ANNA SUTELA

Add-on Stereotactic Core Needle Breast Biopsy

Diagnosis of Non-Palpable Breast Lesions Detected on Mammography or Galactography

Doctoral dissertation

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The purpose of this study was to evaluate 14-gauge stereotactic core needle biopsies (SCNB) obtained by an add-on biopsy device in the diagnosis of mammographically detected non-palpable breast lesions and also to investigate the use of add-on stereotactic device for localizing lesions detected only with galactography. In a study of invasive breast cancers, 14 gauge core biopsies were compared to surgical specimens in the assessment of three clinically important prognostic factors (estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor (HER-2)).

Altogether 221 patients with 231 breast lesions were included in the study of SCNBs and 9 patients were included in the wire-localization study. The learning curve was evaluated by comparing the first five biopsies of each of the five radiologists involved in this study with their later biopsies. During the study period between June 1998 and January 2001, core samples were collected in three different containers as follows: the first sample (the central biopsy) was collected into container A, the second and the third samples (obtained 2 mm from the centre of the lesion) into container B, and all additional samples into container C. Histological evaluation and report were performed for each container separately. After core biopsy, all women whose biopsy result indicated the presence of invasive carcinoma or DCIS (ductal carcinoma in situ) underwent surgical treatment. High-risk lesions, such as ADH (atypical ductal hyperplasia) and radial scars were also surgically resected, as were benign lesions with discordant mammographic findings. All other lesions with benign biopsy results were recommended for mammographic follow-up at 1 year and 2 years.

The results support the existence of a learning curve in the biopsy of microcalcifications. The sensitivity of 100% for masses and 91% for microcalcifications was reached with multiple samples (more than three). Four false negative cases decreasing the sensitivity in microcalcifications were ADH in three cases and there was one lesion with discordant mammographic and SCNB-findings. Stereotactic 14-gauge core biopsy seems to be at least as sensitive as a surgical specimen in the assessment of ER, PR and HER-2. Three cores are needed for reliable assessment of HER-2 after adding CISH and more than 3 cores for PR, possibly due to tissue heterogeneity. For ER sensitivity remained lower, 95%, even in multiple cores, therefore ER-negative cases should be further investigated from surgical specimens.

Stereotactic guided wire-localication can be successfully performed for mammographically invisible intraductal lesions by using galactography in the same session to visualize the target.

The diagnostic accuracy, false negative rate and underestimations of 14-g core biopsies obtained by an add-on stereotactic device in this study are comparable to those obtained by a dedicated prone device according to the literature in the biopsy of non-palpable breast lesions. In addition, an add-on device can be used for wire-localization of mammographically invisible lesions under galactography guidance.

National Library of Medical Classification: WB 379, WK 150, WN 180, WP 815, WP 840, WP 870

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Kuopio, January 2008

Anna Sutela
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADH</td>
<td>Atypical ductal hyperplasia</td>
</tr>
<tr>
<td>ALH</td>
<td>Atypical lobular hyperplasia</td>
</tr>
<tr>
<td>BI-RADS</td>
<td>Breast imaging reporting and data system</td>
</tr>
<tr>
<td>CISH</td>
<td>Chromogenic in situ hybridisation</td>
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<tr>
<td>CNB</td>
<td>Core needle biopsy</td>
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<tr>
<td>DCIS</td>
<td>Ductal carcinoma in situ</td>
</tr>
<tr>
<td>Ductal NOS</td>
<td>Invasive ductal carcinoma, not otherwise specified</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
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<tr>
<td>ER</td>
<td>Estrogen receptor</td>
</tr>
<tr>
<td>ERBB2/HER-2/Neu</td>
<td>Human epidermal growth factor gene</td>
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<tr>
<td>FNA</td>
<td>Fine needle aspiration</td>
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<tr>
<td>FISH</td>
<td>Fluorescence in situ hybridisation</td>
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<tr>
<td>G</td>
<td>Gauge</td>
</tr>
<tr>
<td>HER-2</td>
<td>Human epidermal growth factor</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
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<tr>
<td>LCIS</td>
<td>Lobular carcinoma in situ</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NOS</td>
<td>Not otherwise specified</td>
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<tr>
<td>PR</td>
<td>Progesterone receptor</td>
</tr>
<tr>
<td>pTNM</td>
<td>Pathological Tumor-node-metastasis classification</td>
</tr>
<tr>
<td>SCNB</td>
<td>Stereotactic core needle biopsy</td>
</tr>
<tr>
<td>TDLU</td>
<td>Terminal ductal lobular unit</td>
</tr>
<tr>
<td>TNM</td>
<td>Tumour-node-metastasis classification</td>
</tr>
<tr>
<td>UDH</td>
<td>Unusual ductal hyperplasia</td>
</tr>
<tr>
<td>UICC</td>
<td>International Union Against Cancer</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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LIST OF ORIGINAL PUBLICATIONS

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1. INTRODUCTION

Breast cancer is the most common cancer in women all over the world [1]. Mass screening programmes are capable of detecting increasing number of clinically occult, non-palpable breast lesions. This has created the need to develop minimally invasive, biopsy methods as an alternative for diagnostic surgical biopsy to achieve a definitive diagnosis. Surgical biopsy is costly and associated with a relative morbidity. Imaging guided biopsies can obviate the need for unnecessary surgery in women with benign lesions [2]. In case of malignancy needle biopsy provides special diagnosis and also preoperative determination of histological prognostic factors such as grade, type, and invasion of neoplasm, as well as ER, PR and HER-2 status is often possible. This enables planning of extent of surgery before operation and also planning of neo-adjuvant treatment, if needed. Imaging guided breast biopsy can be performed under local anesthesia; it is quick to perform, and does not cause any deformation or disturbing scarring to the breast [3, 4]. It also reduces the costs for the diagnosis [5-7].

Imaging guided breast biopsy is most often performed under ultrasonographic [4, 7] or stereotactic guidance [8-12] and for specific indications, under MRI guidance [13, 14]. Fine needle aspiration cytology has in most centres been replaced by cutting needles (automated large core needles) [15] and lately directional vacuum assisted devices [16, 17].

For the past decade, stereotactic core needle biopsy (SCNB), first reported by Parker et al [12] has been increasingly used for confirming the histologic diagnosis of non-palpable breast lesions. Several reports of 14-gauge stereotactic core needle biopsy have been published with sensitivities for detection of malignancy ranging
from 87% to 96% [8, 11, 12, 18, 19] and with false negative rates of 3-7% [8, 9, 11, 18]. Almost all of these studies have been performed using a dedicated prone biopsy table. The biopsy with dedicated prone equipment is better tolerated by the patient than biopsy using an add-on device with the patient in a sitting position. With the patient in a prone position, vasovagal reactions and patient motion are eliminated. However, a dedicated biopsy table is expensive and occupies a great deal of space [20, 21].

Add-on unit with conventional mammography equipment is less expensive, does not need extra room and furthermore, the equipments can be used for mammography when not needed for breast biopsy [22]. It may therefore be the first choice for many smaller centres diagnosing breast cancer. Studies for add-on device are scanty. The diagnostic accuracy, the number of cores needed, underestimations, false negative rate, learning curve and prognostic factor assessment need to be investigated with reference to surgical samples or with sufficient follow-up to validate the results obtained with an add-on device for diagnosing non-palpable breast lesions.

Occasionally, surgery is needed to achieve a diagnosis of a breast lesion. Non-palpable lesions can be localized prior surgery by a guide-wire under sonography or stereotactic guidance. Spontaneous clear, serous (yellowish clear) or blood containing discharge secreting unilaterally from one duct orifice is most commonly caused by a benign intraductal papilloma, but in 10-15% carcinoma can be found [23-25]. These lesions are therefore usually regarded as an indication for surgical removal, which is also the treatment for the symptom. The lesions that present with nipple discharge are typically not visible on mammography or ultrasonography, but can be detected on galactography [26, 27]. As a consequence, the usual methods for
guiding biopsy or preoperative localization are not applicable. Retroareolar lesions can be detected perioperatively by methylene dye staining of the duct. Distant lesions may be difficult to localize despite methylene dye staining as the dye quickly diffuses from ducts into breast tissue [28]. This may thus lead to excessive resection or failure to remove the lesion. Also in cases with intermittent discharge where no nipple discharge is detected during the operation, it may be impossible to localize the discharging duct. There is a need for additional methods for localizing distant intraductal lesions and lesions causing intermittent discharge.
2. REVIEW OF THE LITERATURE

2.1. Breast cancer epidemiology

Breast cancer is the most common malignancy in women all over the world and accounts for 23% of all female cancers [1]. According to the Finnish Cancer Register, the annual age-adjusted breast cancer incidence has increased over the past years. During time period 1962-1996 annual incidence increased from 29.9 to 71.9 [29]. Worldwide, the overall annual increase in incidence rate has been 0.5% since 1990. In 2006, the number of new breast cancer cases in Finland was around 4060 [29].

The aetiology of breast cancer is multifactorial. Environmental risk factors are believed to be of greater importance than genetic factors. This is evident from the studies with emigrants, which show that incidence rises following migration from low to high incidence countries [30]. Only around 10% of breast cancer cases in Western countries are thought to be due to a genetic predisposition.

The mortality of breast cancer rose from 1951 to 1990 but has been falling thereafter in most European countries, Australia and in the United States [31]. The reasons for the decline in mortality include widespread mammographic screening, precise diagnosis and effective treatment modalities [32]. The average survival rate in developed countries is 73% whereas in the developing countries it is only 57%. With 411 000 annual deaths, breast cancer is the leading cause of cancer mortality (14%) in women [1].

2.2. Pathogenesis of breast cancer

A definitive progression model from healthy breast tissue to invasive carcinoma has not yet been determined. The preneoplastic potential of benign, proliferative lesions of the breast is still a matter of debate [31] According to the WHO Working
Group, intraductal proliferative lesions are defined as a group of cytologically and architecturally diverse proliferations typically originating from the terminal ductal lobular unit (TDLU) and confined to the mammary duct lobular system. They are associated with an increased risk, albeit of greatly different magnitudes, for the subsequent development of invasive carcinoma [33]. Intraductal proliferative lesions have traditionally been divided into three categories: unusual ductal hyperplasia (UDH), with relative risk of 1.5-2.0 times that for reference population [34, 35], atypical ductal hyperplasia (ADH), relative risk 4-5, range 2.4-13 and ductal carcinoma in situ (DCIS), with relative risk of 8-10 [34]. Currently flat epithelial hyperplasia is defined as a presumably neoplastic intraductal proliferation, whose risk of malignant transformation is still unknown [36].

2.3. Histopathology of carcinomas

Breast cancers are divided into in situ carcinomas and invasive carcinomas according to their capability for infiltrating adjacent tissues and metastasing. Both in situ and invasive breast carcinoma can be divided into subtypes according to their histopathologic properties [33, 37].

2.3.1. Ductal carcinoma in situ (DCIS)

DCIS is a neoplastic intraductal lesion, characterized by increased epithelial proliferation, cellular atypia and an inherent tendency for progression to invasive breast carcinoma [33]. The site of origin for DCIS is the TDLU from where it spreads inside the ductal structures and extends into the epithelium of lobular glands. Detection of DCIS has increased since the introduction of widespread screening mammography in the early 80’s. In current screening programs, 10-30% of all
detected malignancies are DCIS [38, 39]. In mammography, 73-98% of DCIS cases are evident as microcalcifications [40-42]. Lesions that lack mammographic evidence of microcalcifications are either mammographically occult or present as circumscribed or nodular masses, architectural distortions or non-specific densities [41]. If treated, prognosis of DCIS is excellent. Of women diagnosed with DCIS between 1984 and 1989, 1.9% died of breast cancer at 10 years [43].

2.3.2. Invasive ductal carcinoma, not otherwise specified (NOS)

According to the WHO characterisation, invasive ductal carcinoma, not otherwise specified (ductal NOS) is a heterogenous group of tumours that fails to exhibit sufficient characteristics to achieve classification as a specific histological type, such as lobular or tubular carcinoma [33]. It comprises the largest group, 40% to 75% of malignant breast tumours [33, 44]. Typically, the mammographic characteristics of ductal carcinoma include central tumour mass with radiating spicules; associated microcalcifications are common [45]. Ductal carcinoma can also appear as a mammographic density with irregular, nodular or circumscribed contour or as a cluster of microcalcifications [45]. The 5-year relative survival of the patients with ductal carcinoma is 79% [44].

2.3.3. Invasive lobular carcinoma

Invasive lobular carcinoma accounts for 5% to 15% of invasive breast carcinomas [46]. The microscopical growing pattern of invasive lobular carcinoma forms thread-like strands of tumour cells loosely dispersed throughout the fibrous stroma [37]. Both macroscopically and mammographically invasive lobular carcinomas are often poorly delimited and difficult to define [47]. A tendency for
multicentricity [48] and bilaterality [47] has been reported. The average 5-year survival of invasive lobular carcinoma is 86% [49].

2.3.4. Other types of invasive carcinoma

Tubular carcinoma, invasive cribriform carcinoma, medullary carcinoma and mucinous carcinoma are usually histopathologically well differentiated and are mammographically detectable as either a spiculated mass (tubular and cribriform carcinoma) [50, 51] or a well delineated mass (medullary and mucinous carcinoma) [33, 52]. These tumours have a favourable 10-year prognosis ranging from 90% to 100% [53, 54]. Inflammatory carcinoma is a particular form of breast carcinoma with a poor 5-year survival of 18% to 41% [44, 55, 56].

2.4. Histopathology of high risk lesions

High risk lesions are ductal and lobular proliferations that have been shown to have a statistical association with increased risk of subsequent breast cancer or be due to genetic alterations or mutations similar to those present in DCIS or infiltrating carcinoma of the breast [57].

