Minimally invasive approach to neoplastic breast disease

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Minimally Invasive Approach to Neoplastic Breast Disease
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Minimaal invasieve benadering van nieuwvormingen van de borst
(met een samenvatting in het Nederlands)

Proefschrift

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CHAPTER 01

General introduction and thesis outline

CH 01

NEOPLASTIC BREAST DISEASE

The term neoplasia is derived from ancient Greek **νεο** ("neo"; new) and **πλάσμα** ("plasma"; formation) and defines an abnormal growth of tissue. Neoplasms of the breast can be benign (e.g. fibroadenoma), potentially malignant (e.g. ductal carcinoma in situ) or malignant (e.g. invasive breast cancer).

Worldwide every year around 1,100,000 women are diagnosed with **breast cancer** and 410,000 will die of the disease[1–3]. In The Netherlands, breast cancer is the most common form of cancer in women with approximately 14,000 women being diagnosed with invasive breast cancer each year[4]. A woman's risk of having breast cancer over the course of her life is 12-13%. Surgical excision followed by breast irradiation is the first step in the treatment of early stage breast cancer. Further adjuvant treatment decisions regarding chemo- and radiation therapy are predominantly based on the pathology findings in the excision specimen.

Breast conserving surgery aims to provide adequate local control with a cosmetically acceptable result. However, due to the radical resection of the tumour with a margin in combination with postoperative radiotherapy, the cosmetic outcome of breast conserving therapy is often disappointing. The amount of resected breast tissue is an important factor influencing cosmetic outcome. Besides short-term morbidity of surgery due to bleeding and infections[5], cosmetic outcome and long-term morbidity due to (neuropathic) pain[6] and oedema[7] account for impaired quality of life[8].

For optimal management of breast cancer the work of the pathologist is of great importance. Besides the patient's age and general health, the choice of adjuvant systemic treatment mainly depends on tumour characteristics such as tumour diameter, tumour grade, oestrogen receptor status and lymph node status. The vast majority of breast cancers are diagnosed by means of core needle biopsy (CNB). Previous studies have shown that grading of breast cancer varies substantially between CNB and surgical resection specimens[18], probably due to sampling problems with CNB. For this reason grading is performed routinely on resection specimens and not on CNB samples.

Pathologic nipple discharge (PND) is defined as persistent serous or hemorrhagic, spontaneous discharge from a single duct. PND accounts for approximately 5% of surgical

referrals to the breast clinic and is frequently a source of concern for patients[9]. Malignancy rates in patients with PND have been reported up to 23.9%[10]. Patients with PND with negative conventional imaging by mammography and ultrasound have a considerably lower risk. Malignancy rates of approximately 7% have been reported[11,12], with intraductal papillomas accounting for around 45%[13].

There is no consensus about the diagnostic approach to PND. Conventional diagnostic techniques, such as cytology of nipple aspiration fluid or galactography, are not sensitive for diagnosing underlying malignancies. Current diagnostics therefore often result in a series of negative results, leaving the physician with a diagnostic dilemma.

To distinguish between benign and malignant causes of PND, surgery by either microdochectomy or major duct excision, is considered the gold standard. However, since only a minority of patients with non-suspect conventional imaging has cancer, most women will undergo invasive surgery with concomitant risk of complications and effects on cosmesis, breastfeeding and sensitivity of the nipple for a benign cause.

NOVEL TECHNOLOGIES FOR DIAGNOSIS AND TREATMENT OF NEOPLASTIC BREAST DISEASE

The trend towards less radical treatments of breast cancer has led to the exploration of potentially less deforming approaches aiming to preserve the greatest amount of normal breast tissue.

A minimally invasive approach to local treatment is **radiofrequency ablation (RFA)**. This ablative technique was first introduced in breast cancer treatment in 1999 by Jeffrey et al.[14]. RFA uses electrodes to deliver an alternating current that generates ionic agitation, localized frictional tissue heating and cell death[15]. Several studies have indicated the potential of RFA to replace surgical resection as local treatment for small breast cancer[16]. These studies used monopolar electrodes, requiring a cutaneous neutral electrode and a grounding pad to create a closed electrical circuit. This results in a current through the tissue of the patient to the neutral electrode. This method not only requires a large amount of admitted power, it also carries the risk of uncontrolled electrical current paths through the whole body and concordant undesired thermal tissue damage along the path. Consequently, skin

and muscle burns are seen in up to 30% of patients[17]. Bipolar RFA is a technique in which both electrodes are located on one applicator. Consequently, the radiofrequency energy is exclusively applied to the target tissue. This novel bipolar system has not yet been used in mammary tissue.

Adjuvant systemic treatment decision-making for breast cancer is based on the histology of resection specimens. With novel minimally invasive tumour ablation techniques, such as RFA, the preoperative **core needle biopsy (CNB)** specimen may be the only tissue available for assessment of tumour characteristics. The tendency to underscore grading on CNB samples might result in difficulties in decision-making for adjuvant treatment in patients treated by novel ablative treatment methods, but this impact is unknown.

Ductoscopy is a minimally invasive procedure that visualizes the ductal epithelium of the breast via the nipple[19,20]. It can be performed under local anaesthesia in the outpatient clinic. Although it is already used for evaluation of PND, the accuracy of ductoscopy for diagnosing malignancy remains a matter of debate. With addition of a third working channel next to channels for optics and irrigation, interventional ductoscopy was developed. Intraductal biopsy tools[21] and a 'basket'-intervention device can be introduced to acquire tissue and remove the lesion under direct vision[22]. This may not only improve diagnostic accuracy, but can also result in a therapeutic effect. Studies regarding the overall and long-term **therapeutic efficacy of ductoscopy** for patients with PND are however lacking.

Although ductoscopy allows non-invasive removal of non-sessile intraductal lesions like papillomas, current endoscopic interventional methods are suboptimal. Removal is often incomplete, and lesions with non-polypoid morphology cannot be targeted. **Thulium laser** is transmitted through fibers in various diameters, making it suitable for endoscopic procedures such as transluminal treatment of benign prostate hyperplasia[23]. Endoscopic treatment of intraductal neoplasia by transductal laser ablation has not yet been described.

Most breast cancers are thought to arise from ductal epithelium, initiated by an accumulation of multiple molecular alterations that precede phenotypic and architectural changes[24,25]. Evaluating these changes could potentially allow identification of early markers of malignant development, with the intraductal approach by ductoscopy being

an attractive means of accessing ductal epithelial cells. Although ductoscopy has shown to be able to detect malignant lesions that were not visible on conventional imaging[13,26], the role of ductoscopy in breast cancer screening has yet to be fully defined[27]. As is known from the airways, larynx and colon, (pre)malignant epithelial lesions show an aberrant pattern under fluorescent light by which they become detectable[28–30]. The first studies using **autofluorescence** in ductoscopy indicated the possibility to detect malignant lesions *ex vivo,* and its *in vivo* feasibility in patients with PND[31,32]. The breast ductal system has not been evaluated by autofluorescence to detect pathologically confirmed breast cancer precursor lesions.

OUTLINE OF THIS THESIS

In this thesis, novel minimally invasive technologies for diagnosis and treatment of neoplastic disease of the breast are described. It is divided into two parts in which different aspects are addressed.

Part I | Minimally invasive treatment of breast cancer

The first part of the thesis focuses on minimally invasive treatment of breast cancer.

Chapter 2 evaluates the preoperative evaluation of grading on CNB and the impact on clinical risk assessment and patient selection for adjuvant systemic treatment. In **Chapter 3** the optimal power settings to acquire an adequate lesion diameter with bipolar RFA is evaluated, followed by an evaluation of the safety and efficacy of this bipolar RFA technique for the local treatment of small breast carcinomas in women.

Part II | Breast duct endoscopy

The second part of the thesis focuses on breast duct endoscopy (ductoscopy).

Chapter 4 gives a description of the technique and its introduction in The Netherlands. **Chapter 5** systematically reviews the diagnostic accuracy of ductoscopy in patients with pathologic nipple discharge. **In Chapter 6** the therapeutic efficacy of interventional ductoscopy in patients with PND is evaluated. **Chapter 7** describes a novel endoscopic interventional modality. The feasibility and efficacy of endoscopic laser ablation for intraductal breast papillomas is assessed. **Chapter 8** evaluates the feasibility of detection of breast cancer precursor lesions by autofluorescence ductoscopy.

In **Chapter 9** the evidence presented in this thesis is summarized and discussed in the context of recent literature, and future perspectives are given.

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PART I

Minimally invasive treatment of breast cancer

CHAPTER 02

Impact of preoperative evaluation of tumour grade on core needle biopsy on clinical risk assessment and patient selection for adjuvant systemic treatment in breast cancer

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ABSTRACT

Background | Histological characteristics are important when making a decision on adjuvant systemic treatment in breast cancer. Preoperative assessments of core needle biopsy (CNB) specimens are becoming increasingly relevant as novel minimally invasive ablative techniques are introduced, because a surgical specimen is no longer obtained with these methods. The clinical impact of potential underestimation of tumour grade on preoperative CNB on clinical decision-making was evaluated.

Methods | Histological tumour grade was reassessed on CNB and resection specimens from consecutive invasive ductal carcinomas diagnosed between 2010 and 2013. For each patient, the indication for systemic therapy was assessed, based on either CNB or surgical excision, in combination with clinical characteristics and imaging findings. The clinical impact of discordance between tumour grade on CNB versus the resection specimen was assessed.

Results | The analysis included 213 invasive ductal carcinomas in 199 patients. Discordance in tumour grade between CNB and the resection specimen was observed in 64 (30.0 per cent) of 213 tumours (κ = 0.53, 95 per cent c.i. 0.43 to 0.63). A decision on adjuvant treatment based on CNB would have resulted in overtreatment in seven (3.5 per cent) and undertreatment in three (1.5 per cent) of 199 patients. In the undertreated patients, incorrect omission of adjuvant systemic treatment would have increased the predicted 10-year mortality rate by 2.6–5.2 per cent and 10-year recurrence rate by 8.2–15.3 per cent based on the online risk assessment tool Adjuvant!

Conclusion | The substantial discordance in tumour grading between CNB and resection specimens from breast cancer affects the indication for adjuvant therapy in only a small minority of patients with invasive ductal carcinoma. Assessment of tumour grade on CNB is feasible and accurate for the planning of postoperative treatment.

INTRODUCTION

Adjuvant systemic treatment decision-making for breast cancer is based on the histology of resection specimens. Besides the patient's age and general health, the choice of adjuvant systemic treatment mainly depends on tumour characteristics such as tumour diameter, tumour grade, oestrogen receptor (ER) status and lymph node (LN) status.

The Nottingham Grading System (NGS) is derived by scoring tubule formation, nuclear pleomorphism and mitotic frequency1. This grade has been shown to be an independent factor in predicting prognosis and correlates well with 10-year survival[1]. The NGS has been combined with LN status and tumour size to form the Nottingham Prognostic Index[2], which includes NGS and LN status with equal weighting. It has also been incorporated into algorithms and guidelines (such as Adjuvant![3] and the St Gallen consensus recommendations[4]) to determine the indication for adjuvant chemotherapy[5].

Previous studies have shown that grading of breast cancer varies substantially between core needle biopsy (CNB) and surgical resection specimens, with 19–38 per cent discordance in grading. This most commonly leads to an underestimation of grade on CNB, predominantly owing to underscoring of the mitotic count[6–14], as a result of the lower volume of tissue sampled in CNB. The tissue obtained may not include the tumour's growing edge, there may be insufficient tumour present to complete a formal count, and breast carcinomas are known for their heterogeneity in relation to the mitotic index[15,16]. This is why grading is performed routinely on resection specimens and not on CNB samples.

Novel minimally invasive tumour ablation techniques, such as radiofrequency ablation[17] and high-intensity focused ultrasound imaging[18], are being developed as alternatives to surgery. With these techniques, the CNB specimen may be the only tissue available for assessment of tumour characteristics. The tendency to underscore grade on core biopsy samples might result in difficulties in decision-making for adjuvant treatment. The aim of the present study was to estimate the discordance between grading of CNB versus breast resection specimens and its impact on the selection of adjuvant systemic treatment.

METHODS

All consecutive patients diagnosed with invasive ductal cancer on CNB and who subsequently underwent surgical excision of the tumour at the University Medical Centre Utrecht, The Netherlands, between 2010 and 2013 were identified. Patients who had received neoadjuvant therapy were excluded. In the event of multiple tumours in the same patient, and when both a CNB and resection specimen were available from each lesion, each tumour was included separately in the analysis. The need for informed consent was waived by the institutional review board.

Histology

For all tumours, the histological slides from the CNB and surgical specimen were retrieved from the pathology archives. All specimens were formalin-fixed and paraffin-embedded, and tissue sections were stained with haematoxylin and eosin. Reassessment of tumour grade on CNB and surgical specimens was performed according to the NGS by one dedicated breast pathologist, who was blinded to the initial assessment of CNB/surgical specimen grade and biomarker results[1]. A minimum of 10 high-power fields (HPFs) was counted for assessment of mitotic count; if limited tissue was available, extrapolation to 10 HPFs was performed. ER, progesterone receptor (PR) and human epidermal growth factor receptor (HER) 2 status had been assessed routinely by immunohistochemistry according to manufacturer's protocols. According to Dutch guidelines, ER and PR were considered positive if at least 10 per cent of tumour cells stained positive[5]. HER2 protein expression status was scored as described previously, and HER2 amplification status was also assessed[19]. The sentinel nodes had been evaluated with multilevel haematoxylin and eosin staining, and cytokeratin immunohistochemistry. If metastases were present, they were classified as macrometastases (over 2 mm), micrometastases (0.2–2 mm) or isolated tumour cells (less than 0.2 mm in size or fewer than 200 tumour cells)[20]. Macrometastases and micrometastases were considered as pN+ disease, and isolated tumour cells as pN0.

Adjuvant treatment decision

Clinical data were collected retrospectively from the patient reports. The impact of discordance in grading between the CNB and excision specimen on the subsequent indication for systemic treatment was assessed by applying the most recent (2012) national Dutch Institute for Healthcare Improvement (CBO) guidelines[5], which are based on the

St Gallen consensus recommendation[4], and the international online adjuvant systemic treatment decision-making tool Adjuvant![3].

Using the Dutch CBO guidelines, clinical risk assessment (high versus low) is based on patient age, lymph node status, tumour size (pT) and histological grade (Table 1)[5]. Adjuvant systemic treatment is advised in the vast majority of patients with LN (micro/macro) metastases (N+) or histological tumour diameter above 20 mm as these are important indicators of adverse prognosis. In node-negative patients with tumours of 20 mm or smaller further stratification is necessary, often by tumour grade, in order to decide on adjuvant systemic treatment, Adjuvant systemic treatment can be omitted for node-negative disease with tumour size less than 10 mm, except in women aged below 35 years with a tumour grade exceeding 1.

Table 1 | Indication for adjuvant systemic treatment according to Dutch Institute for Healthcare Improvement guidelines[5]

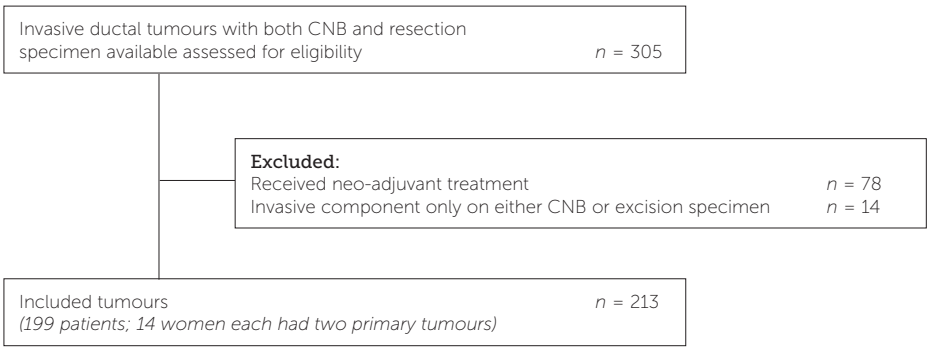
Node status	Indication for systemic treatment	Notes
N+	All patients, unless > 70 years with hormone receptor-negative tumour	Chemotherapy may be considered for fit patients > 70 years with a hormone-negative tumour
Unfavourable N0	Age < 35 years, unless grade I tumour ≤ 1 cm Age ≥ 35 years with tumour 1.1–2 cm, grade ≥ II	If the tumour is 1.1–2 cm in size, grade II, HER-2-negative, but ER and PR > 50 per cent, hormone therapy without chemotherapy may be considered for postmenopausal women
	Age ≥ 35 years with tumour > 2 cm	If HER-2-positive tumour ≥ T1b (0.5–1 cm), treatment may be considered independently of other characteristics. Toxicity and an as-yet uncertain effectiveness should be weighed up against each other

HER, human epidermal growth factor receptor; ER, oestrogen receptor; PR, progesterone receptor.

The online Adjuvant! risk assessment tool (version 8.0)[21] was used to establish the consequence of omitting adjuvant treatment owing to underscoring of tumour grade

on CNB. This software calculates the 10-year mortality and recurrence probability based on the patient's age, tumour size, histological tumour grade, ER status and LN status. It calculates absolute probabilities of survival and disease recurrence, and estimates benefits of adjuvant systemic treatment[3,22]. This model has been validated externally[15,19].

Figure 1 | Study flow chart. CNB, core needle biopsy



Statistical analysis

Normally distributed continuous variables are presented as mean (s.d.) and non-normally distributed data as median (range). The level of agreement between grade on CNB and grade on the surgical specimen was expressed as Cohen's κ; values between 0.00 and 0.20 indicate poor agreement, 0.21 and 0.40 fair agreement, 0.41 and 0.60 moderate agreement, 0.61 and 0.80 substantial agreement, and 0.81 and 1.0 excellent agreement[23]. κ statistics and 95 per cent c.i. were calculated using SPSS® version 16.0 (IBM, Armonk, New York, USA).

Results

A total of 305 invasive ductal breast carcinomas in 291 patients, with both CNB and resection specimens available, were identified. Seventy-eight patients received neoadjuvant treatment, so a total of 78 tumours were excluded from the analysis (Figure 1). Fourteen lesions in 14 patients had only an invasive component on either the CNB or excision specimen and grade could not be compared, so they were excluded. Fourteen women had two primary tumours, which were included as separate samples in the analysis. The

final study cohort comprised a total of 213 biopsies and corresponding excision specimens, from 199 patients. Extrapolation was performed for assessment of grade in five CNB specimens with limited material. Patient and tumour characteristics are shown in Table 2.

Discordance in tumour grade between biopsy and corresponding resection specimen

The overall agreement between grade on the CNB and resection specimen was 149 of 213 (70.0 (95 per cent c.i. 63.8 to 76.1) per cent), corresponding to a κ value of 0.53 (95 per cent c.i. 0.43 to 0.63) (Table 3). Of the tumours assessed as grade 1 on CNB, 23 (35 per cent) of 66 were upgraded to grade 2 or 3 on the resection specimen, whereas 21 (14.3 per cent) of 147 tumours graded 2 or 3 on CNB were downgraded to grade 1 on the resection specimen. Looking at the three constituents of the NGS, the lowest concordance was observed for mitotic frequency, with agreement in 69.0 (62.8 to 75.2) per cent (κ = 0.49, 0.39 to 0.59) (Table 4). Tubule formation and nuclear pleomorphism, on the other hand, showed agreement in 74.2 (68.3 to 80.1) per cent (κ = 0.55, 0.46 to 0.65) and 70.9 (64.8 to 77.0) per cent (κ = 0.50, 0.39 to 0.60).

Large tumours (over 20 mm) showed lower concordance (59 (47 to 70 per cent) and reliability (κ = 0.36, 0.19 to 0.53), whereas for tumours smaller than 10 mm or measuring 11–20 mm grading was concordant in 80 (70 to 90) per cent (κ = 0.67, 0.50 to 0.83) and 73 (63 to 83) per cent (κ = 0.56, 0.39 to 0.72) respectively (Table 4).

There was no difference in agreement for pN0 and pN+ tumours: 69.8 (62.2 to 77.4) per cent (κ = 0.53, 0.41 to 0.65) and 70 (60 to 81) per cent (κ = 0.53, 0.37 to 0.69) respectively (Table 4).

Unifocal tumours showed concordance in 70.6 (63.2 to 78.1) per cent (κ = 0.55, 0.43 to 0.67), whereas multifocal lesions or those with microcalcifications showed agreement in 65 (42 to 88) per cent (κ = 0.43, 0.08 to 0.79) (Table 4).

Table 2 | Baseline, imaging and biopsy characteristics

		No. of tumours* (*n* = 213)
Age (years)†		60 (11)
Menopausal status	Premenopausal	35 (16.4)
	Postmenopausal	149 (70.0)
	Not reported/unclear	29 (13.6)
Imaging	Mammography	210 (98.6)
	Ultrasonography	182 (85.4)
	MRI	62 (29.1)
Tumour focality (imaging)	Unifocal	143 (67.1)
	Multifocal/microcalcifications	17 (8.0)
	Multicentric	51 (23.9)
	Not reported	2 (0.9)
BIRADS score	2	3 (1.4)
	3	16 (7.5)
	4	55 (25.8)
	5	137 (64.3)
	Not reported	2 (0.9)
Biopsy type	Core needle biopsy	199§ (93.4)
	Other (Intact™¶/skin)	3 (1.4)
	Not reported#	11 (5.2)
No. of biopsies†		3.4(1.5)
Biopsy guidance	Ultrasound	180 (84.5)
	Stereotactic	18 (8.5)
	MRI	3 (1.4)
	Other/not reported	12 (5.6)
Surgery	Lumpectomy	133 (62.4)
	Mastectomy	80 (37.6)
Tumour size (mm)‡	Imaging (MRI; ultrasonography if no MRI)	14 (3–60)
	Pathological (excision)	15 (2–81)
Tumour size (excision) (mm)	0–10	64 (30.0)
	11–20	78 (36.6)
	> 20	70 (32.9)
	Not reported	1 (0.5)
ER status (excision)	Positive	178 (83.6)
	Negative	26 (12.2)
	Not reported	9 (4.2)

PR status (excision)	Positive	153 (71.8)
	Negative	51 (23.9)
	Not reported	9 (4.2)
HER2 status (excision)	IHC positive (3+)	14 (6.6)
	IHC negative (0, 1+, 2+)	190 (89.2)
	Not reported	9 (4.2)
Sentinel node (excision)	Tumour-free	120 (56.3)
	Isolated tumour cells	13 (6.1)
	Micrometastases	24 (11.3)
	Macrometastases	50 (23.4)
	No lymph nodes dissected	6 (2.8)

*With percentages in parentheses unless indicated otherwise; values are †mean(s.d.) and ‡median (range). §Of which 21 vacuum-assisted. ¶Biopsy taken with IntactTM Breast Lesion Excision System (Intact Medical Corporation, Natick, Massachusetts, USA)[24]. #Biopsy type not reported; on review all were needle biopsy specimens. BIRADS, Breast Imaging-Reporting and Data System; ER, oestrogen receptor; PR, progesterone receptor; HER, human epidermal growth factor receptor; IHC, immunohistochemistry.

Impact of discordance of grading on clinical risk assessment

According to the Dutch CBO guidelines, for 105 (49.3 per cent) of the 213 tumours there was already an indication for adjuvant systemic treatment of the patient, regardless of tumour grade: 74 (34.7 per cent) were pN+ and 70 (32.9 per cent) had a diameter larger than 20 mm (39 were both pN+ and larger than 20 mm) (Table 5). Of the remaining 108 tumours, where histological grade could be the indication for systemic therapy, three were underestimated as grade 1, whereas seven tumours were overgraded as grade 2 or 3 on CNB, resulting in 9.3 per cent discordance (10 of 108) of clinical risk assessment on CNB compared with the excision specimen, with overall discordance in 4.7 per cent of tumours (10 of 213) (Table 5). This discordance resulted in overtreatment in seven (3.5 per cent) and undertreatment in three (1.5 per cent) of 199 patients.

Assessment of the impact of omitting adjuvant treatment based on CNB assessment in those who were undertreated showed an increase in the Adjuvant! predicted 10-year mortality rate of 2.6–5.2 per cent and an increase in the predicted 10-year recurrence rate of 8.2–15.3 per cent.

Table 3 | Histological grade based on core needle biopsy versus surgical excision specimen assessment

	Core needle biospy			
	Grade 1	Grade 2	Grade 3	Total
Surgical excision				
Grade 1	43 (20.1)	21 (9.9)	0 (0)	64 (30.0)
Grade 2	20 (9.4)	68 (31.9)	3 (1.4)	91 (42.7)
Grade 3	3 (1.4)	17 (8.0)	38 (17.8)	58 (27.2)
Total	66 (31.0)	106 (49.8)	41 (19.2)	213 (100)

Values in parentheses are percentages. Tumours were graded according to the Nottingham Grading System. Overall agreement in tumour grade: 149 of 213 (70.0 (95 per cent c.i. 63.8 to 76.1) per cent), κ = 0.53 (95 per cent c.i. 0.43 to 0.63).

Table 4 | Concordance between tumour grading on core needle biopsy versus surgical excision specimen by Nottingham Grading System components, tumour size, lymph node status and focality on imaging

	Agreement*	κ†	Reported values[2–6]
NGS parameter			
Tubule formation	158 of 213 (74.2; 68.3, 80.1)	0.55 (0.46, 0.65)	55.6–83.7
Nuclear pleomorphism	151 of 213 (70.9; 64.8, 77.0)	0.50 (0.39, 0.60)	57.4–79.6
Mitotic frequency	147 of 213 (69.0; 62.8, 75.2)	0.49 (0.39, 0.59)	58–61
Tumour size (mm)			
0–10	51 of 64 (80; 70, 90)	0.67 (0.50, 0.83)	
11–20	57 of 78 (73; 63, 83)	0.56 (0.39, 0.72)	
> 20	41 of 70 (59; 47, 70)	0.36 (0.19, 0.53)	
Not reported	1		
Lymph node status			
pN0	97 of 139 (69.8; 62.2, 77.4)	0.53 (0.41, 0.65)	
pN+	52 of 74 (70; 60, 81)	0.53 (0.37, 0.69)	
Focality on imaging			
Unifocal	101 of 143 (70.6; 63.2, 78.1)	0.55 (0.43, 0.67)	
Multifocal/microcalcifications	11 of 17 (65; 42, 88)	0.43 (0.08, 0.79)	
Multicentric	35 of 51 (69; 56, 81)	0.48 (0.27, 0.70)	
Not reported	2		

*Values in parentheses are percentages; 95 per cent c.i. †Values in parentheses are 95 per cent c.i. NGS, Nottingham Grading System.

