients with HER2+ cancer were also investigated in subgroups according to the tumour size (HER2+/pT1a vs. HER2+/pT1b), ER-status (HER2+/ER- vs. HER2+/ER+), and the SN ITC status (HER2+/pN0i- vs. HER2+/pN0i+). Survival outcomes of different patient groups were then analysed.

Study specific exclusion criteria for all three studies are provided in Table 3.

4.2 SURGERY

In studies I, II and III BCS or mastectomy was chosen depending on the patient and tumour characteristics in agreement with the patient. The patients underwent either an SNB or ALND, or both. Before the SNB, preoperative lymphoscintigraphy was performed, and the SNs harvested during surgery using a gamma probe and blue dye.

At the time of the study completion ALND was generally performed in patients with SNs contained micro- or macrometastases or even with only ITCs. Level I–II axillary nodes were harvested always when ALND was performed. Level III nodes were dissected when clinically suspicious level II–III axillary nodes were detected during surgery. ALND was performed in patients with axillary metastases diagnosed before surgery. In addition, some of the patients had ALND after an unsuccessful SNB.

4.3 HISTOPATHOLOGICAL METHODS

In all three studies, the breast and SN specimens were sent to the pathology laboratory separately as fresh unfixed specimens and examined histologically by specialised breast pathologists. SN were sliced in multiple sections 1 to 1.5 mm apart. An intraoperative frozen section analysis, including rapid IHC staining based on cytokeratin antibody Cam 5.2. (Becton Dickinson Immunocytometry Systems, San Joes, CA) of the multiple sections, was performed. However, after June 2003 Cyto-nel ultrarapid immunohistochemistry was used (Immuno Diagnostics Oy, Hämeenlinna, Finland). Rest of the SN tissue was formalin fixed, paraffin embedded, and H&E stained in two sections. If no metastases were found, or if ITCs or micrometastasis was found in the SN frozen sections, an IHC staining for cytokeratin was performed, in addition to routine H&E staining. IHC staining was not done when a 2 mm or larger metastasis was found in frozen section analysis. The ALND specimen were sent to the laboratory in formalin. The lymph nodes from the ALND specimen were examined after staining with H&E.

The tumour diameter, histological type and grade, hormone receptor status, HER2-status, and the Ki-67 proliferation index were evaluated. IHC methods were used to evaluate the hormone receptor status and the Ki-67 proliferation index. Over 10% of the cancer cell nuclei staining for the ER or the PgR was considered a positive staining result. Ki-67 antigen expression (the proliferation index) was determined with the MIB-1 monoclonal antibody. The patients were categorised into three categories according to the Ki-67 proliferation index: negative or low (0–19%), intermediate (20–30%), and high (> 30%). Cancer HER2 protein overexpression was evaluated first by IHC. The result was considered positive when the IHC staining result was 2+ or 3+ (on a scale from 0 to 3+). Cancers with a 2+ and 3+ result in IHC were further tested using chromogen *in situ* hybridization (CISH), and whenever HER2/*neu* gene amplification was present, cancer was considered HER2-positive, otherwise HER2-negative. IHC 3+ staining was in some cases considered sufficient evidence for the presence of HER2 amplification without CISH confirmation.

The histological classification and grading were based on the WHO classification (73).

4.4 RADIOTHERAPY

In all three studies postoperative whole-breast RT was given after BCS using tangential fields. The target volume usually covered lower axilla, at least partially. A booster dose of 10 to 16Gy was given to premenopausal women to the breast tumour site. Postmastectomy RT and RT to regional nodes was administered in patients with axillary metastases.

4.5 SYSTEMIC ADJUVANT THERAPY

In studies I, II and III the systemic adjuvant treatments were administered depending on the patient and disease characteristics and according to national and institutional guidelines. In general, patients considered to have a moderate-to-high risk of recurrence were treated with chemotherapy, endocrine therapy, or both. The presence of ITCs in the SNs was not considered an absolute indication for systemic adjuvant therapy. Premenopausal women with ER- or PgR- positive cancer were recommended to receive tamoxifen for five years, and postmenopausal women most often received an aromatase inhibitor for five years. Patients with high risk of recurrence HER2-positive cancer received adjuvant trastuzumab and chemotherapy after May 2005, and a few patients prior to this within the context of a clinical trial (20).

Table 3. Patient inclusion and exclusion criteria in Studies I, II and III. pN0i-/ i+: isolated tumour cell negative/positive lymph node; FNAC: fine needle aspiration cytology; CNB: core needle biopsy; SNB: sentinel lymph node biopsy; ALND: axillary lymph node biopsy; NA: not available.

	Study I		**Study II**		**Study III**	
	n	**%**	***n***	**%**	***n***	**%**
Total (*n*)	1865		1865		1608	
Excluded patients	340		929		1273	
Included patients	1525		936		335	
pT						
pT1a (≥ 1mm to 5mm)					51	15.2
pT1b (≥ 5mm to ≤ 10 mm)					284	84.8
pT1 (≤ 20mm)	1525		936			
pN-status						
pN0i-	935	61.3	861	92.0	311	92.8
pN0i+	70	4.5	75	8.0	24	7.2
pN1mic	166	10.9				
pN1mac	276	18.1				
pN2-3	78	5.1				
Biopsy method						
FNAC	868	56.9	476	50.9		
CNB	657	43.1	407	43.5		
Surgical Biopsy			47	5.0		
NA			6	0.6		
Excluded Patients						
Surgical biopsy or missing biopsy information	83					
No axillary surgery	2		2			
Contralateral breast cancer	190		190			
History of other malignancy	25		25			
Distant metastases at presentation	7		7			
Died of myocardial infarction immediately after surgery	1		1			
Lost to follow-up	32		32			
Sentinel node micro-or macrometastasis			443		100	
ITC in the sentinel node, but micrometastasis in ALND			1			
Upfront ALND without SNB			168			
ALND due to unsuccessful SNB			60			
pT1c (tumour size ≥ 10mm to≤ 20mm)					1094	
Triple-negative breast cancer					19	
Missing steroid hormone receptor status					56	
Other cancer within 5 years					1	
Treated with trastuzumab					3	

4.6 FOLLOW-UP

For all the study patients, follow-up visits after breast surgery were planned at one, three, and five years, and were organised at the Department of Oncology, HUS. Physical examination, blood cell counts, blood chemistry, and bilateral mammography were performed. Whenever considered necessary, breast and axillary ultrasound, bone isotope scan, and CT were also performed. If patient had any concern of breast cancer recurrence an access to additional examinations and visits were organised.

After the first five years, the follow-up continued at the public local health care centres or at private health care providers, which ever was the patient preference. If breast cancer recurrence was suspected, the patient was referred to the HUS for further examinations and treatment.

4.7 STATISTICAL METHODS

In all the studies frequency tables were analysed with the chi-squared test or Fisher's exact test (when the expected $n < 5$), and continuous variables were compared with the Mann-Whitney U-test. In study I binary logistic regression analysis was performed to adjust for the differences between the CNB and the FNAC group characteristics when analysing the incidence of ITC between the pN0i- and the pN0i+ groups.

Distant disease-free survival was calculated from the date of breast surgery to the date of first occurrence of breast cancer metastases outside of the breast or mastectomy area or the regional lymph nodes. Subsequent contralateral breast cancers or other second cancers were not considered as distant disease survival events. Locoregional recurrence-free survival time was calculated from the date of breast surgery to the date of first regional lymph node or ipsilateral breast recurrence. Breast cancer-specific survival was calculated from the date of breast surgery to the date of death considered to result from breast cancer. Patients who died with distant metastases based on clinical, radiological, or autopsy evidence were considered to have died from breast cancer. Overall survival was calculated from the date of surgery to the date of death from any cause-censoring patients who were alive on the date of the last contact. When a patient was lost to follow-up, the date of death was acquired from the Finnish Cancer Registry, but if the date was not available, the patient was censored on the date when lost to follow-up. Patients without such an event on the last date of contact or on the date of death from another cause were censored.