2.4.1. Atypical ductal hyperplasia (ADH)

The diagnosis of ADH is based on morphologic criteria with a cytologic atypia or clonal appearance and structural rigidity and geometric spaces similar to those seen in low-grade DCIS. In ADH, these changes involve only part of a duct space or an area smaller than 2 mm [58, 59]. ADH is the most common high-risk lesion accounting for 5% of all breast biopsies [60]. ADH can coexist with both in situ and invasive carcinomas.
2.4.2. Lobular neoplasia

A spectrum of proliferative changes from ALH to LCIS in the lobule portion of TDLU can be called lobular neoplasia. The distinction between ALH and LCIS is based on the degree of involvement of the lobular units by the characteristic loosely cohesive monomorphic cells [61]. The incidence of LCIS in otherwise benign breast biopsy is between 0.5% and 3.8% [62, 63]. Lobular neoplasia has been thought to be an incidental finding at breast biopsy, without a mammographic correlate [64]. However, it has been noted that it can also appear as a cluster of microcalcifications [65, 66]. Lobular neoplasia is frequently multifocal and bilateral and is associated with increased incidence of malignancy in both breasts [64].

2.4.3. Radial scar

The radial scar and its larger variant, the complex sclerosing lesion, may arise from any of the benign proliferative lesions (adenosis, papilloma, usual and atypical hyperplasia) [57]. In the mammography it appears as an area of architectural distortion without any central mass [67]. Due to the reported association with tubular or ductal carcinoma or high risk lesions [68, 69], surgical excision is usually recommended.

2.5. Prognostic factors of breast cancer

The presence of histologically assessed axillary lymph node metastasis is the single most important predictor of posttreatment breast cancer recurrence and death [70]. Also young age (under 40 years) and tumour size are independent prognostic indicators for breast cancer survival [71]. Survival rates for women with invasive breast cancer are about 90% for tumours smaller than 1 cm, 80% for tumours 1.0 to
1.9 cm, 70% for tumours 2.0 to 4.9 cm and 60% for tumours larger than 5 cm [72]. Tumour staging (Table 1) aids to predict prognosis of breast cancer. It is based on the tumour, nodes and metastases (TNM) classification (Table 2) of International Union Against Cancer (UICC) [73], which can be assessed either clinically (TNM) or pathologically (pTNM).

Table 1. Stage according to UICC classification (2002), with reference to the TNM classification presented in Table 2

<table>
<thead>
<tr>
<th>Stage</th>
<th>T</th>
<th>N</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Tis</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>I</td>
<td>T1</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
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<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIB</td>
<td>T2</td>
<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIIA</td>
<td>T0-2</td>
<td>N2</td>
<td>M0</td>
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<td>M0</td>
</tr>
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<td>T4</td>
<td>N0-2</td>
<td>M0</td>
</tr>
<tr>
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<td>T0-4</td>
<td>N3</td>
<td>M0</td>
</tr>
<tr>
<td>IV</td>
<td>T0-4</td>
<td>N0-3</td>
<td>M1</td>
</tr>
</tbody>
</table>
Table 2. Tumour-Node-Metastasis (TNM) classification of breast tumours according to UICC, 2002

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TX</td>
<td>Primary tumour cannot be assessed</td>
</tr>
<tr>
<td>T0</td>
<td>No evidence of primary tumour</td>
</tr>
<tr>
<td>Tis</td>
<td>Carcinoma in situ</td>
</tr>
<tr>
<td>T1</td>
<td>Tumour 2 cm or less in greatest dimension</td>
</tr>
<tr>
<td>T2</td>
<td>Tumour more than 2 cm but not more than 5 cm in greatest dimension</td>
</tr>
<tr>
<td>T3</td>
<td>Tumour more than 5 cm in greatest dimension</td>
</tr>
<tr>
<td>T4</td>
<td>Tumour of any size with direct extension to chest wall or skin only</td>
</tr>
<tr>
<td>NX</td>
<td>Regional lymph nodes cannot be assessed</td>
</tr>
<tr>
<td>N0</td>
<td>No regional lymph node metastases</td>
</tr>
<tr>
<td>N1</td>
<td>Metastasis in movable ipsilateral axillary lymph node(s)</td>
</tr>
<tr>
<td>N2</td>
<td>Metastasis in fixed ipsilateral axillary lymph node(s) or clinically apparent ipsilateral internal mammary lymph node(s) in the absence of clinically evident axillary lymph node metastasis</td>
</tr>
<tr>
<td>N3</td>
<td>Metastasis in ipsilateral infraclavicular lymph node(s) with or without axillary lymph node involvement; or in clinically apparent ipsilateral internal mammary lymph node(s) in the presence of clinically evident axillary lymph node metastasis; or metastasis in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement</td>
</tr>
<tr>
<td>MX</td>
<td>Distant metastasis cannot be assessed</td>
</tr>
<tr>
<td>M0</td>
<td>No distant metastasis</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastasis</td>
</tr>
</tbody>
</table>

2.5.1. Estrogen receptor (ER) and progesterone receptor (PR) expression

Hormone receptors are proteins that bind and mediate the cellular effects of circulating hormones. Since expression of PR is estrogen regulated, most PR positive carcinomas are also ER positive. Less than 10% of carcinomas are ER negative and PR positive [37]. The expression of estrogen and progesterone
receptors in the tumour tissue predicts a good response to hormonal therapy of breast cancer in both an adjuvant setting and in metastatic disease [74, 75]. Positive ER and/or PR status is also related to significantly prolonged disease-free survival and overall survival [76].

Hormone receptor assessments are increasingly performed by immunohistochemistry. The immunohistochemical method makes it possible to observe the specific cellular site of hormone receptor expression and to distinguish ER/PR activity in carcinoma cells from benign epithelium. The method is applicable both for core samples and surgical specimens [37].

2.5.2. Human epidermal growth factor (HER-2)

Proto-oncogenes are normal genes involved in cell growth and proliferation and their mutated forms promote neoplastic transformation. HER-2, the encoded product of proto-oncogene ERBB2/HER-2/Neu belongs to a human epidermal growth factor receptor (EGFR) family [77, 78]. HER-2 is expressed in a range of normal adult and foetal epithelia and plays an important role of growth and development [79]. In cancer, HER-2 overexpression/amplification has been observed in a variety of tumours including 9 to 30 percent of breast cancers [80-82]. Overexpression of the HER-2 protein, amplification of the HER-2 gene, or both are associated with aggressive behaviour of cancer cells, such as enhanced growth and proliferation and increased invasive and metastatic capabilities [80, 83]. In patients with invasive breast carcinoma, HER-2 amplification has been shown to be associated with a significant reduction in disease-free survival in node-positive patients [80, 84]. This phenomenon has been related to a response to trastuzumab anticancer therapy (a humanised recombinant monoclonal IgG1 antibody against HER-2) [80, 85, 86].
metastatic disease, trastuzumab in combination with chemotherapy has achieved a response rate of 50% and prolonged survival [87]. A significant improvement in both recurrence free survival and in overall survival with trastuzumab has been reported also in adjuvant setting for early breast cancer [88-90].

The majority of HER-2 studies have been performed using HER-2 immunohistochemistry (IHC) [91]. With IHC, there are problems with standardization in slide scoring [92, 93], with a tendency for false positive HER-2 assessments, especially with 2+ results depending on the antibody used [94]. Therefore, it is recommended to confirm IHC positive results by fluorescence in situ hybridisation (FISH) [82], which is the gold standard for HER-2 assessment. The latter method has the advantages of a more objective scoring system, where all non-neoplastic cells within the specimen serve as an internal control [95]. In addition to FISH, there is another relatively new method for detection of gene amplification, chromogenic in situ hybridisation (CISH). CISH uses a peroxidase reaction, which can be viewed with a standard light microscope. It also has built-in internal control and ensures reliable recognition of the invasive carcinoma area by light microscopy. The CISH staining remains in the slides and can be reassessed if necessary [95]. An excellent concordance has been found between CISH and FISH [96-98].

2.6. Breast imaging

Suspicious breast abnormalities should always be investigated by triple assessment: clinical examination, imaging (mammography and ultrasonography) and tissue sampling (needle biopsy) [33, 99].
2.6.1. Mammography

Mammography is the single most important imaging method of breast diseases which can be used for either screening or for clinical purposes. It is the first line imaging method for symptomatic patients [100, 101].

2.6.1.1. Screening mammography

Screening refers to examinations performed regularly on asymptomatic persons. In Europe, this implies a program in which women are systematically invited to participate in mammography imaging according to a population registry with the goal to detect breast cancer at an early, clinically occult stage [28].

Large randomized studies show about a 30% reduction in breast cancer mortality and about a 40% reduction in those receiving screening [102-105]. The decrease in mortality is attributable to a decrease in the size of cancers detected in the screening mammography [106, 107]. Screening is most advantageous in women older than 50 years. However, it has been shown that screening can reduce mortality significantly also in the group of women aged 40-49 years [108].

Recommendations for screening vary on the basis of the trials. The American cancer society has recommended yearly mammographic screening and physical examination beginning at age 40 [109]. The European Union recommends an organized, quality controlled mammography screening programme for women aged 50-69 years at 2-3 years intervals. If screening is offered to women aged 40-49 years, they should be informed about the possible benefits and adverse effects of screening [110]. In Finland, a nationwide screening programme arranged in two year intervals was started for women at the age of 50-59 years in 1987 and gradually implemented [111]. In 2006, a decision to offer mammografic screening was
expanded for women at the age of 50-69 years [112]. The participation rate in 1987-1997 has been high, 89%. The recall rate decreased from 4.7% to 3.3% and the biopsy referral from 1.1% to 0.6% in the first three years [113].

2.6.1.2. Sensitivity of mammography

The sensitivity of mammography to detect cancer ranges from 63% to 98%. In fatty breasts, the sensitivity can be as high as 98% to 100% [101, 114] but in dense breasts, sensitivities as low as 30% to 48% have been reported [101, 114, 115]. The density of breast parenchyma decreases with age, increasing the sensitivity of mammography [116]. Mammography is the most sensitive imaging method at detecting microcalcifications [40].

2.6.1.3. Specificity of mammography

The majority of findings in mammography is non-specific and only permits likelihood statements [117, 118]. In addition to better sensitivity, also specificity (characterization of e.g. density and contours of the lesions) is higher in fatty breasts [114, 117, 118]. Most small, nonpalpable carcinomas present as non-specific changes [108, 119-121].

2.6.1.4. BI-RADS-classification

To improve quality of mammographic reporting and interpretations and to facilitate communication between radiologists and clinicians, Breast Imaging, Reporting and Data system (BI-RADS) was designed by American College of Radiology [122, 123]. The use of BI-RADS-classification gives consistent guidelines to evaluate masses, microcalcifications, architectural distortions, asymmetries and
associated findings as skin or nipple retraction, skin thickening, trabecular thickening, skin lesions and axillary adenopathy and give further management recommendations [122]. A definite diagnosis of a benign lesion (BI-RADS 2) is possible for an oil cyst, a hamartoma, a lipoma, a typically calcified fibroadenoma or an intramammary lymph node. In the case of a low density well circumscribed mass, a benign diagnosis (BI-RADS 2) can be proposed in 98% of the cases [117, 118]. For BI-RADS 3 lesions, short interval follow-up is suggested. BI-RADS 4 and 5 are suspicious for malignancy and should therefore be confirmed by breast biopsy (Table 3).

Table 3. BI-RADS-classification

<table>
<thead>
<tr>
<th>Category</th>
<th>Risk of malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>BI-RADS 0</td>
<td>Incomplete, additional evaluation needed (additional mammographic views, ultrasound)</td>
</tr>
<tr>
<td>BI-RADS 1</td>
<td>Negative, no lesions found, routine follow-up</td>
</tr>
<tr>
<td>BI-RADS 2</td>
<td>Benign, routine follow-up</td>
</tr>
<tr>
<td>BI-RADS 3</td>
<td>Probably benign, short interval follow-up (e.g. 6 months) 2%</td>
</tr>
</tbody>
</table>
| BI-RADS 4     | Suspicious 4A Low suspicion, consider biopsy 6%
|               | 4B Intermediate suspicion: Biopsy 15%
|               | 4C Moderate suspicion: Biopsy 53%
| BI-RADS 5     | Highly suggestive of malignancy: Biopsy 81-91%
| BI-RADS 6     | Known biopsy proven malignancy |

Source: references [120, 122, 124]
2.6.2. Ultrasonography

Currently, ultrasonography is an adjunctive diagnostic tool indicated for evaluation of specific abnormalities detected by either clinical examination or by mammography [125] or for guiding percutaneous biopsy and localization [28]. In the imaging of young women under 30 years, ultrasonography is used as the first imaging study in the evaluation of a palpable mass [28]. Recently, with the introduction of high frequency (>7 mHz) transducers, the differentiation between solid and cystic masses has become more reliable than was the case with older equipment and the characterization of solid masses as benign or suggestive of malignancy can now be established with confidence [126]. Recent studies have demonstrated the ability of breast ultrasonography to detect small, mammographically occult breast carcinomas in women with dense breast tissue [127, 128]. A cancer detection rate of 0.3-0.46% has been reported, similar to that of mammographic screening [127, 128].

2.6.3. Galactography

Spontaneous clear, serous (yellowish, clear), or blood-containing nipple discharge from one duct orifice is an indication for galactography [28]. This kind of discharge is most commonly caused by a benign intraductal papilloma; however carcinoma is found in 10-15% of cases [23-25] Lesions causing nipple discharge are usually not visible on mammography or ultrasonography [26, 27]. In galactography, after injecting contrast media into the secreting duct, orthogonal microfocus magnification mammography images are obtained [129]. Intraductal lesions are classically visualized as a filling defect or a cut-off appearance of the duct. However,
these findings are non-specific and need to be further investigated by core biopsy or by surgical excision to exclude the possibility of cancer.

2.6.4. Magnetic resonance imaging (MRI)

Contrast enhanced MRI with its sensitivity of 88% to 97% is the most sensitive additional imaging method for detecting invasive breast cancer [130, 131] and DCIS [104]. Because of relatively low specificity (30-67%) [130, 131] the use of MRI should be limited to high risk patients and to questions that cannot be reliably answered by conventional imaging methods or by percutaneous biopsy [132].

The use of breast MRI has been reported in a variety of clinical situations. Breast MRI has been shown to be superior to physical examination, mammography and ultrasound in the evaluation of tumor size, multifocality and multicentricity of breast cancer [133, 134], especially in patients with invasive lobular carcinoma [134]. Other indications for breast MRI include the diagnosis of rupture of a silicone implant, differential diagnosis between scar and recurrence after breast conserving therapy or, monitoring of neoadjuvant chemotherapy and the search for the primary tumour in cases of lymph node or distant metastases and suspicion of breast cancer [28]. Although MRI is not suitable for screening of the general population, due to its lower specificity and high cost, encouraging preliminary reports have provided evidence that MRI can be valuable in screening women with a high risk of developing breast cancer [135].