Table 5 | Histological diagnosis based on the core needle biopsy and surgical excision specimen of 213 tumours according to clinically important parameters of the Nottingham Grading System

Lymph node status	Tumour size (mm)	Surgical excision	Core needle biopsy		
			Grade 1	Grade 2–3	Total
pN0	< 10	Grade 1	21 (9.9)	10 (4.7)	31 (14.6)
		Grade 2–3	2 (0.9)	24 (11.3)	26 (12.2)
	11–20	Grade 1	5 (2.3)	7 (3.3)	12 (5.6)
		Grade 2–3	3 (1.4)	36 (16.9)	39 (18.3)
	>20	Grade 1	5 (2.3)	2 (0.9)	7 (3.3)
		Grade 2–3	7 (3.3)	17 (8.0)	24 (11.3)
	All sizes	Total	43 (20.2)	96 (45.1)	139 (65.3)
pN+*	All sizes	Grade 1	12 (5.6)	2 (0.9)	14 (6.6)
		Grade 2–3	11 (5.2)	49 (23.0)	60 (28.2)
		Total	23 (10.8)	51 (23.9)	74 (34.7)
Overall	All sizes	Total	66 (31.0)	147 (69.0)	213 (100)

Values in parentheses are percentages. *Data for lymph node-positive disease are presented for all tumour sizes together because tumour size in this group has no further clinical consequences in adjuvant systemic treatment decisions.

DISCUSSION

This study showed that, despite substantial discordance between histological grading on CNB and the surgical resection specimen, the impact on clinicopathological risk assessment and subsequent advice on adjuvant systemic treatment is limited.

According to Dutch CBO guidelines, assessment of tumour grade on CNB would have led to incorrect omission of systemic adjuvant treatment in only 1.5 per cent of all patients, whereas 3.5 per cent would have received unnecessary adjuvant treatment. Ten-year mortality and recurrence rates predicted by Adjuvant! showed that such undertreatment would have led to an increase in mortality of 2.6–5.2 per cent. Although the effects of overtreatment are harder to calculate, systemic treatment, especially chemotherapy, decreases quality of life and leads to higher rates of morbidity and mortality, besides the psychological effects of potentially unnecessary treatment.

Discordance in grading between CNB and excision specimens was as much as 30.0 per cent, in line with previous findings (ranging from 19 to 38 per cent)6–14. Increased tumour size (over 20 mm) was negatively associated with concordance of tumour grade between CNB and resection specimens, which is also consistent with a previous study11 of 300 tumours, most likely owing to tumour heterogeneity[25,26]. Extrapolation for grading assessment when the amount of material was limited was used only in the case of CNB for tumours smaller than 10 mm, and did not lead to increased misclassification.

Concordance rates for the three individual constituents comprising the NGS were comparable with published data (Table 4). The overall grading agreement (70.0 per cent) was lower than the 72–88 per cent interobserver agreement in studies of grading for excised tumours[27–31], but the clinical impact of discordance on subsequent adjuvant systemic treatment advice was smaller than that from the interobserver variance on excision specimens (4.7 versus 8–15 per cent)[27,31].

Despite their prognostic importance, the concordance for ER expression and HER2 gene amplification on CNB and excision specimens was not analysed. When neoadjuvant treatment is not considered, this is carried out only on the excision specimen in order to keep costs down. Moreover, according to the guidelines, hormone receptor and HER2

status are not used for the initial indication of adjuvant systemic treatment (Table 1)[5]. A study[32] investigating the performance of CNB for determining ER and HER2 status found an overall concordance for ER receptor status of 93.7 per cent[32]. Thus, hormone receptor assays on CNB specimens might be as reliable as those on surgical specimens, and some authors[13,33–35] even recommend that immunohistochemical staining for hormone receptors should be performed routinely on CNB specimens. Overall concordance in HER2 status was found to be 97.8 per cent[32], and the assessment of HER2 status on CNB specimens in breast cancer is considered accurate and reliable[36–38].

The possible impact of molecular multigene assays, such as Oncotype DX® (Genomic Health, Redwood City, California, USA) or MammaPrint® (Agendia, Amsterdam, The Netherlands), on therapeutic decision-making[39] was not analysed here. These tests are intended to be used for women with early-stage (stage I or II), node-negative, ER-positive invasive breast cancer to provide an individualized prediction of the 10-year risk of local recurrence and thus to help guide treatment decision-making[40,41]. Here the assessment of tumour grade on CNB would have led to incorrection omission of systemic adjuvant treatment in 1.5 per cent of the patients, who would all have been candidates for molecular multigene assays that could have had an impact on decision-making[42].

The present results are of importance for emerging therapies, such as thermotherapy, cryoablation and electroporation, as a surgical excision specimen will not be available. These minimally invasive treatment strategies are currently acquiring more importance in the treatment of breast cancer[43]. These techniques are predominantly studied for the treatment of small, unifocal breast tumours[44]. The findings of higher concordance for smaller and unifocal tumours facilitate application of these novel therapies, as the clinical impact of underestimation of tumour grade on CNB will be small. Preoperative grading should be discouraged for tumours with a diameter exceeding 20 mm, taking into account that most of these tumours will be treated surgically rather than by ablation.

The most appropriate endpoint for testing the value and validity of tumour grading is patient outcome. Variation in grading between CNB and excision specimens may not change the prognosis significantly in patients with breast cancer. However, in the absence of long-term survival data, patient outcome was estimated using the online tool Adjuvant!, which has been shown to predict 10-year outcome accurately in a large-scale Dutch validation study[3].

Although the present study demonstrated discordance in tumour grading of invasive ductal carcinoma on preoperative biopsy compared with surgical excision, this had only a minor impact on the decision to administer adjuvant therapy and prognosis. This suggests that the indication for systemic treatment in patients no longer undergoing surgical excision after minimally invasive therapies can be set safely using the CNB grade.

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CHAPTER 03

Radiofrequency ablation of small breast tumours: evaluation of a novel bipolar cool-tip application

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ABSTRACT

Background | Although radiofrequency ablation (RFA) is promising for the local treatment of breast cancer, burns are a frequent complication. The safety and efficacy of a new technique with a bipolar RFA electrode was evaluated.

Methods | Dosimetry was assessed *ex vivo* in bovine mammary tissue, applying power settings of 5-15 W with 10-20 minutes exposure and 3.0-12.0 kJ to a 20-mm active length bipolar internally cooled needle-electrode. Subsequently, in 15 women with invasive breast carcinoma ≤2.0 cm diameter ultrasound-guided RFA was performed followed by immediate resection.

Results | An ablation zone of 2.5 cm was reached in the *ex vivo* experiments at 15 W at 9.0 kJ administered energy. Histopathology revealed complete cell death in 10 of 13 patients (77%); in 3 patients partial ablation was due to inaccurate probe positioning. In 1 patient a pneumothorax was caused by the probe placement, treated conservatively. No burns occurred.

Conclusions | Ultrasound-guided RFA with a bipolar needle-electrode appears to be a safe local treatment technique for invasive breast cancer up to 2 cm. Ways to improve placement of the probe and direct monitoring of the ablation-effect should be the aim of further research.

INTRODUCTION

The trend towards local breast cancer treatment with minimally invasive approaches has led to the introduction of radiofrequency ablation (RFA) as a possible substitute for surgery. RFA uses electrodes to deliver an alternating current that generates ionic agitation, localized frictional tissue heating and cell death [1]. Not the electrode, but the surrounding tissue is the source of heat. The heat transfers conductively to more distant tissue, creating an ellipsoid region of necrotic tissue aligned with the needle-electrode tip. This technique was first introduced for breast cancer treatment in 1999 by Jeffrey et al.[2].

Several feasibility studies have indicated the potential of RFA to replace surgical resection as local treatment for small breast cancer. In initial studies RFA was followed by immediate surgery and subsequent assessment of the tumour cell viability by (immuno)histological examination[3–11]. These studies commonly suggest that RFA might be useful for the local control of small, well-localized breast cancer because of its effective cell destructive ability in a fairly predictable volume of tissue, with a success rate ranging from 84-100% ablation of tumours ≤2.0 cm[3–11]. Ensuing studies with a delayed excision, at intervals ranging from 7 to 202 days, showed similar results[12–16]. All previous studies however, used monopolar electrodes, in either the form of a needle-type or a multi-array umbrella-shaped probe [17]. This method requires a cutaneous neutral electrode and a grounding pad at arm or thigh to create a closed electrical circuit. Aside from causing tissue necrosis around the active electrode, the current flows through the patient to the neutral electrode. Therewith, this method not only requires a large amount of admitted power, but also carries the risk of uncontrolled electrical current paths through the whole body and concordant undesired thermal tissue damage, for example to adjacent organs or skin along the path. Previous studies have indeed shown a relatively high percentage of skin and muscle burns at the site of the grounding pad or pectoral muscle, in up to 30% of patients [8].

An alternative method is a bipolar RFA technique in which both electrodes are located on one applicator. Consequently, the radiofrequency energy is exclusively applied to the target tissue.

Previous clinical studies were able to show that bipolar RFA with internal cooling can be conducted on patients in a safe and uncomplicated manner for tumours in liver and

pancreas[18,19]. A thorough study of the novel bipolar system's dosage/effect relationship in mammary tissue with the objective to obtain an overview of the inducible lesion sizes was however lacking.

The first goal of this study, therefore, was to determine the optimal power settings in a bovine model to acquire an adequate lesion diameter with RFA and to monitor tissue temperature changes during ablation. Subsequently, this study aimed to evaluate the safety and efficacy of this bipolar RFA technique for the local treatment of small breast carcinomas.

METHODS

To determine the optimal power settings to acquire an adequate lesion diameter, first an *ex vivo* study was performed. Subsequently, we continued with the *in vivo* clinical evaluation.

Ex vivo study set up

Fresh bovine mammary tissue of 5x5x5 cm was cut in two equal slices and placed watertight in a temperature bath at 37°C with a BD Lauda thermostat. The RFA procedure started when a tissue core temperature of 35-37°C was reached. Six Luxtron 790 Fluoroptic thermo probes (Luxtron Corporation, Santa Clara (CA), USA) were fixed on an Elastofix bandage at distances of 0.5 cm; 1 cm; 1.5 cm; 2 cm and 2.5 cm from the edge. Hereby, temperatures to a distance of 2.5 cm from the probe were measured.

At intervals of 1 second, energy output and temperature measurements were performed and displayed in real-time. After each ablation procedure, the acquired ablation length and width were measured macroscopically, along with the size of the transition zone.

Radiofrequency ablation procedure

A bipolar radiofrequency ablation system was used (CelonProSurge 150-T20, Olympus Winter&Ibe GmbH, Hamburg, Germany). We used a 15-gauge, bipolar needle-type probe where a current flows exclusively between the two electrodes, separated from each other by an insulator, at the tip of the probe. The conducting part of the bipolar applicator was 20 mm, including both electrodes and the insulator. The high-frequency current flows between the two electrodes at the tip of the bipolar applicator and heats up the tissue surrounding the electrodes. The use of grounding skin pads to close the electrical circuit was therefore dispensable. A peristaltic pump, set at 30 ml/min, circulates sterile saline (at room temperature) solution to provide internal cooling of the probe.

The probe was connected to a resistance controlled automatic power control (APC) unit working at 470 kHz (CelonLab POWER, Olympus Winter & Ibe GmbH, Hamburg, Germany)[20]. This power control unit is able to automatically regulate the optimum power output based on the tissue resistance measured. In this way, the desiccation of the tissue and thus the premature termination of the power output were prevented.

If the impedance increased beyond a limit value, indicating a dehydration status around the electrodes, the power output was automatically reduced, until a stabile resistance value was reached. Then the power was again automatically increased to the pre-set output value. If another significant change in resistance occurred at a later stage in the process, this procedure could be repeated as often as necessary. At power settings <10 W APC was switched off, allowing the device to generate a continuous energy output.

RFA was performed at a pre-set power level. The mean power, the amount of energy emitted and the duration of application were automatically measured and indicated. In the first series, power settings of 5, 7, 10 and 15 Watt (W) were applied for a pre-set period of 10, 15 or 20 minutes.

In the second series, identical power settings were applied, until a pre-set amount of introduced energy was reached; a power of 5 W was applied until 3.0, 6.0 and 9.0 kilojoules (kJ) was reached and powers of 10 and 15 W were applied until 6.0, 9.0 and 12.0 kJ were reached. To enlarge reproducibility and validity, the series with the best results was repeated once.

In vivo study

In a clinical study, a treat and resect protocol was conducted. Adult women with core-biopsy proven invasive ductal or ductolobular breast cancer, less than 2.0 cm in diameter on ultrasound (US) were considered eligible for this study. Only tumours positioned more than 1 cm from the skin and chest wall were included. After 5 patients were included an amendment to the study protocol was made to add magnetic resonance imaging (MRI) to assure tumour size, shape and extent and for comparison with the histological results of RFA. Tumour characteristics such as Bloom Richardson grade, oestrogen- and progesterone receptor status, and HER2 status were determined at core needle biopsy to ensure optimal systemic treatment, since RFA of the tumour precludes these analyses.

Patients with the histopathological diagnosis of carcinoma in situ or lobular carcinoma on core-biopsy, insufficient visualization of the tumour by ultrasound, multiple tumours in the same breast and previous surgery or radiotherapy of the breast were excluded. Also patients with pacemakers or taking anticoagulants were excluded.

This study was approved by the Institutional Review Board. All patients provided written informed consent.

Radiofrequency ablation procedure

For the radiofrequency ablation procedure same devices and settings were used as described above.

RFA was performed under sterile conditions and under general anaesthesia in the operating room. Prior to the RFA procedure, axillary dissection or sentinel lymph node mapping and biopsy were performed by use of technetium (Tc) sulfur colloid peritumoural injection.

After covering the ultrasound transducer with a sterile sheath, the tumour was localized. Subsequently a 5 mm skin incision was made, followed by ultrasound-guided probe insertion (CompactXtreme CX50, Philips Medical Systems, Eindhoven, The Netherlands) with the shaft of the needle-electrode aligned with the scanning plane and as parallel to the chest wall as possible. The correct placement was confirmed in three directions and RFA was performed using intermittent ultrasound monitoring.

Based on our experiences in the prior *ex vivo* study, the generator was set to supply a maximum power output of 15 W and RFA was performed until a total energy of 9.0 kJ was administered, or until the power control unit stopped delivering radiofrequency energy due to a repeatedly steep impedance increase of the ablated tissue (a so-called 'break' or 'roll-off') at short time intervals. Then, while deactivating the resistance control, puncture track coagulation ("track ablation") was performed at 15 W. To minimize thermal injury to the skin, a sterile ice pack was placed on the skin during the ablation procedure.

Immediately following RFA, tumour resection was achieved by local excision or mastectomy, using standard surgical techniques. Resections included the entire ablation volume and a rim of normal breast tissue.

Histopathology

The specimens were submitted fresh to pathology where sectioning of the specimen was performed. At the level of the tumour, the specimen was divided into different parts: one part was formalin fixed, the other part was snap frozen and stored at –80 0C for possible

future studies. Formalin fixed tissue was used for routine histopathological evaluation using conventional haematoxylin eosin (H&E) staining.

Since H&E cannot be used for assessment of cell viability immediately after RFA additional immunohistochemical staining was performed[21]. Therefore, 3 mm parallel paraffin embedded sections were routinely stained with a monoclonal antibody to cytokeratin (CK) 8 (CAM5.2, Becton & Dickinson, Erembodegem-Aalst, Belgium). Staining was developed in 3-3'-diaminobenzidine tetrachloride after application of the peroxidase LSAB kit (Dako Cytomation, Glostrup, Denmark). Finally, sections were counterstained with Mayer's haematoxylin.

Pathologic variables evaluated included viability of invasive tumour cells, marginal clearance and presence and viability of in situ carcinoma. The pathological effects of RFA and cell viability were assessed by one experienced breast pathologist (PvD) at two separate time intervals.

Follow up

Decisions regarding postoperative treatment with adjuvant chemotherapy or hormonal therapy were made according to each patient's risk category and based on the pre-treatment tumour characteristics and size. Adjuvant systemic therapy was administered in 7 patients. Breast irradiation was performed in 9 cases.

Evaluation and analysis

Rates of complete and incomplete ablation were documented descriptively. Statistical analysis was not performed since this was a descriptive phase II study.

RESULTS

Ex vivo optimization of the power settings

A total of 34 *ex vivo* ablations were performed. In the first series, four experiments at power settings of 15 W were performed, with pre-set ablation durations of 5, 10, 15 and 20 minutes. This resulted in total energy outputs of, respectively, 2.3, 4.6, 6.1 and 14.0 kJ. The second series, where ablation at a power setting of 15 W continued until a pre-set amount of introduced energy of 6.0, 9.0 and 12.0 kJ was reached, which was repeated once, resulted in mean ablation durations of, respectively, 7.4, 14.8 and 20.7 minutes. In these ten experiments with power set on 15 W, the minimal cell destructive temperature of 45-60 °C was reached at distances ranging from 1.5 to 2.0 cm distance of the probe-axis.

Power settings of above 10 W share the advantage of APC that changes power according to the tissue impedance in order to postpone tissue dehydration. Figure 1 shows the fluctuating tissue temperatures as a result of this APC.

Ablation procedures at 15 W showed the highest tissue temperatures, with a mean tissue temperature of 101.1°C at 0.5 cm distance of the probe. In these 15 W experiments, the distance from the probe where temperature exceeded the minimal cell destructive temperature of 45-60 °C ranged from 1.5-2.0 cm, indicating an effective ablation zone of at least 1.5 cm distance from the probe.

Figure 1 | Examples of tissue temperature graphs of clinical relevant power settings at 15 W until 9.0 kJ Energy Output is achieved. *MCDT = Minimal Cell Destructive* Temperature

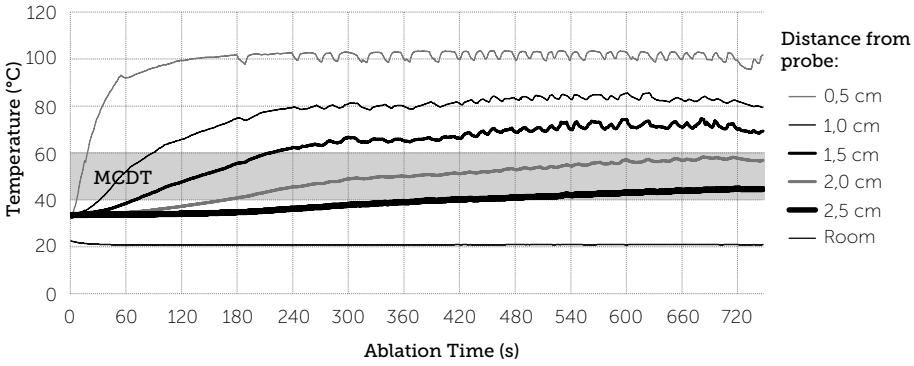
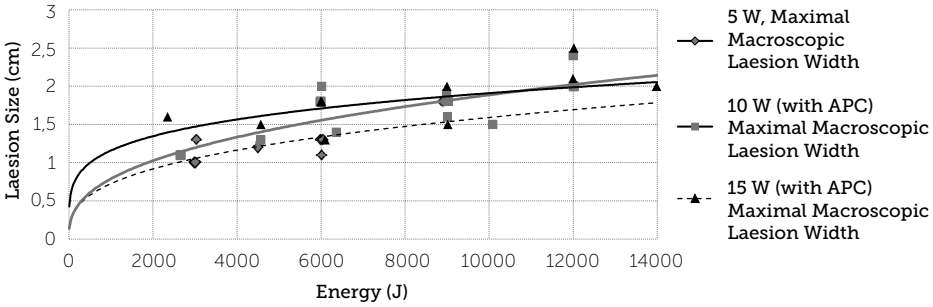


Figure 2 | Dose Effect Curves of Lesion Width



Maximal lesion length ranged from 1.0 cm in 5 W experiments, to 2.5 cm in 15 W experiments. Lesion width was the largest in the 15 W experiments, with a median of 1.8 cm [range 1.3-2.5 cm].

In figure 2, the obtained dose-effect curves present an overview of the relation between acquired lesion sizes for power settings of 5, 10 and 15 W. The desired lesion size of 2.5 cm for T1 breast carcinoma was reached only in 10 and 15 W experiments, when an energy output of 9.0 kJ had been reached.

In vivo study

Between June 2010 and July 2013, 15 postmenopausal women with a median age of 63 years (range 50-76) were included after signing informed consent. Table 1 shows all patient and tumour characteristics.

Preoperative diagnostic imaging with ultrasound showed a median tumour diameter of 11 mm (range 4-17). MRI was performed in 10 patients to assure tumour size, which showed an average tumour diameter of 13 mm (range 5-20). All patients were clinically node-negative.

As determined on core-needle biopsy, 10 ductal and 5 ductolobular tumours were included. Twelve tumours were oestrogen receptor positive and 10 tumours were progesterone receptor positive as well. Six lesions were grade I, 6 lesions grade II and 3 were a grade III. All tumours were HER2 negative.

Table 1 | Patient characteristics

Patient characteristics	***n* = 15**
Age [median, range]	63 years (range 50-76)
Tumour diameter [median, range]	
US	11.0 mm (range 4-17)
MRI (*n* = 10)	13.0 mm (range 5-20)
Breast conserving therapy	12
Mastectomy	3
Histology (assessed on core needle biopsy)	
Ductal carcinoma	10
Ductulolobular carcinoma	5
Tumour grade (Nottingham Grading System)	
I	6
II	6
III	3
ER +	13
PR +	10
Her2Neu +	0

Table 2 shows the characteristics of the RFA procedure. The amount of supplied energy ranged from 2.3 to 11.5 kJ (mean 6.68 kJ $\pm$ 2.51 SD). In only 5 patients the target amount of introduced energy of 9.0 kJ was achieved, and in 1 of these patients the RFA was continued until 11.5 kJ was reached to ablate a little tumour spur. In the 10 other patients ablation was terminated preliminary due to increased tissue resistance. The duration of the RFA in all patients ranged from 6 to 26 minutes (mean 13 $\pm$ 0.2 SD).

During real-life ultrasound monitoring the visibility of the target lesion decreased in all cases due to development of an ill-defined hyperechoic zone around the tip of the probe. Due to incompliance of the tumour, placement of the probe was technically unsuccessful in 2 patients.

Table 2 | Characteristics of the radiofrequency ablation procedure

RF Ablation procedure	*n* = 15
Ablation time [median, range]	16 min (range 6-26)
Energy supplied [median, range]	6.7 kJ (range 2.3-11.5)
Technically feasible procedure	13
Complete ablation	10
Complications	1 pneumothorax

Following RFA, 12 patients underwent a lumpectomy, and 3 a mastectomy. Mastectomy was performed according to patients' preference (*n* = 2) or prior to radiation for Hodgkin's disease (*n* = 1). In 14 cases a sentinel node biopsy was performed, in 1 patient an axillary lymph node dissection. All resections were radical. In 1 patient the sentinel node contained metastases and an axillary lymph node dissection was carried out in a second procedure. Histopathology with H&E and CK8 staining revealed complete cell death of the invasive tumour component in 10 of 13 patients (76.9%) where the RFA procedure was technically successful (figure 3). There was no correlation with the histological tumour type; 2 incompletely ablated tumours were of ductal type, 1 ductolobular. Nor was there a correlation with the fat content of the breast. Incomplete ablation occurred in a fatty breast, a fibroglandular breast and a heterogeneously dense breast (respectively <10%, 10-49% and 49-90% of dense tissue).

The staining methods were in accordance in all patients, except for 1 case where CK8 showed complete ablation whereas H&E showed a partial ablation of 50%.

In 2 patients only partial ablation occurred; in 1 patient the longitudinal shape of the tumour resulted in only 40% ablation of the tumour; in 1 patient a 95% ablation of the invasive tumour was seen on the CK8 staining, possibly due to the underestimation of the tumour on ultrasound; in 1 patient a preliminary increase in resistance occurred, resulting in an early 'roll-off' and partial ablation of 60% (figure 4). In 6 patients DCIS was found; in 1 of them this DCIS was still viable, situated just outside the incompletely ablated invasive tumour component.

In the 2 patients where the probe was misplaced, viable tumour cells with an ablation zone just outside the tumour were seen. In 1 of them misplacement of the probe resulted in a pneumothorax, for which thorax drainage and hospital admission was required. No other complications occurred, in particular no wound infections or burns.

Adjuvant systemic therapy was given in 7 patients, 9 patients received radiation therapy. Median follow-up after surgery was 17 months (range 3-40). One patient developed liver and osseous metastases 34 months after surgery, for which palliative treatment was started. Cosmetic outcome was not assessed in this treat and resect study.

Figure 3 | Completely ablated breast cancer tissue and nonvital DCIS. Paraffin section (1.5 X) HE (a) showing complete ablation of breast cancer tissue and nonvital DCIS (left below) as exemplified by negative CK8 staining (b) showing no viable cells.