Survival was analysed using the Kaplan-Meier method, and survival between groups was compared with the log-rank test. In studies I and II a Cox proportional hazards regression model was used to assess the independent influence of covariables on survival. Variables with a *p*-value less than 0.1 in the univariable survival analysis were entered into a multivariable backward stepwise Cox regression analysis. In studies I and II the Cox regression analysis was performed with and without including systemic adjuvant treatment as a covariate to investigate effect of treatments to survival. In study III due to small number of events, it was not possible to examine the relationship between the outcomes and prognostic factors of interest with the Cox model allowing coefficients to vary over time. Similarly, limited data precluded performing multivariable analyses. Two-sided *p*-values < 0.05 were considered significant. IBM® SPSS® Statistics (SPSS Inc., Chicago, IL) software was used to conduct the statistical analyses.

5 RESULTS

5.1 BIOPSY METHOD AND BREAST CANCER OUTCOME (STUDY I)

PATIENT CHARACTERISTICS

Of the 1,525 cancers 868 (56.9%) were diagnosed with FNAC and 657 (43.1%) with CNB. The patient, tumour and treatment characteristics are summarised in Table 4. ITCs were found in the SNs of 37 (4.3%) patients in the FNAC group and from 33 (5.0%) in the CNB group (p = 0.798).

Table 4. Study I. Patient and tumour characteristic according to biopsy method. CNB: Core Needle Biopsy; FNAC: Fine Needle Aspiration Cytology; ITC: isolated tumour cells; pN0i-/ i+: isolated tumour cell negative/positive sentinel lymph node; ER: oestrogen receptor; PgR: progesterone receptor; Ki-67 (MIB-1): Ki-67 proliferation index determined with MIB-1 monoclonal antibody; HER2: human epidermal growth factor receptor 2; BCS: Breast Conserving Surgery; SNB: Sentinel Node Biopsy; ALND: Axillary Lymph Node Dissection. Reproduced with the permission from European Journal of Surgical Oncology.

		FNAC		**CNB**		
		n = 868	**(%)**	**n = 657**	**(%)**	***p***
Age (years)	Median	59		57		0.002
	SD	11.6		10.6		
Tumour size (mm)	Median	14		12		<0.001
	SD	4		4		
pN stage	pN0i-	509	(58.6)	426	(64.8)	0.069
	pN0i+	37	(4.3)	33	(5.0)	
	pN1mic	100	(11.5)	66	(10.0)	
	pN1mac	171	(19.7)	105	(16.0)	
	pN2-3	51	(5.9)	27	(4.1)	
ITC	pN0i-	509	(58.6)	426	(64.8)	0.798
	pN0i+	37	(4.3)	33	(5.0)	
Tumour histology	Ductal	597	(68.8)	387	(58.9)	<0.001
	Lobular	148	(17.1)	137	(20.9)	
	Other	123	(14.2)	133	(20.2)	
Tumour palpability	Not palpable	238	(27.4)	284	(43.2)	<0.001
	Palpable	628	(72.4)	373	(56.8)	
Histological grade	I	251	(28.9)	267	(40.6)	<0.001
	II	421	(48.5)	288	(43.8)	
	III	189	(21.8)	95	(14.5)	

Multifocality	Unifocal	722	(83.2)	545	(83.0)	0.868
	Multifocal	145	(16.7)	112	(17.0)	
ER-status	Negative	99	(11.4)	59	(9.0)	0.124
	Positive	769	(88.6)	598	(91.0)	
PgR-status	Negative	261	(30.1)	170	(25.9)	0.065
	Positive	604	(69.6)	487	(74.1)	
Ki-67 (MIB-1)	+	471	(54.3)	434	(66.1)	<0.001
	++	196	(22.6)	125	(19.0)	
	+++	193	(22.2)	91	(13.9)	
HER2-status	Negative	688	(79.3)	506	(77.0)	0.221
	Positive	70	(8.1)	40	(6.1)	
Breast surgery	Mastectomy	233	(26.8)	164	(25.0)	0.407
	BCS	635	(73.2)	493	(75.0)	
Axillary surgery	SNB	446	(51.4)	373	(56.8)	0.037
	ALND	422	(48.6)	284	(43.2)	
Radiotherapy	No	152	(17.5)	127	(19.3)	0.367
	Yes	714	(82.3)	529	(80.5)	
Endocrine therapy	No	306	(35.3)	285	(43.4)	0.001
	Yes	558	(64.3)	368	(56.0)	
Chemotherapy	No	567	(65.3)	459	(69.9)	0.062
	Yes	298	(34.3)	196	(29.8)	

Table 5. Study I. Follow up and events in FNAC and CNB groups. CNB: Core Needle Biopsy; FNAC: Fine Needle Aspiration Cytology; BCS: breast conserving surgery. Reproduced with the permission from European Journal of Surgical Oncology.

		FNAC n = 868		**CNB n = 657**		**p**
		n	**(%)**	**n**	**(%)**	
Follow-up (months)	Median	116		113		
	SD	30		24		
Events	Death from any cause	129	(14.9)	62	(9.4)	0.003
	Breast cancer death	40	(4.6)	25	(3.8)	0.461
	Distant metastasis	67	(7.7)	40	(6.0)	0.178
	Regional lymph node recurrence	11	(1.3)	7	(1.0)	0.681
	Local recurrence	34	(3.9)	25	(3.8)	0.814
	Local recurrence after BCS	27	(3.1)	19	(2.9)	
	Local recurrence after mastectomy	7	(0.8)	6	(0.9)	

FOLLOW-UP, EVENTS AND SURVIVAL OUTCOME

The median follow-up time of all patients after surgery was 115 months (9.5 years) and 116 months in FNAC group and 111 months the CNB group. (Table 5)

The events and survival for different survival endpoints are presented in Table 5. In the univariable analysis, the CNB group had more favourable overall survival as compared to the FNAC group (p = 0.003, Figure 1). Nevertheless, the type of biopsy was not associated with breast cancer-specific survival (p = 0.461), distant disease-free survival (p = 0.178), regional lymph node recurrence-free survival (p = 0.681), or local recurrence-free survival (p = 0.814) in univariable analyses.

Nonetheless, the CNB lost its statistical significance for overall survival in the multivariable analysis (p = 0.718). Instead, a high pN category (pN2 or pN3 vs. pN0; p <0.001; HR = 2.98, 95%CI 1.90-4.69), tumour palpability (p = 0.004; HR = 1.77, 95%CI 1.19–2.62), and young age at diagnosis (p <0.001; HR = 1.09, 95%CI 1.07–1.10) were significantly associated with unfavourable overall survival in the multivariable analysis. (Table 6) When the multivariable analysis for overall survival was repeated including chemotherapy as a further covariable, the results remained essentially similar (data not shown).

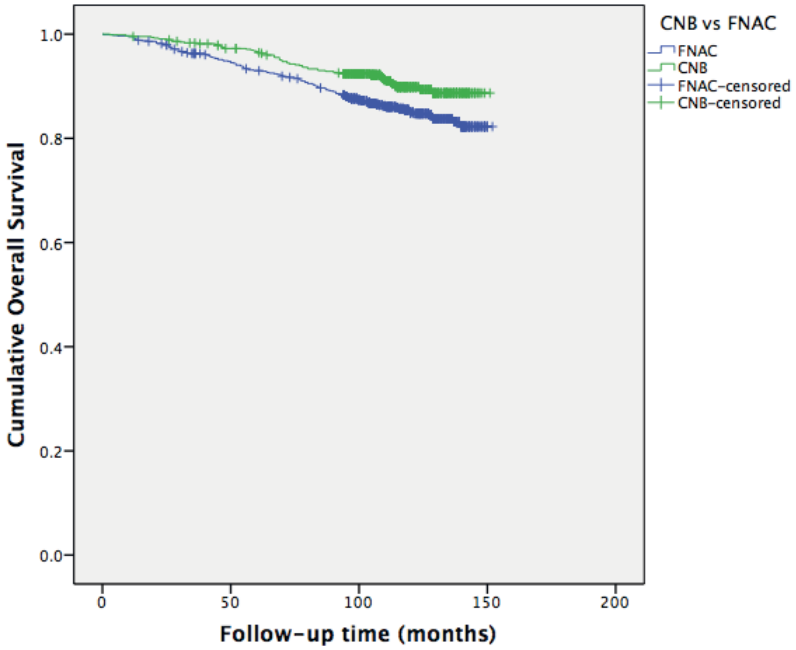

Figure 1. Study I. Overall survival CNB vs. FNAC, p = 0.003. Reproduced with the permission from European Journal of Surgical Oncology.