2.7. Imaging guided breast biopsy

Traditionally, open surgical breast biopsy has been the gold standard in diagnosing breast lesions; non-palpable lesions undergo open biopsy after
ultrasonography or mammography guided wire localization. The costs and relative morbidity associated with the surgery in conjunction with detection of large number of non-palpable breast lesions on screening programmes have lead to the development of percutaneous imaging guided breast biopsy methods to replace surgical biopsies. Imaging guided breast biopsy is most often performed under ultrasonographic [4, 7, 136] or stereotactic guidance [8-12] and for specific indications, under MRI guidance [13, 14]. Complications are rare, <2% for core biopsies [137, 138] and 4% for vacuum assisted biopsies [139]; the complications include bleeding, hematomas, vasovagal reactions and infections. Early work with percutaneous breast biopsy involved fine needle aspiration (FNA) which has in most centres been replaced by cutting needles (automated large core needles) [15] and recently by directional vacuum assisted devices [16, 17, 28].

2.7.1 Ultrasonographic guidance

Ultrasonographic guidance may be regarded as the first line method for percutaneous biopsy of lesions visible by ultrasonography in terms of real time visualisation of the needle, patient comfort, lack of ionizing radiation, and low cost [4, 7]. With the help of ultrasonography, access to all areas of breast and axilla, even in small breasts can be reached [4, 7]. With ultrasonography guided 14-gauge biopsies, sensitivities and specificities of 100% have been reported [4, 140]. However, microcalcifications and a small subset of masses cannot be reliably detected by using ultrasonography [28, 141, 142]. The ultrasonography guided biopsies of tiny lesions and deep localizations may be complicated and time-consuming and stereotactic guidance in these situations may be preferable [28, 141].
2.7.2. Stereotactic guidance

Stereotaxis is based on the principle that the precise localization of the lesion in three dimensions can be calculated on the basis of its parallax shift of position on two angled images [17, 28]. At the start of the procedure, a non-angle scout image is obtained to document the accurate positioning of the lesion. Two angled images, 15° to the right and left along horizontal axis are then obtained. A cursor is placed over identical target areas on the angled images to allow the software to calculate x, y and z-axes [17, 28].

There are two major types of stereotactic devices: add-on devices, which are attached to ordinary mammography units to convert it into a biopsy guidance system and dedicated prone biopsy devices [11, 143]. With the add-on device, the biopsy procedure is usually performed with the patient in the sitting position. When not being used for biopsies, the mammographic unit can be used for normal diagnostic purposes. In the dedicated prone biopsy table, the patient lies on the table with the breast protruding through a hole in the table. Prone tables are more expensive than add-on units and require more room but have several advantages including more working space for the operating physicist and less likelihood of patient movement due to patient discomfort and vasovagal reactions [20-22]. Conventional or digital spot mammographic imaging is available for both types of stereotactic devices. Digital imaging reduces the biopsy time and this may increase the likelihood of a successful procedure [4]. Stereotactic guidance is the preferred biopsy type for microcalcifications but it can be used for all types of mammographic lesions [141].

After the biopsy of microcalcifications, specimen radiography needs to be obtained to confirm that the lesion in the target has been biopsied [144].
2.7.3 MRI guidance

MRI-guidance equipments for breast biopsy are commercially available [13, 14], however these devices are not yet in widespread use. In addition, MRI-guided biopsy requires much time and personnel [145]. Therefore, when a suspicious lesion is detected on MRI, it should be re-evaluated by ultrasonography and mammography and if detected, it should be biopsied under guidance of these methods [145]. MRI-guidance is usually performed with stereotactic devices, which usually consist of three main components: a compression mechanism, an imaging coil, and an aiming device [13, 14]. Core needles and vacuum devices have been used [13, 14] with high technical success rates. After the biopsy, a clip marking of the biopsy site is recommended for further mammographic follow-up and for possible adjunctive surgery [146].

2.7.4. Fine needle aspiration (FNA)

Fine needle aspiration has been widely used since the early 80’s in the diagnosis of breast lesions [147]. In FNA, cells of the tumour tissue are aspirated for cytological analysis using a 20-25-gauge (G) needle [15]. FNA is cost-effective, minimally invasive and rapid. At institutions where highly experienced on-site cytopathologists are available, sensitivity of 100% and specificities of 96-100% have been reported [148]. However, FNA is unable to reliably distinguish invasive carcinoma from in situ carcinoma [148, 149]. The false negative rate is relatively high, with lobular carcinoma false negative rates of 32% have being published [150, 151]. Also high numbers of unclear samples have been reported; in a multicenter study 152 of 429 samples (35%) were insufficient [152]. FNA is a useful tool for diagnosing cysts if ultrasonography fails to reveal typical cyst findings. If the needle tip proves to be
within the lesion in ultrasonography and the aspiration is unsuccessful, a solid tumour must be suspected [28]. Ultrasonography-guided FNA can also be used for diagnostic work-up of enlarged lymph nodes [123].

2.7.5. Core needle biopsy (CNB)

CNB, first reported by Parker et al. has largely replaced FNA since the early 90’s in the diagnosis of palpable and non-palpable breast lesions [12]. An automated long throw 14-G needle is most often used to obtain samples which are 20 mm long and 2 mm wide, weighting approximately 17 mg [11, 153-155]. In these systems, a cutting needle is “shot” rapidly through a breast lesion. After each pass the needle with the core is removed and the needle must be reinserted for the next biopsy. In studies comparing 14-, 16-, and 18-gauge needles, the diagnostic accuracy increased significantly with increasing needle size [153, 154]. Cores acquired with a 14-gauge needle allow histopathologic analysis of the tumour tissue, including tumour grade. There are also some studies assessing hormone receptor status and HER-2 from core samples [156, 157]. Validation studies have revealed 91-97% agreement between stereotactic 14-gauge core biopsies and surgery [8, 11, 19, 158] and 100% agreement between US-guided 14-gauge biopsies and surgery [4]. The recommendation of obtaining five cores for masses and a minimum of five cores for microcalcifications has been defined for the biopsy of non-palpable breast lesions using a stereotactic prone device [18, 158] and a minimum of 4 cores using US-guidance [159].
2.7.6. Vacuum assisted biopsy

Vacuum-assisted biopsy devices are attached to a vacuum, which draws tissue into a side hole in the probe. Then a rotating cutter advances over the tissue, cuts a core from the breast and withdraws the specimen into the system. A vacuum can also be used for suction of blood from the biopsy cavity during the procedure. Multiple 11-gauge specimens with the average weight 100 mg each, can be obtained by rotating the needle sequentially around its axis [16, 17, 28].

2.8. Imaging guided preoperative localization

Despite of development of percutaneous imaging guided biopsy methods there are limitations that need to be taken into consideration e.g. the possibility of false negative diagnoses and histopathological underestimations of ADH and DCIS-lesions [160]. Lesions causing nipple discharge are usually visible only in galactography and need surgical biopsy both for diagnosis of the lesion and for the treatment of the symptom (discharge) [25]. Therefore an imaging guided localization and surgical excision remains as an alternative for achieving diagnosis of breast lesions.

2.8.1. Percutaneous wire localization

For example, for lesions that are too close to the chest wall, or in very thin breasts, it may be impossible to perform stereotactic biopsy and if these lesions are not visible on ultrasonography, the surgical biopsy under guidewire guidance may have to be considered [28]. For guidewire localization, mammography with a perforated or marked compression plate, stereotaxy or ultrasonography are most often used. MRI-guidance is possible for lesions visible only in MRI [28]. According to an extensive literature review, mammographically directed needle localized breast
biopsies had miss rates of 0-17.9% (mean, 2.6%) [161]. Experience and co-operation between radiologists, surgeons and pathologists are essential for successful procedures [162].

2.8.2. Preoperative galactography with methylene dye

According to literature 20%-33% of the surgical biopsies, where the localization of the intraductal lesion was based on diagnostic galactography only had no finding to explain the discharge [25, 163]. Preoperative galactography performed immediately before surgery, using 1:1 ratio of contrast medium and methylene dye improved lesion detection to 100% [25]. However, lesions situated deep within small peripheral ducts may be difficult to localize with methylene dye, which quickly diffuses throughout the breast hindering precise localization for both the surgeon and the pathologist [28] and therefore additional localization methods may be beneficial.
3. AIMS OF THE STUDY

The main purpose of this study was to evaluate the reliability of 14-gauge stereotactic core needle biopsy (SCNB) obtained by an add-on biopsy device in the diagnosis of mammographically detected non-palpable breast lesions in terms of diagnostic accuracy, number of false negative cases and underestimations of ADH and DCIS. In addition, the aim was to evaluate the use of an add-on stereotactic device aided by galactography for preoperative wire-localization of lesions visible only in galactography.

Specific aims were:
1. To evaluate the rate of technically successful biopsies as a function of operator experience.

2. To determine the number of cores required for adequate histopathologic diagnosis.

3. To compare 14-gauge SCNB with surgery in the assessment of prognostic factors of breast cancer (ER, PR and HER-2) and to determine the number of cores needed for assessment of these factors.

4. To assess the feasibility and diagnostic performance of stereotactic guided galactography aided wire or coil localization of breast lesions in patients with spontaneous unilateral nipple discharge.
4. PATIENTS AND METHODS

4.1. Patient selection and study design

Early in 1998, stereotactic equipment for core needle breast biopsy became available in Kuopio University Hospital in Eastern Finland with a catchment area of 251,000 people. Between April 1998 and January 2001, all patients referred to our hospital for suspicious, mammographically detected non-palpable breast lesions (small mass lesions, architectural distortions and microcalcifications) that could not be detected by using ultrasonography were scheduled for stereotactic guided breast biopsy and were enrolled into this study (study I).

During the study period between June 1998 and January 2001, stereotactic 14-gauge core samples were collected in three different containers as follows: the first sample (the central biopsy) was collected into container A, the second and the third samples (obtained 2 mm from the centre of the lesion) into container B, and all additional samples were placed into container C. Histological evaluation and report were performed for each container separately (study II).

For invasive cancers, estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth-factor receptor (HER-2) were assessed by immunohistochemistry for each container separately and for the surgical sample. All HER-2 results 2+ and 3+ were scored by chromogenic-in-situ-hybridisation (CISH). In the comparisons of different containers and also of surgical specimens, all cases with invasive cancer in at least two containers and in the surgical specimen were included in the study (study III).

In addition, between January 2002 and December 2003, all consecutive patients with spontaneous, clear, serous or bloody unilateral nipple discharge, normal
mammographic and ultrasonographic findings (if achievable) and a filling defect $\geq 3$ cm from the nipple noted on a diagnostic galactography were scheduled for an additional galactography examination and immediate stereotactic wire localization or coil placement prior to surgery using the filling defect as the target (study IV).

Between April 1998 and December 2003, altogether 230 patients with 240 lesions were evaluated in these four studies (Fig. 1).

4.1.1. Studies I and II

Between April and June 1998, all patients referred for SCNB, were included in study I for assessment of the learning curve. After this pilot phase, during study II period, 661 patients with mammographically detected suspicious breast lesions were referred to the hospital. Ultrasound guided core needle biopsy was performed in a total of 449 patients. Altogether 212 patients with 220 lesions were scheduled for SCNB. Of those, 15 patients were excluded. In seven patients, the location of the lesion was high, near the axillary fossa or so close to the thoracic wall and pectoral muscle that it could not be reached by the stereotactic equipment. These kinds of lesions were excised surgically. In two (1%) patients, the biopsy had to be terminated after the first pass because of a vaso-vagal reaction. In five patients, the samples were mistakingly all placed in one container instead of three containers, and therefore no separate analysis could be performed. In addition, one patient died from unrelated causes before any follow-up examination. The remaining 197 patients (mean age 56 years, range 32-88 years) with 205 breast lesions (97 mass lesions and 108 clusters of microcalcifications) were evaluated in study II. Eight patients had two lesions: in seven patients there were two lesions ipsilaterally and one patient had a lesion in each breast.
Altogether 221 patients (mean age 56 years, range 22-88 years) with 231 breast lesions (113 mass lesions and 118 clusters of microcalcification) were eligible for study I assessing the learning curve effect. Ten patients had 2 lesions: in 9 patients there were 2 lesions ipsilaterally and one patient had a lesion in each breast.

Twentyfour patients (26 lesions) were examined between April and June 1998, and 197 patients (205 lesions) between June 1998 and January 2001. These 197 patients were the same as those in study II (Fig.1).

Altogether 149 (73%) of the lesions in study II were found from screening mammograms. Forty nine (24%) lesions were found from mammograms obtained because of a symptom (lump, pain or eczema somewhere in the breasts) and 7 (3%) lesions from mammograms obtained because the patient was receiving hormone replacement therapy.

4.1.2. Study III

The material of study III comprises of a subgroup of the material of study II (Figure 1). In study II, there were a total of 54 patients with invasive carcinoma in the surgical specimen. Of these, 13 patients with invasive carcinoma detected in none or in only one container in SCNB were excluded. Eleven patients had either ductal carcinoma in situ (DCIS) (n=7), atypical ductal hyperplasia (ADH) (n=3) or benign findings (n=1) in the three containers and 2 patients had invasive carcinoma only in one container. Altogether 41 patients (mean age 61 years, range 42-82 years) with invasive cancer in at least two containers were included in study III. In three cases, the first container (A) did not contain invasive carcinoma, in three cases the second container (B) did not contain invasive carcinoma and in one case no invasive carcinoma was detected from the third container (C). Altogether 23 (56%) of the 41
lesions were found from screening mammograms. Seventeen (42%) lesions were found from mammograms obtained because of a symptom (lump, pain or eczema somewhere in the breasts) and 1 (2%) lesions from mammograms obtained because the patient was receiving hormone replacement therapy. Mammographically, there were 35 (85%) masses and 6 (15%) clusters of microcalcifications.