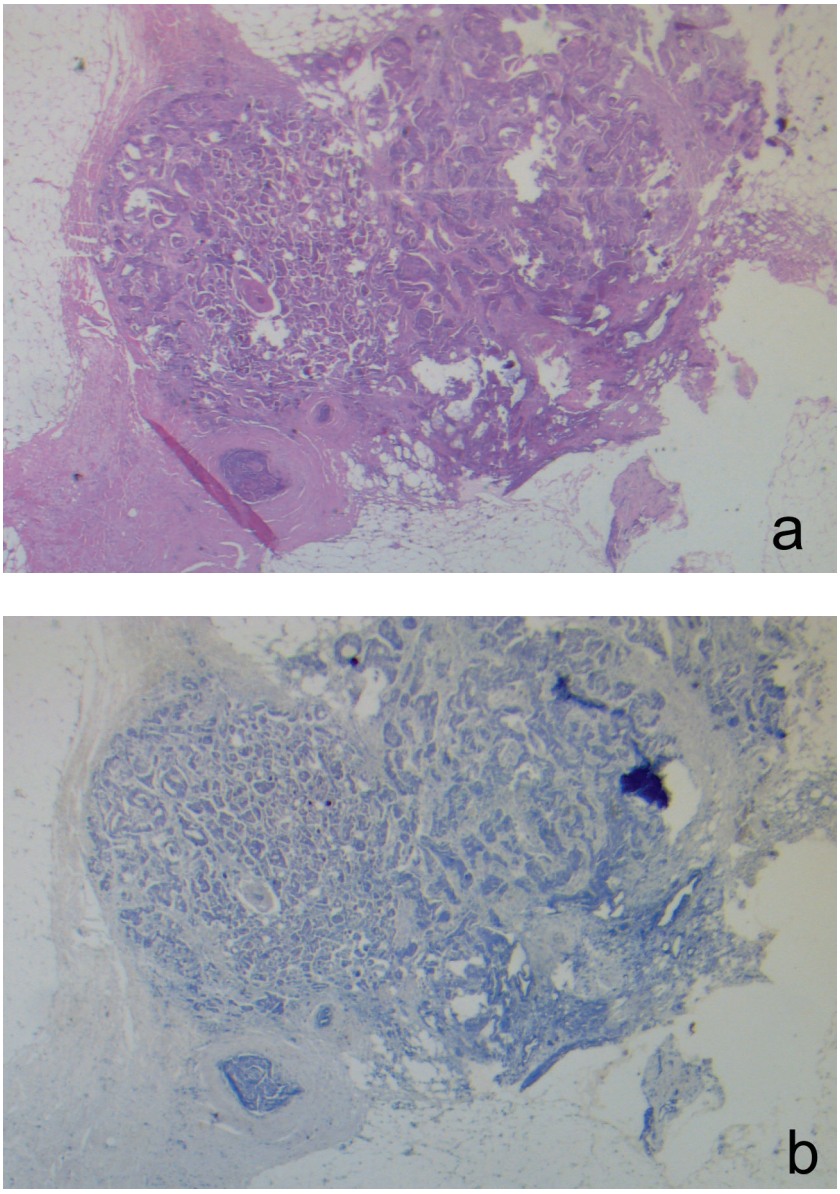
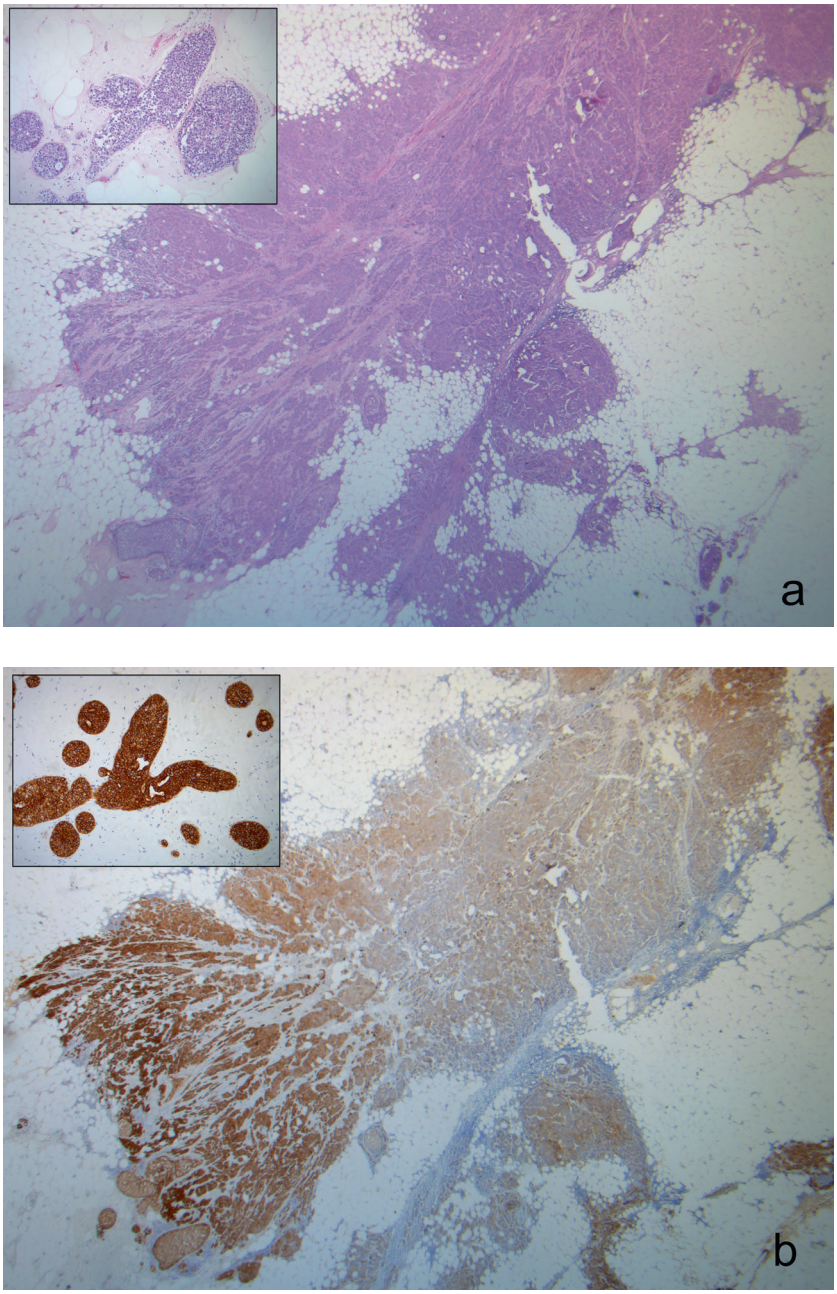


Figure 4 | Partially (60%) ablated (ductal) breast tumour. Paraffin section (1.5 X) HE (a) and CK8 immunohistochemistry (b) showing transition from viable breast cancer tissue and DCIS (10 X insertion) on the left to nonvital tumour cells on the right.



DISCUSSION

Our study is the first study using a bipolar internally cooled needle-electrode for the local treatment of breast tumours. In this phase II study we found that RFA with this novel electrode is capable of causing complete tumour cell death without causing any burns. Histopathology with H&E and CK8 staining revealed complete cell death of the invasive tumour component in 10 of 13 (76.9%) patients in which the RFA was technically successful.

This success rate is in line with previous reports using RFA techniques with a monopolar needle-type electrode reporting complete ablation rates of 70-93% and burn complication rates of 0-12%[6, 7, 9, 10, 22]. The use of multiple-array umbrella-type probes resulted in slightly higher complete ablation rates but also in higher burn complication rates, respectively 86-100% and 0-30%[3–5, 8, 16]. In our study no burns occurred. Complications were seen in 1 patient, due to misplacement of the probe. We thereby strongly recommend the procedure to be performed by an expert radiologist.

Our study once again subscribes that tumour selection, based on tumour size and shape is of major importance for effective ablation, since two incomplete ablations were due to the shape of the tumour. Also tumour extent and compliance should be assessed. In our series we encountered 1 patient with viable DCIS just outside the completely ablated invasive component. This highlights an important potential selection problem. Findings of invasive carcinoma, DCIS and lymphatic foci beyond 1 cm from the edge of the dominant mass have been reported in up to nearly 50% of the tumours[23]. Pre-treatment assessment of mammography findings, MRI washout kinetics, ER status and quantity of DCIS in the index tumour has the potential to accurately identify breast cancer of limited extent, but up till now it is not clear what is the best tool to select these tumours[24]. Due to a solid tumour, probe positioning centrally in the tumour, was abortive in 2 patients, even resulting in a pneumothorax in one. Difficult positioning with comparable needle-type probes has been described previously, where Wiksell et al. also reported a pneumothorax[10, 25]. Although these misplacements can easily be recognized during the procedure and should in future situations lead to immediate conversion to surgery, they do pose the question whether working with multiple probes, positioned alongside the tumour, should be considered. This will avoid the central placement of the probe inside the tumour; hereby not only solving the problem of an incompliant tumour, but also making the accuracy of probe

positioning less important. Furthermore it will prevent the theoretical possibility of needle track seeding, where tumour cells attached to the needle applicator can be disseminated through the breast, resulting in new tumour formation somewhere along the applicator track[21].

In our study, real-time monitoring was performed by ultrasound and feedback of tissue resistance. Ultrasonography during RFA or directly afterwards did not provide an accurate measurement of the histological zone of complete coagulative necrosis. Real-time MRI monitoring has been proposed as an option before, but has already shown drawbacks with non-complete tumour ablation[26]. In what way follow up after RFA will come about is another subject that needs to be addressed. In a small series with 8-24 days delayed excision after RFA MRI enhancement after RFA showed correlation with pathologically viable tumour cells in the excision specimen[12]. This possible usefulness of MRI for the assurance that all cancerous cells have been histologically destroyed might be more expanded by the introduction of novel technologies, such as ultra-high field (7 tesla) MRI[27]. Another modality that has been studied to evaluate RFA is Doppler ultrasonography with perfusion software and contrast agent injection (DUPC). In a study of ten breast lesions Lamuraglia et al. found DUPC to be suitable for determining tumour destruction in lesions that are hypervascularised[28]. Core needle biopsy to confirm complete ablation or absence of residual or recurrent disease has been used in most RFA studies where surgical resection after RFA was omitted[29, 30]. Nevertheless, this carries the risk of sampling error, even when multiple biopsies are taken, especially in the relatively larger tumours.

We only found viable DCIS in 1 case where the invasive index tumour had been incompletely ablated. However, Hayashi et al. reported a 23% incidence of small foci of viable disease outside the completely ablated index tumour suggesting that RFA alone would be insufficient and lead to a high rate of local recurrence[13]. This is similar to studies that have identified unsuspected foci of carcinoma after lumpectomy[31]. Adjuvant radiation treatment will be required in patients treated with RFA as well as conventional breast conserving therapy.

Cosmetic outcome is an important issue in breast-conserving treatment. It is not yet known whether RFA of breast cancer leaves a permanent scar or induces fat necrosis, both of which could be more or less pronounced than those resulting from a standard

lumpectomy. With a mean follow-up of 15 months Oura et al. reported excellent breast cosmetic results after RFA in 43 patients (83%), good in 5 (12%), and fair in 3 (6%)[29]. However, also cases of granulomatous mastitis after radiation therapy post-RFA have been reported[30], whereas others instilled depo-medrol in the ablation cavity to prevent inflammatory fat necrosis at RFA site[25].

Drawback of our study could be the underestimation of the ablation, due to the immediate excision of the ablated mass. Earashi et al. found greater degenerative changes in their series with (20-201 days) delayed excision, an indication of the possible continuing cell death after the RFA procedure due to the effects of local vessel thrombosis and subsequent necrosis of the surrounding tissue[32]. Also, we used very strict assessment criteria, were even the finding of a single viable tumour cell was demarcated as 'incomplete ablation'; In other studies findings of 5% viable tumour cells would be scored as a successful procedure[22]. As a marker for non-viability after RFA we used immunostainings for CK8, shown to be a reliable marker after RFA[12, 21]. Cytokeratins are epithelium-specific intermediary filaments and CK 8 is cleaved at an early step in apoptosis, so it can be used to test for cell viability[33, 34]. In our study CK8 staining and H&E were concordant in all but 1 patient; in all cases of negative CK8 the H&E showed complete morphological cell death as well, again indicating that the CK8 can be used as a reliable marker.

Summarizing, our results demonstrate that radiofrequency ablation with a bipolar internally cooled needle-electrode can be used as a safe minimally invasive treatment technique for small invasive breast cancer. These findings are important since it is a new and promising method whereby (skin) burns associated with monopolar techniques can be prevented. However, improving probe placement and monitoring of the completeness of tumour ablation remains the primary goal of studies in the nearby future before surgical specimen resection can be safely omitted.

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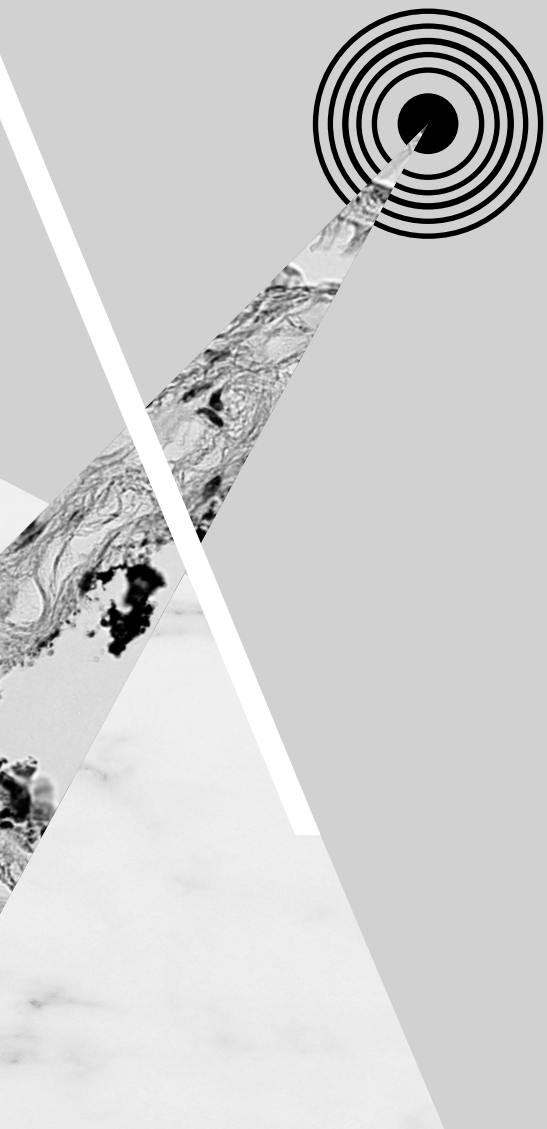
PART II

Breast duct endoscopy



CHAPTER 04

Ductoscopy for pathologic nipple discharge

Introduction of a novel technique in the Netherlands

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ABSTRACT

Pathologic nipple discharge is a symptom that frequently causes female patients to visit the outpatient breast clinic. In the vast majority of cases, the symptom is caused by a benign intraductal lesion. The options for diagnosis and treatment have long been limited; surgery was frequently the treatment of choice. With the advent of breast ductoscopy, a micro-endoscopic procedure, it is possible to visualise abnormalities in the ductal system. Tissue for histopathological investigation can be retrieved from the duct and the condition can be treated. The patient with nipple discharge is consequently prevented from having to undergo an invasive and fairly 'blindly' executed procedure under general anaesthesia. The miniscule dimensions of the duct in which the technique is carried out pose the greatest challenge to the further development of the ductoscope.

NOVEL TECHNIQUES

Description of the novel technique

Ductoscopy is a micro-endoscopic technique enabling direct access to the ductal system via the nipple (figure 1). The first ductoscopy was performed in the early 90s, but was not feasible for clinical use. Recent development in technology has led to thinner optics with improved image qualities. Integration of a working channel next to channels for optics and irrigation makes histologic tissue sampling and therapeutic intervention possible.

In 2011 ductoscopy was introduced in the University Medical Center Utrecht for evaluation of women with pathologic nipple discharge at the outpatient clinic. Ductoscopy is performed using the 6000 pixels 0.55mm optic ("LaDuScope T-flex", Polydiagnost GmbH, Pfaffenhofen, Germany) inserted into a 1.15 mm outer diameter polyshaft (PD-DS-1015, Polydiagnost), displayed with 60x magnification on a video monitor.

After periareolair local infiltration anaesthesia the productive lactiferous duct orifice in the nipple is dilated with a salivary duct probe (Karl Storz, Tuttlingen, Germany). Meanwhile upward traction of the nipple is used, in order to straighten the subareolar ducts to facilitate cannulation. An introduction port (SoLex-Nipple-Expander®, Polydiagnost) is placed into the duct orifice through which the ductoscope is introduced. Sterile saline infusion is used for ductal distension. Endoscopic exploration is performed starting in the fluid-producing duct.

Via the 0.45 mm working channel, a 'basket' device (Saliva, 3-times-wrathed, diameter 380 µm, Polydiagnost) can be introduced, by which intraductal polypoid lesions such as papillomas can be extracted. This integrated working channel provides a broadening in the applicability of the ductoscope, although its miniscule dimensions pose the greatest challenge to further developments.

Acquiring tissue for histopathological assessment is not always possible, due to obstructing lesions blocking basket passage. Also for flat epithelial lesions the basket device is not useful.

Therefor we are currently working on the development of a novel biopsy-instrument and

investigating the possibility of laser-ablation.
When transductal removal is not possible, the lesion can be marked by dye or a wire, followed by targeted surgical excision (figure 2). This minimizes tissue excision, as surgical resection is no longer 'blindly' executed.

Why is there a need for a novel technique?

Conventional diagnostics in women with pathologic nipple discharge include mammography and ultrasound. In only a minority of women, these studies result in the suspicion of malignancy. Mostly however, conventional imaging leaves the physician with a series of negative results.

Due to the persistent commodious symptoms and fear for underlying malignancy, surgical excision is frequently performed, by either microdochectomy or major duct excision. These are invasive procedures, performed under general anaesthesia. Moreover, they are fairly 'blindly' executed, risking that the resected tissue does not contain the underlying cause of symptoms. Furthermore, surgery carries a risk of complications and effects on cosmesis, breast-feeding and sensitivity of the nipple.

With ductoscopy the ductal epithelium of the breast can be visualized via the nipple. Besides its diagnostic ability it can also be used as a therapeutic modality, performed under local anesthesia in the outpatient clinic. Adding ductoscopy to the evaluation of patients with pathologic nipple discharge may hereby result in reducing surgery and associated costs and complications.

For what indication is it used?

Ductoscopy is indicated for patients with pathologic nipple discharge without an underlying cause on conventional imaging with mammography and breast ultrasound. Pathologic nipple discharge is defined as unilateral, spontaneous, discharge from a single duct and is among the top 3 breast complaints for women vising the breast clinic. Around 6% of women visiting the breast clinic have symptoms of pathologic nipple discharge. Every year around 3500 women in the Netherlands will present with this complaint.

This incommodious symptom is often caused by a benign intraductal lesion, of which intraductal papillomas occur most frequent. Ductoscopy is feasible in nearly all patients

Figure 1 | (a,b) Ductoscopic images of a normal breast duct. (c,d) Ductoscopic images of an intraductal papilloma.

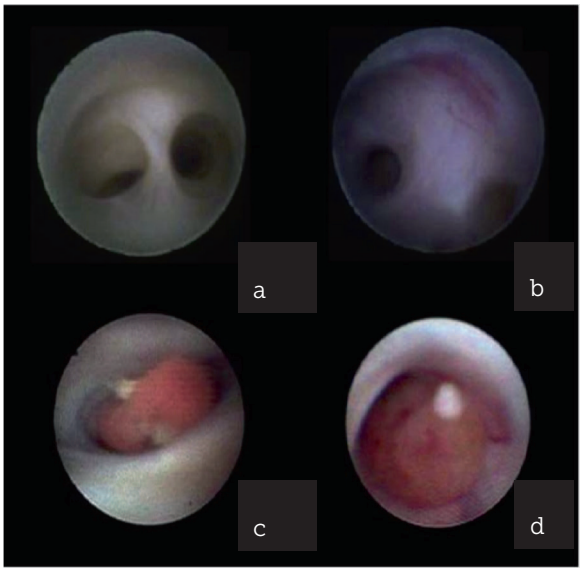
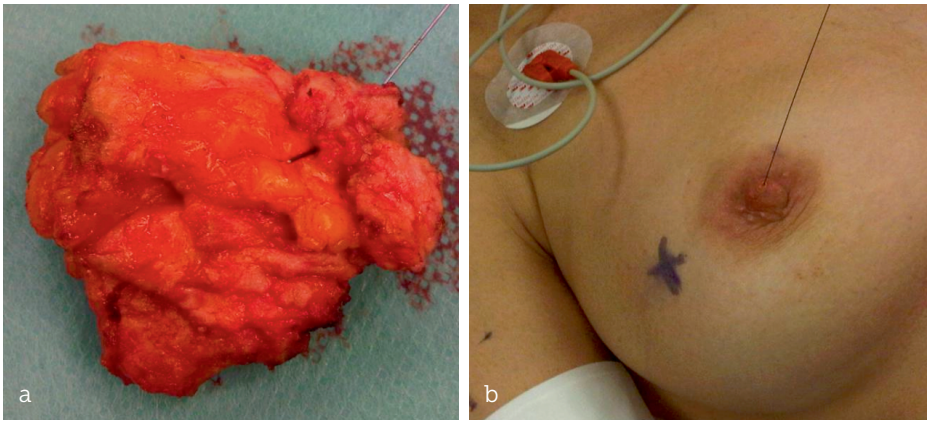


Figure 2 | Targeted surgical excision (a) after ductoscopic wire-marking (b) of a visualized intraductal papilloma.



with pathologic nipple discharge and can be used for the evaluation and treatment of this breast complaint[1,2].

In case of inverted nipple or previous breast surgery, ductoscopy is not feasible due to cannulation problems. Large, solid lesions (>1 cm), clearly visible on breast ultrasound, are not suitable for endoscopic approach, due to the risk of malignancy.

What problem does it solve?

Ductoscopy facilitates minimally invasive diagnosis and treatment of patients with pathologic nipple discharge, thereby obviating the need for surgery. Ductoscopy-guided surgery improves the conventional, fairly 'blindly' executed surgical procedure.

What is known about its efficacy?

In small patient cohorts the sensitivity of ductoscopy for finding intraductal lesions was 55-58%[3,4].

Other studies show the possibility of minimally invasive treatment of the visualized intraductal abnormalities in up to 90%[1,2].

Due to the included learning curve of the physician, the results of a pilot study in 22 patients undergoing ductoscopy probably show an underestimation of the efficacy of ductoscopy. In 2 out of 22 patients cannulation was technically not feasible due to inverted nipple. In 14 patients the underlying cause of symptoms could be visualized; in 9 the lesion could be removed. In only 4 of them tissue collection was possible and histopathological assessment could be performed.

Women in whom ductoscopy was technically not feasible (2/22), or in whom abnormalities could not be detected (6/22) or removed (5/22), were offered surgery. Nine of these 13 patients opted for surgery. Overall 13 patients (59%) had been successfully treated (8/22) or reassured (5/22) by ductoscopy, waiving the need for surgery. To evaluate the cost-effectiveness of ductoscopy the University Medical Center Utrecht will commence a prospective study.

Is the technique difficult to learn?

For a surgeon experienced in endoscopy, the technique of ductoscopy is rather easy to

learn. Beyond the point of approximately 10-15 procedures a surgeon is able to successfully perform the procedure.

Future perspectives

Ductoscopy may be performed in every hospital in the Netherlands. Nowadays ductoscopy is only performed in the University Medical Center Utrecht.

With the development of novel techniques ductoscopy will be able to prevent invasive diagnostics and treatment of patients with pathologic nipple discharge. In the future, ductoscopy may also be of use in diagnosis and treatment of patients with malignant or premalignant breast disease[1,4]. Ductoscopy provides a new approach for diagnostic molecular markers, therapeutic agents and laser ablation. Various divisions in the University Medical Center Utrecht cooperate in studying the opportunities of the intraductal approach.

Table 1 | The benefits and disadvantages of ductoscopy in pathologic nipple discharge.

Benefits	Disadvantages
Procedure can be performed under local anaesthesia in the outpatient department	Acquiring tissue for histopathological assessment not always feasible*
Minimally invasive procedure without need for skin incision	Transductal removal not always possible†
Procedure has no consequences for symmetry and cosmetic outcome of the breast	Not feasible after previous surgery of the nipple or inverted nipple
Provides the possibility of lesion marking, resulting in targeted surgical excision; also in radiological occult abnormalities	

* This disadvantage may be obviated by the development of novel biopsy device.
† Transductal laser-ablation is evaluated *ex vivo* to obviate this disadvantage

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CHAPTER 05

Diagnostic accuracy of ductoscopy in patients with pathologic nipple discharge; a systematic review and meta-analysis

submitted

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ABSTRACT

Objective | This systematic review and meta-analysis was designed to evaluate the diagnostic accuracy of ductoscopy in patients with PND.

Background | For diagnosis of pathologic nipple discharge (PND), invasive surgery is the gold standard. Only a minority of patients with non-suspect conventional imaging has cancer. Ductoscopy is a minimally invasive alternative for evaluation of PND.

Methods | We systematically searched electronic databases for studies addressing ductoscopy in patients with PND. Two classification systems were assessed. For DS_{any}, all visualized ductoscopic abnormalities were classified as positive, whereas for DS_{susp}, only suspicious findings were considered positive. After checking heterogeneity, pooled sensitivity and specificity of DS_{any} and DS_{susp} were calculated.

Results | The search yielded 4542 original citations of which twenty studies were included in the review. Malignancy rates varied from 0 to 27%. Twelve studies, including 1994 patients, were eligible for meta-analysis. Pooled sensitivity and specificity of DS_{any} were 94% (95% CI, 88-97%) and 47% (95% CI, 44-49%), respectively. Pooled sensitivity and specificity of DS_{susp} were, respectively, 50% (95% CI, 36-64%) and 83% (95% CI, 81-86%). Heterogeneity between studies was moderate to large for sensitivity (DS_{any} I^2 = 17.5%, DS_{susp} I^2 =37.9%) and very large for specificity (DS_{any} I^2 = 96.8%, DS_{susp} I^2 = 92.6%).

Conclusions | Ductoscopy detects about 94% of all underlying malignancies in patients with PND. However, ductoscopy does not allow for reliable discrimination between malignant and benign findings. Since most visualized lesions are benign, optimization of ductoscopic histologic sampling remains warranted. If histology of a visualized lesion cannot be acquired through ductoscopy, surgery is indicated to exclude the risk of underlying malignancy.

BACKGROUND

Pathologic nipple discharge (PND), defined as spontaneous single duct nipple discharge, is responsible for approximately 5% of surgical referrals to the breast clinic[1]. Malignancy rates up to 23.9% have been reported in patients with PND[2], but these studies were dated, selected only surgical patients or included patients with suspect findings on imaging[2–7]. Modern studies including patients with PND and negative conventional imaging by mammography and ultrasound have shown considerably lower malignancy rates of 3-7%[3,8–11].

To evaluate the nature of PND, mammography and ultrasound are commonly used, sometimes supplemented with MRI, as well as galactography and/or cytology of nipple aspiration fluid (NAF) or ductal lavage. In patients with PND without signs of malignancy on conventional diagnostic examination by mammography and ultrasound, the added value of a breast MRI is limited[4,12]. Investigations by galactography and cytological analysis of NAF or ductal lavage lack sensitivity and usually do not reveal the underlying pathology[2,4,13–15]. Using these diagnostic modalities therefore often result in a series of negative results.

There is no consensus on the diagnostic approach to PND, but surgery, either by selective duct excision (microdochectomy) or major duct excision, is considered the gold standard[16]. This is, however, an invasive procedure with concomitant risk of complications[17], and possible effects on cosmesis, breast-feeding potential and sensitivity of the nipple. Ductoscopy is a minimally invasive procedure that visualizes the ductal epithelium of the breast via the nipple[14,18]. It can be performed under local anesthesia in the outpatient clinic. Although it is frequently used for evaluating PND[19], the accuracy of ductoscopy in patients with symptoms of PND remains a matter of debate. Several studies reported promising results, but included heterogeneous patient populations, rating systems, and prior diagnostics[20]. And while some studies reported a significant correlation between intraductal morphological findings and histological diagnosis[21,22], others found this only for papillomas[23–26].

The aim of this study was therefore to systematically review the literature on, and if possible to perform a meta-analysis to determine the diagnostic accuracy of, ductoscopy in the diagnosis of malignancy in patients with PND.