Table 6. Study I. Univariable and multivariable Cox proportional hazards regression survival analysis for overall survival. CNB: Core Needle Biopsy; FNAC: Fine Needle Aspiration Cytology; pN0i-/ i+: isolated tumour cell negative/positive sentinel lymph node; ER: oestrogen receptor; PgR: progesterone receptor; Ki-67 (MIB-1): Ki-67 proliferation index determined with MIB-1 monoclonal antibody HER2: Human epidermal growth factor receptor 2; HR: hazard ratio. Reproduced with the permission from European Journal of Surgical Oncology.

		Univariate				Multivariate			
			95% CI for HR				95% CI for HR		
		HR	**Lower**	**Upper**	***p***	**HR**	**Lower**	**Upper**	***p***
Age (years)		1.08	1.07	1.09	<0.001	1.09	1.07	1.10	<0.001
Tumour size (mm)		1.05	1.03	1.09	0.001	1.00	0.96	1.04	0.98
CNB vs. FNAC		0.63	0.47	0.86	0.004	0.94	0.69	1.29	0.71
pN category	**pN0i-**								
	pN0i+	1.54	0.85	2.78	0.15	2.54	1.38	4.65	0.003
	pN1mic	0.82	0.49	1.37	0.45	1.22	0.72	2.05	0.46
	pN1mac	0.97	0.65	1.45	0.88	1.10	0.73	1.67	0.64
	pN2-3	3.07	2.00	4.69	<0.001	2.98	1.89	4.69	<0.001
Breast tumour site	**Lower Lateral**								
	Central	1.50	0.86	2.62	0.15				
	Upper Medial	1.02	0.64	1.62	0.93				
	Lower Medial	0.82	0.46	1.47	0.51				
	Upper Lateral	0.70	0.46	1.08	0.10				
Palpability		2.22	1.56	3.15	<0.001	1.77	1.19	2.62	0.004
Histological grade		1.53	1.26	1.85	<0.001	1.30	0.96	1.64	0.10
Tumour histology	**Ductal (ref)**								
	Lobular	1.09	0.77	1.55	0.62				
	Other	0.79	0.53	1.21	0.29				
Tumor multifocality		1.12	0.78	1.61	0.54				
ER-status (positive vs. negative)		0.56	0.39	0.81	0.002	1.30	0.83	2.05	0.25
PgR-status (positive vs. negative)		0.75	0.56	1.02	0.06				
Ki-67 (MIB-1) (+ vs ++ vs +++)		1.29	1.09	1.53	0.002	1.14	0.89	1.44	0.29
HER2-status (positive vs. negative)		0.87	0.49	1.54	0.65				
Chemotherapy (given vs not given)		0.76	0.55	1.05	0.09				

5.2 PROGNOSTIC VALUE OF ISOLATED TUMOUR CELLS IN SENTINEL LYMPH NODES IN pT1 BREAST CANCER (STUDY II)

ITC FINDINGS AND PATIENT CLINICOPATHOLOGICAL CHARACTERISTICS

There were 861 (92%) ITC-negative (pN0i-) and 75 (8%) ITC-positive (pN0i+) patients. No statistically significant differences were observed between the pN0i- and pN0i+ -groups in patient age, tumour histology, histological grade, the Ki-67 proliferation index, or hormone receptor status. Albeit, patients with pN0i+ cancer had slightly larger median tumour diameter as compared to patients with pN0i- disease (13 *vs* 12 mm, respectively; $p = 0.021$). Patient and tumour characteristics are shown in Table 7.

SURGERY AND SYSTEMIC ADJUVANT TREATMENTS

Patients with pN0i+ cancer underwent more often mastectomy and had an ALND, and they were treated more frequently with systemic adjuvant therapies as compared to the patients with pN0i- disease (Table 8).

FOLLOW-UP, EVENTS AND SURVIVAL OUTCOME

The patients with pN0i- cancer had a longer median follow-up time than patients with pN0i+ cancer (9.5 *vs* 9 years, respectively; $p = 0.016$). Altogether 47 (5.0%) patients were diagnosed with distant metastases during the follow-up: 39 (4.5%) out of 861 patients in the pN0i- group, and 8 (10.7%) out of 75 patients in the pN0i+ group. Ten-year distant disease-free survival was 95.3% in the pN0i- group and 88.8% in the pN0i+ group (univariable Cox regression hazard ratio [HR] 2.53, 95% CI 1.18–5.41; $p = 0.017$; Figure 2). In a multivariable Cox proportional hazards regression analysis, ITC findings were found to associate statistically significantly with worse distant disease-free survival (HR 2.34, 95% CI 1.09–5.04; $p = 0.029$) together with a larger tumour diameter and a high Ki-67 proliferation index (Table 9).

Table 7. Study II. Patient and tumour characteristics stratified by the presence of ITCs in the sentinel lymph nodes. pN0i-/ i+: isolated tumour cell negative/positive sentinel lymph node; FNAC: Fine Needle Aspiration Cytology; CNB: Core Needle Biopsy; Ki-67 (MIB-1): Ki-67 proliferation index determined with MIB-1 monoclonal antibody; HER2: Human epidermal growth factor receptor 2; NA: Not Available. Reproduced with the permission from Springer Nature.

	Number of pN0i- patients *n* = 861 *n* (%)		**Number of pN0i+ patients *n* = 75 *n* (%)**		***p***
Age at diagnosis (years)					
Median	58		57		0.262
Range	27–92		35–87		
Tumour Size (mm)					
Median	12		13		0.021
Range	1–20		1–20		
Biopsy method					
FNAC	439	(51.0)	37	(49.3)	0.788
CNB	374	(43.4)	33	(44.0)	
Surgical Biopsy	42	(4.9)	5	(6.7)	
NA	6	(0.7)	0	(0.0)	
Tumour Histology					
Ductal	546	(63.4)	50	(66.7)	0.519
Lobular	155	(18.0)	15	(20.0)	
Other	160	(18.6)	10	(13.3)	
Histological Grade					
I	340	(39.5)	25	(33.3)	0.262
II	359	(41.7)	39	(52.0)	
III	151	(17.5)	11	(14.7)	
NA	11	(1.3)	0	(0.0.)	
ER-status					
Negative	96	(11.1)	8	(10.7)	0.888
Positive	761	(88.4)	67	(89.2)	
NA	4	(0.5)			
PgR-status					
Negative	267	(31.0)	19	(25.3)	0.286
Positive	587	(68.2)	56	(74.7)	
NA	7	(0.8)			
Ki-67 (MIB-1)					
0–19%	539	(62.6)	48	(64.0)	0.746
20–30%	166	(19.3)	12	(16.0)	
>30%	143	(16.6)	14	(18.7)	
NA	13	(1.5)	1	(1.3)	
HER2-status					
Negative	675	(78.4)	55	(73.3)	0.424
Positive	52	(6.0)	4	(5.3)	
NA	134	(15.6)	16	(21.3)	

Table 8. Study II. Treatments in pN0i- and pN0i+ groups. pN0i-/ i+: isolated tumour cell negative/positive sentinel lymph node; BCS: Breast Conserving Surgery; SNB: Sentinel Node Biopsy; ALND: Axillary Lymph Node Dissection; NA: Not Available. Reproduced with the permission from Springer Nature.

	Number of pN0i- patients ***n* = 861 (%)**	**Number of pN0i+ patients** ***n* = 75 (%)**	***p***
Breast Surgery			
Mastectomy	144 (16.7)	24 (32)	<0.001
BCS	717 (83.3)	51 (68)	
Axillary Surgery			
SNB	842 (97.8)	13 (17.3)	<0.001
SNB and ALND	19 (2.2)	62 (82.7)	
Radiotherapy			
No	150 (17.4)	20 (26.7)	0.040
Yes	710 (82.5)	54 (72.0)	
NA	1 (0.1)	1 (1.3)	
Endocrine Therapy			
No	480 (55.7)	16 (21.3)	<0.001
Yes	377 (43.8)	58 (77.3)	
NA	4 (0.5)	1 (1.4)	
Chemotherapy			
No	747 (86.8)	53 (70.7)	<0.001
Yes	112 (13.0)	21 (28.0)	
NA	2 (0.2)	1 (1.3)	

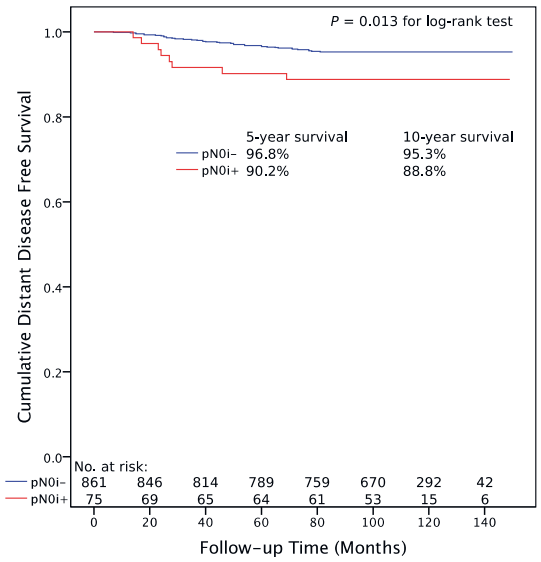

Figure 2. Study II. Distant disease-free survival pN0i- vs. pN0i+. Reproduced with the permission from Springer Nature.