According to pathological TNM-classification, 37 (90%) of the tumours were classified as T1 and 4 (10%) as T2. Axillary lymph nodes were classified as N0 in 32 (78%) of the cases, N1 in 7 (17%) of the cases and N2 in 2 (5%) of the cases. According to the histopathological grading, there were 17 (42%) grade I tumours, 21 (51%) grade II tumours and 3 (7%) grade III tumours.

4.1.3. Study IV

Altogether 9 consecutive patients (mean age 54 years, range 34-75 years) with spontaneous, clear, serous or bloody unilateral nipple discharge, normal mammographic and ultrasonographic findings (available in 5 patients) and a filling defect noted on a diagnostic galactography were included in the study (Fig.1).
### Patients/Lesions Table

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Studies</th>
<th>Count</th>
</tr>
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<tbody>
<tr>
<td>April 1998</td>
<td>Study I (221/231)</td>
<td>24/26</td>
</tr>
<tr>
<td>June 1998</td>
<td>Study II (197/205)</td>
<td>197/205</td>
</tr>
<tr>
<td>Jan 2001</td>
<td>Study III (41/41)</td>
<td>9/9</td>
</tr>
<tr>
<td>Jan 2002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dec 2003</td>
<td>Study IV (9/9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>230/240</td>
</tr>
</tbody>
</table>
4.2. Approval of the ethics committee

The study was approved by the ethical committee of Kuopio University Hospital. Informed consent was obtained from all patients.

4.3. Mammographic work-up

A complete mammographic imaging work-up, including magnification images was carried out for all lesions. A comparison with prior mammograms was performed, if achievable. Mammographic findings were categorized either as masses, which included masses with or without microcalcifications, or as clusters of microcalcifications. The size of the lesion was determined as the greatest diameter of the lesion measured directly from the mammogram, and was reported in mm. The classification of the mammographic findings was independently performed by two radiologists with 8 and 9 years of experience in breast imaging who were blinded to the histological results. In study I, stereotactic images of unsuccessful biopsies were retrospectively analysed to confirm correct targeting of the lesions.

All lesions were retrospectively categorized according to the standardized Breast Imaging Reporting and Data System BI-RADS recommended by the American College of Radiology. In cases of discrepancy, a consensus reading was done.

4.4. Stereotactic core biopsy

All biopsies were performed using a regular mammography machine (Planmed, Helsinki, Finland) and a conventional add-on stereotactic biopsy device (Planmed, Helsinki, Finland) with the patient in an upright seated position (Fig.2.). Film-screen system (Kodak MIN-R 2000) was used. A delay due to film processing was approximately 2.5 minutes for each film. Biopsies were obtained with an automated
biopsy gun (C.A. Bard, Covington, GA) with a 22 mm throw and a 14-gauge needle. The biopsy procedures were undertaken by one of five radiologists all with 4-6 years of experience of performing breast biopsies.

Figure 2. An add-on stereotactic biopsy device with an automated needle.

Four of the radiologists had no previous clinical experience with stereotactic breast biopsies prior this study. The remaining radiologist in order to learn the technique, had participated in 3 add-on stereotactic core needle biopsy procedures under the direct supervision of an attending radiologist at another breast clinic that used equipment similar to the hospital, where this study was carried out, in addition to practising the technique with a phantom. The other 4 radiologists performed their first three biopsies under his direct supervision. All five radiologists performed the
subsequent biopsies (after the first 3) independently. The mean number of biopsies was 46 (range 11-94) per radiologist (study I).

In the beginning of the biopsy procedure, the procedure was explained to the patient, who was then positioned for the scout images. The patient was given the opportunity to hear the noise of the biopsy gun prior to procedure. The biopsy direction was chosen by the performing radiologist, after reviewing diagnostic films, taking into consideration the best visibility of the lesion in craniocaudal or lateral orientation and the shortest biopsy route to the lesion. Once the targeted lesion was located on a scout image, the compression was tightened and a pair of stereotactic images was obtained with 15° angulation for each projection. The target was identified and marked on both images. The skin over the lesion was cleaned and anesthetized. The core biopsy needle was inserted to the biopsy gun, which was then set to the holder of the stereotactic device. A 2-3 mm incision was performed to the skin and the needle tip was inserted through the skin at the zero location. For mass lesions, after localizing the lesion, the first needle pass was targeted to the centre of the lesion. Routine pre-fire stereotactic views were obtained to confirm the position of the needle. The needle-tip location was modified, if needed, to ensure a central position was achieved. The following 3-4 needle passes were obtained in a clock-wise manner 2 mm from the centre. Additional needle passes were obtained after individual consideration, depending on the lesion distribution. For microcalcifications there was more variability in needle placement. The intent was to target the most suspicious area for needle biopsy. If the calcifications were tightly clustered, the first pass was obtained either by targeting the centre of the lesion or by selecting a particularly distinctive calcification that could be reliably discerned on the two stereotactic images. Subsequent passes were planned according to the
geography of the calcifications.

4.5. Specimen radiography

For lesions evident as microcalcifications without any mass, the presence of microcalcifications in the biopsy material was confirmed from radiography of the specimen or from the histopathologic report. The practise of routine specimen radiography was initiated in May, 2000. Radiography was performed for 51/118 (43%) specimens taken from lesions of microcalcifications in study I and 48/108 (44%) specimens from lesions of microcalcifications in study II.

4.6. Histopathologic analysis

Handling of the histological specimens was standardized. Core samples were immediately placed into 10% neutral formalin and sent to the Department of Pathology, where they were kept in fixative for two to four hours before routine processing overnight.

One of five pathologists all with 7 to 20 years, mean 12 years, of experience with breast pathology analysed the contents of each container separately for the presence of (1) invasive carcinoma, (2) DCIS, (3) radial scar, (4) ADH, or (4) a specific benign diagnosis. The same pathologist analyzed all three containers of each lesion and he was not blinded to the study design.

The fresh surgical specimen was X-rayed to ensure that the lesion was in the sample with tumour free margins. Then the specimen was measured, painted and sliced within the next half an hour. After overnight formalin fixation, the specimens were cut, processed and embedded in paraffin on the following day. Malignant tumours were classified according to the TNM classification system and histological
grading was performed according to the Nottingham modification of the Bloom and Richardson method.

4.6.1. Immunohistochemistry (IHC) and chromogenic in situ hybridisation (CISH)

For invasive carcinoma, hormone receptor, HER-2 and CISH stainings were reviewed by the same consultant pathologist with seven years’ experience in breast pathology. Double reading was performed for HER-2 staining. In cases of discrepancy, a consensus reading was done.

For ER and PR, the proportion of nuclear staining was quantified from 0 to 100%. Staining < 10% was then designated as negative and ≥ 10% as positive. For HER-2, the Clinical Trial Assay (CTA) system was used to grade the degree of membrane staining. No staining or membrane staining observed in less than 10% of tumor cells was given a score of 0. A faint /barely perceptible membrane staining detected in more than 10% of the tumor cells was scored as 1+ and a weak to moderate membrane staining in >10% was graded as 2+. Strong complete membrane staining in >10% of the tumor cells was graded as 3+ which was a threshold for HER-2 positivity. All 2+ and 3+ results were further investigated by CISH to detect gene amplification.

4.7. Preoperative galactography-aided stereotactic wire or coil localization (Study IV)

Diagnostic galactography was performed in patients with unilateral bloody, serous, or clear nipple discharge by using standard techniques [129].
For patients with an intraductal filling defect that was not visible on mammography or ultrasound, an additional galactography examination was performed before surgery.

The secreting duct was cannulated with the patient in the supine position with the ipsilateral arm resting behind the head. First, the nipple and the periareolar area were cleansed with an ethanol swab. The orifice of the secreting duct was localized using slight periareolar pressure to produce discharge. The ducts were cannulated by insertion of a blunt sterile metallic cannula, (30-G curved or straight cannula; Galactography Kit, MDTECH, USA) measuring 20 mm in length, gently into the secreting duct. The cannula was then connected to small-volume extension tubing with a 1 ml syringe filled with water-soluble, non-ionic contrast material. The duct was filled with 0.2-2 ml of contrast material by gentle hand injection until resistance was felt, the patient experienced discomfort or pain, or there was backflow of the contrast material at the nipple. The cannula and the syringe were fixed securely in place with a bandage to enable administration of additional contrast material, if necessary, and to minimize contrast material leakage. The patient was aided in the transfer from the examination table to the mammography unit. Standard orthogonal screen-film magnification images were obtained from the retroareolar area to verify that the correct duct had been cannulated and to further locate the filling defect.

The add-on stereotactic device was set in place on the mammography unit. The screen-film magnification images just obtained were used to locate the fenestration on the compression plate. Two scout images were first obtained from the target area. Local anesthetic was injected and the breast lesion localization needle with the wire (n=8) (DuaLok, Bard, Covington, USA) or a coil (n=1) (MReye Breast lesion needle localization coil, Cook, Bjaeverskov, Denmark) was targeted to the filling defect under
stereotactic guidance. A new pair of scout images was taken to ensure the localization of the needle tip, which was corrected, if necessary. When the needle tip was correctly within the lesion, a wire or coil was passed through the location needle and the needle was pulled out. After the procedure, standard craniocaudal and mediolateral mammographic views were obtained to verify the correct location of the wire and the lesion for the surgeon. All procedures were performed by one of two radiologists immediately preoperatively, except for a single coil localization which was performed one week prior the surgery. On the operation day, the coil was localized with the wire under ultrasonography guidance. At surgery, periareolar incision to enter the retromamillary area was performed and an excision was made containing the tip of the wire with 1 to 2 cm of the surrounding area.

4.8. Treatment and follow-up

The histopathology of each lesion was reviewed in conjunction with the mammographic findings and the clinical history in a multidisciplinary meeting with a radiologist, a pathologist, a plastic surgeon and an oncologist to plan further management of the patient. All of the cases with the galactography aided wire or coil localization were also reviewed in this multidisciplinary meeting to confirm that the lesion identified by galactography was in the surgical sample.

After core biopsy, all women whose biopsy result indicated the presence of invasive carcinoma or DCIS underwent surgical treatment. High-risk lesions, such as ADH and radial scars, were also surgically resected, as were benign lesions with discordant mammographic findings. All other lesions with benign biopsy results were recommended for mammographic follow-up at 1 year and 2 years.
5. STATISTICS

In general, continuous variables were tested for normal distribution with the Kolmogorov-Smirnov 1-sample test. The Mann-Whitney U test for continuous variables with non-normal distributions was used for group comparisons in the case of two independent samples and the Wilcoxon Signed Ranks Test in the case of two related samples. Chi-square test was used for dichotomized discrete variables. Differences were considered statistically significant if the p value was less than 0.05.

5.1. Studies I and II

The 95% confidence intervals in study II are approximations of the confidence intervals (preferred when proportions are near unity or zero) based on formulas given in Fleiss et al [164]. The other analyses were performed with the statistical package SPSS for Windows, Version 11.5. (SPSS, Inc., Chicago, IL).

In studies I and II, the calculations were performed separately for mass lesions and microcalcifications. The sensitivity and specificity values with 95% confidence intervals, and the overall accuracy as well as positive (PPV) and negative (NPV) predictive value of the stereotactic core biopsy, were determined using the results of surgical samples and mammographic follow-up as a reference standard (study II).

5.1.1. “Strict” and “working” analysis

Sensitivity, specificity and overall accuracy were separately calculated in two categories (study II). (1) “Strict” analysis - Strict sensitivity was defined such that any stereotactic core biopsy diagnosis of a non-malignant lesion that after surgical excision proved to be malignant was considered as a false negative. (2) “Working”
analysis - Working sensitivity was defined such that a stereotactic core biopsy diagnosis of ADH or radial scar (high risk lesions) was considered as a true positive if the final surgical sample corresponded to the result or showed malignancy (DCIS or invasive carcinoma), and a false-positive if the diagnosis in the pathology report of surgical excision was benign [165].

5.1.2 Determinations

5.1.2.1. False negative rate

False negative rate was determined by dividing the number of cases that were negative by the method (core biopsy or surgical sample) and positive by the other (reference) method by all positive cases of the reference method.

5.1.2.2. Malignancy rate

Malignancy rate was calculated by dividing the number of malignancies in each BI-RADS category by the number of lesions in each category.

5.1.2.3. Adequate samples

Samples were considered adequate 1) for microcalcifications, if microcalcifications were detected either on specimen radiography or in the histopathology of clusters of microcalcification and 2) for mass lesions if histopathologic diagnosis in concordance with the mammographic appearance was obtained.
5.1.2.4. Technically successful biopsies

In study II, achieving adequate samples, as described above, was considered as technically successful.

5.1.2.5. Technically unsuccessful biopsies

Samples were considered technically unsuccessful 1) for microcalcifications, if there were no microcalcifications detected either on specimen radiography or on histopathology of clusters of microcalcifications and 2) for mass lesions, if histopathologic diagnosis was not concordant with the mammographic appearance of the lesion. In addition, the biopsies that yielded a false negative histologic result were considered as technically unsuccessful. Also biopsies that had to be repeated were considered as technically unsuccessful even if the re-biopsy was successful.

5.1.2.6. Underestimated ADH and DCIS lesions

Underestimated ADH lesions were defined as lesions that yielded ADH at core needle biopsy and carcinoma at surgery [166]. Underestimated DCIS lesions were defined as lesions that yielded DCIS at core biopsy and invasive carcinoma at surgery [166].

5.1.2.7. Learning curve

In the assessment of the learning curve, the rate of technically successful biopsies as a function of operator experience was calculated cumulatively for all 5 individual radiologists, separately for the first 5 independently performed biopsies of each radiologist and for all subsequent cases and also separately for the first 5
clusters of microcalcifications and for the first 5 mass lesions for each individual radiologist (study I).

5.2. Study III

Statistical differences in the proportions of positive and negative cases between the dichotomised ER, PR and HER-2 scores in core samples and surgical specimen were evaluated using McNemar’s non-parametric paired proportions test. Intertechnique differences were tested by the kappa statistics.

Containers were dichotomized as negative if all cores in the container were negative and positive if at least one of the cores was positive for ER, PR and HER-2. Container combinations were designated accordingly; negative if all containers were negative and positive if at least one of the containers was positive.