METHODS

Search strategy

A systematic search of the Pubmed, Embase and Cochrane Library databases was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[27]. The search query was limited to February 2nd 2015 and included synonym terms within the index test (i.e. ductoscopy), Supplement 1. To minimize the risk of missing relevant studies we did not include synonyms for the target condition or reference standard in the search strategy.

Data collection and analysis

Selection of studies

After removal of duplicates, all identified studies were screened by title and abstract. Full-text papers were retrieved when studies evaluated ductoscopy, reported original data, and were written in English, German, French or Dutch. Studies reporting ductoscopic findings and histopathological outcome after surgery in patients presenting with PND were included and classified as "surgical reference studies". In some studies, negative or non-suspect ductoscopic findings were not followed by surgery. In these cases, clinical follow-up was used as a surrogate reference standard. We defined these studies as "selective follow-up studies".

Original studies performing ductoscopy were eligible for inclusion in the systematic review. Studies had to report ductoscopic outcome in patients with PND or a heterogenic cohort in which test performance was documented separately for each group, and use histology (by surgical excision or endoscopic tissue collection) and/or follow-up as a reference test. Studies reporting small series (fewer than 25 patients), studies including patients with pre-operative diagnosis of breast cancer, overlapping studies (only the most recent article or the article reporting the data of interest was included) or studies other than original clinical trials (e.g. congress abstracts, reviews, editorials, case reports, *ex vivo* studies, protocols) were excluded.

A cross-reference check was performed to assess the quality of the search and to identify eligible additional studies not identified by the primary search. Two authors (L.W. and J.S.) independently performed study selection, quality assessment

and data extraction. Any differences were resolved by mutual agreement. Any disagreement was resolved through re-evaluation by a third author (A.W.), blinded to the outcome of previous assessment.

Quality assessment

The quality of eligible studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS- 2) questionnaire[28]. The QUADAS-2 was tailored to our analysis, as described in the guideline (Supplemental 2a)[28], and applied to a random sample from the available studies to evaluate inter-rater agreement. Finally, all included studies were evaluated (Supplemental 2b).

Data extraction and management

We extracted the following data for each study: study period, inclusion criteria and sample size, together with patients' age and technical and procedural characteristics.

For meta-analysis two different definitions were used to classify ductoscopic findings. For DS_{any}, any visualized finding at ductoscopy was classified as positive, in case of normal ducts ductoscopy was classified as negative. For DS_{susp}, ductoscopically suspicious findings were classified as positive[29–31]. Suspicious ductoscopic findings were defined as duct wall irregularities, epithelial thickening, inflammatory changes, web, stricture, red patches or fronds. Normal ducts, intraductal debris or polypoid lesions were defined as non-suspicious. Malignancy at histopathology or during follow-up was considered a positive outcome of the reference test. Malignancy was defined as ductal carcinoma in situ (DCIS) or invasive breast carcinomas. Intraductal (solitary or multiple) papillomas, atypical ductal hyperplasia (ADH), fibroadenomas, mastopathy, cysts and fibrosis were considered inconspicuous findings.

The primary endpoints for this systematic review were the sensitivity and specificity of DS_{any} and DS_{susp}. Sensitivity DS_{any} was defined as the proportion of women with a positive ductoscopy among all women subsequently diagnosed with malignancy at histology or follow-up. Specificity DS_{any} was defined as the proportion of women with a negative ductoscopy among all women with benign outcome of histology or follow-up.

For DS_{susp} sensitivity was defined as the proportion of women with suspect findings at

ductoscopy among all women diagnosed with malignancy at histology or follow-up. Specificity of DS_{susp} ductoscopy was defined as the proportion of women with non-suspect ductoscopy among all women with benign outcome of histology or follow-up.

In order to construct 2 x 2 contingency tables, data on the number and morphological characteristics of ductoscopic findings and outcomes of histology or follow-up were extracted. If, due to the specific classification system used or due to the lack of a detailed description, data-extraction was not possible, the study was excluded from the analysis.

A stratified analysis of surgical reference studies versus selective follow-up studies was performed to evaluate impact of differential outcome on sensitivity and specificity of DS_{any}.

Statistical analysis

From the contingency tables sensitivity, specificity and corresponding 95% confidence intervals (CIs) were calculated for DS_{any} and DS_{susp} of individual studies. Heterogeneity was assessed by the I^2 statistic (i.e. estimated proportion of unexplained inter-study variance) prior to pooling. Random effects models, using a restricted maximum likelihood estimator, were used in case of large inter- study variance (I^2 > 25%) to calculate a pooled estimate. Otherwise, fixed effects modeling was applied. Results were reported in accordance with the PRISMA recommendations[32].

RESULTS

Results of the search

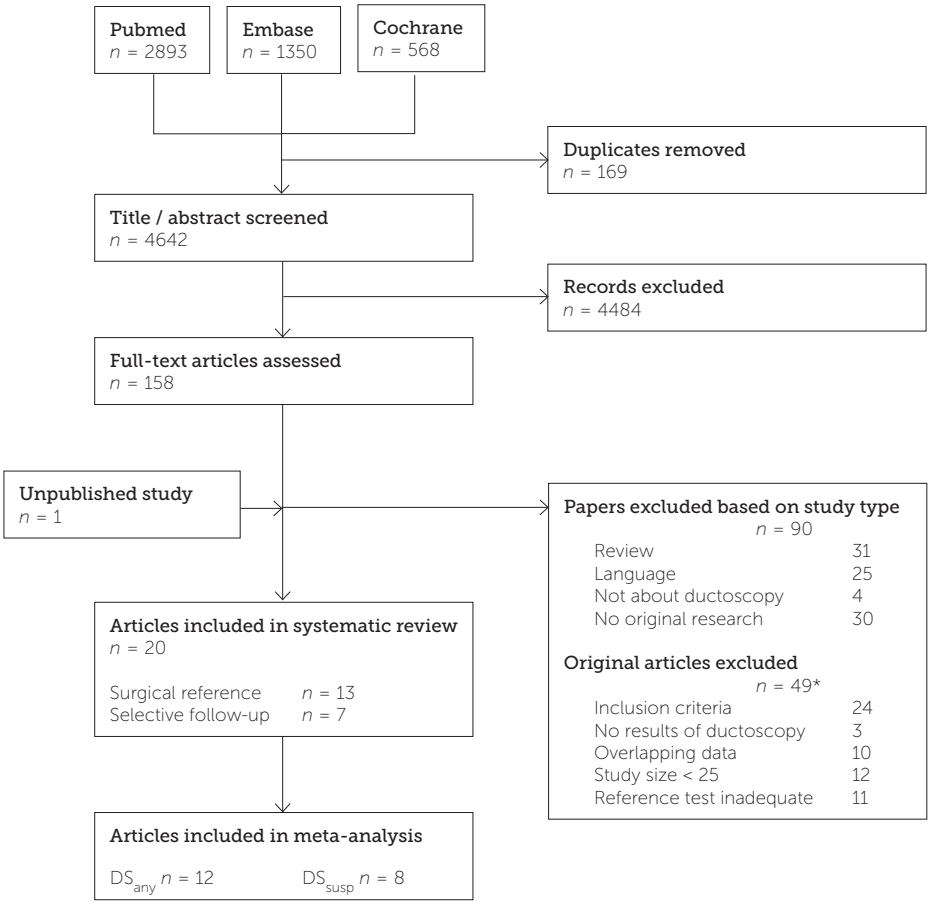
The search query resulted in 4811 citations. After filtering duplicates, 4484 citations were excluded based on title and abstract (Figure 1). The full text of 158 studies was retrieved for detailed review. Cross-reference check of these studies identified no additional studies. Ninety studies were excluded after full-text assessment due to language (*n* = 25) and type of article (31 reviews, 13 editorials, letters or notes, eight congress abstracts, five case reports, three *ex vivo* studies, one study protocol), while four studies did not address ductoscopy. Forty-nine original studies were excluded after full-text assessment, some for more than one reason; 24 studies included a different population (patients without PND, eg. patients with malignancies or high risk of breast cancer) or the results of PND patients in a heterogenic cohort were not (separately) reported, 12 studies included fewer than 25 patients, 11 studies used a different or inadequate reference test, 3 studies did not report results of ductoscopic findings, and 10 studies used the same patient cohort.

Finally, we included 13 surgical reference studies (with histology as the reference standard in all patients) [22,24,26,33–42], and six selective follow-up studies (with surgical confirmation for patients with suspicious ductoscopic findings and follow-up in patients with normal/nonsuspect ductoscopic results) [43–48](Table 1). Unpublished results of the recent prospective study by the authors themselves were included as well [Waaijer et al., unpublished](11).

Quality assessment

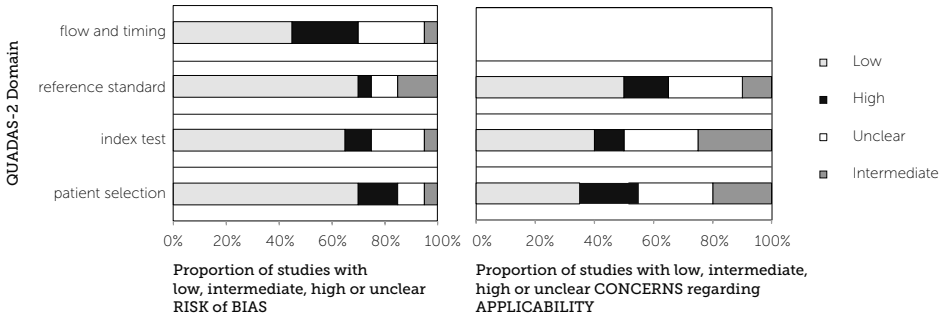
The assessment of methodological quality showed that the overall quality of the included studies was acceptable (Figure 2, Supplemental 2). The inter-rater agreement of the modified QUADAS-2 tool was fair, with an agreement rate of 0.69 (95% CI, 0.59-0.79). Seventy percent of studies were classified as having low risk of bias in patient selection. Other studies included patients with different or poorly described prior diagnostic work-up. Low risk of bias in the index test was found in 65% of studies. Non-defined, or even non-reported ductoscopic scoring systems were the most common reason for risk of bias in index test. For reference standard, a low risk of bias was found in 70% of all studies. Higher risks were mainly caused by non-reported methods of tissue acquiring or poorly reported follow-up. A high, intermediate or unclear risk of bias in flow and timing was assessed in 55% of all studies, mostly due to exclusion of unsuccessfully cannulated patients or due

Figure 1 | Study flow diagram



* Original articles could be excluded for more than one reason

Figure 2 | Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2)
Graphical display for the risk of bias and the applicability concern regarding the included studies using the QUADAS-2 tool.



to an inadequate or unclearly described study flow. High risk of bias in patient selection, index test and reference standard was found in respectively 15% [36,40,45], 10% [38,40], and 5% [46] of the included studies.

Regarding applicability, concerns were mostly raised by studies including only a selected group of patients who were already referred for surgery, or, in case of retrospective studies, had undergone both ductoscopy and surgery. The index test was clearly described in most studies, but on its conduct and interpretation very little data was supplied. The histopathological outcome was mostly clearly described, but data on conduct of follow-up was limited.

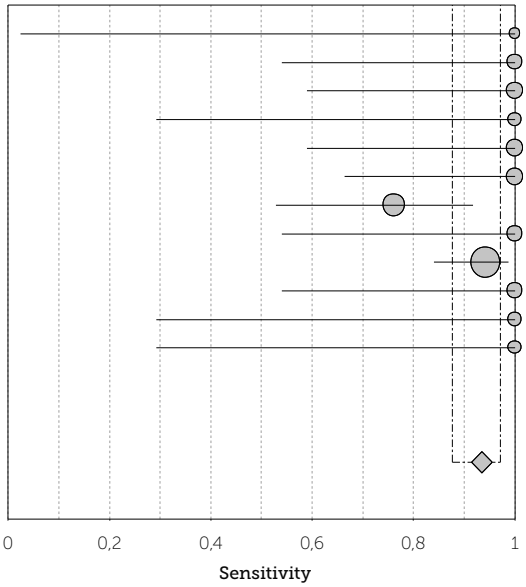
Characteristics of the included studies

Ten studies were prospective cohort studies (OCEBM level 2), six were retrospective cohort studies (OCEBM level 3) and the study design of four studies was not mentioned (OCEBM level unknown) (Table 1).

Histology was acquired mostly by surgery (microdochectomy or major duct excision). Ductoscopic collection of histological tissue was performed in four studies [11,44,46,48]. Three studies used a 'basket'-device (Polydiagnost, Pfaffenhofen, Germany), which is feasible in non-sessile polypoid lesions [11,44,46].

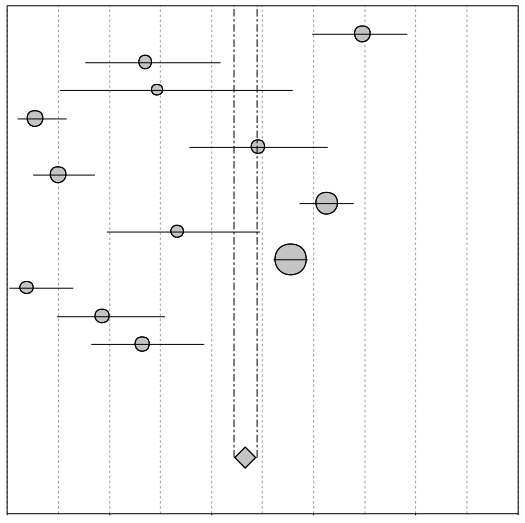
Figure 3 | Forest plot and pooled estimates of DS_{any}.

Sensitivity DS_{any} was defined as the proportion of women with a positive ductoscopy among all women subsequently diagnosed with malignancy at histology or follow-up. Specificity DS_{any} was defined as the proportion of women with a negative ductoscopy among all women with benign outcome of histology or follow-up.



	Sensitivity (95% CI)	
Bender et al 2009	1,00	(0,03 - 1,00)
Denewer et al 2008	1,00	(0,54 - 1,00)
Deshmane et al 2010	1,00	(0,59 - 1,00)
Dietz et al 2002	1,00	(0,29 - 1,00)
Fackler et al 2009	1,00	(0,59 - 1,00)
Fisher et al 2011	1,00	(0,66 - 1,00)
Kamali et al 2014	0,76	(0,53 - 0,92)
Khan et al 2011	1,00	(0,54 - 1,00)
Liu et al 2008	0,94	(0,84 - 0,99)
Moncrief et al 2005	1,00	(0,54 - 1,00)
Simpson et al 2009	1,00	(0,29 - 1,00)
Waaijer et al 2015	1,00	(0,29 - 1,00)

Pooled Sensitivity = 0,94 (0,88 to 0,97)
Chi-square = 13,33; df = 11 (p = 0,2721)
Inconsistency (I-square) = 17,5 %

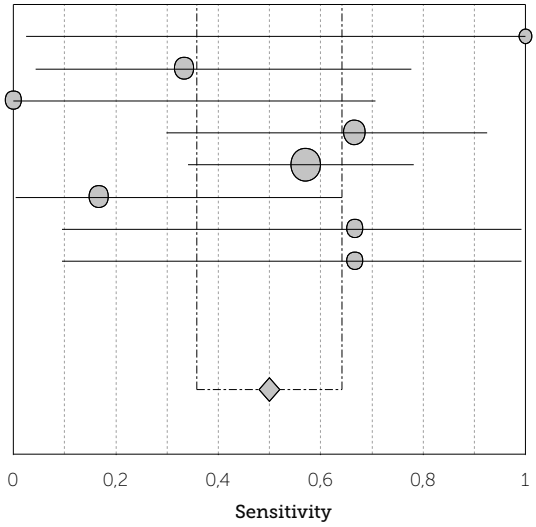


	Specificity (95% CI)	
Bender et al 2009	0,70	(0,60 - 0,78)
Denewer et al 2008	0,27	(0,15 - 0,42)
Deshmane et al 2010	0,29	(0,10 - 0,56)
Dietz et al 2002	0,06	(0,02 - 0,12)
Fackler et al 2009	0,49	(0,36 - 0,63)
Fisher et al 2011	0,10	(0,05 - 0,17)
Kamali et al 2014	0,63	(0,57 - 0,68)
Khan et al 2011	0,33	(0,20 - 0,50)
Liu et al 2008	0,56	(0,52 - 0,59)
Moncrief et al 2005	0,04	(0,00 - 0,13)
Simpson et al 2009	0,19	(0,10 - 0,31)
Waaijer et al 2015	0,26	(0,17 - 0,39)

Pooled Specificity = 0,47 (0,44 to 0,49)
Chi-square = 340,88; df = 11 (p = 0,0000)
Inconsistency (I-square) = 96,8 %

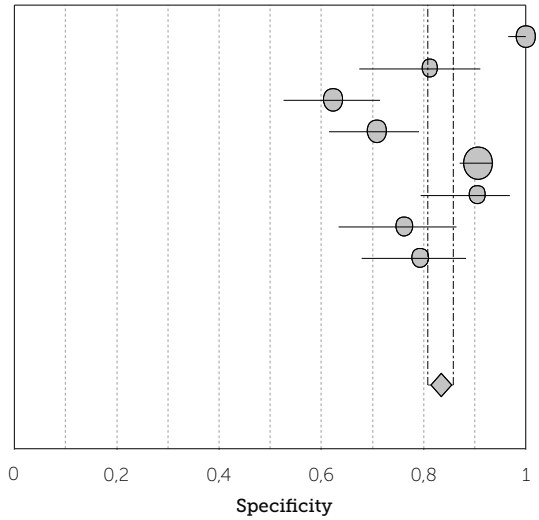
Diamond, pooled estimate; unbroken line; 95% confidence interval. Sensitivity pooled by fixed-effect model (Mantel-Haenszel), Specificity pooled by random effect model (DerSimonian-Laired)

Figure 4 | Forest plot and pooled estimate of DS_{susp}.
Sensitivity for DS_{susp} was defined as the proportion of women with suspect findings at ductoscopy among all women diagnosed with malignancy at histology or follow-up. Specificity of DS_{susp} was defined as the proportion of women with non-suspect ductoscopy among all women with benign outcome of histology or follow-up.



	Sensitivity (95% CI)	
Bender et al 2009	1,00	(0,03 - 1,00)
Denewer et al 2008	0,33	(0,04 - 0,78)
Dietz et al 2002	0,00	(0,00 - 0,71)
Fisher et al 2011	0,67	(0,30 - 0,93)
Kamali et al 2014	0,57	(0,34 - 0,78)
Moncrief et al 2005	0,17	(0,00 - 0,64)
Simpson et 2009	0,67	(0,09 - 0,99)
Waaijer et al 2015	0,67	(0,09 - 0,99)

Pooled Sensitivity = 0,50 (0,36 to 0,64)
Chi-square = 11,26; df = 7 (p = 0,1275)
Inconsistency (I-square) = 37,9 %



	Specificity (95% CI)	
Bender et al 2009	1,00	(0,96 - 1,00)
Denewer et al 2008	0,81	(0,67 - 0,91)
Dietz et al 2002	0,62	(0,53 - 0,71)
Fisher et al 2011	0,71	(0,61 - 0,79)
Kamali et al 2014	0,91	(0,87 - 0,94)
Moncrief et al 2005	0,91	(0,79 - 0,97)
Simpson et 2009	0,76	(0,63 - 0,86)
Waaijer et al 2015	0,79	(0,68 - 0,88)

Pooled Specificity = 0,83 (0,81 to 0,86)
Chi-square = 94,92; df = 7 (p = 0,0000)
Inconsistency (I-square) = 92,6 %

Diamond, pooled estimate; line; 95% confidence interval. Sensitivity and specificity pooled by random effect model (DerSimonian-Laired).

Table 1 | Study characteristics

Study (year)	Country	Study period	Study population	Study Design	Ductoscope size (manufact)	Reference standard	Composite reference standard
Surgical reference studies							
Fisher et al. (2011) [33]	USA	2006-2007	PND	Retrospective	0.9 mm (Acuity)	Microdochectomy	NA
Hahn et al. (2009) [61]	Germany	2007-2008	PND without abnormal imaging findings amenable to biopsy	Prospective	0.9-1.1 mm (Karl Storz)	Microdochectomy	NA
Denewer et al. (2008) [22]	Egypt	2005-2006	PND without palpable lesions or imaging abnormalities	Prospective	NM	Microdochectomy	NA
Dietz et al.(2002) [24]	USA	1999-2001	PND	Retrospective	0.9-1.2 mm (Acuity)	Microdochectomy	NA
Moncrief et al.(2005) [26]	USA	1996-2003	PND	Retrospective	0.9 mm (Acuity)	Microdochectomy	NA
Simpson et al.(2009) [35]	Canada	2004-2008	PND	Prospective	0.7 mm (FiberTech)	Microdochectomy	NA
Deshmane et al.(2010) [36]	India	2004-2007	PND undergoing DS and DS-directed surgery	Retrospective	1.1 mm (NM)	Microdochectomy	NA
Dooley et al.(2002) [37]	USA	2000-2001	PND	NM	0.9 mm (Acueity)	Microdochectomy	NA
Ohlinger et al.(2014) [38]	Germany	2006-2009	PND	Prospective	0.9 mm (Karl Storz)	Microdochectomy	NA
Tekin et al.(2009) [39]	Turkey	2002-2007	PND without imaging abnormalties	NM	1 mm (Karl Storz)	Microdochectomy	NA
Albrecht et al.(2013) [40]	Germany	2002-2006	PND and / or abnormal diagnostic findings	Retrospective	0.55-1.30 mm Karl Storz and PolyDiagnost	Open biopsy or microdochectomy	NA
Beechey-Newman et al.(2005) [62]	England	NM	PND undergoing diagnostic surgery	Prospective	0.95-1.1 mm (Polydiagnost)	Microdochectomy or major duct excision	NA
Sauter et al.(2005) [42]	Canada	NM	PND with/without (suspect) imaging abnormalities	Prospective	0.9 mm (Acueity)	NM	NA

Selective follow-up studies							
Khan et al.(2011) [43]	USA	2005-2009	PND	Prospective	0.9 mm (Acueity)	Polypoid lesion: microdochectomy	No intraductal lesion: FU
Kamali et al.(2014) [44]	Turkey	2005-2010	PND	Retrospective	0.55-1.1 mm (Polydiagnost)	- Polypoid lesion: DP - Unsuccessful DP and/ or positive cytology: microdochectomy	Negative DS AND cytology: FU
Fackler et al.(2009) [45]	USA	NM	PND or atypical lavage cytology*	Prospective	0.9 mm (Acuity)	Positive DS: microdochectomy	Negative DS: FU
Bender et al.(2009) [46]	Turkey	2006-2008	PND	Prospective	0.55-1.1 mm (Polydiagnost)	Positive cytology: microdochectomy Non-sessile polypoid lesions: DP Sessile polypoid lesions/ MPs and negative cytology: microdochectomy	Negative DS AND negative cytology: FU
Liu et al.(2008) [47]	China	1997-2005	PND	NM	0.72 mm (Fujikura Co.) 0.45-0.8 mm (Polydiagnost)	Abnormal DS, positive cytology, clinical/ radiological abnormalities: microdochectomy or lumpectomy	Negative DS, cytology AND imaging: FU
Waaijer et al.(2015) [11]	Netherlands	2010-2014	PND, BIRADS ≤ 3 or histologically proven benign	Prospective	1.15 mm (Polydiagnost)	- Suspect DS or patient's request: microdochectomy or major duct excision - Non-sessile polypoid lesions: DP	Non-suspicious findings or papillomas: FU
Matsunaga et al.(2009) [48]	Japan	1993-2007	PND without specific findings on X or US**	NM	0.72 mm (FiberTech)	Surgery or intraductal biopsy	No surgery or biopsy: FU

Manufact: manufacturer, DS: ductoscopy, MP: multiple papilloma, PND: pathologic nipple discharge, NM: Not mentioned, FU: Follow-up, DP: ductoscopic papillomectomy. * Women of the Program for Breast Cancer Early Detection and Prevention, if they had symptoms of PND or atypical lavage cytology. ** Indication for DS was based on cytologic and galactographic findings, which were not described in further detail

Table 2 | Patient characteristics and outcome

Study	Age, years (range)	Study size, no ¶	Successful cannulation, no (%)	Histology acquired, no (%)	Follow-up, no (%)	Missing reference, no (%)	Papilloma, no (%)	Malignancy$^{\Phi}$ at initial histology, no (%)	Malignancy$^{\Phi}$ at FU, no (%)	Total malignancy$^{\Phi}$, no (%)***
Surgical reference studies										
Fisher et al. (2011) [33]	‡ 48 (22-80)	121	119 (98)	121 (100)	0 (0)	0 (0)	64 (53)	7 (6)	NA	7 (6)
Hahn et al. (2009) [61]	† 51.7 (20-71)	33	33 (100)	33 (100)	0(0)	0 (0)	22 (67)	2 (6)	NA	2 (6)
Denewer et al. (2008) [22]	† 46 (20-75)	54	54 (100)	54 (100)	0(0)	0 (0)	18 (33)	6 (11)	NA	6 (11)
Dietz et al.(2002) [24]	† 52 (14-82)	121*	105 (88)#	121 (100)	0(0)	0 (0)	83 (69)	5 (4)	NA	5 (4)
Moncrief et al.(2005) [26]	† 48.7 (28–83)	68*	59(87)*	68 (100)	0(0)	0 (0)	37 (54)	6 (9)	NA	6 (9)
Simpson et al.(2009) [35]	(25-76)	65	63 (95)*	62 (95)	0(0)	3 (5)	52 (80)	3 (5)	NA	3 (5)
Deshmane et al.(2010) [36]	NM	26	24 (92)	24 (92)	0(0)	2 (8)	11 (42)	7 (27)	NA	7 (27)
Dooley et al.(2002) [37]	(18-27)	27	26 (96)	26 (96)	0(0)	1 (4)	NM	2 (7)	NA	2 (7)
Ohlinger et al.(2014) [38]	† 52.2 (19-86)	247	214 (87)	214 (87)	23 (9)	33 (13)	110 (45)	11 (4)	NA	11 (4)
Tekin et al.(2009) [39]	† 45.3 (17-71)	34	30 (88)	20 (59)	0 (0)	14 (41)	9 (26)	3 (9)	NA	3 (9)
Albrecht et al.(2013) [40]	† 53.3 (21-78)	100*	98*(98)	100*(100)	0(0)	0 (0)	44 (44)	11 (11)	NA	11 (11)
Beechey-Newman et al.(2005) [62]	NM	50	50 (100)	33 (66)	0(0)	17 (34)	18 (36)	0 (0)	NA	0 (0)
Sauter et al.(2005) [42]	† 48.4 (25-81)	40	40 (100)	39 (98)	0(0)	1 (3)	18 (45)	3 (8)	NA	3 (8)
TOTAL‡			**94%**	**98%**	**0%**	**3%**	**45%**	**7%**		**7%**

Table 2 | continued

Selective follow-up studies										
Khan et al.(2011) [43]	† 47.5	59	50 (85)	38 (64)	19 (32)	2 (3)	24 (41)	6 (10)	0 (0)[a]	6 (10)
Kamali et al.(2014) [44]	‡ 42.7 (14-83)	456*	383* (84)	157 (34)	196 (43)	103 (23)	97 (21)	21 (5)	0 (0)[b]	21 (5)
Fackler et al.(2009) [45]	† 48 (26-75)	64**	NM	36 (56)	28 (44)	0 (0)	27 (42)	7 (11)	0 (0)[c]	7 (11)
Bender et al.(2009) [46]	‡ 49.2 (24-80)	126	102 (81)	32 (25)	71 (56)	24 (19)	30 (24)	1 (1)	0 (0)[d]	1 (1)
Liu et al.(2008) [47]	‡ 43 (13-80)	1093*	NM	436 (40)	489 (45)	168 (15)§	232 (21)	51 (5)	1 (0)[e]	52 (5)
Waaijer et al.(2015) [11]	† 47 (20-72)	82	71 (87)	36 (44)	45 (55)	1 (1)	23 (28)	3 (4)	1 (1) [f]	4 (5)
Matsunaga et al.(2009) [48]	NM	323	323 (100)	235 (73)	88 (27)	0 (0)	155 (48)	80 (25)	NM[g]	80 (25)
TOTAL‡			**85%**	**44%**	**44%**	**3%**	**28%**	**5%**		**5%**

Values are in number of patients with percentages in parentheses unless indicated otherwise; † outcome presented in mean ‡ outcome presented in median..
¶ In number of patients in whom ductoscopy was performed, including unsuccessful cannulation. * = Outcome reported in number of ductoscopies. ** = Outcome reported in number of ducts. ᵠ Malignancy: invasive carcinoma or ductal carcinoma in situ (DCIS). *** Total malignancy: malignancy outcome at histology and FU. # Cannulation was given in number of patients, whereas the other rates of this study are reported in number of ductoscopies. § all 157 patients with positive ductoscopic findings were lost to follow-up. IQR: Interquartile range. NM: not mentioned. NA: not applicable. PE: physical examination. X: mammography. US: ultrasound. [a] FU by repeat PE at 3, 6, 12 months, X at 12 months, followed by annual PE and X. Median FU 38 months (range, 7-63 months). [b] FU by PE, US, X and other diagnostic procedures. [c] minimum FU of 2 years. [d] FU duration NM. [e] mean FU 19 months. [f] median follow-up of 17 months (range, 3-45 months). [g] minimum FU of 5 years.