Table 9. Study II. Univariable and multivariable survival analyses for distant disease-free survival. pN0i-/ i+: isolated tumour cell negative/positive sentinel lymph node; ER: Oestrogen Receptor; PgR: Progesterone Receptor; Ki-67 (MIB-1): Ki-67 proliferation index determined with MIB-1 monoclonal antibody; HER-2: Human epidermal growth factor receptor 2. Age, Ki-67 and the histological grade were entered as continuous variables. Eighteen patients had one or more missing values in the final steps of the analyses. Reproduced with the permission from Springer Nature.

	Univariable analysis			Multivariable analysis		
	HR	**95% CI for HR**	***p*-value**	**HR**	**95% CI for HR**	***p*-value**
Age (Years)	1.00	0.98–1.03	0.960			
Tumour Size (mm)	1.13	1.06–1.21	0.001	1.08	1.01–1.16	0.033
pN0i- vs pN0i+	2.53	1.18–5.41	0.017	2.34	1.09–5.04	0.029
Histological Grade	2.48	1.66–3.70	<0.001	1.34	0.77–2.33	0.302
Tumour Histology						
Ductal (reference)						
Lobular	0.55	0.21–1.40	0.208			
Other	1.25	0.63–2.50	0.518			
Tumour Multifocality	1.18	0.50–2.79	0.699			
ER-status	3.23	1.70–6.12	<0.001	1.04	0.43–2.49	0.934
PgR-status	2.20	1.24–3.89	0.007	1.51	0.82–2.77	0.183
Ki-67 (MIB-1)	2.37	1.71–3.30	<0.001	2.16	1.54–3.04	<0.001
HER2-status	3.46	1.59–7.56	0.002	1.76	0.76–4.08	0.415

Altogether 104 (11.1%) patients died during the follow-up: 92 (10.7%) out of the 861 patients in the pN0i- group and 12 (16.0%) out of the 75 patients in the pN0i+ group. Ten-year overall survival was 89.2% in the pN0i- group and 83.8% in the pN0i+ group (univariable Cox regression HR 1.67, 95% CI 0.92–3.06; $p = 0.094$; Figure 3.). In a multivariable Cox regression analysis, the presence of ITCs was an independent predictor of worse overall survival (HR 2.42, 95% CI 1.32–4.46; $p = 0.005$) together with higher age at diagnosis and the high Ki-67 proliferation index (Table 10).

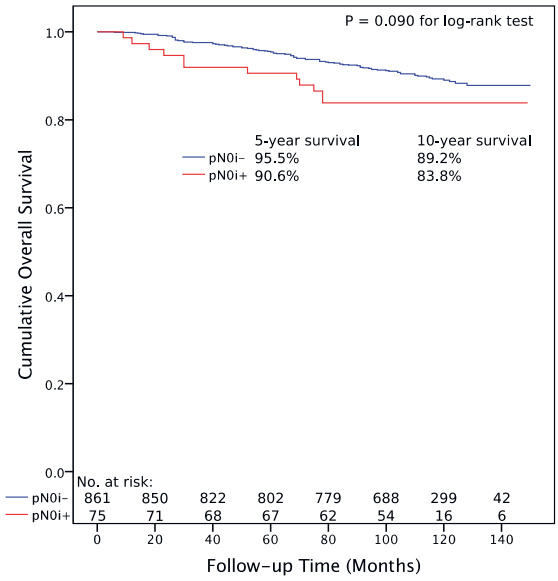

Figure 3. Study II. Overall survival pN0i- vs. pN0i+. Reproduced with the permission from Springer Nature.

Table 10. Study II. Univariable and multivariable survival analyses for overall survival. pN0i- / i+: isolated tumour cell negative/positive sentinel lymph node; ER: Oestrogen Receptor; PgR: progesterone Receptor; Ki-67 (MIB-1): Ki-67 proliferation index determined with MIB-1 monoclonal antibody; HER2: Human epidermal growth factor receptor 2. Age, Ki-67 and the histological grade were entered as continuous variables. Nineteen patients had one or more missing values in the final steps of the analyses. Reproduced with the permission from Springer Nature

	Univariable analysis			Multivariable analysis		
	HR	**95% CI for HR**	***p*-value**	**HR**	**95% CI for HR**	***p*-value**
Age (years)	1.09	1.07–1.11	<0.001	1.09	1.08–1.11	<0.001
Tumour Size (mm)	1.06	1.01–1.11	0.011	1.03	0.98–1.08	0.299
pN0i- vs pN0i+	1.67	0.92–3.06	0.094	2.42	1.32–4.46	0.005
Histological Grade	1.34	1.03–1.74	0.030	1.09	0.76–1.57	0.646
Tumour Histology						
Ductal (reference)						
Lobular	0.94	0.56–1.58	0.803			
Other	0.84	0.49–1.45	0.538			
Tumour Multifocality	0.93	0.48–1.78	0.814			
ER-status	1.49	0.89–2.53	0.158			
PgR-status	0.94	0.61–1.45	0.777			
Ki-67 (MIB-1)	1.32	1.04–1.67	0.024	1.41	1.11–1.79	0.005
HER2-status	0.42	0.13–1.34	0.142			

Only 27 (26%) of the 104 deaths were considered to have resulted from breast cancer (20 and 7 in the pN0i- and pN0i+ groups, respectively). The characteristics of patients with ITCs in the SNs and who died of breast cancer during the follow-up are provided in Table 11. Ten-year breast cancer-specific survival was as high as 97.6% in the pN0i- group and 90.1% in the pN0i+ group (univariable Cox regression HR 4.29, 95% CI 1.81–10.15; $p = 0.001$; Figure 4). In the multivariable Cox proportional hazards regression analysis including factors provided in Table 10, the presence of ITCs was an independent prognostic factor for unfavourable breast cancer-specific survival (HR 4.13, 95% CI 1.75–9.77; $p = 0.001$) together with a high Ki-67 proliferation index (HR 2.93, 95% CI 1.86–4.60; $p < 0.001$).

A locoregional recurrence was diagnosed in 50 (5.3%) patients (47 [5.5%] and 3 [4.0%] in the pN0i- and pN0i+ groups, respectively). Ten-year locoregional recurrence free survival was 94.2% in the pN0i- group and 95.8% in the pN0i+ group (log-rank $p = 0.652$).

The above-mentioned survival analyses were performed without considering the treatment-related variables (the types of surgery and systemic adjuvant treatments administered). The results remained largely unchanged with respect to the prognostic importance of ITCs in multivariable analyses for distant disease-free survival, overall survival, and breast cancer-specific survival, when the type of surgery and the adjuvant treatments given were included in the multivariable models (data not shown).

Table 11. Study II. Characteristics of the patients with ITCs in the SN who died of breast cancer. SNB: Sentinel Node Biopsy; ALND: Axillary Lymph Node Dissection; RT: Radiotherapy; ER: Oestrogen receptor; PgR: Progesterone Receptor; HER2: Human epidermal growth factor receptor 2; RT: radiotherapy.