Sensitivities of the individual containers (A, B and C) and container combinations (A+B, A+B+C) for the assessment of ER, PR and HER-2 were calculated. In the calculations of sensitivity, the “optimal reference” was used, which was considered positive if at least one of the containers or the surgical specimen was positive and negative if all three samples and the surgical specimen were negative.
6. RESULTS

A minimum of 4 samples (mean 7) were obtained from each patient (study I, II and III). The numbers of biopsies performed by each individual radiologist were 94, 31, 12, 83 and 11 (study I). One patient (0.5%) suffered from a vaso-vagal reaction leading to the termination of the biopsy procedure after three passes. The rebiopsy was performed without complications. Infections and hematomas requiring further treatment were not noted in these studies. One biopsy failed because of patient movement (0.5%). The repeat biopsy was accomplished successfully.

Final diagnosis according to surgery (n=95) and follow-up (n=110) was malignant in 86 (42%) cases (54 invasive carcinomas and 32 cases of DCIS) and benign in 119 (58%) cases (includes two cases of radial scars).

Categorization of the lesions according to the BI-RADS classification is in Table 4.
TABLE 4. Number of malignancies in surgical samples and core samples of 97 mass lesions and 108 clusters of microcalcifications and malignancy rate (%) according to the mammographic appearance of lesions classified using BI-RADS categorization (Study II)

<table>
<thead>
<tr>
<th></th>
<th>Number of lesions</th>
<th>Number of malignant lesions</th>
<th>Malignancy rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>surgery/core sample</td>
<td>surgery/core sample</td>
<td></td>
</tr>
<tr>
<td>Mass lesions</td>
<td>97</td>
<td>39 / 40</td>
<td>40 / 41</td>
</tr>
<tr>
<td>BI-RADS 2</td>
<td>0</td>
<td>0 / 0</td>
<td>-</td>
</tr>
<tr>
<td>BI-RADS 3</td>
<td>13</td>
<td>0 / 0</td>
<td>0 / 0</td>
</tr>
<tr>
<td>BI-RADS 4</td>
<td>59</td>
<td>16 / 16</td>
<td>27 / 27</td>
</tr>
<tr>
<td>BI-RADS 5</td>
<td>25</td>
<td>23 / 24</td>
<td>92 / 96</td>
</tr>
<tr>
<td>Microcalcifications</td>
<td>108</td>
<td>47 / 43</td>
<td>43 / 40</td>
</tr>
<tr>
<td>BI-RADS 2</td>
<td>2</td>
<td>0 / 0</td>
<td>0 / 0</td>
</tr>
<tr>
<td>BI-RADS 3</td>
<td>22</td>
<td>0 / 0</td>
<td>0 / 0</td>
</tr>
<tr>
<td>BI-RADS 4</td>
<td>62</td>
<td>28 / 24</td>
<td>45 / 39</td>
</tr>
<tr>
<td>BI-RADS 5</td>
<td>22</td>
<td>19 / 19</td>
<td>86 / 86</td>
</tr>
<tr>
<td>Total</td>
<td>205</td>
<td>86 / 83</td>
<td>40 / 41</td>
</tr>
</tbody>
</table>

6.1. SCNB histology (Study II)

Histopathological analysis of core samples revealed carcinoma in 83 (40%) of the 205 lesions in the whole material, ADH in 3 (1.5%), radial scar in 1 (0.5%) and a benign diagnosis in 118 (58%) samples.

Of the 97 mass lesions (17 with microcalcifications and 80 without microcalcifications), the stereotactic core sample revealed malignancy in 40 (41%) lesions (invasive carcinoma in 38 cases and DCIS in two cases), radial scar in 1 (1%) lesion and a benign diagnosis in 56 (58%) lesions of which one yielded only benign breast tissue and was classified as inadequate. No DCIS underestimations were
detected. There were no false negative results among the 96 diagnostic core samples. Instead, one false positive result occurred. In this one special case, the main diagnosis was a radial scar in core samples, but there was a mild suspicion of tubular carcinoma. The final surgical diagnosis was a radial scar. The result of a repeated retrospective blinded reading of the core samples from this lesion was also radial scar. Altogether 13 of 17 (77%) mass lesions with microcalcifications turned out to be malignant.

Of the 108 lesions which were evident as clusters of microcalcification without any mass, stereotactic core sample revealed malignancy in 43 (40%) cases (invasive carcinoma in 12 and DCIS in 31), ADH in 3 (3%) cases, and a benign diagnosis in 62 (57%) cases, four of which were inadequate (4%). All three cases with a core sample diagnosis of ADH proved to be malignant (two cases of DCIS and one invasive carcinoma) at surgery. These three cases were categorized as false negative according to the strict analysis (Fig.3). In the fourth strict false negative case, the lesion was surgically operated because of discordance between the mammographic finding and the histopathologic diagnosis of fibrocystic disease in the core samples, and subsequently invasive lobular carcinoma was detected. This was the single false negative case in the material according to the working analysis (Fig. 4). The strict false negative rate was 4.7% (4 of 86) and the working false negative rate was 1.1% (1 of 88). Among the 31 clusters of microcalcifications indicating DCIS at core samples, surgery revealed invasive ductal carcinoma in 7 (23%).
Figure 3. (a) Craniocaudal mammogram obtained in a 36-year-old woman shows a cluster of microcalcifications (arrow) classified as BI-RADS category 4. (b) Lateral mammogram at core sample shows a cluster of microcalcifications (arrow); the diagnosis was ADH. At surgery, histologic grade 3 invasive ductal carcinoma was revealed.
The first screening mammogram and magnification mammogram obtained in a 49-year-old woman revealed a 20-mm-diameter cluster of microcalcifications (arrow) classified as BI-RADS category 4 in the upper lateral quadrant of the right breast. Diagnosis at core sample was fibrocystic disease. Because of the discordance between the mammographic finding and the histopathologic diagnosis at core-needle biopsy, the patient underwent surgical excision. LCIS, ALH, fibrocystic disease and a small 0.5 x 0.3 cm focus of invasive lobular carcinoma was found. (a) Mediolateral oblique view. (b) Area of interest.

6.2. Diagnostic performance of stereotactic core needle biopsy (Study II)

The diagnostic performance of SCNB for the 3 different containers is shown in Table 5.
TABLE 5. Diagnostic performance of add-on stereotactic core needle breast biopsy by “strict” and “working” analysis with reference to the final clinical diagnosis; 205 breast lesions (97 masses and 108 clusters of microcalcifications, Study II)

<table>
<thead>
<tr>
<th></th>
<th>Strict analysis</th>
<th>Working analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity %</td>
<td>Specificity %</td>
</tr>
<tr>
<td>Container A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>66 (31/47)</td>
<td>100 (61/61)</td>
</tr>
<tr>
<td>Mass</td>
<td>90 (35/39)</td>
<td>98 (57/58)</td>
</tr>
<tr>
<td>Total</td>
<td>77 (66/86)</td>
<td>99 (118/119)</td>
</tr>
<tr>
<td>Container A + B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>91 (43/47)</td>
<td>100 (61/61)</td>
</tr>
<tr>
<td>Mass</td>
<td>95 (37/39)</td>
<td>98 (57/58)</td>
</tr>
<tr>
<td>Total</td>
<td>93 (80/86)</td>
<td>99 (118/119)</td>
</tr>
<tr>
<td>Container A + B + C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>91 (43/47)</td>
<td>100 (61/61)</td>
</tr>
<tr>
<td>Mass</td>
<td>100 (39/39)</td>
<td>98 (57/58)</td>
</tr>
<tr>
<td>Total</td>
<td>95 (82/86)</td>
<td>99 (118/119)</td>
</tr>
</tbody>
</table>

Note.—OA = overall accuracy, MC = microcalcifications
See text for definition of “strict” and “working” analysis.
Malignant cases confirmed by subsequent surgical resection. Benign lesions followed up clinically and mammographically, mean follow-up time 24 months. Numbers in parentheses were used to calculate the percentages. Inadequate samples (n=5) were classified as true negative.
6.2.1. The strict analysis

The sensitivity of the first sample was 77% (90% for masses and 66% for microcalcifications). The result for the first sample was false negative significantly more often for microcalcifications (n=16) than for mass lesions (n=4, p=0.01). Combined results of containers A and B, i.e. three samples, yielded higher sensitivity than the first sample alone: the sensitivity increased to 95% for mass lesions (p=0.196) and to 91% for microcalcifications (p<0.001). Multiple samples reached a sensitivity of 100% for mass lesions. For microcalcifications, multiple samples did not improve the sensitivity (91%) (Table 5).

6.2.2. The working analysis

The sensitivity of the first sample was 79% (90% for mass lesions and 70% for microcalcifications). Three samples yielded a sensitivity of 94 % (95 % for mass lesions and 94% for microcalcifications). For the combined results of containers A, B and C, a sensitivity of 99% (100% for mass lesions and 98% for microcalcifications) was reached (Table 5).

6.2.3. Verification of microcalcifications

In the samples of clusters of microcalcification (108 lesions) histopathological evidence of calcium was detected in 57 (53%) lesions in container A, in 81 (75%) lesions in containers A and B combined and in 95 (88%) lesions in at least one of the three containers. Radiography was performed on samples taken from 48 (44%) of all the 108. The radiography revealed calcifications in at least one sample in 46 (96%) lesions. In 9 clusters of microcalcifications (8 patients) there were no calcifications seen either in specimen radiographs or in the histopathological analysis. Details of
these 9 lesions are included in the paragraph “Technically unsuccessful biopsies” (study I). In study I, radiography was performed for 43% (51/118) of the samples obtained from lesions of microcalcifications.

6.3. The rate of successful biopsies correlated with the experience of each radiologist (Study I)

The rates of technically successful biopsies for the 5 individual radiologists separately for microcalcifications and mass lesions are shown in Table 6. For microcalcifications, the rate of successful biopsies was lower for the first 5 biopsies than for the subsequent cases and this was true for all radiologists: 75% (18/24) for the first 5 biopsies and 88% (79/90) for the subsequent biopsies (p=0.335). For mass lesions the rates of successful biopsies were equal: for the first 5 biopsies 96% (22/23) and for the subsequent biopsies 96% (79/82) (p=1.0).
Table 6. The rate of technically successful biopsies in percentages separately for the first five and subsequent biopsies of each radiologist (Study I). Number of patients are in parentheses

<table>
<thead>
<tr>
<th>Radiologist</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>all</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcalcifications</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>first five biopsies</td>
<td>80 (4/5)</td>
<td>80 (4/5)</td>
<td>80 (4/5)</td>
<td>40 (2/5)</td>
<td>100 (4/4)</td>
<td>75 (18/24)</td>
</tr>
<tr>
<td>subsequent biopsies</td>
<td>95 (37/39)</td>
<td>91 (10/11)</td>
<td>-</td>
<td>80 (32/40)</td>
<td>-</td>
<td>88 (79/90)</td>
</tr>
<tr>
<td>Masses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>first five biopsies</td>
<td>100 (5/5)</td>
<td>80 (4/5)</td>
<td>100 (4/4)</td>
<td>100 (5/5)</td>
<td>100 (4/4)</td>
<td>96 (22/23)</td>
</tr>
<tr>
<td>subsequent biopsies</td>
<td>96 (43/45)</td>
<td>100 (7/7)</td>
<td>-</td>
<td>97 (29/30)</td>
<td>-</td>
<td>96 (79/82)</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>first five biopsies</td>
<td>100 (5/5)</td>
<td>80 (4/5)</td>
<td>100 (5/5)</td>
<td>80 (4/5)</td>
<td>100 (5/5)</td>
<td>92 (23/25)</td>
</tr>
<tr>
<td>subsequent biopsies</td>
<td>94 (84/89)</td>
<td>91 (21/23)</td>
<td>75 (3/4)</td>
<td>87 (64/75)</td>
<td>100 (3/3)</td>
<td>91 (175/194)</td>
</tr>
</tbody>
</table>
6.3.1. Technically unsuccessful biopsies

Technically unsuccessful biopsies occurred in 21 (9.6%) of the 219 independently performed biopsies; these included more frequently lesions of microcalcifications: 15% (17/114) compared with 4% (4/105) of mass lesions (p=0.004). For mass lesions, normal breast tissue (n=3) or fatty tissue (n=1) was detected on histopathology. In 13 of 17 technically unsuccessful lesions of microcalcifications, there were no microcalcifications detected in the specimen radiographs or on the histopathologic report. Four of the 17 lesions of microcalcifications were false negative at histological examination and were classified technically unsuccessful although there were calcifications on specimen radiography or histopathology.

6.3.1.1. Surgical excision

Five of 13 lesions with no microcalcifications detected in the specimen radiographs or on the histopathology were surgically excised. In three of these lesions, the histologic finding of core biopsy was DCIS or invasive ductal carcinoma, which was confirmed at surgery. Two lesions were benign (fibrosis with microcalcifications). In one mass lesion with inadequate histopathology in core samples, surgery revealed fibrosis.

6.3.1.2. Rebiopsy

Four clusters of microcalcifications underwent successful rebiopsy with a benign diagnosis and microcalcifications on histopathology or on specimen radiography. In 2 lesions, rebiopsy was unsuccessful and no microcalcifications were seen either on
specimen radiography or on histopathology. One mass lesion was successfully rebiopsied with a diagnosis of fibrocystic disease.

6.3.1.3. Follow-up

There were two clusters of microcalcifications with unsuccessful biopsies that were not surgically excised or rebiopsed. In the follow-up, these lesions as two lesions with unsuccessful rebiopsies have remained stable. One mass lesion had disappeared at the 6-month follow-up. The remaining mass lesion was still unchanged at the 28-month follow-up mammography.