In selective follow-up studies, patients were selected for follow-up based on negative ductoscopy [11,43,45,48]. In 3 studies negative cytology of either NAF and/or ductal lavage was a second prerequisite [44,46,47].

Twenty studies included in the systematic review reported on 3189 ductoscopies in 3144 patients with PND. The number of analyzed ductoscopies in each study ranged from 27 to 1093 (Table 2). Cannulation success rate ranged from 88 to 100%. Malignancy rates ranged from 0 to 27%. Papillomas, calculated only from surgical reference studies, ranged from 26 to 80%.

Twelve studies including 1994 ductoscopies were included in the meta-analysis. In total, 151 (7.6%) malignancies were found. The respective pooled sensitivity and specificity of DS_{any} were 94% (95% CI, 88–97%) and 47% (95% CI, 44–49%), respectively (Figure 3). Statistical analyses of heterogeneity revealed I^2 = 17.5% and I^2 = 96.8%, respectively. Eight studies with 933 ductoscopies including 52 (5.6%) malignancies were eligible for the DS_{susp} analysis (Supplemental 3) [11,22,24,26,33,35,44,46]. Pooled sensitivity and specificity of DS_{susp} were 50% (95% CI, 36–64%) and 83% (95 CI, 81–86%) respectively (Figure 4). Heterogeneity analyses indicated degrees of between-study heterogeneity for the pooled sensitivity (I^2 = 37.9%) and for the pooled specificity (I^2 = 92.6%).

Stratified analysis (surgical reference versus selective follow-up)

Studies were stratified by differential outcome (surgical reference versus selective follow-up). Follow-up reference ranged from a median of 17 months to a minimum follow-up of 5 years (Table 2). Median successful cannulation in selective follow-up studies was 85% (range, 81-100%) versus 94% in the surgical reference studies (range, 87-100%). In selective follow-up studies histology was acquired in a median of 44% (range, 25-73%) and follow-up in a median of 44% (range, 27-56%) of all patients. In surgical reference studies histological outcome was acquired in a median 98% (range, 59-100%).

Malignancy was diagnosed in a median of 7.0% (range, 0-27%) of all patients in surgical reference studies. In selective follow-up studies malignancy was found in a median of 5.0% (range, 1-25%) of all patients. Most malignancies in the selective follow-up studies (median 4.5%, range 1-25%) were diagnosed at initial histology.

Stratified analysis did not modify the sensitivity of DS_{any}. Pooled sensitivity of surgical reference studies was 100% (95% CI, 90-100 %, I^2 = 0.0%) versus 91% of selective follow-up studies (95% CI, 83-96 %, I^2 = 37.5%). In contrast, pooled specificity of surgical reference studies was 12% (95% CI 9-16%, I^2 = 79.2%) versus 56% (95% CI, 53-58%, I^2 = 89.6%) in selective follow-up studies.

DISCUSSION

This is, to our knowledge, the first systematic review and meta-analysis evaluating diagnostic accuracy of ductoscopy in patients with PND. The search yielded 4542 original citations of which twenty studies were eligible for the review, including a total of 3144 patients. Meta-analysis classifying any intraductal lesion visualized by ductoscopy as a positive finding (DS_{any}) was possible in twelve studies, including a total of 1994 cases, and resulted in a pooled sensitivity of 94% (95% CI, 88–97%) and specificity of 47% (95% CI, 44–49%). In order to find the optimal cut-off point a second classification scale was analyzed, in which only suspicious ductoscopic findings were classified as a positive finding (DS_{susp}). For this analysis a total of eight studies including 933 cases were eligible, resulting in pooled sensitivity and specificity of 50% (95% CI, 36–64%) and 83% (95% CI, 81–86%), respectively. Statistical investigation revealed that heterogeneity in both DS_{any} and DS_{susp} was moderate to large for sensitivity and very large for specificity. Although ductoscopy was capable of visualizing about 94% of lesions, the specificity was low. The cut-off point assessed in the DS_{susp} classification resulted in increased specificity at the cost of unacceptably low sensitivity. These results imply that, in order to exclude malignancy in patients with PND and positive ductoscopy, histological diagnosis remains necessary. To minimize the number of surgical interventions, acquiring histologic tissue is preferably done endoscopically. Currently, tissue collection of polypoid lesions can be performed with a commercially available 'grabbing' biopsy clip or a so-called 'basket' device (Polydiagnost), resulting in reliable histopathological diagnosis [11,49,50]. Unfortunately this device is not available for the USA market. Intraductal needle biopsy devices (e.g. JN Biopsy Needle, Hakko Company, Tokyo, Japan) can be used for flat epithelial lesions and have been used in research, but are relatively short (45.5 mm) and moreover not commercially available in neither Europe nor the USA [48,51]. For intraductal lesions in which endoscopic tissue diagnosis is not possible, surgery therefore remains inevitable. Due to the high sensitivity and the low incidence of malignancy in patients with PND, the negative predictive value of DS_{any} ranged from 98 to 100%. We therefore conclude that surgery can be avoided in case of negative ductoscopy. The malignancy rate of the twenty included studies ranged from 0 to 27%, but only two studies demonstrated malignancy rates above 11% [36,48]. These studies both scored high regarding risk of bias and applicability concerns (Figure 2, Supplemental 2). Deshmane et al. included 26 patients with PND without mentioning previous diagnostic work-up [36].

Matsunaga et al. included patients with bloody nipple discharge without specific findings on mammography or ultrasound, but used cytology and galactography as an indication for ductoscopy, without further defining these indications[48]. As cytology and galactography in PND have reported low sensitivity with higher specificity[2], the high malignancy rates of the two discussed studies are most likely based on the selection of included patient population, which is possibly not applicable to our research question.

To investigate this possible distorted selection of patients, this review also included studies using follow-up as a reference standard in selected patients (selective follow-up studies). Selective follow-up was described for patients with negative ductoscopic (and cytological) findings or in patients with endoscopically acquired histological tissue diagnosis of benign intraductal papillomas. Stratified analysis of the two differential reference designs (surgical reference versus selective follow-up) showed a difference in cannulation rate, which indicates a possible selection bias in the studies describing surgical reference in all patients. No difference in median malignancy rate was found (7.0% in surgical reference versus 5.0% in selective follow-up studies). The selective follow-up studies showed low rates of malignancy in the conservative cohort. These findings suggest that the used selection criteria in the selective follow up studies might indeed be capable of triaging patients for surgery or conservative treatment. Stratified analysis showed an increased specificity in the selective follow up studies of 56% versus 12% in surgical reference studies. Since the overall rate of diagnosed malignancies is comparable, missed malignancies in the follow-up group are not likely. In the study design where selected criteria will lead to surgery (i.e. selective follow-up studies) the physician might use this criterion with more caution, leading to a relatively high rate of false-positives in the surgical studies where all patients undergo surgery.

The main limitation of this review is the heterogeneity in interpretation of the index test (ductoscopy) between the different studies. The used morphological category scales vary and are likely to be subjective and observer-dependent. This impairs the comparison and pooling of the different studies. Due to this wide range or variability we have analyzed two arbitrary choices for cut-off values of positive ductoscopy, DS_{any} and DS_{susp}. We suggest to classify endoscopic appearances according to one universal scale, as has been proposed and validated previously; the superficial type, polypoid solitary type, polypoid multiple type, and combined type [29,30].

Another limitation is the poorly reported information on inclusion methods, prior diagnostic work-up and on patient characteristics, raising concerns regarding applicability and hampering quality assessment and data extraction (Figure 2). Despite this, only two studies showed outliers concerning malignancy prevalence [36,48].

For this study, only DCIS and invasive breast cancer were classified as positive (malignant) outcome. As a consequence, diagnoses of atypical ductal hyperplasia, atypical lobular neoplasia or papilloma (with or without atypia) were left out of the analysis, despite their impact regarding breast cancer risk. However, the risk and management of these findings when visible on conventional imaging is not unambiguous. The long-term risk of such lesions without concurring abnormalities on imaging remains to be clarified.

Evaluation of other diagnostic techniques was not an aim of this review. Previous studies in women with PND have shown that radiological investigations, including ductography and MRI, lack sensitivity [4,15,16]. The cytological examination of nipple fluid has proven to be unhelpful [52], although Liu et al. reported slight improvement in diagnostic performance of ductoscopy when cytological findings were included [47]. Also, ductal lavage fails to yield adequate specimens for reliable cytological diagnosis [53]. However, the use of proteomic biomarkers in serum or methylation in nipple aspiration fluid or ductal lavage appears to present a promising alternative [45,54–58]. Also imaging enhancement techniques such autofluorescence might assist in optimization of diagnostic accuracy [59,60].

By the introduction of transductal intervention devices, ductoscopy may not only be used as a diagnostic technique but also to attain symptom resolution [11,50]. In this way, ductoscopy may be able to lead to a reduction in surgical procedures for a benign cause or for symptom resolution. As an invasive procedure can be omitted, both patient and hospital costs might benefit.

CONCLUSIONS

This systematic review and meta-analysis demonstrates that ductoscopy has very high sensitivity but low specificity in diagnosing malignancy in patients with PND. Recognizing the limitations of our systematic review and meta-analysis, we conclude that ductoscopy can be used for triage to minimize the need for invasive and expensive surgery. Analysis of different study designs showed that surgery may be safely avoided in case of negative ductoscopy or endoscopically acquired benign histology. However, with current diagnostic accuracy of ductoscopy and the number of underlying malignancies, acquiring histology of visualized intraductal lesions, either endoscopically or surgically, remains inevitable. Future research and investment should focus on additional techniques, such as micro-endoscopic biopsy devices, to improve diagnostic accuracy.

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Supplemental 1 | Systematic search

BOX 1 | Search Syntaxes

Syntax "Medline"
((((ductoscopic[Title/Abstract]) OR ductoscopy[Title/Abstract]) OR ductoscope[Title/Abstract])) OR ((((((((nipple[Title/Abstract]) OR breast[Title/Abstract]) OR mammary[Title/Abstract])) OR breast[MeSH Terms]) OR breast cancer[MeSH Terms])) AND ((((((((((((((endoscopic[Title/Abstract]) OR endoscopy[Title/Abstract]) OR natural orifice transluminal endoscopy[Title/Abstract]) OR NOTE[Title/Abstract]) OR micro-endoscopy[Title/Abstract]) OR microendoscopy[Title/Abstract]) OR micro-endoscopic[Title/Abstract]) OR microendoscopic[Title/Abstract]) OR micro-endoscope[Title/Abstract]) OR microendoscope[Title/Abstract]) OR endoscope[Title/Abstract])) OR natural orifice transluminal endoscopy[MeSH Terms]) OR endoscopy[MeSH Terms]) OR endoscope[MeSH Terms]))

Syntax "Embase"
ductoscopic:ab,ti OR ductoscopy:ab,ti OR ductoscope:ab,ti OR (nipple:ab,ti OR breast:ab,ti OR mammary:ab,ti OR 'breast cancer':ab,ti AND (endoscopic:ab,ti OR endoscopy:ab,ti OR 'natural orifice transluminal endoscopy':ab,ti OR note:ab,ti OR 'micro endoscopy':ab,ti OR microendoscopy:ab,ti OR 'micro endoscopic':ab,ti OR microendoscopic:ab,ti OR 'micro endoscope':ab,ti OR microendoscope:ab,ti OR endoscope:ab,ti)) AND [embase]/lim NOT [medline]/lim

Syntax "Cochrane"
ductoscopic OR ductoscopy OR ductoscope OR ((nipple OR breast OR mammary OR "breast cancer") AND (endoscopic OR endoscopy OR "natural orifice transluminal endoscopy" OR note OR "micro endoscopy"OR microendoscopy OR "micro endoscopic" OR microendoscopic OR "micro endoscope" OR microendoscope OR endoscope))

CH 05

Search syntax for ductoscopy in the Pubmed database, Embase database and Cochrane library.

Supplemental 2a | Quality assessment of the included studies

QUADAS-2 Tool Signaling Questions [28]

A. Evaluation of bias

1. Patient selection – Could the selection of patients have introduced bias?
RISK: LOW/INTERMEDIATE/HIGH/UNCLEAR
- a. Was a consecutive or random sample of patients enrolled?
- b. Was a case-control design avoided?
- c. Was selection bias avoided by including patients with pathologic nipple discharge and comparable previous diagnostic work-up?

2. Index test (ductoscopy) – Could the conduct or interpretation of the index test have introduced bias?
RISK: LOW/INTERMEDIATE/HIGH/UNCLEAR
- a. Was the ductoscopy outcome interpreted without knowledge of the histologic outcome?
- b. Was the ductoscopic visual scoring system specified?

3. Reference standard (histology or follow-up in a defined selection of patients) – Could the reference standard, its conduct or its interpretation have introduced bias?
RISK: LOW/INTERMEDIATE/HIGH/UNCLEAR
- a. Was histology used as a reference standard?
- b. When histology was not used as a reference standard in all patients, was follow-up performed in the other group of patients?

4. Flow and timing
RISK: LOW/INTERMEDIATE/HIGH/UNCLEAR
- a. Was the ductoscopy performed within one month before histology?
- b. Did all patients receive a reference standard?
- c. Were all patients included in the analysis?

B. Evaluation of applicability

1. Patient selection – Is there concern that the included patients do not match the review question?
CONCERN: LOW/INTERMEDIATE/HIGH/UNCLEAR
- a. Was the patient group studied matching with the review question?
- b. Were patients included before they were referred for surgery, and not when they were already referred for surgery?

2. Index test (ductoscopy) – Is there concern that the index test, its conduct, or interpretation differ from the review question?
CONCERN: LOW/INTERMEDIATE/HIGH/UNCLEAR
- a. Was the same interpretation of visual findings used in every patient?
- b. Was ductoscopy performed by an experience operator?

3. Reference standard (histology or follow-up) – Is there concern that the target condition as defined by the reference standard does not match the review question?
CONCERN: LOW/INTERMEDIATE/HIGH/UNCLEAR
- a. Was histology or follow-up in a defined selection of patients used as reference standard?

Supplemental 2b | Quality assessment of the included studies

The risk of bias for the included articles and the level of concern regarding the applicability of included articles are shown.

Study (Ref)	Year of publication	Risk of bias				Applicability concerns		
		Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Fisher et al. [33]	2011	☺	☺	☺	☺	☺	☺	☺
Hahn et al. [61]	2009	☺	☺	☺	☺	☺	☺	☺
Denewer et al. [22]	2008	☺	☺	☺	☺	☺	☺	☺
Dietz et al. [24]	2002	☺	☺	☺	☺	☺	☺?	☺
Moncrief et al. [26]	2005	☺	☺	☺	☺	?	☺	☺
Simpson et al. [35]	2009	☺	☺	☺	☺	☺?	☺?	☺
Deshmane et al. [36]	2010	☹	?	☺	☺	?	?	☺
Dooley et al. [37]	2002	☺	?	☺	?	☺	☺?	☹
Ohlinger et al. [38]	2014	☺	☹	☺	☺?	?	☹	☺
Tekin et al. [39]	2009	☺	☺?	?	☹	☺?	☹	☹
Albrecht et al. [40]	2013	☹	☹	☺	☹	☹	?	?
Beechey-Newman et al. [62]	2005	?	?	☺	?	?	?	?
Sauter et al. [42]	2005	?	☺	☺?	?	☹	☺?	?
Khan et al. [43]	2011	☺	☺	☺	☺	☺	☺	☺
Kamali et al. [44]	2014	☺	☺	☺	☹	☺?	☺	☺?
Fackler et al. [45]	2009	☹	☺	☺?	?	☹	☺?	?
Bender et al. [46]	2009	☺?	☺	☹	?	☺?	☺	☹
Liu et al. [47]	2008	☺	?	☺	☹	?	?	☺?
Waaijer et al. [11]	2015	☺	☺	☺	☺	☺	☺	☺
Matsunaga et al. [48]	2009	☺	☺	?	☹	☹	?	?

Ref, reference. ☺ = low risk. ☺? = intermediate risk. ☹ = high risk. ? = unclear risk.

Supplemental 3 | Categories of index test (ductoscopy) and reference standard (histology) and re-classification used for analysis of sensitivity and specificity of DS_{any} and DS_{susp} .

Study	Index Test Categories	§DS_{any}	#DS_{susp}	Reference Standard Categories	Malignancy
Fisher et al. (2011) [33]	inflammatory changes	+	+	malignant (DCIS, ADH, LCIS)*	+
	debris or web	+	+	papillomas	
	papilloma	+		benign or ductectasia	
	normal				
Denewer et al. (2008) [22]	ductal thickening	+	+	invasive carcinoma	+
	papillary lesions	+		DCIS	+
	normal			ADH	
				atypical papilloma	
				papilloma	
				hyperplasia	
				normal	
Dietz et al.(2002) [24]	obliterated duct	+	+	invasive carcinoma	+
	papilloma	+	+	DCIS	+
	debris, web, discharge	+	+	ADH	
	papillary lesion	+		papilloma	
	narrow lumen	+		hyperplasia	
	narrow lumen / bleeding	+		benign non-proliferative	
	normal				
Moncrief et al.(2005) [26]	injected	+	+	cancer	+
	red patches	+	+	ADH	
	fronds	+	+	papilloma	
	papilloma	+		hyperplasia	
	narrow	+		non proliferative	

Supplemental 3 | continued

Simpson et al.(2009) [35]	superficial spreading	+	+	invasive ductal carcinoma	+
	difficult to classify	+	+	DCIS	+
	solitary polypoid	+		ADH	
	multiple polypoid	+		papilloma with atypical hyperplasia	
	combined	+	+	papilloma	
	normal			no papilloma, no atypia	
Deshmane et al.(2010) [36]	abnormal	+	NP	invasive carcinoma	+
	normal			DCIS	+
				papilloma	
Khan et al.(2011) [43]	significant finding	+	NP	DCIS	+
	minimal finding			papilloma	
				hyperplasia	
				normal or non-proliferative	
Kamali et al.(2014) [44]	normal (incl debris)			invasive carcinoma	+
	ductal calibration changes	+		DCIS	+
	ductal epithelial color differences	+		ADH	
	wall irregularities (incl intraluminal irregular mass)	+	+	papillary	
	solitary papilloma	+		benign	
	multiple papilloma	+			
	other	+			
Fackler et al.(2009) [45]	significant findings (papillomatous growths)	+	NP	malignant	+
	insignificant abnormalities			benign	

Supplemental 3 | continued

Bender et al.(2009) [46]	solitary papilloma	+		DCIS	+
	multiple papillomas	+		papilloma with ADH	
	epithelial surface abnormality	+	+	papilloma	
	debris **	-			
	normal				
Liu et al.(2008) [47]	intraductal abnormalities	+	NP	malignancy (incl DCIS)	+
	Negative (incl. normal or minimal findings)			ADH	
				papilloma	
				hyperplasia	
				fibrocystic or ductectasia	
				hematoma	
				cysts	
Waaijer et al.(2015) [11]	epithelial (suspect / nonsuspect)	+	+	invasive carcinoma	+
	polypoid	+		DCIS	+
	miscellaneous (fibrotic duct or debris)	+		papilloma	
	negative			benign	
				normal	

§DS; considered a positive ductoscopic finding in the analysis. #DS_{susp}: considered a positive ductoscopic finding in the threshold-analysis. *LCIS was not separately described and was also included in "malignancies". ** Debris was considered 'inconspicuous' and was treated by FU.

CHAPTER 06

Interventional ductoscopy reduces the amount of surgical procedures in patients with pathologic nipple discharge

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ABSTRACT

Background | For definitive diagnosis and treatment in women with pathologic nipple discharge (PND), surgery is the intervention of choice. Ductoscopy has been reported to improve diagnostics, but as an interventional procedure it may also reduce the need for surgery. This study evaluated the therapeutic efficacy of ductoscopy in patients with PND.

Methods | A prospective study on ductoscopy was conducted in 82 consecutive patients with PND, not suspect for malignancy on routine diagnostic workup. Intraductal lesions were removed by ductoscopic extraction. Surgery was performed in case of suspect ductoscopic findings or upon patient's request. Therapeutic efficacy was determined by cannulation success, detection and removal rates, symptom resolution and surgery avoided.

Results | Ductoscope introduction was successful in 71 (87%) patients, with abnormalities visualized in 53 (65%), being mostly polypoid lesions (*n* = 29). In 27 of the 34 attempted ductoscopic extractions, the lesion could be removed. Twenty-six (32%) patients underwent surgery, in 56 (68%) surgery was avoided. Forty (49%) of these patients no longer experienced symptoms of PND after median follow-up of 17 months (range, 3-45 months), 14 (17%) experienced insufficient therapeutic effect and in 2 (2%) the outcome was unknown. (Pre)malignancy was diagnosed in 5 (6.1%) patients; 2 had been missed at ductoscopy and 2 at initial surgery after ductoscopy.

Conclusions | Interventional ductoscopy is technically feasible and may help to avoid surgery in the majority of patients. Since endoscopic removal of intraductal lesions is not always possible and malignancies can be the underlying cause of PND, optimization of ductoscopic instruments remains warranted.

INTRODUCTION

Pathologic nipple discharge (PND) is defined as spontaneous, unilateral bloody or serous discharge during non-lactational period and is responsible for approximately 5% of surgical referrals to the breast clinic[1]. As PND may be associated with breast cancer, numerous women with PND undergo exploratory surgery, such as major duct excision or microdochectomy to rule out malignancy and to treat symptoms. However, the vast majority of these women will undergo this invasive procedure for a benign cause since evaluation of patients with PND without imaging abnormalities renders a diagnosis of cancer in only 3-6% of the cases[2–5]. Higher risk of carcinoma up to even 23% has been reported, but these studies included merely patients undergoing surgery or patients with malignant findings on imaging[5–8].

Most women will therefore undergo invasive surgery with concomitant risk of complications and effects on cosmesis, breast-feeding and sensitivity of the nipple for a benign cause, being mostly (57-69%) papilloma[2,9,10]. Furthermore, lesions are frequently found at some distance from the nipple orifice[9,11]. As a consequence, blind surgical excision may lead to non-removal of peripheral lesions[5]. Cytology of nipple aspiration fluid has been proposed as a preoperative diagnostic tool, but predictive values are disappointing (i.e. positive predictive value of 50-55% and a negative predictive value of 76-76.5%)[12,13].

Ductoscopy, a minimally invasive intraductal approach enabling direct access to the ductal system via the nipple[14–16], has proven to be a useful tool in the diagnostic evaluation of patients with nipple discharge[17]. Moncrief et al. identified 97.3% of pathologically diagnosed papillomas at endoscopy[9].

With improving imaging qualities and addition of a third working channel next to channels for optics and irrigation, the first interventional ductoscopy was developed. In order to improve diagnostic accuracy, intraductal biopsy tools[18–20] and a 'basket'-intervention device was introduced to acquire tissue and remove the lesion under direct vision[21–23]. Bender et al. demonstrated ductoscopy to be a promising tool, by showing that, in a cohort of 22 solitary papillomas, 21 could be successfully removed endoscopically[22]. Studies regarding the overall and long-term therapeutic efficacy of this attractive minimally invasive approach for patients with PND were however lacking.

The aim of our study was, therefore, to evaluate therapeutic efficacy of intervention ductoscopy in patients with PND without suspicious imaging findings.

METHODS

Patients

We conducted a prospective cohort study of 82 consecutive patients who presented with PND to the University Medical Center Utrecht (UMCU), The Netherlands, between May 2010 and May 2014 (Figure 1). PND was defined as spontaneous, single duct nipple discharge during non-lactational period, persistent for more than 3 months. The appearance of the discharge was serous, sanguineous, or serosanguineous.

Prior to ductoscopy, standard diagnostic work-up was performed in all patients (Table 1), including a complete history and physical examination, recent (≤ 3 months) imaging (mammography, ultrasonography, and/or magnetic resonance imaging (MRI)), and/or core needle biopsy if indicated[24]. None of the patients were suspected for malignancy after diagnostic work-up. In 50 patients cytological evaluation of the nipple fluid had been performed by the referring hospital.