Patient	Age	Type of Axillary surgery	Tumour Size	Histo-logical Grade	Histo-logical Type	Ki-67	ER	PgR	HER-2	RT	Endocrine Therapy	Chemo-therapy
1	79	SNB+ALND	20mm	2	Lobular	>30%	-	-	-	No	No	No
2	56	SNB+ALND	19mm	2	Lobular	<20%	+	+	-	Yes	Yes	No
3	62	SNB+ALND	19mm	2	Lobular	<20%	+	+	-	Yes	Yes	No
4	47	SNB+ALND	15mm	3	Ductal	>30%	+	+	-	Yes	Yes	Yes
5	70	SNB+ALND	17mm	3	Ductal	>30%	-	-	-	Yes	No	No
6	66	SNB only	10mm	2	Ductal	>30%	+	-	NA	Yes	No	Yes
7	74	SNB only	15mm	2	Other	>30%	+	+	NA	Yes	Yes	No

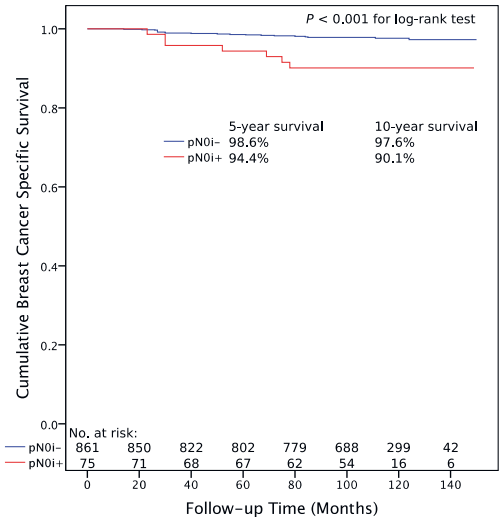

Figure 4. Study II. Breast cancer-specific survival pN0i- vs. pN0i+. Reproduced with the permission from Springer Nature.

5.3 LONG-TERM SURVIVAL OUTCOMES OF PATIENTS WITH SMALL (≤ 1 CENTIMETRE) NODE-NEGATIVE HER2-POSITIVE BREAST CANCER NOT TREATED WITH ADJUVANT ANTI-HER2-TARGETED THERAPY (STUDY III)

PATIENT AND TUMOUR CLINICOPATHOLOGICAL CHARACTERISTICS

No statistically significant differences were found between the patients with HER2+ (n = 44) cancer and those with ER+/HER2- (n = 291) cancer regarding the patient age, the tumour size, the axillary lymph node status (pN0i- or pN0i+), tumour histology, or tumour focality. Patients with HER2+ cancer had more often tumours with a high histological grade and a high Ki-67 proliferation index (p < 0.001). In the HER2+ group there were 33 (75%) patients with ER-positive tumours. They also received more often adjuvant chemotherapy. (Table 12)

Table 12. Study III. Patient and tumour characteristics and treatments according to patient groups. HER2: Human epidermal growth factor receptor 2; pN0i-/ i+: isolated tumour cell negative/positive lymph node; ER: oestrogen receptor; PgR: progesterone receptor; Ki-67 (MIB-1): Ki-67 proliferation index determined with MIB-1 monoclonal antibody; BCS: breast conserving surgery; SNB: sentinel node biopsy; ALND: axillary lymph node dissection; NA: Not Available. Reproduced with the permission from Karger Publishers.

	HER2+		**ER+/HER2-**		**All patients**		
	n	**%**	***n***	**%**	***n***	**%**	***p***
	44	**13.1**	**291**	**86.9**	**335**	**100.0**	
Age at diagnosis (years)							
Median	55		58		58		
Range	35–83		31–91		31–91		0.36
Tumour size							
pT1a (≥ 1mm to 5mm)	9	20.5	42	14.4	51	15.2	
pT1b (≥ 5mm to 10 mm)	35	79.5	249	85.6	284	84.8	0.30
Axillary lymph node status							
pN0i-	40	90.9	271	93.1	311	92.8	
pN0i+	4	9.1	20	6.9	24	7.2	0.54
Tumour histology							
Ductal	35	79.5	183	62.9	218	65.1	
Lobular	4	9.1	60	20.6	64	19.1	
Other	5	11.4	48	16.5	53	15.8	0.08
Histological grade							
1	8	18.2	173	59.5	181	54.0	
2	9	43.2	102	35.1	121	36.2	
3	15	34.1	11	3.8	26	7.8	<0.001
NA	2	4.5	5	1.7	7	2.1	
ER-receptor							
Negative (<10%)	11	25.0	0	0.0	11	3.2	
Positive (>10%)	33	75.0	291	100.0	324	96.7	<0.001
PgR-receptor							
Negative (<10%)	25	56.8	61	21.0	86	25.7	
Positive (>10%)	19	43.2	229	78.7	248	74.0	<0.001
NA	0	0.0	1	0.3	1	0.3	
Ki-67 (MIB-1)							
0–19%	14	31.8	240	82.5	254	75.8	
20–30%	20	45.5	35	12.0	55	16.4	
>30%	10	22.7	15	5.2	25	7.5	<0.001
NA	0	0.0	1	0.3	1	0.3	
Breast surgery							
BCS	33	75.0	237	81.4	270	80.6	
Mastectomy	11	25.0	54	18.6	65	19.4	0.31

Axillary surgery							
SNB	38	86.4	241	82.8	279	83.3	
SNB+ALND	3	6.8	23	7.9	26	7.8	
ALND	3	6.8	27	9.3	30	9.0	0.83
Adjuvant endocrine therapy							
No	29	65.9	218	74.9	247	73.7	
Yes	15	34.1	70	24.1	85	25.4	0.30
NA	0	0.0	3	1.0	3	0.9	
Adjuvant chemotherapy							
No	31	70.5	284	97.6	94	94.0	
Yes	13	29.5	6	2.1	19	5.7	<0.001
NA	0	0.0	1	0.4	1	0.3	
Systemic adjuvant therapy (endocrine- and/or chemotherapy)							
No	21	47.7	220	75.6	241	71.9	
Yes	23	52.3	71	24.4	94	28.1	<0.001
Adjuvant radiation therapy							
No	13	29.5	55	18.9	68	20.3	
Yes	31	70.5	235	80.7	266	79.4	0.10
NA	0	0.0	1	0.3	0	0.3	

FOLLOW-UP AND SURVIVAL OUTCOME

The median follow-up time for all patients was 9.7 years (range 0.5–12.5 years) after the date of primary breast surgery. In the subsets of patients with HER2+ cancer and those with ER+/HER2- cancer the median follow-up times were 9.2 years (range, 1.2–12 years) and 9.8 years (range, 0.5–12.5 years), respectively *(p = 0.08)*.

The survival events during the follow-up are presented in Table 13. Ten-year distant disease-free survival was 84.0% in the HER2+ group and 98.2% in the ER+/HER2- group, (p < 0.001, Figure 5). In addition to the cancer HER2-status, also the histological grade 3 and a high Ki-67 proliferation index were associated with an increased risk of distant recurrence in univariate analysis (Table 14).

Table 13. Study III. Breast cancer events. HER2: human epidermal growth factor receptor 2; ER: oestrogen receptor. Reproduced with the permission from Karger Publishers.

	HER2+ n=*44* ***n (%)***	**ER+/HER2- n=*291*** ***n (%)***	***p***
Events			
Locoregional recurrence	2 (4.5)	18 (6.2)	0.67
Distant metastases	7 (15.9)	5 (1.7)	<0.001
Breast cancer death	5 (11.4)	4 (1.4)	<0.001
Death from any cause	7 (15.9)	24 (8.2)	0.10

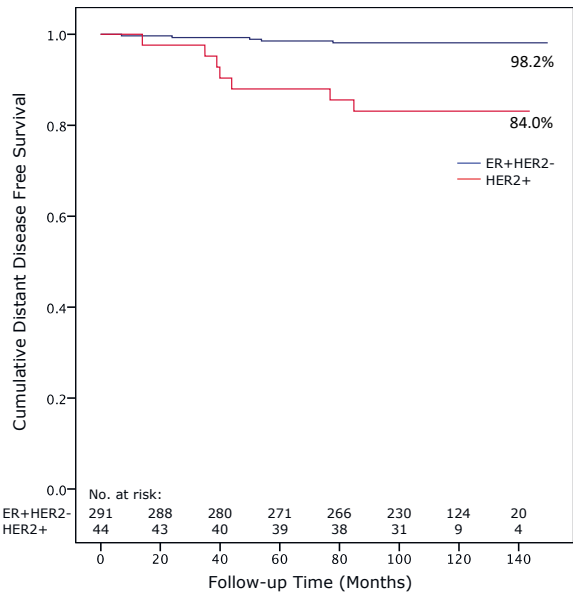

Figure 5. Study III. Kaplan-Meier survival plot for distant disease-free survival (p < 0.001) in the HER2+ and ER+/HER2- patient groups. Reproduced with the permission from Karger Publishers.