6.3.2. Technically successful and unsuccessful biopsies in different BI-RADS categories

In the comparison of unsuccessful biopsies in different BI-RADS categories for mass lesions, the rate was highest in the BI-RADS 3 category (11.8%). For BI-RADS 4 microcalcifications the rate of unsuccessful biopsies was 18.6%. These lesions were smaller (mean 10 mm, range 3-45 mm) than BI-RADS 5 microcalcifications (mean 17 mm, range 4-30 mm), but equal to BI-RADS 3 microcalcifications (mean 10 mm, range 8-15 mm) (Table 7).
Table 7. The lesion size in successfully and unsuccessfully biopsied lesions in different BI-RADS categories; 229 lesions (113 masses and 116 clusters of microcalcifications, Study I)

<table>
<thead>
<tr>
<th>BI-RADS Category</th>
<th>Masses</th>
<th>Microcalcifications</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lesion size (mm)</td>
<td>lesion size (mm)</td>
<td>lesion size (mm)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>mean</td>
</tr>
<tr>
<td>BI-RADS 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all</td>
<td>17</td>
<td>10</td>
<td>3 - 19</td>
</tr>
<tr>
<td>successful</td>
<td>15</td>
<td>88</td>
<td>11</td>
</tr>
<tr>
<td>unsuccessful</td>
<td>2</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>BI-RADS 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all</td>
<td>68</td>
<td>12</td>
<td>4 - 37</td>
</tr>
<tr>
<td>successful</td>
<td>66</td>
<td>97</td>
<td>12</td>
</tr>
<tr>
<td>unsuccessful</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>BI-RADS 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all</td>
<td>28</td>
<td>11</td>
<td>7 - 23</td>
</tr>
<tr>
<td>successful</td>
<td>28</td>
<td>100</td>
<td>11</td>
</tr>
<tr>
<td>unsuccessful</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Note.- BI-RADS 2 lesions (n=2) have not been included in the table.
6.4. Treatment and follow-up (Studies I and II)

Surgery was performed for 110 of the 231 lesions, and this revealed 18 benign and 92 malignant lesions, including one mass with a change detected at the 1 year follow-up. Mammographic follow-up was performed for 122 lesions. The mean mammographic follow-up time was 24 months (range 6-39 months). Altogether 83% (101/122) of the patients were followed-up for 20 months and 64% (78/122) for 24 months. No patients were unavailable for follow-up, but one patient died and two patients moved to another area after adjunctive 6-month follow-up mammography. A mammographic change occurred in 2.5% (3 of 122) of the lesions. One mass lesion with a core needle biopsy diagnosis of fibroadenoma had grown in the follow-up; the diagnosis was fibroadenoma also at surgery. In addition, growth was noted in one cluster of microcalcifications and in one mass lesion at follow-up mammography. On rebiopsy, the initial diagnosis of fibrocystic disease was confirmed in both cases. No change has been noted in the remaining 119 lesions.

6.5. ER, PR and HER-2 assessments (Study III)

6.5.1. Comparison of core samples with the surgical specimen

The agreement between core samples and surgical specimens was high: 83% for ER, 88% for PR, 88% for HER-2 (IHC) and 93% after adding CISH. Discordant cases tended to be positive for core samples, but negative for surgery: 5 of 5 discordant cases were positive in core samples for PR, 5 of 7 for ER, 4 of 5 for HER-2 after IHC and 3 of 3 for HER-2 after IHC and CISH, respectively. However, these differences did not reach statistical significance (Table 8).

Cases showing 2+ or 3+ HER-2 overexpression by immunohistochemistry (9 (22%) surgical specimens and 19 (46%) core samples) were further investigated by
CISH. HER-2 gene amplification was detected in 6 cases according to core biopsies but only in three cases for surgical specimens.

When comparing the prognostic assessment based on the surgical specimens to that based on the core samples, 5 false negative assessments were made for ER (14%) and PR (15%) and 3 false negative assessments for CISH-positive HER-2 (50%). When comparing prognostic assessments made from core samples with assessments made from surgical specimens, 2 false negative assessments were encountered for ER (6%), and none for PR and HER-2. Proportions of immunohistochemically PR-positive and ER-positive cells from all malignant cells in core samples (highest score) were significantly higher than in surgical specimens (for ER 74% vs. 63%; p=0.031, for PR 62% vs. 50%; p=0.001) (Fig. 5. a and b).
Table 8. Agreement, number of discordant cases and percentages of positive stainings in core samples compared to surgical specimen. (Study III, 41 cases of invasive carcinoma)

<table>
<thead>
<tr>
<th></th>
<th>Agreement</th>
<th>Disagreement</th>
<th>Statistical difference between methods</th>
<th>Positive stainings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Core - /</td>
<td>Core + /</td>
<td>Containers (A+B+C)</td>
<td>Surgical specimen</td>
</tr>
<tr>
<td></td>
<td>Surgery +</td>
<td>Surgery -</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>κ</td>
<td>n</td>
</tr>
<tr>
<td>ER</td>
<td>34/41</td>
<td>83</td>
<td>0.39</td>
<td>2/41</td>
</tr>
<tr>
<td>PR</td>
<td>36/41</td>
<td>88</td>
<td>0.69</td>
<td>0</td>
</tr>
<tr>
<td>HER-2 (IHC)</td>
<td>36/41</td>
<td>88</td>
<td>0.69</td>
<td>1/41</td>
</tr>
<tr>
<td>HER-2 (IHC +CISH)</td>
<td>38/41</td>
<td>93</td>
<td>0.64</td>
<td>0</td>
</tr>
</tbody>
</table>

ER = estrogen receptors, PR = progesterone receptors, HER-2 = HER-2 over-expression, CISH = chromogenic in situ hybridisation. IHC = immunohistochemistry. Statistical differences between methods were calculated using McNemar’s non-parametric paired proportions test.
Figure 5. Proportions of immunohistochemically ER-positive (a.) and PR positive (b.) cells from all malignant cells on core samples (highest score) versus surgical specimens. Dashed line: x=y
6.5.2. Comparison of containers of cores

When dichotomized as positive or negative, ER assessments for all three containers were concordant in 41/41 (100%) of the cases ($\kappa=1.0$) and PR and HER-2 assessments in 35/41 (85%) of the cases ($\kappa=0.66$).

The proportions of ER and PR positive cells relative to all malignant cells in each container are shown in figure 6. The containers of cores show a high correlation for PR. In discordant cases the proportions are near the cut off value. For ER more heterogeneity was detected between different containers. Sensitivities for the detection of ER, PR and HER-2 were individually calculated for the three containers and container combinations (Table 9). The central core (container A) did not differ markedly in sensitivity from the more peripheral cores (containers B and C) in the assessment of ER, PR and HER-2. With three cores sensitivity was 95% for ER, 92% for PR, 95% for HER-2 (IHC) and 100% after adding CISH. With more than three cores sensitivities of 95% for ER, 100% for PR, 98% for HER-2 (IHC) and 100% after adding CISH were reached.
Figure 6. Proportions of immunohistochemically ER-positive (a.) and PR-positive (b.) cells from all malignant cells in different core samples. Dashed line indicates the cut-off value of 10% for positive and negative cases.
Table 9. Sensitivity (%) of different containers and container combinations for the assessment of ER, PR and HER-2 (Study III, 41 patients)

<table>
<thead>
<tr>
<th>Container</th>
<th>ER</th>
<th>PR</th>
<th>HER-2</th>
<th>CISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>95 (36/38)</td>
<td>92 (35/38)</td>
<td>89 (34/38)</td>
<td>95 (18/19)</td>
</tr>
<tr>
<td>B</td>
<td>97 (36/37)</td>
<td>92 (34/37)</td>
<td>92 (35/38)</td>
<td>100 (19/19)</td>
</tr>
<tr>
<td>C</td>
<td>95 (38/40)</td>
<td>95 (38/40)</td>
<td>88 (35/40)</td>
<td>90 (18/20)</td>
</tr>
<tr>
<td>A + B</td>
<td>95 (36/38)</td>
<td>92 (35/38)</td>
<td>95 (39/41)</td>
<td>100 (20/20)</td>
</tr>
<tr>
<td>A + B + C</td>
<td>95 (39/41)</td>
<td>100 (41/41)</td>
<td>98 (40/41)</td>
<td>100 (20/20)</td>
</tr>
</tbody>
</table>

ER = estrogen receptors, PR = progesterone receptors, HER-2 = HER-2 overexpression, CISH = chromogenic in situ hybridisation. Number of patients are in the parentheses.

6.6. Galactography aided stereotactic wire localization (Study IV)

On galactography, 7 solitary and 2 multiple filling defects were detected. The localization procedure was well tolerated by the patients, and no complications were noted. All patients that underwent stereotactic-guided wire or coil localization were operated by surgical excision of the diseased duct segment. Surgery and subsequent histopathological analysis revealed 1 ductal carcinoma in situ (DCIS), 4 solitary intraductal papillomas, 2 cases of intraductal papillomatosis, 1 case of mastitis with granulomatous tissue and 1 fibrocystic disease with intraductal papillary proliferation. In 8 patients, a coexisting ductal ectasia was noted (Table 10). All patients remained free of nipple discharge at one year follow-up after surgery.
Table 10. Patient and lesion characteristics in nine patients with nipple discharge (Study IV)

<table>
<thead>
<tr>
<th>Patient n:o</th>
<th>Age years</th>
<th>Type of discharge</th>
<th>Duration of discharge months</th>
<th>Mammography</th>
<th>Ultrasound</th>
<th>Galactography</th>
<th>Distance of lesion from the nipple cm</th>
<th>Histological diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>bloody</td>
<td>-</td>
<td>normal</td>
<td>-</td>
<td>amputation of the duct</td>
<td>3.5</td>
<td>papilloma</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>bloody, intermittent</td>
<td>8, intermittent</td>
<td>normal</td>
<td>normal</td>
<td>solitary filling defect, ductectasia</td>
<td>3.0</td>
<td>papillomatosis</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>bloody</td>
<td>6</td>
<td>normal</td>
<td>normal</td>
<td>amputation of the duct, ductectasia</td>
<td>3.0</td>
<td>papillomatosis</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>bloody</td>
<td>1</td>
<td>normal</td>
<td>-</td>
<td>multiple filling defects, ductectasia</td>
<td>4.5</td>
<td>chronic mastitis</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>clear</td>
<td>48</td>
<td>normal</td>
<td>-</td>
<td>amputation of the duct, ductectasia</td>
<td>5.0</td>
<td>papilloma</td>
</tr>
<tr>
<td>6</td>
<td>51</td>
<td>bloody</td>
<td>6</td>
<td>normal</td>
<td>normal</td>
<td>solitary filling defect, ductectasia</td>
<td>6.0</td>
<td>DCIS</td>
</tr>
<tr>
<td>7</td>
<td>75</td>
<td>bloody</td>
<td>4</td>
<td>normal</td>
<td>normal</td>
<td>ductectasia</td>
<td>4.0</td>
<td>papilloma</td>
</tr>
<tr>
<td>8</td>
<td>45</td>
<td>serous</td>
<td>6</td>
<td>normal</td>
<td>-</td>
<td>multiple filling defects, ductectasia</td>
<td>3.0</td>
<td>papilloma</td>
</tr>
<tr>
<td>9</td>
<td>55</td>
<td>bloody</td>
<td>10</td>
<td>normal</td>
<td>normal</td>
<td>amputation of the duct, ductectasia</td>
<td>3.5</td>
<td>fibrocystic disease</td>
</tr>
</tbody>
</table>
One malignancy was detected: micropapillary DCIS, high grade, with two foci of microinvasion (Table 10: patient n:o 6). Surgery was performed under guide wire and methylene dye guidance (Fig. 7 A-C). Because of the large, high grade DCIS and unclear resection margins, subsequent skin sparing mastectomy with immediate reconstruction was performed.

**Figure 7.** 51-year-old woman with brownish bloody discharge from left breast for 6 months.

a. Craniocaudal galactography image revealed a duct with multiple branches and an ectatic duct segment with a 3 mm filling defect 6 cm from mamilla (arrow)

b. Enlargement of area of interest (arrow) in a

c. Immediately after galactography, the craniocaudal mammogram revealed adequate stereotactic wire localization, which was performed with ectatic duct as the target (arrow)
7. DISCUSSION

This study focuses on the validation of an add-on stereotactic guidance method for performance of 14-gauge core needle breast biopsies of non-palpable breast lesions and for additional use of the add-on stereotactic device for localising lesions detected only with galactography.

7.1. Equipment

Compared to dedicated devices used when the patient is prone, add-on biopsy has been thought to be more vulnerable to patient movement and vaso-vagal collapses because of the sitting position of the patient. In the present study, only one (0.5%) of 205 biopsy procedures had to be terminated because of a vaso-vagal reaction. Another two patients were excluded from the study because the procedure had to be interrupted after only one pass. The results of this study are consistent with those of Caines et al [167] who reported vaso-vagal attacks in 1.6% of their procedures, and of Wunderbaldinger et al [22], who reported vaso-vagal collapses leading to termination of the biopsy procedure in 2% of the procedures.

Prone tables have also been advocated over add-on units because the lying horizontal position is thought to minimize patient movement [20]. Patient movement may make the biopsy procedure more time consuming, and with re-positioning, increase the radiation dose. In the present study, only one biopsy failed because of patient movement (0.5%). The repeat biopsy was accomplished successfully.

The current study was performed with conventional film-screen technology with an average of 2.5 min time delay due to development of each film. Digital imaging
reduces the biopsy time and this may further decrease the incidence of vasovagal reactions and the possibility of patient movement [168].

7.2. Classification of mammographic findings

Core samples were taken from all the lesions referred for biopsy, and BI-RADS classification was performed in retrospect. Consequently, some patients with benign or probably benign lesions underwent stereotactic core biopsy in this study. Core biopsy was performed for two BI-RADS 2 lesions and 35 BI-RADS 3 lesions, with no malignancies being detected in these samples. These results are similar to the previous reports of a very low incidence of malignancy (0.5-2%) detected among lesions categorized as probably benign [169-171] (Table 3). Malignancy was present in 36% of BI-RADS category 4 lesions and in 89% of BI-RADS category 5 lesions which also is in concordance with the literature [120, 124]. These results support the point of view that biopsy is indicated only for category 4 and 5 lesions.

Pleomorphic microcalcifications within a solid mass need special attention and should be considered suspicious for malignancy [172]. In this study 77% of mass lesions with microcalcifications were malignant.