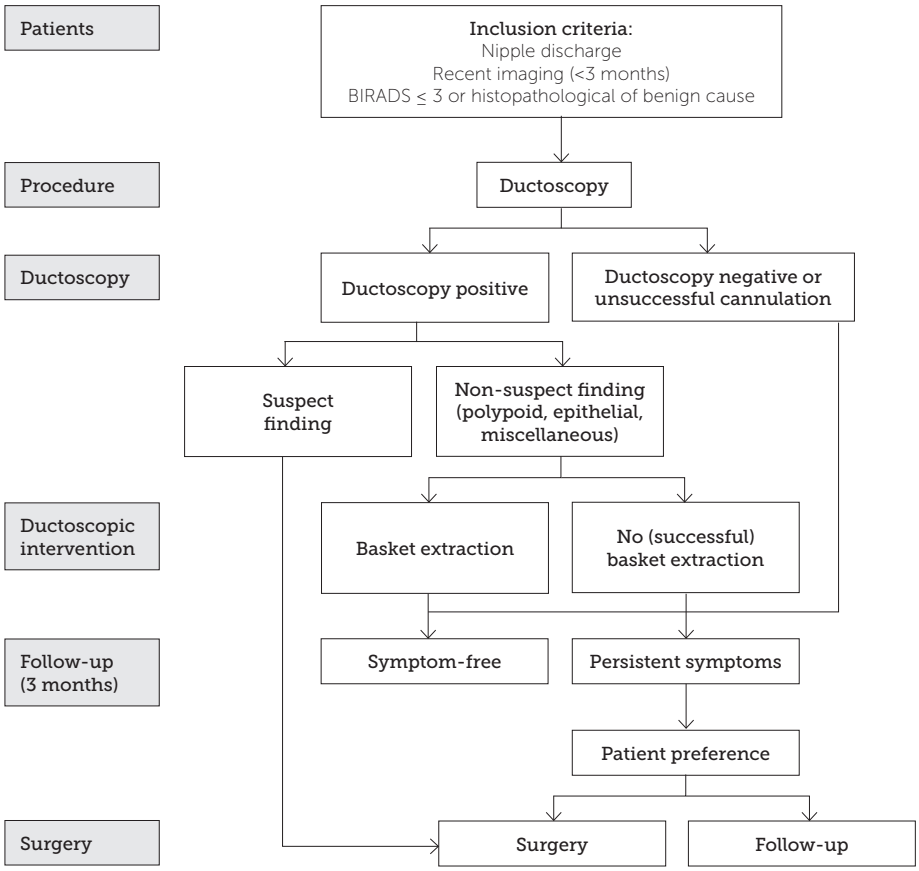
Patients with final assessment category according to the American College of Radiology Breast Imaging Reporting and Data System (BIRADS) ≤ 3, or histological proof of a benign cause underwent ductoscopy. Imaging and/or pathology performed elsewhere were revised by radiologists and pathologists at the UMCU.

Informed consent was obtained from all patients according to the Law on Medical Treatment Agreement (WGBO), following comprehensive information on content, expected course, potential complications/risks and prognosis of this treatment.

Ductoscopy system

The ductoscopic procedure was conducted in an outpatient setting in the UMCU. Endoscopy was performed using the 6000 pixels 0.55mm optic ("LaDuScope T-flex", Polydiagnost GmbH, Pfaffenhofen, Germany) inserted into a 1.15 mm outer diameter polyshaft (PD-DS-1015, Polydiagnost). This provides a separate irrigation channel for saline-infusion and a working channel accommodating ductoscopic extraction of papillary polypoid lesions, for which a grasping endobasket ("Saliva", 3-times-wrathed, diameter 380 µm) was used. The ductoscope had a working length of 80 mm, a 0° angle direct view and a field vision of 70°, and was gas sterilized.

Figure 1 | Chart of the study protocol



Cannulation

Prior to ductoscopy, the nipple and areola of the breast were disinfected with 70% ethanol. After periareolair local infiltration anaesthesia with 1% lidocaine a salivary duct probe (Karl Storz, Tuttlingen, Germany) size 0000 to 1 and an obturator (Polydiagnost) were used for dilatation of the productive lactiferous duct orifice in the nipple. Upward traction of the nipple was used for straightening of the subareolar ducts in order to facilitate cannulation. The introduction port (SoLex-Nipple-Expander®, Polydiagnost) was placed into the duct

orifice through which the ductoscope was introduced. Once this was achieved cannulation was considered successful.

Inspection of the ductal system

After initial intraductal infusion of 4 ml lidocaine, sterile saline was used for ductal distension. Endoscopic exploration was performed starting in the fluid-producing duct. The major lactiferous ducts and segmental branches were visualized in an orderly fashion until the scope could not be advanced any further. Images of ductal abnormalities were recorded. All ductoscopic procedures were performed by a single surgeon (AW), with an experience of >10 procedures prior to the start of this study[25].

A modified version of the previously proposed macroscopic classifications was used[26–28]. Lesions were classified as *polypoid, epithelial or miscellaneous* and any of these lesions was considered a positive finding at ductoscopy. *Polypoid* lesions were defined as localized expansive lesions, smooth surfaced without haemorrhage or atypical vessels. *Epithelial* lesions were defined as superficial spreading lesions, accompanied by no obvious elevations and mostly without haemorrhage or atypical vessels. Subtype is the *suspect epithelial* lesion, which was defined as fissured surfaced lesions being red, friable or displaying atypical vessels. The group of *miscellaneous* lesions included intraluminal debris and fibrotic tissue, defined as a sudden duct ending (blind-ending duct) without an extraductal pressing cause. When no intraductal abnormalities were found, ductoscopy was considered negative.

Histopathological diagnoses were divided into 1) *malignancies*, including (in situ) ductal and lobular carcinoma 2) *papilloma*, consisting of intraductal papilloma or papillomatosis and 3) *miscellaneous*, including benign epithelial lesions or fibrotic tissue, ductal hyperplasia, apocrine metaplasia, fibroadenomas, mastopathy, cysts, ductectasia or periductal fibrosis. An accepted positive result for pathology was the presence of any of these lesions in the, ductoscopically or surgically acquired, tissue specimen.

Interventional procedure

In patients with intraductal polypoid lesions, ductoscopic extraction was attempted. For this purpose, the basket was moved forward distally to the lesion, opened, rotated, and moved back to extirpate and remove the lesion. Removal of (a part of) the intraductal lesion

was considered successful (partial) ductoscopic extraction. Initial diameter and remnant diameter after basket extraction were estimated. Removal was considered complete when less than 10%, and partial when 10% or more lesion remained.

Pain assessment

For patients' evaluation of the procedure in the last 18 consecutive patients of this cohort, a paper questionnaire was used to asses pain intensity on a visual analogue scale (VAS) of 0 (no pain) to 10 (maximum pain)[29] for lidocaine infiltration anaesthesia, cannulation, and endoscopic examination of the ducts.

Surgery

All patients with suspicious epithelial lesions surgery were treated surgically. To all remaining patients surgery was offered (either targeted excision when visible on imaging or microdochectomy and/or major duct excision) and left up to the patient's discretion.

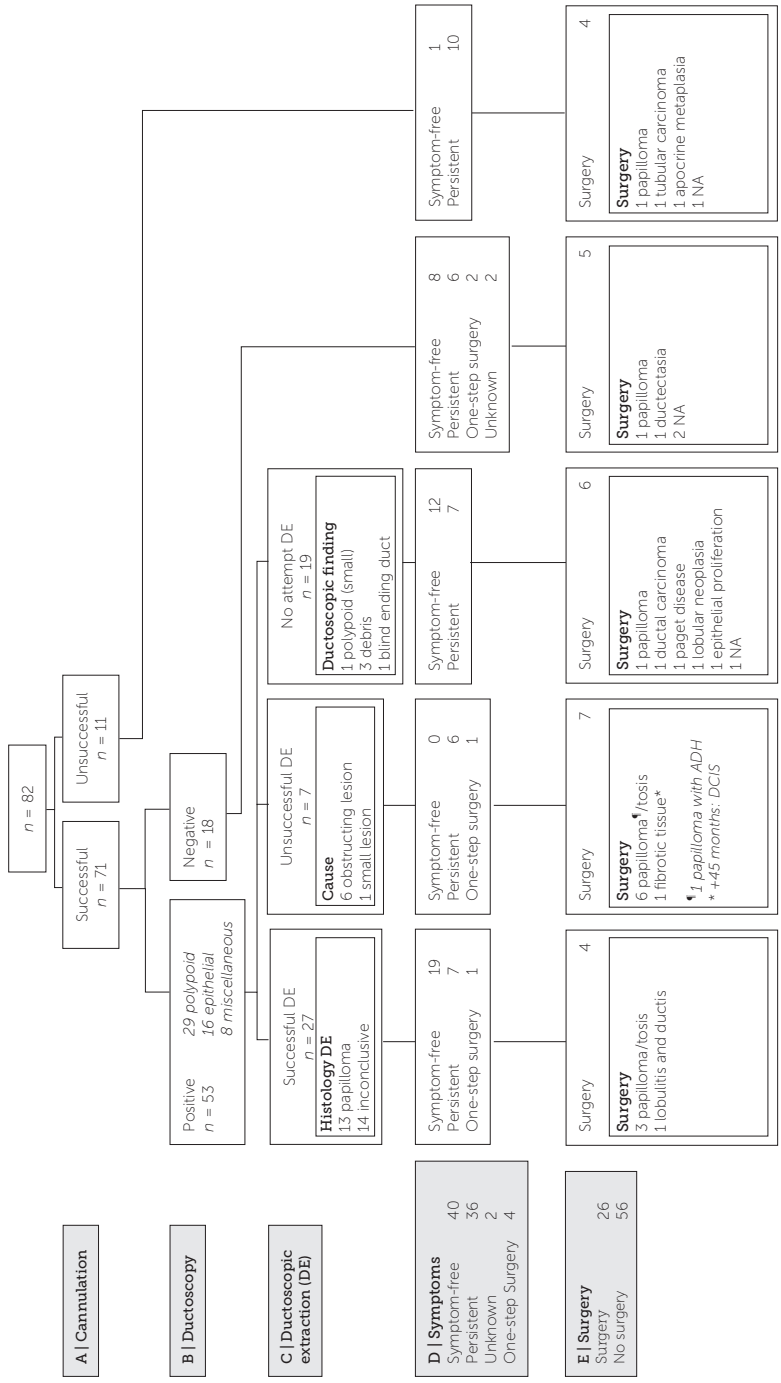
Follow-up

The effect of treatment on symptoms was evaluated in all patients during outpatient clinic visits or by telephone consultations after 2-4 weeks and subsequent follow-up in case of on-going symptoms or surgery. In May 2014 all patients were approached in order to acquire information on symptoms, if patients had undergone surgery elsewhere and occurrence of new lesions. Complications were ranked according to the Clavien-Dindo Classification[30].

Statistical analysis

Primary endpoint was therapeutic efficacy, determined by cannulation success, lesion detection and removal rates, symptom resolution and surgery avoided. All statistical analyses were performed using SPSS statistical software version 22.0. Normally distributed continuous variables were presented as means (standard deviation), and as medians (range) if otherwise. Categorical data were described using absolute and relative frequencies.

Figure 2 | Results flow-chart



RESULTS

Ductoscopy was performed in 82 women with a mean age of 47 years (range 20-72 years) (Table 1). Duration of nipple discharge ranged from 0.5 to 108 months, with a mean duration of 18.2 months. For logistical reasons, one patient underwent ductoscopy before the 3 months of symptoms. Patients described the colour of the discharge as haemorrhagic in 68 (83%). Nipple abnormalities were reported in 5 patients, i.e. reversible nipple retraction (*n* = 4) and skin lesions *(n* = 1). Median follow up of 17 months (range 6-45 months) was obtained for 78 (95%) patients. Three patients had died and 1 patient was lost to follow-up. All deaths were non breast-related, and these patients had not undergone surgery nor was there a diagnosis or indication of breast cancer before their death.

Cannulation and inspection of the ductal system

Ductoscopic results are shown in the flow chart in Figure 2. The ductal system was cannulated successfully in 71 (87%) of all patients (Figure 2a). In 11 patients (13%) ductoscopy failed at the dilatation part of the procedure, either because of too narrow (*n* = 7) or unidentifiable (*n* = 1) duct orifices, or due to nipple retraction (*n* = 3).

Ductoscopy was positive in 53 (65%) patients (Figure 2b). The abnormalities found were polypoid lesions (*n* = 29), epithelial abnormalities (*n* = 16) and miscellaneous (*n* = 8) lesions.

In twelve (12.6%) patients false routes occurred. In 3 patients this prevented cannulation. Despite the false route, a lesion could be subsequently removed by ductoscopic extraction in 4 patients. Eight of 12 patients with false routes had persistent symptoms, 4 were symptom-free (of which 3 had underwent ductoscopic lesion extraction).

Ductoscopic extraction

Due to lesion's size or type (very small or obstructing large polypoid lesions, epithelial abnormalities and intraductal debris are not suitable for basket extraction), ductoscopic extraction was not attempted in 19 of 53 visualised intraductal abnormalities (Figure 2c). For results regarding the interventional procedure, only patients in whom ductoscopic extraction was attempted (*n* = 34) were included in the calculations.

In 27 (79%) of 34 attempts, the observed intraductal lesion could be removed (Figure 2c).

Table 1 | Patient characteristics and imaging at baseline

	Ductoscopy (n = 82)	
Mean age ± SD (years)	47 ± 11 (range, 20-72)	
Mean duration of symptoms ± SD (months)	18.2 ± 26.0 (range, 0.5-108)	
Median duration of follow-up (months)	17 (range, 3-45)	
	n	%
Side nipple discharge		
Unilateral	75 (39 left: 36 right)	91.5
Bilateral	7	8.5
Aspect nipple discharge		
(Sero)sanguineous	68	82.9
Other	14	17.0
Nipple retraction/skin lesions		
Yes	5	6.1
No	77	93.9
Previous surgery on ipsilateral breast		
Yes	12	14.6
No	70	85.4
BIRADS		
1	31	37.8
2	36	43.9
3	13	15.9
4	2	2.4
Mammographic abnormalities (Defined as a mass, indeterminate/suspicious calcifications, or architectural distortion)		
Present	11	13.4
Not present	69	84.1
No mammogram performed	2[¶]	2.4
Ultrasonic abnormalities (Defined as a mass or intraductal mass[es])		
Present	25	30.4
Not present	55	67.0
No ultrasound performed	2[#]	2.4

MRI abnormalities (Defined as a mass or suspicious enhancement pattern)		
Present	5	6.1
Not present	5	6.1
No MRI performed	72	87.8
Cytology performed		
Yes	50	61.0
No	32	39.0
Core needle biopsy		
Yes	15	18.3
Yes, showing no abnormalities	2	2.4
No biopsy performed	65	79.3
Follow-up		
Lost to follow-up	1	1.2
Deceased	3	3.6
One-step surgery*	4	4.9

SD: standard deviation. * In case of one-step surgery the ductoscopic therapeutic efficacy was unknown.

¶Based on patients' age (< 30 years) mammography was replaced by MRI. #Patients with a BIRADS 1 score on mammography. For logistic reasons ultrasound was not performed.

Lesion removal was complete in 21 and partial in 6 removals. Seven (21%) of 34 attempts failed, caused by either problematic basket passage (*n* = 6) or lesion dimensions being too small to be grasped with the basket (*n* = 1). In 13 (38%) of the 34 attempts, a sufficient amount of tissue for adequate pathologic assessment could be collected. All were diagnosed as intraductal papilloma at histopathology. Reason for failure of pathologic assessment was insufficient tissue quantity and intraductal loss of the removed tissue.

Pain assessment

For infiltration of lidocaine and for cannulation mild pain was reported, with median VAS scores of 3 and 2, respectively (range, 0-8 in both). During ductoscopy pain was reported to be moderate, with a median VAS score of 3.5 (range, 1-7).

Therapeutic efficacy

Six patients (7%) had unknown therapeutic efficacy, due to immediate post-ductoscopy surgery (*n* = 4), death before therapeutic efficacy was assessed (*n* = 1) or loss to follow-up (after negative ductoscopy) (*n* = 1) (Figure 2d). After a median follow-up of 17 months (range, 3-45 months) post-ductoscopy 40 (49%) patients no longer experienced symptoms of PND without having surgery. The remaining 36 (44%) patients had persistent PND symptoms after ductoscopy.

Of the 27 patients in whom a lesion had been successfully removed, symptom resolution was effectuated in 19. In 12 of the 40 symptom-free patients, only ductal flushing with saline had been used in the endoscopic appearance of epithelial lesions (*n* = 7), debris (*n* = 3), a small papilloma (*n* = 1) and a blind-ending duct (*n* = 1). In 8 symptom-free patients ductoscopy had been negative and in one patient cannulation was unsuccessful.

Surgery

Surgery was performed in 26 (32%) of 82 patients, after a median interval of 3 months (range, 0-8 months) (Figure 2e).

Four patients underwent one-step-surgery immediately after negative ductoscopy (*n* = 2) or unsuccessful ductoscopic extraction (*n* = 2). Other indications for surgery were ductoscopic findings of suspicious epithelial lesions (*n* = 3) or patient's preference, i.e. symptom relieve (*n* = 10), patient reassurance (*n* = 9).

Surgery was performed by either major duct excision (*n* = 17), microdochectomy (*n* = 2), a combination of both (*n* = 3) or excision biopsy (*n* = 4). Surgery was performed 'blindly', without imaging guidance, except for the excision biopsies. Here ultrasound-localization (*n* = 3) or ductoscopy-assisted wire-placement was used as guidance (*n* = 1), as described previouly[31].

Correlation between ductoscopic and histological findings

A total of 36 (44%) patients had tissue diagnosis acquired from either ductoscopic extraction (*n* = 13) and/or surgery (*n* = 26) (Table 2).

Of the 29 polypoid lesions, histology was acquired in 21, showing papilloma in 20 and benign epithelial changes (lobulitis and ductitis) in one patient. A total of 23 papillomas were histologically diagnosed, 20 of which had been visualised as a polypoid lesion by ductoscopy. In cases where histology was acquired from both ductoscopic extraction and surgical excision (*n* = 2) these were in concordance, revealing intraductal papilloma.

Atypical ductal hyperplasia (ADH) was found In 1 of 23 papillomas diagnosed. Due to the 10 mm lesion diameter measured on ultrasound, ductoscopy was performed in the operation room. A large polypoid lesion obstructing the duct was endoscopically visualized. Ductoscopic extraction with the basket was not possible and the patient was converted to surgery. Histopathology confirmed the preoperative CNB diagnosis of intraductal papilloma, but also revealed a focus of ADH.

The surgical specimen of 3 of the 3 suspect epithelial lesions on ductoscopy contained malignancy (Table 2); one suspect lesion was diagnosed as lobular neoplasia (or lobular in situ carcinoma).

After a median follow-up of 17 months (range, 3-45 months) a total of 5 (6.1%) patients had been diagnosed with invasive breast cancer (*n* = 3), DCIS (*n* = 1) or lobular neoplasia (*n* = 1) or (Table 3), of which 3 had been correctly identified as suspect by ductoscopic evaluation (Table 3). Malignancies were identified surgically at a median interval of 5 months (range, 3-45 months) after initial ductoscopy, either following suspect findings at ductoscopy (*n* = 3) or at follow-up (*n* = 2).

One malignancy was missed both at ductoscopy and initial surgery; ductoscopy showed a white-yellow duct obstruction indicative of fibrotic tissue, which was confirmed at microdochectomy. However, at breast reduction 45 months after initial ductoscopy and microdochectomy, extensive DCIS was found. Although the presence of this malignancy at the time of ductoscopy and initial surgery is unknown, for this study it was counted as missed malignancy. Both ductoscopy and initial surgery failed to diagnose two malignancies.

Complications

Three women (3.7%) developed mastitis in the examined breast for which antibiotic treatment (*n* = 3) or surgical incision (*n* = 1) were initiated. In 2 patients, mastitis was diagnosed and treated by the general practitioner, without cell culture. Upon patient's explicit request ductoscopy was performed in one patient during lactation in the presence of a galactocele. Four days after ductoscopy she presented to the UMCU with mastitis and an abscess for which surgical incision was performed and oral antibiotics were given based on the cultured staphylococcus aureus bacteria.

In the 26 patients who underwent surgery, iatrogenic injury to the nipple resulting in an areola scar (*n* = 1), necrosis of the nipple (*n* = 1), postoperative mastitis (*n* = 1) or wound infection (*n* = 1) occurred, resulting in a 15% postoperative complication rate. Although complications occurred more frequently after surgery, complications with a Clavien-Dindo classification as high as grade 3a were only observed after ductoscopy (*n* = 1).

Table 2 | Ductoscopic appearance of lesions in relation to worst pathologic finding (in specimen of ductscopic extraction and/or surgery). Tissue diagnosis was acquired in 36 patients.

	Histopathology					Total
	Papilloma(/-tosis)	(In situ) malignancy	Benign lesion / fibrosis	Normal breast tissue	No material obtained	
Ductoscopy positive						
Polypoid lesion	20¶	0	1	0	8	29
Epithelial lesion	1	2	2	0	11	16
Suspect		(2)	(1)			(3)
Miscellaneous	0	0	1	0	7	8
Fibrotic duct			(1)*		(1)	(2)
Intraductal debris					(6)	(6)
Ductoscopy negative	1	0	2	2	13	18
Unsuccessful cannulation	1	1	1	1	7	11
Total	**23**	**3**	**7**	**3**	**46**	**82**

¶ One polypoid lesion was histopathologically diagnosed as intraductal papilloma with a focus of atypical ductal hyperplasia (ADH).

*One malignancy was diagnosed at breast reduction surgery 45 months post-ductoscopy, while histology of the major duct excision 1-month post-ductoscopy in this patient showed fibrotic tissue. For this table this patient was scored under 'fibrotic tissue'.

Table 3 | Patients diagnosed with (pre)malignancies at ductoscopy or follow-up

Age	Past medical history	Haemorrhagic discharge?	Imaging	Cytology ND	Ductoscopic findings	Outcome (+time post-ductoscopy)
49	Fibroadenoma ipsilateral	Yes and scaly, red-coloured epidermis of the nipple	NAD	Atypical cells	Suspect epithelial: Intramammilar friable red-coloured ductal lining	Biopsy: Paget disease Lumpectomy (+3 months): Paget disease
58	Unknown variance BRCA-carrier	Yes	NAD	Highly atypical epithelial cells	Suspect epithelial: Retromammilar circular, friable red-coloured ductal wall	Major duct excision (+2 weeks): ductectasia Re-excision for persistent PND (+5 months): ductal carcinoma (1 mm) and DCIS
67	None	Yes and nipple retraction	NAD	N/A	Suspect epithelial: Constricted duct with atypical epithelial aspect	Major duct excision (+2 months): lobular neoplasia
61	Anticoagulant therapy	Yes and nipple retraction	NAD	NAD	Unsuccessful cannulation	Imaging (+5 months): 15 mm lesion Lumpectomy (+5 months): tubular carcinoma 5 mm and intracystic papillary carcinoma 17 mm
36	Excised fibroadenoma ipsilateral	Yes	NAD	Papillary epithelial cells	Miscellaneous: White-yellow duct obstruction, suspect for fibrotic tissue	Microdochectomy (+1 month): fibrotic tissue Reduction mammoplasty (+45 months): incidental finding of extensive DCIS

N/A = Not applicable. NAD = No abnormalities detected

DISCUSSION

As PND may be associated with breast cancer, numerous women with PND undergo exploratory surgery to rule out malignancy and to treat symptoms. However, the vast majority of these women will undergo this invasive procedure for a benign cause since evaluation of patients with PND without imaging abnormalities renders a diagnosis of cancer in only 2-6% of the cases[2–5]. Ductoscopy has emerged as a minimally invasive approach to identify intraductal neoplasia, but more recent intraductal interventional techniques have been introduced. Although there have been several reports regarding the diagnostic accuracy for intraductal changes, there are no data on the overall and long-term therapeutic efficacy of ductoscopy for the treatment of PND. We have effectuated such an analysis, because adding ductoscopy to the evaluation of these patients may aid in non-surgical treatment and selection for surgery, possibly improving the quality of life whilst reducing costs and complications. The results of this analysis, based on a prospective cohort of 82 patients, indicate that interventional ductoscopy is technically feasible for the diagnosis and treatment of patients with PND without suspicious findings on imaging and leads to avoidance of surgery in the majority of patients.

A 87% cannulation rate as shown in the present study is in accordance with previously reported rates of 81-100% in women with PND[22,32–35]. With intraductal abnormalities visualized in 75% of the 71 successfully cannulated breasts (65% of all 82 patients), our positive ductoscopy rate was higher then previous reports of 41-65% in studies of 54-1093 successfully cannulated breasts [22,27,33]. Possible explanation could be the use of more advanced imaging technology.

This is the first study focusing on the therapeutic effect of ductoscopy and prevention of unnecessary surgery for patients with PND with low suspicion of malignancy (BIRADS ≤ 3 or benign histology). The reported 79% successful lesion removal rate in the 34 ductoscopic extraction attempts, with symptom resolution in 70%, is in accordance with the 85% success rate in the 26 solitary papillomas that Bender et al. attempted to extract, which led to symptom resolution in 95% of the patients in their hands[22]. Kamali et al., on the other hand, reported a successful ductoscopic extraction in only 47% of 47 solitary papillomas, with symptom resolution in 29%, but they also included patients with significant indications of malignancy and/or palpable masses by conventional radiography[34]. Nevertheless,

further improvement of the therapeutic efficacy of current interventional techniques is welcomed. Patients diagnosed with an intraductal papilloma suffering from persistent PND after successful (partial) ductoscopic extraction were scheduled for transductal laser ablation in an upcoming feasibility trial in the UMCU.

After a median follow-up of 17 months, 40 (53%) of the examined patients no longer experienced PND symptoms after ductoscopy not followed by surgery. In only 19 (50%) of these patients, ductoscopic lesion extraction had been performed. This could point to self-limiting disease or a therapeutic effect of ductoscopic saline flushing. In 12 of 19 patients in whom ductoscopic extraction of the intraductal, mostly epithelial, abnormality was not attempted, symptom resolution occurred within 3 months. This strengthens the suggestion of therapeutic effect of ductal saline lavage. Since we have performed re-ductoscopies without signs of intraductal fibrosis we do not think that fibrosis is a likely result of ductoscopy. Symptom persistence in the other patients might be explained by the intraductal abnormality remaining in situ despite lavage, or the lesion causing symptoms, e.g. an intraductal papilloma, not being visualized. False routes without ductoscopic extraction did not appear to effectuate symptom resolution.