Ten-year locoregional recurrence-free survival was 95.4% in the HER2+ group and 93.4% in the ER+/HER2- group (p = 0.66). Age over 50 and tumour multifocality were associated with an increased risk of locoregional recurrence in univariate analysis (Table 14). Ten-year breast cancer-specific survival was 82.6% in the HER2+ group and 98.9% in the ER+/HER2- group (p < 0.001). Nine patients died due to breast cancer (Table 15). Ten-year overall survival was 78.5% in the HER2+ group and 91.7% in the ER+/HER2- group (p = 0.09, Figure 6).

Table 14. Kaplan-Meier 10-year survival rate estimates. pN0i-/ i+: isolated tumour cell negative/positive lymph node; HER2: human epidermal growth factor receptor 2; ER: oestrogen receptor; PgR: progesterone receptor; Ki-67: Ki-67 proliferation index determined with the MIB-1 monoclonal antibody. *Seven patients had missing cancer histological grade. Reproduced with the permission from Karger Publishers.

	Locoregional recurrence-free survival		**Distant disease-free survival**		**Breast cancer-specific survival**		**Overall survival**	
	10-year survival estimate	***p***	**10-year survival estimate**	***p***	**10-year survival estimate**	***p***	**10-year survival estimate**	***p***
Age (years)								
<50	87.2 %		95.8 %		98.6 %		92.7 %	
>50	95.5 %	0.01	96.4 %	0.79	96.8 %	0.42	90.4 %	0.40
Tumour size								
pT1a (≥ 1mm to ≤ 5mm)	87.4 %		96.0 %		94.0 %		90.1 %	
pT1b (≥ 5mm to 10 mm)	94.8 %	0.06	96.4 %	0.89	97.8 %	0.58	90.4 %	0.66
pN-status								
pN0i-	93.2 %		96.3 %		97.3 %		90.6 %	
pN0i+	100.0 %	0.22	95.7 %	0.85	95.7 %	0.54	87.5 %	0.46
HER2-status								
ER+/HER2-	93.4 %		98.2 %		98.9 %		91.7 %	
HER2+	95.4 %	0.66	84.0 %	<0.001	82.6 %	<0.001	78.5 %	0.09
ER-status								
ER-	90.9 %		100.0 %		100.0 %		100.0 %	
ER+	93.8 %	0.70	96.2 %	0.51	97.7 %	0.60	90.0 %	0.32
PgR-status								
PgR-	91.4 %		94.1 %		93.4 %		90.0 %	
PgR+	94.5 %	0.34	97.1 %	0.21	98.3 %	0.18	90.2 %	0.70
Tumour histology								
Other	90.4 %		98.0 %		100.0 %		91.5 %	
Lobular	93.2 %		95.1 %		94.3 %		86.5 %	
Ductal	94.7 %	0.53	96.2 %	0.70	97.1 %	0.89	91.0 %	0.84
Histological grade*								
1	94.8 %		97.7 %		97.6 %		94.1 %	
2	94.6 %		96.5 %		96.1 %		84.2 %	
3	84.4 %	0.09	84.6 %	0.01	88.5 %	0.01	88.5 %	0.12
Ki-67 (MIB-1)								
0–19 %	93.8 %		98.0 %		99.2 %		91.7 %	
20–30 %	94.4 %		90.8 %		90.0 %		83.1 %	
>30 %	90.7 %	0.9	91.7 %	0.02	91.3 %	0.01	91.3 %	0.40

Table 15. Patients and their characteristics who died due to breast cancer. pT1a: tumour size ≥ 1mm to ≤ 5mm; pT1b: tumour size ≥ 5mm to ≤ 10 mm; pN0i-/ i+: isolated tumour cell negative/positive sentinel lymph node; HER2: human epidermal growth factor receptor 2; ER: oestrogen receptor; SNB: sentinel lymph node biopsy; BCS: breast conserving surgery; ALND: axillary lymph node dissection; DM: distant metastasis.

Patient	Age	pT	pN	HER2-status	ER-status	Surgery	Systemic treatment	Time to DM (months)
1	56	pT1b	pN0i-	-	+	BCS+SNB	No	74
2	77	pT1a	pN0i-	-	+	BCS+SNB	No	24
3	85	pT1b	pN0i-	-	+	BCS+SNB	Endocrine therapy	85
4	58	pT1b	pN0i-	-	+	MASTECTOMY+SNB	No	50
5	71	pT1a	pN0i-	+	+	MASTECTOMY+ALND	No	39
6	52	pT1b	pN0i-	+	+	BCS+SNB	No	39
7	66	pT1b	pN0i+	+	+	BCS+SNB	Chemotherapy	14
8	37	pT1b	pN0i-	+	+	BCS+SNB	Endocrine therapy and chemotherapy	35
9	53	pT1b	pN0i-	+	+	MASTECTOMY+ALND	Endocrine therapy	40

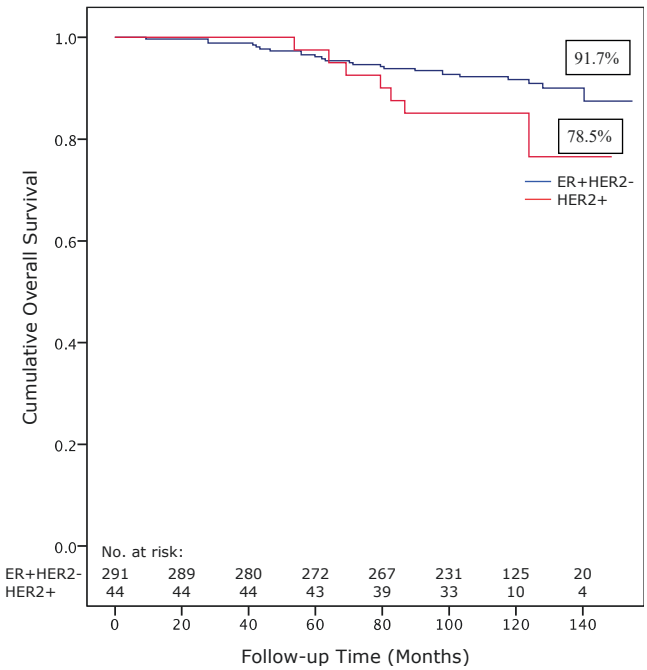

Figure 6. Study III. Kaplan-Meier survival plot for overall survival (p = 0.09) in the HER2+ and ER+/HER2- patient groups. Reproduced with the permission from Karger Publishers.

Subgroups of the HER2+ patient group were compared according to tumour size (pT1a vs. pT1b), the ER-status (ER- vs. ER+), and the pN-stage (pN0i- vs. pN0i+). Patients with pN0i+, pT1b and ER+ breast cancer seemed to have worse distant disease-free survival, but the observed differences were not statistically significant (Table 16). In addition, no statistically significant differences were seen between the subgroups in other survival end points neither.

Table 16. Distant disease-free survival in the HER2+ group patients according to the subgroups. HER2: human epidermal growth factor receptor 2; pT1a: tumour size ≥ 1mm to ≤ 5mm; pT1b: tumour size 6–10mm; pN0i-/ i+: isolated tumour cell negative/positive sentinel lymph node; ER, oestrogen receptor. Reproduced with the permission from Karger Publishers.

HER2-positive cancer, subgroup	Distant disease-free survival					
	No. of patients	No. of events	%	5-year survival %	10-year survival %	*p*
pT1a vs. pT1b						
pT1a	9	1	11.1	100.0	88.9	
pT1b	35	6	17.1	85.7	82.9	0.60
pN0i- vs. pN0i+						
pN0i-	40	6	15.0	90.0	84.9	
pN0i+	4	1	25.0	75.0	75.0	0.50
ER- vs. ER+						
ER-	11	0	0.0	100.0	100.0	
ER+	33	7	21.1	84.8	78.7	0.10

6 DISCUSSION

6.1 BIOPSY METHOD AND BREAST CANCER OUTCOME

THE INCIDENCE OF ISOLATED TUMOUR CELLS

It was concluded that, CNB was neither associated with an increased rate of ITC findings nor inferior survival when compared with FNAC. There are only some prior studies in agreement with our results focusing on the association between the type of preoperative biopsy method and the axillary lymph node status (18,69,185).