7.3. Diagnostic accuracy of core needle biopsies with an add-on device (Studies I and II)

7.3.1. Strict and working accuracy

With multiple (>3) cores a strict sensitivity of 95%, strict specificity of 99% and overall accuracy of 98% was achieved; the results are similar with those obtained with the prone devices (Table 11). The strict false negative rate was 4.7 % (4 of 86) for the surgically confirmed malignancies. In previous studies, a false negative rate of
4.4% (range, 2.9-6.7%) has been reported, which is comparable to the results of this study [8, 9, 11, 18, 173]. All four false negative core needle biopsy diagnoses in this study were evident as clusters of microcalcifications, and three of them were ADH. In clinical practise, ADH can be calculated as a true positive as was done in working calculations, where high sensitivity of 99%, specificity of 99% and overall accuracy of 99% were achieved. In working calculations there was only one false negative case detected. Surgery of this cluster of microcalcifications was performed because of discordance between the BI-RADS 4 mammographic appearance and the histopathologic diagnosis of fibrocystic disease at core-needle biopsy. Invasive lobular carcinoma was found. In previous studies, up to 63.6% of the lesions with discordant stereotactic core biopsy and mammography results revealed carcinoma in subsequent surgery; the discrepancy is an indication for surgery [174-178]. If one wishes to find possible cancers in discordant cases, then it is important to compare core biopsy histology with the imaging results in each case [175, 179].

A high strict specificity of 99% was achieved in this study in accordance with the studies of prone biopsy devices [165]. Only one false positive lesion was detected. In this particular case, the main diagnosis was a radial scar at core-needle biopsy, but there was a mild suspicion of tubular carcinoma. The final surgical diagnosis was a radial scar. The diagnosis was assured by repeated retrospective blinded reading of the core specimens. A malignant diagnosis in a core needle biopsy followed by a benign surgical diagnosis is usually not considered as false positive [138]. It can be explained by either complete removal of the lesion by the core needle biopsy or incomplete surgical excision.

It has to be clarified, that there were 5 lesions in the material classified as true negative, that were biopsied technically unsuccessfully with no subsequent rebiopsy
or surgery. Instead of mammographic follow-up, these lesions should have been surgically excised. The decision was made in the multidisciplinary meeting and was not in accordance with the original study protocol.

7.3.2. Underestimation of ADH and DCIS

ADH is known to underestimate cancer and warrants surgical excision [3, 160, 176, 180]. In a series of 1032 lesions diagnosed by core biopsy, Meyer et al [176] detected 18 ADH lesions, 10 (56%) of which were malignant at surgery. Similar results have been reported by others [3, 10, 177, 180-182]. In this study core needle biopsy underestimated the presence of carcinoma in all (3/3) the ADH-lesions.

Seven of 33 DCIS diagnosed by SCNB proved to be invasive ductal carcinoma at surgery. This underestimation rate of 21% is comparable with previous studies with figures ranging from 16 to 35%. [3, 10, 136, 183, 184].

Since most lesions attributable to ADH or DCIS contain microcalcifications, histologic underestimates at percutaneous biopsy are most frequently encountered in lesions with microcalcifications [180, 182].

7.3.3. Number of cores

There is a recommendation of five cores for masses and a minimum of five cores for microcalcifications in the biopsy of non-palpable breast lesions with dedicated prone equipment [18, 158]. A minimum of four specimens should be obtained with ultrasonography guided core-needle breast biopsy and a 14-gauge needle [159]. The experience in this study with an add-on device is in line with these recommendations. Strict accuracy after a single sample had been removed was 90%, after 3 samples this rose to 97% and after more than 3 (multiple) samples were removed, to 98%. For
masses the strict sensitivity after a single sample had been removed was 90%, after three samples this rose to 95% and after multiple samples were removed 100% was reached. However, these results do not provide data to indicate on how many more than three samples are sufficient. For microcalcifications the strict sensitivity was lower; 66% after a single sample and 91% after three samples, respectively. The access to multiple samples did not provide any improvement in the strict sensitivity (91%) though an acceptable working sensitivity of 98% was reached. This is again explained by the three cases in which core needle diagnosis remained ADH even after multiple samples, and surgery revealed carcinoma. The reported strict sensitivity rates for different numbers of cores acquired with a prone biopsy table and an add-on device are shown in Table 11.

One of the limitations of these studies (I - III) was, that the number of cores obtained was not standardised. If the number had been standardised and the samples collected in separate containers each as in previously published studies performed using a prone device, it would have been possible to determine the minimum number of cores needed to attain a reliable histologic diagnosis. The decision to place the cores in three different containers instead of for example five containers was partly based on economical considerations.

The other important limitation of these studies was that the pathologists who interpreted the samples were not blinded to the study design, nor were they required to analyze the samples in alphabetical order (that is, to record an interpretation for sample A before looking at specimen B, and then to record an interpretation for sample B before looking at sample C). This kind of study design creates the opportunity for observer bias from the pathologist.
<table>
<thead>
<tr>
<th>Study</th>
<th>No. of lesions</th>
<th>Needle gauge</th>
<th>Biopsy position</th>
<th>No. of cores</th>
<th>Strict sensitivity %</th>
<th>Histologic agreement %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parker 1990 (1)</td>
<td>103</td>
<td>14+16+18</td>
<td>sitting (71 %), prone (29 %)</td>
<td>3-4</td>
<td>87</td>
<td>ND</td>
</tr>
<tr>
<td>Elvecrog 1993 [8]</td>
<td>100</td>
<td>14</td>
<td>prone</td>
<td>≥ 5</td>
<td>94</td>
<td>96</td>
</tr>
<tr>
<td>Liberman 1994 [158]</td>
<td>145</td>
<td>14</td>
<td>prone</td>
<td>1</td>
<td>ND</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>ND</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>ND</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>ND</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>ND</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>ND</td>
<td>97</td>
</tr>
<tr>
<td>Brenner 1996 [18]</td>
<td>230</td>
<td>14</td>
<td>prone</td>
<td>1</td>
<td>87</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>94</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>96</td>
<td>ND</td>
</tr>
<tr>
<td>Rich 1999 [185]</td>
<td>500</td>
<td>14</td>
<td>prone</td>
<td>2</td>
<td>80</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>81</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>83</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>84</td>
<td>ND</td>
</tr>
<tr>
<td>Wunderbaldinger 2002 [22]</td>
<td>200</td>
<td>14</td>
<td>sitting (50 %), prone(50 %)</td>
<td>≥ 6</td>
<td>96</td>
<td>ND</td>
</tr>
</tbody>
</table>

Note: - ND= no data
7.3.4. Technically unsuccessful biopsies

The majority of the technically unsuccessful biopsies (13/21) in Study I occurred with microcalcifications. For lesions evident as microcalcifications, the calcifications need to be identified on specimen radiographs; if they are not evident, then rebiopsy is clearly warranted [179, 186]. One limitation of this study was that specimen radiographs were available only in 44% of the core biopsies (Study II). The presence of microcalcification was mainly confirmed from the histopathology of the cores. Dahlstrom et al [187] noted that calcifications of < 100 µm assessed histologically were not visible on specimen radiography and may thus not represent the calcifications seen mammographically. Stomper et al [188] reviewed mammographic and histopathologic features of 27 breast cancers that presented mammographically as noncalcified masses. The histopathology revealed that 41% of these lesions exhibited microscopic calcifications in the tumours or in adjacent tissue. Microcalcifications 50-100 µm are seen histopathologically and microcalcifications > 150 µm can be seen on mammography. The histopathologic size of the calcium particles was not measured in the present study.

Thus, if biopsies of microcalcifications would have been continued until positive specimen radiography in every case the number of unsuccessful biopsies would undoubtedly have been smaller and the diagnostic accuracy higher. With an add-on device, it is impossible to verify that the core sample contains microcalcifications during the biopsy procedure without a specific specimen radiography cabine. The patient should be released from the biopsy device which also should be taken off from the mammography machine to be able to use the equipment for imaging the core samples. The continuation of the procedure again, if the samples did not contain microcalcifications, takes time and may be impossible because the possible
hematoma of the biopsy site may make microcalcifications invisible, and also because of the timetables of the hospital. With a dedicated prone device these problems may be avoidable, because the separate mammography machine can be used for specimen radiography during the biopsy procedure. Nevertheless, specimen radiography is considered as a necessity and this study also emphasizes the advantages of specimen radiography for all lesions with microcalcifications. In this study, failure to retrieve microcalcifications occurred in 11% (13/114) of lesions, a result similar to that of Jackman et al who used a prone device [179].

Visualization of microcalcifications on specimen radiography does not necessarily guarantee adequate sampling and correct diagnosis. In study I, in four of the 17 technical failures of microcalcification cases, there were microcalcifications detected on specimen radiography or in histopathology of the cores, but the histopathologic diagnosis was false negative. On the other hand, in 13 cases no microcalcifications were detected in the samples, but in three of these lesions, the histologic finding of core samples was DCIS or invasive ductal carcinoma, which was confirmed at surgery.

The comparison of technically unsuccessful biopsies in different BI-RADS categories revealed that the biopsy of BI-RADS 4 microcalcifications was the most demanding, a result in agreement with recent report using a prone device [179]. In this study, these lesions were smaller than BI-RADS 5 microcalcifications. Also appearance of BI-RADS 4 microcalcifications is often amorfic with round or ‘flake’ shaped calcifications that are small or hazy in appearance [60] whereas BI-RADS 5 calcifications have more conspicuous linear, branching of pleomofic appearance [122]. Liberman et al noted that the failure to retrieve calcifications was significantly more likely in small (5 mm or smaller) lesions and in calcifications with amorphous
Special attention should be paid during the biopsy of BI-RADS 4 microcalcifications that there is truly representative sampling (Table 7).

Mass lesions were a minority among unsuccessful lesions (n=4). Normal breast tissue was the core biopsy diagnosis in three of these cases and fatty tissue in one case. One of these lesions was removed surgically (fibrosis) and one was re-biopsied (fibrocystic disease). One lesion disappeared by the time of 6-month follow-up. The remaining mass lesion was unchanged at the 28-month follow-up mammography.

Verkooijen et al [137] in their multicenter study of 984 stereotactic core biopsies performed for mammographically suspicious non-palpable lesions found that in 5 of 30 (17%) core biopsy results of normal breast tissue, malignancy was detected at subsequent surgical excision. They calculated that the predictive value of normal breast tissue is 83% (95% CI 65-94%) and they thus recommended repeated biopsy or surgical excision if core biopsy diagnosis is benign tissue.

7.3.5. Operator experience (Study I)

There are many studies analysing learning process of different medical procedures, which have shown improvement in outcome as the physicians gain experience [189, 190].

The existence of a learning curve for stereotactic breast biopsy has been suggested previously [18, 180, 191]. All these studies have been performed using dedicated equipment with the patient in the prone position. The biopsy procedure may be more challenging for the performing radiologist if an add-on stereotactic device is in use with the patient in a sitting position and thus vulnerable to vasovagal reactions and movement.
In their multi-institutional study of stereotactic 14-G core biopsies, Brenner et al. [18] found a higher frequency of failing to diagnose the lesion when the procedure was still novel (defined as the first 20 cases per institution) than during later experience. Liberman et al. [191] detected a significantly lower technical success rate for the first 5 cases of each radiologist than for subsequent cases (83.3%, versus 95.3%, p<0.02) as well as for the first 20 cases compared to subsequent cases (90%, versus 95.9%, p<0.05) in their extensive retrospective analysis of 923 stereotactic core biopsies. The false negative rate was higher for the first 15 cases than for subsequent cases (p<0.06). For 11-gauge vacuum-assisted biopsy, a significantly lower technical success rate was also seen for the first 5 cases than for subsequent cases (85% versus 96.3%, p<0.05) and for the first 15 cases compared to subsequent cases (90.0% versus 96.5%, p=0.03). In the study entitled “Cancers not diagnosed at stereotactic 14-gauge core needle biopsy”, 4/5 (80%) of the cases in which inaccurate needle placement led to failure to diagnose cancer occurred during the first 9 months after the procedure had been introduced [180]. In a study of stereotactic guided 14-gauge vacuum-assisted biopsy, a higher frequency of failure to retrieve calcifications during the first 4 months of experience compared to the second 4 months was noted [192]. The frequency of imaging-histologic discordance was significantly higher in the first 2 years than in later years (5.0% versus 2.7%, p<0.04) [175]. Liberman et al [144] found in their study of radiography of microcalcifications that experience is a strong contributing factor in calcification retrieval, which is supported by the results of this study. Technically unsuccessful biopsies occurred significantly more often for microcalcifications than for mass lesions (p=0.004) and there was a tendency towards a lower rate of successful biopsies for the first 5 biopsies compared to for subsequent cases, but the difference
was not statistically significant (75% versus 88% p=0.335). For mass lesions, there was no learning curve effect detected. In the present study the performing radiologist decided the needle placement for clusters of microcalcifications after individual consideration. The intent was to target the most suspicious area for needle biopsy.

Stereotactic images of unsuccessful biopsies were retrospectively analysed. The targeting of the lesions seemed to be correct. The difficulty of sampling microcalcifications reflects the geometry and histologic heterogeneity of these lesions [193], which makes the biopsy challenging. It seems that the targeting and retrieval of calcifications is more successful after gaining experience of estimating these lesions. These findings also emphasize the importance of supervision, especially for the biopsy of microcalcifications. To improve the calcification retrieval rate, experience in performing biopsies of microcalcifications should be gained under the guidance of a qualified mentor, also with respect to vacuum assisted biopsies [191]. Three guided biopsies are too few.

One limitation of Study I was, that two of the radiologists performed rather few biopsies, which prevented a more detailed analysis of steepness of the learning curve.

7.3.6. Follow-up

Only recently has long-term follow-up information about benign lesions diagnosed by stereotactic core needle biopsy been published. Lee detected mammographic change at 6-55 months (mean, 20 months) in 21 of 298 cases (7%), in which two (0.7%) malignancies were noted [194]. Jackman et al undertook an extensive follow-up study in which 307 (99%) of 310 lesions with benign diagnosis at 14-gauge stereotactic core biopsy were followed-up mammographically and reported
26 (9%) cases with progression noted in the follow-up mammography at 6-85 months (mean, 55 months), two (0.7%) of which were malignancies [160]. In the large multi-institutional study of core needle biopsy with clinical and imaging follow-up in 2456 patients, Parker and colleagues noted a change in 27 lesions, 5 (2%) of which were malignant [20]. In a follow-up study of 752 lesions biopsied by 11-gauge stereotactic vacuum device, 3 lesions exhibited progression and 3 additional lesions were surgically removed but with only confirmation of benign diagnoses; no carcinomas were detected during 6-67 months (mean, 24 months) follow-up [139]. In this study, 3 out of 122 patients have displayed progression in the follow up mammography (mean, 24 months), but no malignancies have been detected. The follow-up time may not yet be sufficient, since the optimal length of the follow-up remains controversial [194, 195].