After ductoscopy, 56 (68%) of all patients did not require further surgery, being either symptom-free or reassured sufficiently. However, for definitive reassurance, ductoscopy should provide for an adequate distinction between the different natures of a lesion. In this report the histology of all macroscopically suspect findings showed malignancy, whereas of the benign appearing polypoid lesions 20 of 21 were histologically confirmed as papillomas. Despite the therapeutic design of this study with lack of histology in 56% of patients, its results imply a correlation between endoscopic appearance of intraductal lesions and final histologic diagnosis as has been proposed previously[9,26]. Previous authors reported a significant correlation with correct ductoscopic identification in 73-97.3% of the pathologically diagnosed papillomas and a positive predictive value of 73-92%[9,33,34,36]. Comparing ductoscopy for the diagnosis of PND with standard investigation methods, Kamali et al. reported a 72% sensitivity for papillomas at ultrasound, 62.9 % at mammography, 81.4% at galactography, and 86.6% (the highest rate) at ductoscopy[34].

In order to increase the diagnostic yield, Shen et al. combined ductoscopy in 259 women with symptoms of PND (regardless of malignancy suspicion and imaging status) with

cytological analysis of the ductal washings[37]. This ductal washing technique yielded an average of 5,000 cells per washed duct, as compared with 50 in nipple aspirate fluid alone. In 36% of cases they visualized a papillary lesion with an overall 83% positive predictive value of ductoscopy, which improved to 86% when combined with cytological analysis of endoscopic ductal lavage. The surgical pathology of 11 patients revealed DCIS, of whom eight had abnormal ductal findings on mammary ductoscopy with normal mammograms and physical exams in six of them.

Regarding the diagnostic value of ductoscopy for malignancy, a study in 1048 patients reported a 94.2% sensitivity of ductoscopy for detection of malignant lesions, but with low specificity[27]. Also other authors concluded that there are no visual characteristics that can accurately predict a final diagnosis of malignancy[38]. Therefore a device to facilitate adequate intraductal biopsy and subsequent diagnosis seems indispensable for future ductoscopic diagnosis of malignancy, especially considering that all malignant findings ductoscopically visualised were epithelial lesions, i.e. not suitable for basket extraction. For tissue collecting in polypoid lesions the current basket device is useful, with removal in 79% of the attempts. However, to improve tissue collection for pathologic assessment better tissue grip with the basket would be welcomed.

In our cohort 3 lesions were diagnosed as a solid mass measuring more than 10 mm on ultrasound, and ductoscopy visualised an obstructing intraductal polypoid lesion for which basket extraction was unsuccessful and the patient was converted to surgery. One solid mass, measuring 10 mm diameter, had been diagnosed as intraductal papilloma on initial ultrasound-guided CNB, but was upstaged to papilloma with ADH in the excision specimen. Upstaging of pure intraductal papillomas on CNB due to atypia or malignancy on excision has been reported in up to 16.4%[39,40], partly depending on the type and size of biopsy used[41]. Previous authors therefore recommended excision of all papillomas diagnosed on CNB[39,42]. These studies, however, included lesions initially diagnosed on CNB, inherent to BIRADS $\geq$ 3, often being larger. In two studies Cheng et al. and Chang et al. reported that upgrading to malignancy was significantly more frequent with increased size on ultrasound for lesions larger than 1.2 respectively 1.5 cm[43,44]. Irrespective of the possible risk of underlying atypia or malignancy, our results show that transductal basket extraction of lesions larger than 10 mm on ultrasound is not feasible. Therefore, to treat PND caused by papillomas larger than 10 mm, we recommend surgical excision.

Although the inclusion criteria of the here reported study (BIRADS ≤ 3 and/or benign histopathology) focused on avoiding malignancies, a total of 5 patients (6.1%) were diagnosed with breast cancer, lobular neoplasia or DCIS. This result is in accordance with the previously reported 3-6% in breast clinic referred women with PND[2–4], and higher than the 0-2% reported among the patients with PND with negative physical examination, mammography and ultrasonography[4,45]. Whether initial surgery would have diagnosed these malignancies is questionable. In 2 patients subsequent surgery found nothing but benign disease, with malignancy only being diagnosed at follow-up. Malignancies have been shown to be localized in the periphery of the breast[46]. In addition, in a study of galactograms it was found that 70% patients with carcinomas presenting with nipple discharge had lesions further than 2 cm from the nipple[11]. Conventional surgery by major duct excision or microdochectomy is therefore likely to miss these lesions because only the proximal ducts, usually closer than 3 cm, are removed. This is underlined by the higher incidence of multiple lesions identification by ductoscopy, suggestive of underestimation of the true causes of bleeding from deeper lesions which would not be identified by classic blind retroareolar resection[47].

Although our results indicate the need for alertness in patients with PND, with a systematic approach the risk of underlying carcinoma can likely be further defined, whereas benign pathology can be diagnosed and treated without surgery. In current practice, either surgical excision or follow-up is advised. Long-term yearly follow-up visits and imaging as part of follow-up protocols are associated with high costs and increased patient anxiety. Due to the incommodious symptoms of PND and the fear of underlying malignancy, patients frequently opt for surgical excision instead of follow-up. Recognizing the limitations of our study, we suggest a ductoscopic approach for patients with PND with BIRADS ≤ 3 and/or benign, representative histopathology, except for patients with irreversible nipple retraction, skin abnormalities, or with lesions > 10 mm on ultrasound. For polypoid lesions, ductoscopic extraction can be performed. In case of suspect epithelial findings or unsuccessful ductoscopic extraction without prove of benign histology, surgery is indicated. In case of benign histology, non-suspect epithelial lesions or unsuccessful cannulation, choice between follow-up or surgery can be left at patient's discretion. In patients with negative ductoscopy, further follow-up can be waived.

Rather than subjecting all patients with PND to operative intervention this approach will

aid in patient selection and non-surgical treatment. Pain associated with ductoscopy was reported to be moderate, in accordance with previous reports[48]. Adding ductoscopy to the evaluation of patients with PND may hereby result in reducing surgery and associated costs and complications whilst improving the quality of life.

In conclusion, this study has shown that minimally invasive interventional ductoscopy is technically feasible and can serve as an important tool for the diagnosis and treatment of patients with PND. Moreover, it may obviate surgery in the majority of patients with PND. Since endoscopic removal of intraductal lesions is not always possible and malignancies can be the underlying cause of PND, optimization of ductoscopic instruments remains warranted.

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CHAPTER 07

Endoscopic laser ablation of intraductal neoplasia of the breast

submitted

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ABSTRACT

Purpose | Ductoscopy is a minimally invasive endoscopic approach of the milk ducts of the breast via the nipple. Besides diagnosis in women with pathologic nipple discharge (PND), ductoscopy allows non-invasive removal of intraductal lesions with a stalk like papillomas. Removal, however, is often incomplete and flat lesions cannot be targeted. This first-in-man study evaluated the feasibility and safety of breast endoscopic thulium laser ablation for treatment of intraductal papilloma.

Patients and Methods | Dosimetry was assessed in mastectomy specimens, applying power settings of 1-5 W with 100 ms pulsed exposure to a 375-µm outer diameter thulium fiber laser. Subsequently, in three patients with persistent PND, laser ablation of histologically proven intraductal papilloma was performed.

Results | Endoscopic view was maintained during ductoscopic laser ablation at 1-3 W. Increasing power to 4-5 W caused impaired vision due to shrinkage of the main duct around the ductoscope tip. Histology revealed localized ablation of the duct wall. In all patients, laser ablation of the intraductal papilloma was feasible. No complications occurred. Symptom resolution was effectuated in all patients with a median follow-up of 5 (range, 4 - 7) weeks.

Conclusions | This first-in man study shows that laser ductoscopy is technically feasible, can be performed under local anaesthesia and is capable of complete local ablation of intraductal papillomas. Short-term follow up confirmed its therapeutic effectiveness. This encourages further studies on endoscopic laser ablation of flat ductal cancer precursor lesions to prevent cancer.

INTRODUCTION

Pathologic nipple discharge (PND) is defined as spontaneous, single duct nipple discharge and is responsible for approximately 5% of surgical referrals to the breast clinic[1]. As PND might be associated with breast cancer, numerous women with PND undergo exploratory surgery to rule out malignancy and to treat symptoms. Breast cancer, however, is found in a minority (3-7%) of the referred women with PND and no other signs of malignancy at physical or additional examination[2–6], while benign intraductal papillomas are found in 53-84%[6–10].

The minimally invasive endoscopic approach with ductoscopy enables direct access to the ductal system via the natural orifices of the nipple[11–13] and has been proven to be a useful tool in the evaluation of patients with PND[6,14]. Moreover, intervention ductoscopy has shown to allow transductal minimally invasive removal of intraductal papillomas[6]. Therefore, interventional ductoscopy has the potential to replace surgery in patients with PND but no signs of malignancy during diagnostic workup.

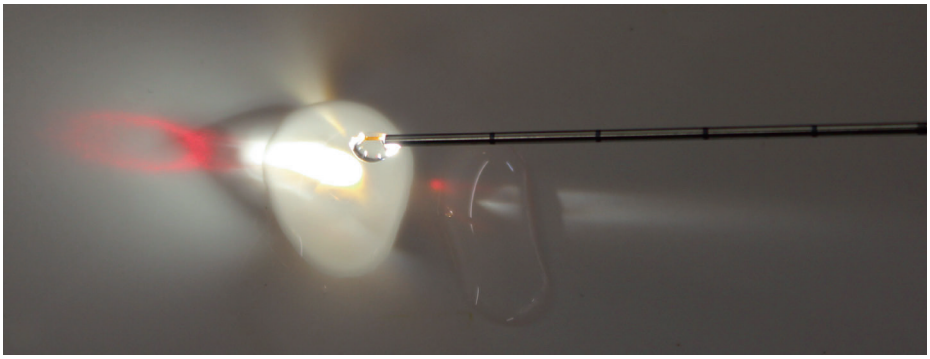
Current endoscopic interventional methods, however, are suboptimal. The 'basket'-intervention device[6,15–17] is not feasible for all lesion types nor for complete lesion removal since basically the lesion is torn of at the level of the stalk[6]. Other intraductal biopsy devices are not commercially available[18,19]. In our recent cohort study basket extraction was feasible in only a selected group in 41% of 82 patients with polypoid lesions. Symptom resolution has been limited to 70% of the 27 patients in whom a lesion could be removed[6]. Flat intraductal cancer precursor lesions cannot be targeted.

In the current study we investigate the therapeutic possibilities of transductal laser ablation through the ductoscope in patients with PND in order to enhance the therapeutic effectiveness of ductoscopic treatment.

Thulium laser has a 2-µm wavelength and is transmitted through fibers in various diameters making it suitable for endoscopic procedures. It is used mostly in minimally invasive (transluminal) treatment of benign prostate hyperplasia[20]. The superficial heat production results in a localized tissue ablation zone, minimizing the risk of thermal damage in underlying tissue[21].

The primary aim of our study was to determine the feasibility and safety of thulium laser in ductoscopic procedures. In addition, complications and therapeutic effect were analysed.

Figure 1 | Tip of the laser fiber presented through a ductoscope (Karl Storz) with the red aiming beam. Stripe marks on the ductoscope represent 1 cm. The tip of the laser fiber can only be moved back and forth through the axis of the working channel. Aiming of the laser needs to be performed by manoeuvring the ductoscope.



METHODS

To determine the optimal power settings for acquiring an adequate lesion diameter, an *ex vivo* study was performed. Subsequently, we evaluated *in vivo* patients with PND.

Ex vivo study

Mastectomy specimens from patients with a diagnosis of breast carcinoma or high-risk of breast cancer who underwent a prophylactic or therapeutic mastectomy between May 2013 and November 2014 were recruited for the study. Mastectomy specimens from patients with previous surgery or radiotherapy of the breast were excluded since this might impair cannulation. This *ex vivo* study was approved by the Internal Review Board of the University Medical Center Utrecht. Since this *ex-vivo* study did not interfere with conventional diagnosis, the need for informed consent was waived.

All mastectomy specimens were examined freshly within 1 hour after surgical resection. The specimen was placed and stitch fixated onto a dome-shaped pad to spread the milk ducts for easier passage of the ductoscope. Salivary duct probes (Karl Storz, Tuttlingen, Germany) size 0000 to 1 and an obturator (Polydiagnost GmbH, Pfaffenhofen, Germany) were used for dilatation of the duct orifices in the nipple. The introduction port (SoLex-Nipple-Expander®, Polydiagnost) or a custom-made introduction port compatible with the Storz endoscope was placed into the duct orifice through which the ductoscope was introduced.

For the *ex vivo* procedure a 0.55 mm optic (LaDuScope T-flex, Polydiagnost) inserted in a 1.15 mm outer diameter Polyshaft (PD-DS-1015, Polydiagnost), or a Storz miniature endoscope (Erlangen, Karl Storz) with incorporated fiberoptic light transmission and outer diameter of 1.1 mm was used. Both devices are semi-flexible and have a separate irrigation channel for saline-infusion and a working channel (diameter 0.45 mm) via which the fiber was introduced.

Ductoscopic exploration was performed while avoiding the breast quadrant containing malignancy in order to prevent interference with patient diagnostics. At depths of approximately 4 cm from the nipple orifice with clear view and normal appearing ducts,

a bare-tipped single use low OH silica fiber (Tobrix, Waalre, The Netherlands) with a core diameter of 200 µm and an outer diameter of 375 µm (Figure 1) was introduced through the working channel. Laser energy was delivered using 2.0 µm thulium laser generator (Revolix Junior; LISA Laser Products, Katlenburg, Germany) at stepwise increased power settings of 1 to 5 W with single pulses of 100-1000 ms. The aiming beam was used at 85% for precise focusing at the duct wall.

In order to facilitate precise histological correlation in the last three specimens a marking wire (n = 2) (PD-AC-0061, Polydiagnost) or colour marker (n = 1) (sterile Black Eye Endoscopic marker™, The Standard, Korea) was placed under direct vision, through the working channel of the ductoscope at the level of the ablation. Ductoscopic images were recorded. All *ex vivo* ductoscopy procedures were performed by the same physician (LW). After the *ex vivo* ductoscopy procedure the specimens were submitted fresh to pathology, where the margins of the specimen were inked. In specimens with intraductal dye-marking different colours were used in order to prevent interference with the intraductal dye-mark. The specimen was sliced in 5-mm slices, searched for the marked area and macroscopic ablation lesions. At the level of the laser ablation where a marking wire or colour marker had been placed, the specimen was totally embedded at a transversal plane to acquire a trans sectional view of the duct. All tissue was formalin fixed and used for routine histopathological evaluation using conventional haematoxylin eosin (HE) staining. At the level of the tumour the specimen was embedded according to standard procedure.

Pathologic variables evaluated included viability of the ablated cells and size of the ablation zone. The histological effects of laser ductoscopy were assessed by a dedicated breast pathologist (PvD).

In vivo study

Laser ductoscopy was conducted in three women in a clinical setting. All patients had initially been evaluated for symptoms of PND with standard work-up and conventional ductoscopy. At initial ductoscopy a polypoid lesion had been visualized, histologically diagnosed as intraductal papilloma by tissue acquiring with (partial) basket extraction. All patients suffered from persistent daily up to weekly symptoms of PND after the initial ductoscopy.

Informed consent was conducted for all patients according to the Law on Medical

Treatment Agreement (WGBO), preceded by comprehensive information on the content, expected course, potential complications/risks and prognosis of this treatment.

For the *in vivo* ductoscopy procedure the Polydiagnost endoscope, which is gas-sterilized and has a working length of 100 mm and a 0° angle direct view, was used in all patients. For the laser ablation procedure the previously described devices were used. The ductoscopic procedure was performed under sterile conditions in the outpatient clinic under local anaesthesia.

Prior to ductoscopy the nipple and areola of the breast were disinfected with 70% ethanol. After periareolair local infiltration anesthesia with 1% lidocaine the productive lactiferous duct orifice in the nipple was dilated and the ductoscope was introduced. Following intraductal infusion of 4 ml lidocaine, sterile saline was used for ductal distension. Vital parameters (blood pressure, heart rate, saturation) were continuously measured by a bedside monitor under anesthesiologist supervision. In case of intolerable pain intravenous analgesia or sedation was administered.
Pain was scored by the Visual Analogue Scale for pain (pain VAS) before the start of the ductoscopy, during ductal examination with the ductoscope, during laser ablation and after ending the ductoscopy.

Ductoscopic exploration was performed starting in the fluid-producing duct. The major lactiferous ducts and segmental branches were visualized in an orderly fashion until the scope could not be advanced any further. Images of ductal abnormalities were recorded. When the known intraductal papilloma was encountered, extraction with the basket device was attempted. When basket extraction was unsuccessful or incomplete (i.e. intraductal lesion remnant), the laser fiber was inserted into the working channel and connected to the generator. Under direct vision of the scope pulsed ablation of the intraductal lesion was performed. This was started at 1 W, increasing power up to 5 W. The procedure was ceased according to the operator's judgement when the lesion was completely ablated or showed a coagulated surface. All ductoscopic procedures were performed by one dedicated breast surgeon (AW) with wide experience of > 140 conventional ductoscopy procedures.

Follow up

Decisions regarding adjuvant chemotherapy or hormonal therapy for patients in the *ex vivo* study were made according to standard protocol. Patients in the *in vivo* study were followed up prospectively with consultations after 2 days, 2 weeks and 4 weeks after laser ductoscopy.

RESULTS

Ex vivo feasibility and optimization of the power settings

A total of thirteen mastectomy specimens from ten patients were studied. Cannulation of at least one duct could be achieved in nine specimens (82%) in eight patients. Due to technical problems in two specimens laser ablation was not performed. Laser ablation was executed in the remaining seven specimens, using single pulses of 100-1000 ms at 1, 2, 3, 4 and 5 W. In five specimens multiple ablations were performed at different locations in a single duct system. In two specimens where more than one duct orifice was cannulated, ablations were performed in multiple ducts.

Endoscopic view was maintained during laser ablation with 1-3 W. At 3 W shrinkage of a small ductal branch could be achieved (Figure 2). Increasing power to 4-5 W caused impaired vision due to shrinkage of the main duct around the ductoscope tip.

Histology *ex vivo* study

Showing a coloured segment of the ductal system or an intraductal wire, the marking wire and colour marker both aided in macroscopic localisation of the treated duct, but the colour marker was also identifiable microscopically (Figure 3).

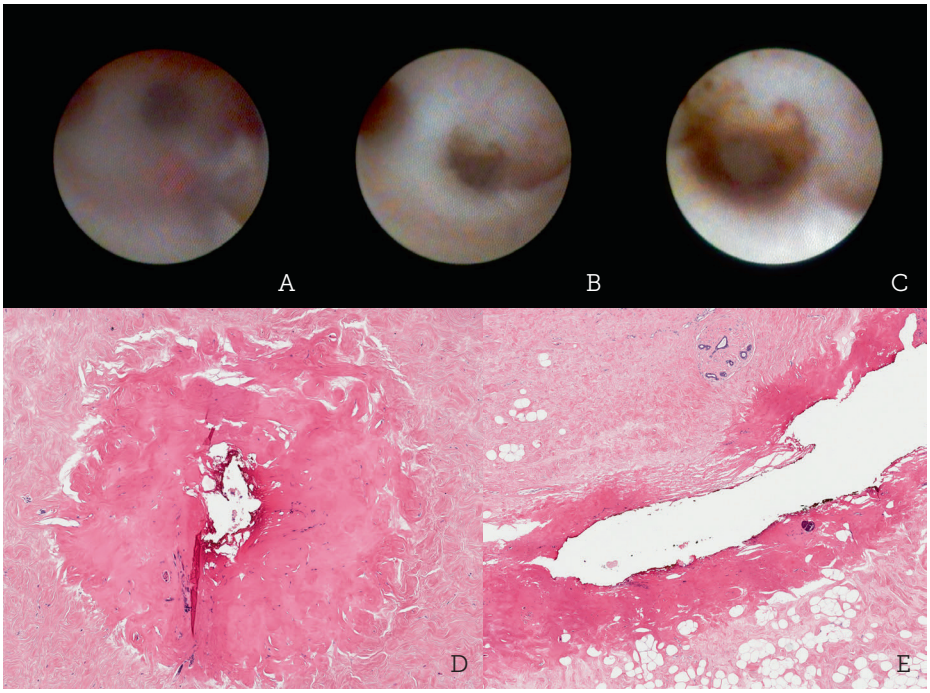
Macroscopically no ablation effects could be identified. Microscopically, in six (85.7%) of seven laser-treated mastectomy specimens one or more areas with ablation effects were localized. In one patient the location of ablation could not be found, although endoscopically the ablation effect was clearly visible.

An example of the pattern and depth of thermal damage after a resection with thulium laser is shown in Figure 2. Median thermal damage depth was 1.0 mm, ranging from 1.0 to 2.0 mm. Based on the properties of endoscopic-control and low penetration depth, transductal thulium laser ablation was deemed safe for *in vivo* use at settings of 1-5 W.

In vivo study

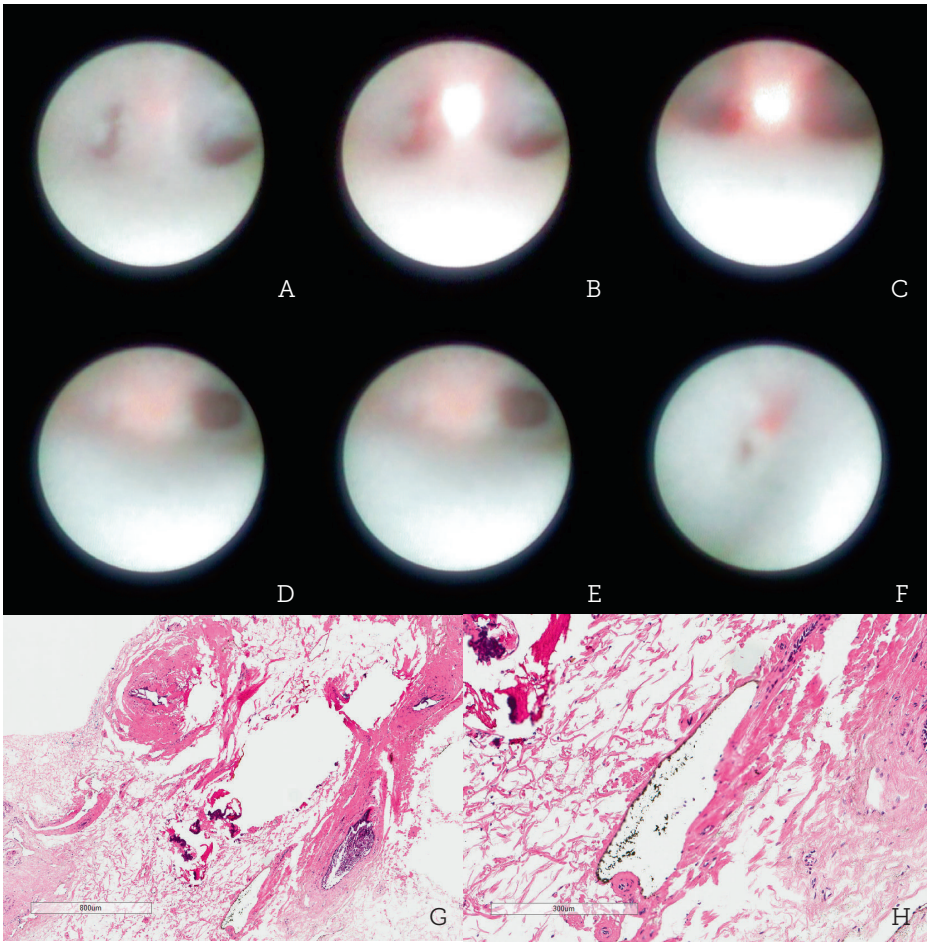
Between March 2015 and April 2015 three women with a median age of 44 (range, 43-50) years were included after obtained informed consent. Table 1 shows patient and imaging characteristics.

Figure 2 | *Ex vivo* transductal laser ablation with 3 W resulting in shrinkage of a small ductal branch, followed by histological assessment.



A. Trifurcation of ducts with the tip of the laser fiber approaching from bottom right, aiming for middle duct.
B. Laser ablation at 3 W leading to shrinkage of the middle ductal branch with endoscopic ablation effect
C. Close-up of ablated middle duct
D. Histology (HE, 5x). Axial coupe of the duct showing completely destroyed ductal epithelium and deeply eosinophylic surrounding stroma as sign of ablated breast tissue, with a central increase in intensity.
E. Histology (HE, 2x). Transversal section of an ablated duct, showing the typical ablation histology

Figure 3 | Ex vivo transductal laser ablation at 3 W shrinkage of a ductal branch, followed by histological assessment where the ablation could be precisely correlated by intraductal dye-marking. After removal of the optic, dye marker was inserted through the polyshaft.



A. Duct trifurcation.
B. Laser fiber tip appearing from above.
C. Laser ablation at 3 W.
D. Disappearing duct on the left.
E. Ongoing ablation.
F. Shrinkage of the ductal branch on the right.
G. Histology (HE) overview with ablated zone.
H. Histology (HE) of ablated duct with intraductal black marking locating the ablated duct.v

Table 1 | Patient and imaging characteristics *in vivo* study

	Patient 1	Patient 2	Patient 3
Initial presentation			
Findings initial ductoscopy	Solitary polypoid lesion	Solitary polypoid lesion	Solitary polypoid lesion
Basket extraction	Partial removal	Partial removal	Partial removal
Histological diagnosis	Intraductal papilloma	Intraductal papilloma	Intraductal papilloma
Duration of symptoms at initial ductoscopy (months)	7	48	6
Side nipple discharge	Right	Left	Left
Aspect nipple discharge	serosanguineous	serosanguineous	serosanguineous
Presentation at laser ductoscopy			
Age	43	50	44
Interval initial ductoscopy (months)	6	10	24
Frequency nipple discharge	Daily	Daily	Daily -weekly
Spontaneous nipple discharge	Yes	Yes	Yes
Productive duct orifices, no	1	1	1
Mammographic abnormalities	No	No	Dilated duct
Ultrasonic abnormalities	No	No	No
MRI abnormalities	5 mm diameter non-suspect lesion in dilated duct	NA	Dilated duct
Follow-up (weeks)	7	5	4

In all patients the previously diagnosed intraductal papilloma was encountered. It was featured as an obstructing polypoid lesion at distances of 3 to 5 cm from the nipple. In two patients partial basket extraction was performed, providing tissue diagnosis of intraductal papilloma in both (Figure 4).