However, it has found to be challenging to evaluate the effect of the type of breast tumour biopsy on the rate of ITCs in the regional lymph nodes. In randomised trials the biopsy method had no effect on the rate of H&E detected SN metastases but influenced the incidence of IHC only-detected metastases (186,187). In these studies, a needle biopsy was compared with an excisional biopsy (186), or FNAC was compared with a CNB and an excisional biopsy (187). The latter study concluded that IHC only detected SN metastases were more common in patients after CNB compared to those after FNAC despite adjustment for potential confounding factors in a multivariable analysis. Accordingly, the authors speculated that the IHC only detected SN tumour deposits might represent artificial findings without clinical significance. Nevertheless, in our study no distinction between the IHC only detected and H&E detectable SN findings was made, and, therefore, the studies are not directly comparable. However, in general, ITCs are very seldom detected without immunohistochemistry, whereas most micrometastases can be found also in H&E-stained tissue. Moreover, modern adjuvant treatments are effective and may eradicate small tumour deposits released at the time of the biopsy, further confounding comparisons between the recent and the older studies.

BREAST CANCER OUTCOME

Most studies agree that tumour cells are displaced into the needle track at the time of the needle biopsy (188,58,57), but the clinical significance of these cells is still controversial. Most previous studies have focused on the local recurrence rate after biopsy, but insufficient data are available on the effects of the biopsy method on breast cancer outcome. In the present study, a better overall survival was observed in patients who underwent CNB compared to

patients with FNAC in the univariable analysis. Nonetheless, in multivariable analysis the difference was not seen any more, suggesting that the difference in overall survival was explained by other factors, such as age at the time of the diagnosis and the lymph node status. In addition, no difference in breast cancer-specific and distant disease-free survival was seen between the groups even in the univariable survival analyses.

We are aware of only one other study on this topic that has addressed overall survival. Similarly in that study, the type of biopsy did not influence the local recurrence rate or overall survival during a median follow-up of 6.5 years (62). Neither the present study nor the previous ones have revealed a convincing association between the biopsy method and the local recurrence rate (62,63).

A few case reports and small case series have described patients with breast cancer recurrence at the biopsy site (59,189). The need to excise the CNB needle track at breast surgery and on the role of RT to prevent local recurrence especially after skin-sparing mastectomy are being debated. Some recommending the surgical resection of the needle track, especially when no RT is planned (190), while others do not (191).

6.2 PROGNOSTIC VALUE OF ISOLATED TUMOUR CELLS IN SENTINEL LYMPH NODES

The conclusion of the present study is that ITC findings in SNs in patients with pT1 node-negative breast cancer is an independent predictor for distant recurrence. Moreover, ITC findings were found to be associated with unfavourable overall survival, breast cancer-specific survival and distant disease-free survival. Yet, ITC findings were not predictive for locoregional recurrences. The association of ITCs with inferior survival outcomes was observed both in univariable and multivariable analyses, regardless of whether the types of treatments administered were included in the multivariable models or not. Of note, the patients with ITC findings in SNs had generally received more often any systemic adjuvant therapy than those who did not have ITCs, suggesting that the present estimates regarding the prognostic significance of the ITCs may be even conservative. Patients without ITCs in the SNs had excellent 10-year distant disease-free and breast cancer-specific survival rates.

Previous studies have yielded controversial conclusions regarding the prognostic significance of ITCs in early-stage breast cancer. While some studies have considered ITCs to have prognostic significance (12–14,81,87), others have not found such an association (15–17,83,192,193).

The MIRROR study was a cohort study investigating the clinical outcome of breast cancer < 3cm in size with ITC findings or micrometastases in regional

lymph nodes. They compared the outcome of patients with node-negative (pN0i-) no-adjuvant therapy to a cohort with ITCs and no-adjuvant therapy. They concluded that during a median follow-up of 5.1 years patients with ITCs had significantly worse disease-free survival compared to pN0i- patients. The results remained significant even after adjusting for different patient and tumour characteristics. (14)

Nonetheless, in the American College of Surgeons Oncology Group (ACOSOG Z0010) trial with patients having T1-2N0M0 breast cancer IHC detected occult SN metastases did not have effect on overall survival during a median of 6.3 years of follow-up. They concluded that patients with H&E negative but with IHC detected occult metastases in SNs did not cause reduced overall survival in univariable and multivariable analysis compared to those diagnosed without occult metastases. In addition, occult metastases were not associated with an increased risk of recurrence. Nevertheless, the ACOSOG Z0010 trial have had some criticism, especially because the amount of patients lost to follow-up was rather large. (16)

The controversial results may, in part, be explained with the good short-term prognosis in pN0 breast cancer, and the relatively small number of survival events in the present study. However, in the present and in a previous study in this patient population (13), the number of survival events continues to increase during a longer follow-up (194). Thus, it seems likely that to draw firm conclusions on the prognostic importance of ITCs in the SNs long follow-up times are needed.

When investigating the diagnostics and treatment of the axilla in breast cancer, distant disease-free survival and overall survival are the relevant end-points to study, but not loco-regional recurrence. Radiotherapy and systemic treatments will most often eliminate the possible residual disease in the axilla. The results of the present study with a long follow-up time, suggest strongly that finding ITCs in the SN is associated with an increased risk of distant recurrences. The mechanisms of cancer cell seeding that leads to distant recurrence may be multiple. The exact mechanisms are still incompletely understood. Rather, the presence of ITCs might imply non-indolent biology of cancer, suggesting that besides the SNs, the tumour may have seeded cancer cells elsewhere in the body, which may manifest as metastatic disease only after a long latency period. Nonetheless, it is known that extensive surgical treatment of the axilla does not appear to result in survival benefits in patients with ITCs in their SNs (144,195–197).

In node-negative breast cancer, administration of systemic adjuvant therapy has traditionally depended on tumour characteristics such as the size, the nodal status, tumour grade, the Ki-67 proliferation index, the hormone receptor status, and the HER2-status. More recently, biological subtypes,

characterised with gene expression profiling, or approximated with surrogate immunohistochemical profiles, have been suggested as prognostic and predictive tools in the decision-making for adjuvant systemic therapy (97). The criteria in the Finnish National guidelines recommend systemic adjuvant treatment in patients with recurrence risk of 10% or higher during a 10-year follow-up. In the present study, seven (9.3%) out of the 75 patients with pN0i+ cancer died of breast cancer, and eight (10.7%) others had distant metastases, adding up to a rate of 20% during a median follow-up time of 9.5 years.

In the MIRROR study systemic adjuvant therapy significantly improved survival of patients with ITCs compared to those without adjuvant therapy (14). Collectively, our data suggest that adjuvant systemic therapy should be considered for patients with ITC findings.

6.3 ISOLATED TUMOUR CELLS IN PATIENTS WITH NEOADJUVANT THERAPY

Everything mentioned above about the prognostic value of ITCs is valid in patients treated with upfront surgery. The more current question and interest of research is the importance of low-volume residual disease in SN, that is ITCs and micrometastases, and breast cancer outcome after neoadjuvant chemotherapy. A retrospective study showed that inferior long-term survival is seen in patients with residual ITCs and micrometastases in SNs (198). Significantly worse overall survival and approximately 2-fold risk of death was reported in patients with ypN0i+ and ypN0mi stages compared to those with ypN0i- disease during a 5-year follow-up. The survival was also analysed in subgroups according to biological subtypes. In the triple-negative and the ER-/HER2+ groups the SN ITCs and micrometastases were strongly predictive for unfavourable prognosis. In conclusion, the investigators ended up recommending use of IHC staining in SNs after neoadjuvant chemotherapy to detect even minimal nodal residual disease. ITCs and micrometastases are also regarded as an indication for ALND after neoadjuvant chemotherapy, at least so far. (199) Unlike in patients with upfront surgery, SN ITCs micrometastases and ITCs represent residual disease after neoadjuvant treatment reflecting treatment resistance (200,201).

6.4 PROGNOSIS OF HER2-POSITIVE pT1a-bN0M0 BREAST CANCER

BREAST CANCER OUTCOME IN HER2+ VS. ER+/HER2- PATIENTS

In the current study, HER2 expression was seen as prognostic factor for unfavourable survival in patients with small node-negative (pT1a-bN0) cancer. A moderately large absolute difference of 14.2 percentage points was seen in the 10-year distant disease-free survival in patients with HER2+ cancer compared to patients with ER+/HER2- cancer (84.0% vs. 98.2%). Likewise, patients with HER2+ cancer had an inferior 10-year breast cancer-specific survival compared to patients with ER+/HER2- cancer. These outcomes were worse in the HER2+ group patients even though they received more often adjuvant chemotherapy, although adjuvant trastuzumab was given in none of them. In general, patients with ER+/HER2- pT1a-bN0 tumours had an excellent 10-year survival, although only 24% of them received any kind of systemic adjuvant therapy.