7.4. Accuracy of ER, PR and HER-2 assessments (Study III)

Detailed preoperative histopathologic information is useful for patients who would benefit from neoadjuvant chemotherapy and also for prognostic purposes. With concordance of 83% for ER, 88% for PR and for HER-2 (IHC) and 93% after adding CISH, the results of this study showed good correlation with assessments from core biopsies of those from surgical specimens. There are only a few published studies which have compared the assessment of prognostic factors of core samples to surgical specimens (Table 12). These publications are in line with the results of the present study. However, the heterogeneous expression of prognostic factors in tumour tissue has been suspected to be a confounding factor in the estimation of cores [157, 196] and therefore the surgical sample has remained the gold standard in any assessment of ER, PR and HER-2.
Table 12. Agreement and disagreement for ER, PR and HER-2 between core biopsies and surgical specimens. Review of the literature (Study III)

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<tbody>
<tr>
<td>Number of patients</td>
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<td>64</td>
<td>44</td>
<td>103</td>
<td>100</td>
<td>100</td>
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<td>Needle gauge</td>
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<td>11-14</td>
<td>14-18</td>
<td>14</td>
<td>14</td>
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<tr>
<td>Mean tumour size</td>
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<td>-</td>
<td>1.7 cm</td>
<td>palpable</td>
<td>1.4 cm</td>
<td>2.7 cm</td>
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<td>ER</td>
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<tr>
<td>Agreement %</td>
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<td>-</td>
<td>98</td>
<td>90</td>
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<tr>
<td>Disagreement %</td>
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<td>8</td>
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<td>PR</td>
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<td>Agreement %</td>
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<td>82</td>
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<td>Disagreement %</td>
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<td>19</td>
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<tr>
<td>HER-2</td>
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<td>5</td>
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Note. - ER = estrogen receptors, PR = progesterone receptors, HER-2 = HER-2 over-expression, NA = not available
7.4.1. ER

A study of 51 core samples and subsequent surgical specimens reported that in the surgical specimen, the ER staining was less profound in the centre than on the edges of the tumour. The same tendency was not noted on the microscopy of cores. It was argued that this might be due to either homogenous fixation of the cores or there could be a higher chance of sampling the peripheral part of a tumour using core biopsy [201]. In this study, the first needle pass that was always targeted to the centre of the tumour did not differ from the more peripheral needle passes. This may be due to the small tumour size (12 mm, range 5-27 mm) which promotes rather homogenous fixation also for the surgical samples. In addition, immunoreactivity for both ER and PR was significantly higher in core biopsies than surgical samples, which may reflect the better preservation of the antigens with rapid fixation achievable with the cores. In the comparison of proportions of positive ER staining, some heterogeneity was noted between individual cores (Fig 6a). When comparing values dichotomized as positive or negative, the three different containers were concordant in all cases for ER.

7.4.2. PR

In the comparison of proportions of positive staining for PR, heterogeneity between different cores was even less obvious than that seen with ER. However, for PR, there were 6 discordant cases between containers, the majority (4) of which were near to the chosen cut-off value of ≥ 10% (Fig. 6b). The commonly used cut-off points of 10% to 20% for positive and negative ER and PR assessments are somewhat arbitrarily selected. Harvey et al [202] reported that including patients with
as few as 1-10% weakly ER-positive cancer cells led to significantly improved response to endocrine therapy.

7.4.3. HER-2

Tumours with HER-2 overexpression or amplification have been noted to respond to trastuzumab therapy, which lately has been increasingly used as an adjuvant treatment for early breast cancer [88-90]. Reliable HER-2 assessment is crucial in order to select the true HER-2 positive patients to receive this possibly life-saving therapy and not to unnecessarily expose non-responders (HER-2 negative patients) to the potentially serious adverse effects [89, 90].

Because of the tendency for false positive results [94], ICH 2+ and 3+ results have been recommended to have further confirmation by FISH or CISH for detection of gene amplification [96, 203]. HER-2 overexpression or amplification is detected in 9 to 30 percent of breast cancers [80]. According to results of this study, HER-2 immunopositivity (3+) was suspected in 24% of the core biopsies, but after CISH-confirmation, HER-2 gene amplification proved to be present in 15% of the cases, a result similar to other reports.

7.4.4. Number of cores for reliable assessment of ER, PR and HER-2

With three cores (containers A and B) sensitivity of 100% was reached for HER-2 after adding CISH and with more than three cores, sensitivities of 100% for PR and 95% for ER were achieved. There were two cases where ER-assessment was positive in surgical specimens but negative in core samples. The probable explanation for the discordance in these two cases was that handling of the cores had not been optimal and may have lead to receptor destruction. Thus, in ER-
negative cases, the receptor status should be confirmed from the surgical sample. As far as I know there are no published studies in which hormone receptor and HER-2 assessments have been performed from systematically obtained, separate core samples to be able to determine the number of cores needed for reliable assessments of these factors.

7.4.5. False negative rates for ER, PR and HER-2 assessment

In this study, core samples seem to be at least as sensitive as surgical specimens in the assessment of ER, PR and HER-2. A similar trend has been detected by some other authors [156, 157, 197-199, 204] (Table 12). In the comparison of core samples and surgical specimens, false negative rates for surgical specimens were 14% in the assessment of ER, 15% in the assessment of PR and 50% in the assessment of HER-2 by CISH. Gene amplification was detected by CISH in 6 core samples, whereas only 3 surgical samples indicated gene amplification. Thus 50% of the HER-2 positive cases would have remained undetected if HER-2 assessment by CISH had been restricted to surgical samples as is the routine in most centres. With respect to the core biopsies, false negative rates were lower (6%, 0% and 0%, respectively). Interestingly, Wood et al [200] in a recent study achieved contradictory results for PR and HER-2 immunohistochemistry (Table 12). However, in their study HER-2 results were not confirmed by FISH or CISH. It is well known that a large range of factors affect IHC staining results including factors related to specimen handling and fixation, techniques used in antigen retrieval and staining and interpretations of stainings. In this context, it is important for each laboratory to know its own IHC staining results for both core biopsies and surgical specimens to be able to use the most sensitive method.
The main limitation of this study is the small number of invasive cancers decreasing the statistical power. Because the intention was to investigate tumour heterogeneity and to define the number of cores needed for reliable ER, PR and HER-assessments, only cases with invasive cancer both in at least two containers and in surgical specimen were included. In any case, with respect to diverse results on the field, larger, standardized studies are needed.

7.5. Galactography aided guide-wire or coil localization (Study IV)

Unilateral spontaneous bloody or clear nipple discharge from a single duct may be the first sign of breast carcinoma [23-25]. Therefore it has been considered an indication for surgical resection of the pathological duct area. Galactography is a method of choice for detecting intraductal pathology. In the absence of abnormalities in physical examination, mammography or ultrasonography, the conventional preoperative localising methods (palpation, ultrasonography or stereotactic guided wire localization) cannot normally be used.

Galactography alone may not provide adequate guidance for surgery. Baker et al. [163] reported that in 6 of 30 cases the abnormalities identified with galactography could not be confirmed in the surgical pathology. This is particularly the case for deep lesions, where the position of the galactographic lesion imaged in the compressed breast may be different in the non-compressed supine breast on the operating table.

Traditionally, surgical resection has been performed with perioperative methylene dye marking of the secreting duct. The duct may also be visualized galactographically immediately preoperatively by using a combination of contrast media and methylene dye [28]. Retromamillary tumours can usually be easily found with these techniques, but more proximal lesions may be difficult to detect in the
operating theatre, especially in large breasts. Methylene dye quickly diffuses from the ductal system into breast tissue [28], and this may potentially lead to over-excessive excisions. In cases of small breasts or benign findings, overly large excisions may not be acceptable because of the possible deformity of the nipple area complex. With mammographic wire localization immediately after galactography, the filling defect can be marked for the surgeon to avoid incomplete or excessive resection. In addition, the presence of a wire in the surgical sample may help the pathologist in locating the lesion.

Lesions causing intermittent discharge are often difficult to localize. If there is no discharge during the operation, the pathologic duct may remain undetected. In case of known intermittent discharge, stereotactic guided localization of the filling defect with a coil immediately after a successful galactography may be beneficial despite of more time-consuming procedure and the use of ionizing radiation. The coil is readily marked with a wire for further surgery, when needed. In the present study a single coil localization of a lesion causing intermittent discharge was performed one week prior to surgery. On the operation day, the coil was successfully localized with a wire under ultrasonography guidance; the localization would have also been possible under stereotactic guidance. More cases are needed to validate this method.

Rissanen et al. [205] reported a series of 52 patients with abnormal nipple discharge. An echogenic intraductal tumour was ultrasonographically visualized in 36 (69%) of the cases: eighty percent of papillomatous lesions, 58% of other benign lesions and 20% of malignant lesions were identified. If pathology is detected in the ultrasonographic assessment, then the primary localization of the lesion can also be performed by ultrasonography to avoid unnecessary ionising radiation. In the present study, ultrasonography was performed for 5 patients, all with normal findings.
7.6. Vacuum assisted biopsies; Future aspects

This study was performed before the vacuum-assisted biopsy device was introduced in clinical practice in Kuopio University Hospital. In recent years, stereotactic biopsy has been increasingly performed with the assistance of novel devices, such as vacuum-assisted 11-gauge needles, that acquire a larger volume of tissue. Compared with 14-gauge core-needle biopsy, 11-gauge vacuum-assisted device has been noted to be advantageous in calcification retrieval [178, 179, 186], to have a lower frequency of histological underestimation [206, 207] and has been shown to lead to a lower rebiopsy rate [177]. In a retrospective analysis of 1701 consecutive nonpalpable microcalcification lesions, Jackman et al [179] noted that failure to retrieve microcalcifications occurred in 1% (19/1423) of lesions with 11-gauge vacuum assisted biopsy, in 4% (4/96) of lesions with 14 -gauge vacuum assisted biopsy and in 16% (30/182) of lesions with 14-gauge core needle biopsy. Nowadays vacuum assisted 11- gauge biopsy is considered as state-of-the-art in the biopsy of microcalcifications. With twelve specimens maximum diagnostic yield is achieved [208].

For mass lesions, the diagnostic accuracy of 14-g core biopsies is very high for both prone and add-on devices [8, 20, 22, 158], as verified also in the present study. Therefore, core biopsies are likely to remain the primary biopsy method for mass lesions.

Expensiveness is a disadvantage of the vacuum system: the disposable material costs 10 – 20 times more than the corresponding material of the 14-g stereotactic device.

In addition to its validation with an add-on device, it would be interesting to know if results equal to or even better than those obtained using 14-gauge core biopsies
for ER, PR and HER-2 assessment can be achieved by means of vacuum assisted biopsies with their larger sample size. However, value of vacuum biopsy in prognostic factor assessment may remain reduced, because it is most often performed for microcalcifications with malignancy usually detected at preinvasive stage [209]. At present, hormone reseptor and HER-2 assessments have influence in treatment decisions for patients with invasive carcinoma only.

Recently promising results have been reported with the use of vacuum assisted biopsy both to diagnose and treat intraductal lesions causing nipple discharge. Dennis et al. [210] described a technique in which an ultrasonography-guided mammotome biopsy was performed immediately after galactography. The discharge resolved in the majority (97.2%) of the 38 patients after the biopsy. Guenin [211] performed stereotactic vacuum-assisted biopsy immediately after galactography in 5 patients; in all of them benign papilloma was diagnosed. In four of those patients nipple discharge ceased after the biopsy, one patient underwent surgical excision for atypia detected within the papilloma. These new techniques are promising in the diagnosis and treatment of solitary lesions. Multiple lesions, if they are located in distant spots or in many duct branches, may require surgery for complete removal.
8. SUMMARY AND CONCLUSIONS

The main purpose of this study was to evaluate an add-on stereotactic guidance method for the performance of 14-gauge core needle breast biopsies of non-palpable breast lesions by means of investigating the learning curve and number of cores needed to achieve a reliable diagnosis. In a study of invasive cancers, different numbers of core samples were compared to surgical specimens in the assessment of ER, PR and HER-2. A total of 221 patients with 231 breast lesions were included in these studies. As a whole, the add-on stereotactic device proved to be well comparable with the published results of dedicated prone device in terms of diagnostic accuracy, false negative cases and underestimations of histologic diagnosis (DCIS and ADH). In a subgroup of 41 invasive cancers, core samples seemed to be at least as sensitive as surgical specimens in the assessments of ER, PR and HER-2.

This study also describes galactography-aided stereotactic wire localization preoperatively in 9 patients with spontaneous clear, serous or bloody nipple discharge.

On the basis of the present study, the following conclusions can be drawn:

1. The results of this study support the existence of a learning curve in the biopsy of microcalcifications, in which the majority of technically unsuccessful biopsies occurred. More than three mentor-guided biopsies are needed.
2. More than three samples are needed for a histologic diagnosis of a mass lesion. For microcalcifications, an acceptable sensitivity is reached with more
than three samples, if the tendency for the core biopsy to underestimate ADH lesions is taken into account and these, as well as mammographically and histopathologically discordant lesions, are surgically excised.

3. Stereotactic 14-gauge core biopsy seems to be at least as sensitive as surgery in assessment of ER, PR and HER-2. Three cores are needed for reliable assessment of HER-2 after adding CISH and more than 3 cores for HER-2 (IHC) and PR, possibly due to tissue heterogeneity. For ER sensitivity remained lower, 95%, even in multiple cores, therefore ER-negative cases should be further investigated from surgical specimens.

4. Galactography-aided stereotactic wire or coil localization can be successfully used to localize intraductal lesions not detected on mammography or ultrasonography.
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APPENDIX: ORIGINAL PUBLICATIONS
Kuopio University Publications D. Medical Sciences