TREATMENT OF pT1a-bN0M0 BREAST CANCER

While cancer HER2 expression is an established prognostic factor for inferior survival in breast cancer in general (119,202,203), there is controversy whether patients with HER2+ pT1a-bN0M0 breast cancer should be routinely treated with systemic adjuvant therapy and especially with anti-HER2-targeted therapy. Numerous previous studies have concluded that also patients with subcentimetre, node-negative HER2+ tumour have an unfavourable prognosis when not treated with systemic adjuvant therapy, and, therefore, could derive survival advantage from systemic adjuvant therapy including trastuzumab (128,129,176–179). Yet, other studies addressed that the prognosis of subcentimetre HER2+ breast cancer is so excellent, and routine use of systemic adjuvant therapies is therefore not indicated (131,132). The St. Gallen consensus panel recommends adjuvant chemotherapy and anti-HER2 therapy for patients with HER2-positive stage I pT1bN0 breast cancer, but for patients with pT1aN0 cancer the recommendation is to evaluate it case by case (160). In Finland, the HER2-positive pT1bN0 patients has been treated with trastuzumab already for years. In addition, treatment of very young patients or patients multifocal HER2-positive pT1aN0 cancer should be evaluated individually.

Due to the small number of events, the present study data cannot provide a recommendation about systemic adjuvant therapy use for patients with pT1aN0 breast cancer. There were only nine such patients in the current series and only one event due to distant recurrence in the HER2+/pT1a patient group. The effect of anti-HER2 therapy has not been evaluated in clinical trials in patients with pT1aN0 cancer. Nevertheless, if anti-HER2 would be administered, a shorter duration than the standard one-year treatment might be considered in these patients. Furthermore, the potential reduction in the risk of recurrence versus treatment associated risks such as risk of cardiotoxicity should be carefully evaluated.

Randomised clinical trials investigating the treatment effect of systemic adjuvant therapy and especially anti-HER2 therapy in the prognosis of patients with HER2+ pT1a-bN0M0 breast cancer would most probably provide essential information. But, due to the relatively low incidence of HER2+ subcentimeter tumours and the very low numbers of survival events even during a 10-year follow-up, it seems challenging to conduct such a trial. Therefore, meta-analyses including series like the present one seems worthwhile to perform. Quite recently a meta-analysis concluded that treating HER2+ pT1a-bN0M0 patients with trastuzumab significantly improves overall survival and distant recurrence free survival. However, administration of chemotherapy varied between the studies and therefore the role trastuzumab cannot be validated (204).

While further evidence is awaited, individual patient and tumour characteristics should be carefully taken into consideration, since a small tumour size alone may provide incomplete information for the clinical decision-making. In agreement with the present study, also previous studies have reported unfavourable outcomes in patients with especially ER-positive HER2+ tumours (133,203). It is hypothesised whether the role of a possible crosstalk between HER2- and hormone receptor may result in decreased effect of endocrine therapy, thus leading to a worse survival outcome also in subcentimetre tumours.

After the publication of the study III, the AJCC published an edited TNM-classification. In addition to the clinical and pathological stage also new prognostic stage of breast cancer was introduced in the 8th edition of TNM-classification (75). In this novel classification, biological factors including tumour grade, ER-, PgR- and HER2-status were included in addition to the previous anatomic factors (Table 17). This new prognostic staging has indeed found to provide more accurate evaluation of prognosis. (205)

Table 17. Clinical prognostic stage according to American Joint Committee on Cancer (AJCC) 8th Edition in tumours <20mm and nodal status not larger than micrometastasis. (64)

TNM	Grade	HER2-status	ER-status	PgR-status	Clinical prognostic stage
Tis N0 M0	Any	Any	Any	Any	0
T1*N0 M0 T0 N1mi M0 T1* N1mi M0	G1	Positive	Positive	Positive	1A
				Negative	1A
			Negative	Positive	1A
				Negative	1A
		Negative	Positive	Positive	1A
				Negative	1A
			Negative	Positive	1A
				Negative	1B
	G2	Positive	Positive	Positive	1A
				Negative	1A
			Negative	Positive	1A
				Negative	1A
		Negative	Positive	Positive	1A
				Negative	1A
			Negative	Positive	1A
				Negative	1B
	G2	Positive	Positive	Positive	1A
				Negative	1A
			Negative	Positive	1A
				Negative	1A
		Negative	Positive	Positive	1A
				Negative	1B
			Negative	Positive	1B
				Negative	1B

*T1 includes Tmi *T1 includes Tmi

6.5 STRENGTHS AND LIMITATIONS OF THE STUDY

The strengths of the study include the long follow-up time and the population-based setting (Studies I, II, III). In study I, the large size of the series allowed performing of multivariable analyses with the main prognostic factors as covariables.

The SNs were analysed centrally using a standardised protocol, and the criteria for ITC detection remained unaltered during the study (Studies I, II, III). In another study from our institute, the breast pathologists re-analysed the SNs histopathological findings from a part of the present patient population, and found only a very low rate of false-positive ITC findings (206), reflecting a high quality of pathology.

Most of the patients with ITC-positive cancer underwent a completion ALND, and patients with micrometastases or macrometastases in the non-sentinel nodes could be excluded from the study. Therefore, the ITC-positive patients in this study truly had pN0i+ cancer, a patient population that may currently be difficult to identify, since at present patients with ITCs do not undergo ALND.

The main limitation of the study is that the analyses were based on a prospectively followed up cohort of patients, and not on a clinical trial population with previously defined inclusion and exclusion criteria and study procedures (Studies I and II). Study III was a retrospective analysis of this prospectively collected data. The observational nature of the studies makes it also challenging to assess the effect of the given systemic therapies on prognosis.

In study II, there was relatively large sample size, although the number of patients with ITCs in the SNs was relatively small. Similarly, the number of patients with HER2+ tumours (Study III) was small. Also, the event rates in studies II and III were low. Therefore, there was neither enough statistical power to allow performing multivariable analysis nor a reliable subgroup analysis in Study III.

The systemic adjuvant treatment was not standardised throughout the study period (Studies I, II and III). However, patients with HER2+ breast cancer received more systemic adjuvant treatments than the patients with ER+/HER2- cancer and, yet, HER2 expression was strongly associated with unfavourable distant disease-free survival (Study III).

A small proportion of the patients with an SNB have undetected metastases in the axilla due to false negative findings (9). Axillary dissection was carried out substantially more frequently in the subset of patients with pN0i+ cancer than among those with pN0i- disease, and, therefore, undetected axillary macrometastases, if any, were present more likely in the pN0i- group than in the pN0+ group, which may have influenced the observed survival difference between the groups.

6.6 FUTURE ASPECTS

In the future, the role of gene assays will increase in determining the systemic adjuvant treatment of early breast cancer. In addition, during the last decade research about the meaning of detecting circulating plasma tumour DNA (ptDNA) has increased. It is speculated that with the help of ptDNA one can detect microscopic residual disease after surgery and identify patients in a higher risk of recurrence and even analyse response to adjuvant therapy and thus reduce overtreatment (207). With non-invasive "liquid biopsy" and help of circulating cell-free DNA it is possible also to identify specific mutations which can give information e.g. therapy resistance and help in detecting metastatic breast cancer earlier (208).

7 CONCLUSIONS

I. In the diagnosis of breast cancer CNB does not increase the incidence of ITCs in the SNs when compared to FNAC. Compared to FNAC, CNB increases neither local-regional nor distant breast cancer recurrences and does not have an adverse influence on survival.

II. Presence of ITCs in SNs are associated with an increased risk of distant recurrence in patients with pT1N0M0 breast cancer. Accordingly, ITCs should be considered in the decision-making for the need of systemic adjuvant therapy.

III. Patients with HER2+ T1a-bN0M0 breast cancers have an unfavourable distant-disease free survival when adjuvant anti-HER2-targeted treatment is not administered. However, more data are needed about cancers 5 mm or smaller in diameter.

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