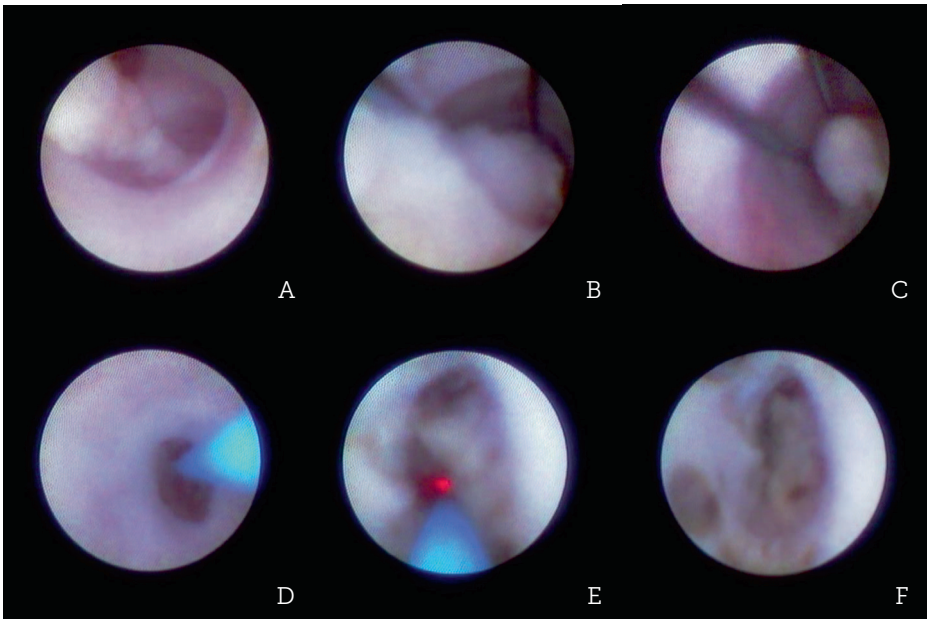
Laser ablation with 50 ms pulses at 1 W showed no visual ablation effects. Gradual increase in power up to 5 W and pulses of 1000 ms led to shrinking of the papilloma (Figure 4)

In one patient, following laser ablations up to 5W removal of the ablated lesion was performed with the basket (Figure 5). Histological effects of laser ablation on the papilloma were assessed (Figure 6).
Highest reported VAS pain during the procedure was 5. In two patients intravenous analgesia was administered by propofol and alfentanil.

Table 2 shows the characteristics of ductoscopy and laser treatment procedure. The mean ductoscopically estimated lesion size was 6 mm (range 4 - 6 mm). Duration of laser ablation ranged from 3.48- 5.96 minutes (median, 3.57 minutes). In all patients the applied power was gradually increased up to 5 W. The mean total energy supplied was 1030 (range 440 - 1995) J. Minimal vapour around the fiber tip without visual impairment was observed.

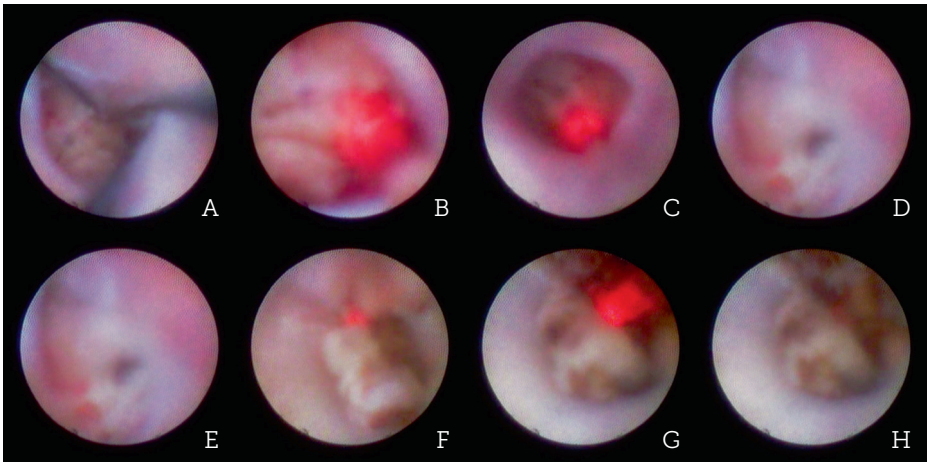
Symptom resolution was effectuated in all patients with a median follow-up of 5 (range, 4 - 7) weeks. No complications occurred. No effects on cosmesis were seen.

Figure 4 | *In vivo* laser ablation of an intraductal papilloma causing pathologic nipple discharge.



A. The known intraductal papilloma.
B. Basket extraction of the papilloma. No abnormalities are seen peripherally from the lesion.
C. Partial extraction of the papilloma with the basket device.
D. Introduction of the laser fiber for ablation of the lesion remnant.
E. Aiming beam providing precise focus.
F. Final result of intraductal laser ablation showing complete ablation of the papilloma surface and also showing ablation effects on the duct wall.

Figure 5 | *In vivo* laser ablation of a solitary intraductal papilloma causing pathologic nipple discharge.



A. Intraductal papilloma appearing as a polypoid lesion obstructing the duct. Basket extraction is attempted, but passage of the lesion is not possible and extraction cannot be performed.
BCD. Laser ablation of the papilloma. Tip of the laser fiber with aiming beam appearing from below.
E. Dark coloured spot on the papilloma representing the ablation effect at 2W
FG. Continued ablation.
F. Final result of intraductal laser ablation showing complete ablation of the papilloma surface.

Figure 6 | Histology (HE) of the lesion remnant that was removed by ductoscopic extraction after laser ablation, showing intraductal papilloma with severe coagulation artefacts.

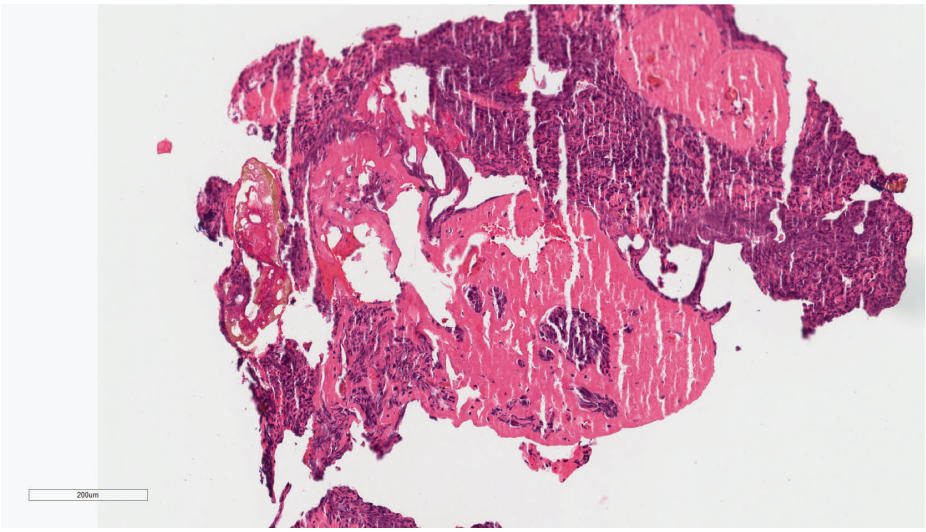


Table 2 | Characteristics of the ductoscopy procedure

	n = 3
Technically feasible procedure, no	3
Duration total procedure*, min [median, range]	70, (50-70)
Ablation time, min [median, range]	3.57 (3.48- 5.96)
Energy supplied, J [median, range]	655 (440 – 1995)
Pain score (VAS)**, maximum without intravenous analgesia	4 (1 – 5)
Intravenous analgesia administered, no of patients	2
Complications, no	0

* Measured from injection of local anaesthesia until end of the ductoscopy procedure
** Pain scores were measured before ductoscopy, at ductoscopy, during laser ablation and after the procedure

DISCUSSION

The aim of this study was to assess the safety and feasibility of ductoscopic laser treatment for intraductal papillomas causing PND. After establishing technical feasibility and optimization of power settings *ex vivo*, our *in vivo* study showed that ductoscopy with transductal thulium laser ablation is capable of local ablation of intraductal papillomas. Our first-in-man results show that endoscopic laser ablation can be performed safely under local infiltration anaesthesia without side effects and can effectuate symptom resolution.
Previous studies have touched upon the therapeutic consequences of diagnostic tissue collecting via ductoscopy but also showed its limitations. The used biopsy devices are not yet commercially available[18,19]. Lesion extraction with the "basket device" can inherently not always achieve complete removal and is restricted to a selected group of polypoid lesions. Although the procedure has been suggested previously[22,23], data on laser ductoscopy were not reported before.

The main advantage of ductoscopy for localization and treatment of intraductal neoplasms are reduction of the need for surgery for a benign cause, conservation of breast tissue and nipple function, better cosmesis and fewer complications (approximately 5% wound complications for microdochectomy and major duct excision[24], 15% for microdochectomy in our hands, unpublished results[6]).
Before this novel minimally invasive treatment strategy can be implemented in a routine clinical manner, it is important that the benign nature of the intraductal lesion causing the symptoms can be reliably assessed. There are contradictory views about the correlation between intraductal visual observations and histological diagnosis. While some studies reported a significant correlation[25,26], others found no specific data except for gross morphological abnormalities such as papillomas[9,27–29]. Although the sensitivity of ductoscopy for all (both benign and malignant) intraductal lesions in patients with PND with non-suspicious imaging is reported to be as high as 88-99%, specificity remains questionable at 20-85%[7–9,26,30]. Our study was performed in a selected group of patients with histologically proven benign intraductal papillomata after earlier partial basket extraction. The current basket device is useful for tissue diagnosis in polypoid lesions, with successful (partial or complete) removal in 63-79% of attempts[6,28]. For superficial epithelial lesions or small or obstructing polypoid lesions basket extraction is not feasible[6,15–17]. For ductoscopic diagnosis and assessment of suitability for laser ablation therefore a commercially available

device to facilitate adequate intraductal biopsy and subsequent diagnosis is eagerly awaited. Diagnostic accuracy may also be improved with advanced imaging techniques such as molecular imaging with optical coherence tomography (OCT)[31] or autofluorescence[32,33] or by complementing ductoscopy with assessment of cytology or promoter methylation of ductoscopy-directed ductal (brush) lavage fluid[34–37].

As sensitivity of directed breast imaging improves, the amount of patients with PND and negative imaging should decrease, as should the likelihood of malignancy within this population. Among modern series of patients presenting with PND with negative physical examination and both negative mammography and diagnostic ultrasound, the incidence of malignancy on duct excision was 2-4%, leading the authors to advise a conservative approach[5,38]. Moreover, due to lesions in the periphery of the breast being missed, conventional surgery in the form of major duct excision or microdochectomy is not the perfect diagnostic standard[24,39]. To treat benign but incommodious symptoms of PND ductoscopic diagnosis and intervention with basket extraction and/or subsequent laser ablation provides a promising minimally invasive approach. Also for symptomatic selective treatment of affected ducts laser ductoscopy may be of use; the use of higher power levels caused shrinkage and thereby complete occlusion of the main duct. A potential future application may be the complete ablation of the ductal epithelium or ablation of precursor lesions as a form of primary prevention of breast cancer.

In conclusion, this first-in-man study has shown that laser ductoscopy is technically feasible and can serve as an important adjuvant tool for minimally invasive treatment of intraductal papillomas in patients with PND without imaging abnormalities. A large-scale prospective study is needed to further demonstrate this potential of this novel approach including analysis of cost-effectiveness. Since endoscopic diagnosis of intraductal lesions is not always possible, optimization of ductoscopic diagnostic instruments is warranted.

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CHAPTER 08

Detection of breast cancer precursor lesions by autofluorescence ductoscopy

in preparation

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ABSTRACT

Background | Autofluorescence is an image enhancement technique used for detection of cancer precursor lesions in pulmonary and gastrointestinal endoscopy. This study evaluated the feasibility of addition of autofluorescence to ductoscopy for the detection of breast cancer precursor lesions.

Methods | An autofluorescence imaging system, producing real-time computed images combining fluorescence intensities, was coupled to a conventional white light ductoscopy system. Prior to surgery, ductoscopy with white light and autofluorescence was evaluated under general anaesthesia in women scheduled for therapeutic or prophylactic mastectomy. Endoscopic findings in both modes were compared, marked and correlated with histology of the surgical specimen.

Results | Four breast cancer patients and four high-risk women, with a median age of 47 years (range, 23-62) were included. In autofluorescence mode two intraductal lesions were seen in two breast cancer patients, which had an increase in the red-to-green fluorescence intensity compared with the surrounding tissue. One lesion had initially been missed by white light ductoscopy, but was clearly visible in subsequent autofluorescence mode. One endoscopic finding was classified as suspicious by white light, but was negative in autofluorescence mode with normal histology.

Conclusions | This study demonstrates for the first time the *in vivo* feasibility of autofluorescence ductoscopy to detect pathologically confirmed breast cancer precursor lesions in both breast cancer patients and high-risk women that were occult under white light.

INTRODUCTION

Worldwide every year around 1,100,000 women are diagnosed with breast cancer and 410,000 will die of the disease[1–3]. Hereditary breast cancer accounts for up to 5-10% of all breast cancers with two high-penetrance genes (*BRCA1* and *BRCA2*) responsible for about 16% of the familial risk of breast cancers, associated with a 60-80% lifetime risk of developing breast cancer[4–7]. The ultimate prevention in these women is bilateral prophylactic mastectomy[8]. Unfortunately, this is accompanied by complications[9], and serious cosmetic and psychical consequences[10]. Moreover, the 20-40% of BRCA1/2 patients who would have never developed breast cancer have been grossly overtreated[4–7]. Regrettably, stratification within the high-risk group remains elusive and more sensitive screening methods are warranted, although primary prevention with less radical treatment methods would be the ultimate solution. Therefore, new screening techniques must be developed that identify individuals on the path towards cancer but still in the pre-invasive phase.

Most breast cancers are thought to arise from the ductal epithelium[11,12]. An appealing approach would be to target breast cancer precursors originating from the epithelial lining of the breast ducts through ductoscopy, a micro-endoscopic technique that gains direct access to the ductal system via the natural orifices in the nipple. This endoscopic approach by ductoscopy has already been proven useful for diagnosis and treatment in patients with pathologic nipple discharge[13,14] and has shown to be able to detect malignant lesions not visible on conventional imaging[15,16]. The role of ductoscopy in breast-cancer screening and breast conservation surgery has yet to be fully defined[17], but the first studies using autofluorescence in ductoscopy indicated the feasibility and the possibility to detect malignant lesions[18,19]. The former study was an *ex vivo* study, the latter a technical *in vivo* feasibility study in three patients not aimed at detecting lesions, and without taking material for pathological evaluation. (Pre)malignant epithelial lesions show an aberrant pattern under fluorescent light by which they become detectable, as is already known from the airways, larynx and colon[20–22]. However, the breast ductal system had not been evaluated before by autofluorescence to detect pathologically confirmed precursor lesions.

From a prospective feasibility study in patients affected by breast cancer and in women with a known mutation in *BRCA1* or *BRCA2,* we report for the first time the *in vivo* feasibility

of autofluorescence to detect (white light occult) breast cancer precursor lesions by autofluorescence ductoscopy, confirmed by histology of the subsequently performed mastectomy.

METHODS

Patients

A prospective observational phase II cohort study was conducted in adult women who underwent either therapeutic or prophylactic mastectomy in the University Medical Center Utrecht, The Netherlands, between October 2014 and May 2015 (Table 1).

Two cohorts were included. The therapeutic cohort consisted of 4 female patients undergoing a mastectomy for recently diagnosed invasive breast cancer or ductal carcinoma in situ (DCIS). The prophylactic cohort consisted of 4 women undergoing prophylactic mastectomy for increased risk of breast cancer. The first cohort hypothetically carries multiple precursor lesions and serves as a reference for the autofluorescence ductoscopy technique; the second is the index population that will provide information about the diagnostic value of this technique in high-risk patients.

Patients with previous surgery or radiotherapy of the breast were excluded. This study was approved by the Institutional Review Board. All patients provided written informed consent.

Table 1 | Ductoscopic, radiologic, and patient characteristics of the two cohorts

Node status	Therapeutic Mastectomy	Therapeutic Mastectomy
	n = 4	*n* = 4
Mean age (range), years	50 (range, 29-62)	44 (range, 23-61)
Spontaneous nipple discharge	No	No
No	4	4
Ductoscopy side*		
Left	2	3
Right	2	1

*In bilateral prophylactic mastectomy the ductoscopy was performed unilateral, in the most accessible duct orifice for cannulation. Reported here is the ductoscopy side where cannulation was successful.

White light and autofluorescence ductoscopy

Study procedures were conducted immediately prior to mastectomy, and were all performed under sterile conditions and under general anaesthesia in the operation room. Salivary duct probes (Karl Storz, Tuttlingen, Germany) size 0000 to 1 and an obturator (Polydiagnost GmbH, Pfaffenhofen, Germany) were used for dilatation of one of the duct orifices in the nipple. The introduction port (SoLex-Nipple-Expander®, Polydiagnost) or a custom-made introduction port compatible with the Storz endoscope was placed into the duct orifice through which the ductoscope was introduced. A 0.55 mm optic (LaDuScope T-flex, Polydiagnost) was inserted in a 1.15 mm outer diameter Polyshaft (PD-DS-1015, Polydiagnost) or a Storz miniature endoscope (Erlangen, Karl Storz) with incorporated fiberoptic light transmission and outer diameter of 1.1 mm, was used. Both devices are semiflexible and have a separate irrigation channel for saline-infusion, and a working channel (diameter 0.45 mm).

The ductoscope was coupled via a custom-made eyepiece to an autofluorescence endoscopic imaging system (OncoLIFE®, Xillix Technologies Corporation, British Columbia, Canada, now Pinpoint®, Novadaq Technologies Corporation, Ontario, Canada). Method of operation was described previously by Douplik et al. [18]. Briefly, white-light and autofluorescence images were recorded using 6.3-mW broadband light and 5.3-mW blue band (390 to 450 nm), respectively. In other organs, premalignant tissues have a reduced green autofluorescence relative to normal tissues when excited by blue light; normal tissue appears as cyan, while abnormal tissue is shown red coloured[21,23]. In autofluorescence mode the central 16-12 pixels are averaged over four frames and continuously displayed as a numerical color value (NCV). The higher the NCV, the lower the autofluorescence intensity, which has been associated with neoplasia[24].

First, standard (white-light mode) ductoscopy was performed. Whenever suspicious findings were encountered, we switched to autofluorescence mode by hand switch or foot pedal. When no suspicious findings were encountered under white light, the entire duct was examined by autofluorescence ductoscopy.

White light findings were classified as normal (no visual abnormality) or abnormal (irregularity of the ductal lining such as redness, hyper vascularity, swelling, thickening, as well as nodular or polypoid lesions). In autofluorescence, a green colour was classified as normal, while areas showing red

colour with decreased autofluorescence were classified as abnormal. In autofluorescence mode NCV values were continuously monitored.

In the first seven patients, the ductoscopy procedure was performed via one single duct orifice, in order to limit operation time. In the 8th case, multiple ducts were examined. In the therapeutic cohort ductoscopic exploration was performed in the breast quadrant containing malignancy in order to encounter the previously established lesion. In the prophylactic cohort the duct orifice that was easiest to cannulate was chosen and only one breast was examined. In case of an abnormality in the studied duct, 1-2 ml colour marker (sterile Black Eye Endoscopic marker™, The Standard, Korea) was placed through the working channel of the ductoscope after removal of the optic, in order to facilitate precise histological correlation. When no abnormalities were found, the most extensively examined duct was marked. Images of ductal abnormalities were recorded. All ductoscopy procedures were performed by the same physicians (AW, CP).

Following the ductoscopy procedure a conventional (therapeutic or prophylactic) mastectomy was performed.

Pathology

Mastectomy specimens were submitted fresh to pathology, where the margins of the specimen were inked with non-black colours to avoid interference with the intraductal dye-mark. The specimen was sliced in 5-mm slices, and scrutinized for the marked area and macroscopic lesions. At the level of the colour marker, the specimen was totally embedded at a transversal plane to acquire a trans sectional view of the duct. All tissue was formalin fixed and used for routine histological evaluation using conventional haematoxylin eosin (HE) staining. At the level of the tumour the specimen was embedded according to standard procedure

All intraductal abnormities were described. Assessment of the surgical specimens was performed by one dedicated breast pathologist (PD) blinded to the endoscopic results.

Follow up

Decisions regarding postoperative treatment with adjuvant chemotherapy or hormonal therapy were made according to usual protocols based on patients' risk category and based on the tumour characteristics, size and stage.

Evaluation and analysis

Primary endpoint was the technical feasibility, determined by cannulation success and findings of intraductal abnormalities. Endoscopic findings in white light, autofluorescence and NCVs were correlated to final histology of the surgical specimen. Differences in endoscopic findings under white light and autofluorescence ductoscopy were described.

Table 2 | Endoscopic findings per patient

Age	Ducts cannulated, no	Cohort	Histology (excision specimen)	White light	Autofluorescence	NCV max (autofluorescence mode)	Histology marked duct
62	1	Therapeutic	DCIS	+ Friable intraductal duct lining depositions or debris. Distance to nipple: 4-5 cm	-	0.10	-
23	1	Prophylactic BRCA2	No (pre) malignancy	-	-	0.25	-
61	1	Prophylactic BRCA1	No (pre) malignancy	-	-	0.13	-
47	1	Therapeutic	Multicentric IDC and DCIS	+ Red-coloured flat lesion of ductal lining. Distance to nipple: 7 cm	+	0.43	+ Ductal hyperplasia
41	0	Prophylactic BRCA1	No (pre) malignancy	NA	NA	NA	NA
62	1	Therapeutic	ILC and LCIS	-	-	0.18	-
29	1	Therapeutic	Multicentric DCIS and focal micro-invasive carcinoma	A. - Intraductal polypoid lesion initially missed by white light ductoscopy. Distance to nipple: 4 cm B. + Friable duct lining depositions. Distance to nipple: 4 cm	A. + B. -	A. 1.53 B. 0.53	Ductal hyperplasia and apocrine metaplasia
52	1	Prophylactic BRCA1	No (pre) malignancy	-	-	0.20	-

DCIS: ductal carcinoma in situ. IDC: invasive ductal carcinoma. ILC: invasive lobular carcinoma. LCIS: lobular carcinoma in situ. NA = not applicable, due to unsuccessful cannulation no ductoscopy could be performed.

RESULTS

Table 1 shows patient and imaging characteristics of the 8 included patients. Duct cannulation and subsequent ductoscopic exploration was successful in 7 of 8 (88%) women. In one patient undergoing prophylactic mastectomy cannulation failed due to narrow duct orifices.

Ductoscopic examination time, from cannulation to termination of the procedure ranged from 20 to 45 min (mean, 30 min). This is in line with conventional ductoscopy, additional time for autofluorescence examination ranged from 5 to 8 minutes.

Breast cancer patients

In three of four breast cancer patients solitary (n = 1) or multiple (n = 2) intraductal abnormalities were visualised in with light and / or autofluorescence mode. Table 2 shows the characteristics of the lesions found. Normal appearing ducts in white light coloured green in autofluorescence mode, corresponding with low NCVs (Figure 1a). In one breast cancer patient, three friable intraductal abnormalities (debris) were visualised in white light mode. In autofluorescence mode these abnormalities where shown green-coloured with NCVs < 0.10 (Figure 1b). Histology of the examined duct showed the intraductal marker, confirming the correct localization (Figure 2a). Histology showed no abnormalities (Figure 2b).

In another patient, a haemorrhagic, red-coloured epithelial lesion was seen under white light, showing normal green colour in autofluorescence mode with maximum NCVs of 0.43 (Figure 3). Histology of the duct showed the intraductal marker, confirming the correct localization, with ductal epithelial hyperplasia and epithelial damage.

In another patient, an intraductal polypoid lesion appearing as irregular protrusion into the ductal lumen, with colours similar to the surrounding ductal tissue, was initially missed in white light ductoscopy. In autofluorescence mode the same lesion was displayed as a red-coloured intraductal polypoid lesion clearly contrasting the surrounding ductal tissue, with maximum NCVs of 1.53 (Figure 4a). In the same ductal tree, another abnormality was visualised (Figure 4b); friable ductal wall adhesions seen in white light, showed no clear red-colour in autofluorescence mode, but did show maximum NCVs of 0.53. Histology of

the examined ductal system shows the intraductal black dye-marking with both apocrine metaplasia and ductal hyperplasia (Figure 5). Since the dye-marker coloured the complete ductal system, more precise correlation of the two separate intraductal abnormalities was not possible.

Prophylactic mastectomies

In none of the patients undergoing prophylactic mastectomy abnormalities were found with either white light or autofluorescence ductoscopy. This was confirmed by histology of the studied ducts, showing no abnormalities.

Follow-up

Following ductoscopy, all patients underwent mastectomy. All resections were radical. Sentinel node biopsy was performed in all patients undergoing therapeutic surgery. In one patient the sentinel node contained a micro-metastasis. Adjuvant systemic therapy and radiation therapy was given in one patient. Median follow-up after surgery was 4 months (range, 2-6). In all patients of the prophylactic cohort, immediate reconstruction was performed. In one patient undergoing prophylactic mastectomy with tissue expanders for subsequent reconstruction, bilateral necrosis of skin and nipple occurred, for which surgical necrotectomy was performed. No other complications occurred. Perforation of one or more ducts occurred in four patients. Although this may limit endoscopic view (n = 1), it is without consequence for the patient. Subsequently inserted intraductal marker macroscopically showed extraductal diffusion from the ducts with perforations, without impairing microscopic localization.

Figure 1a | Left: normal appearing duct in white light. Right: same duct in autofluorescence mode, showing green colour and corresponding low NCV.

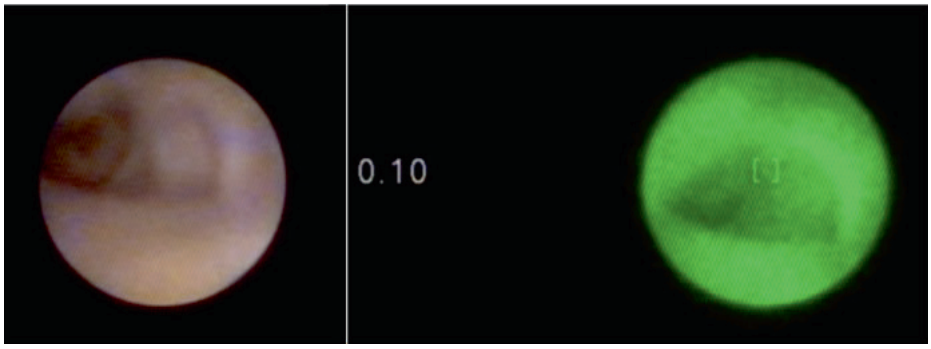


Figure 1b | Upper and lower left: intraductal abnormalities in white light.
Upper and lower right:showing the same abnormalities in autofluorescence mode, with green colour and corresponding low NCV.

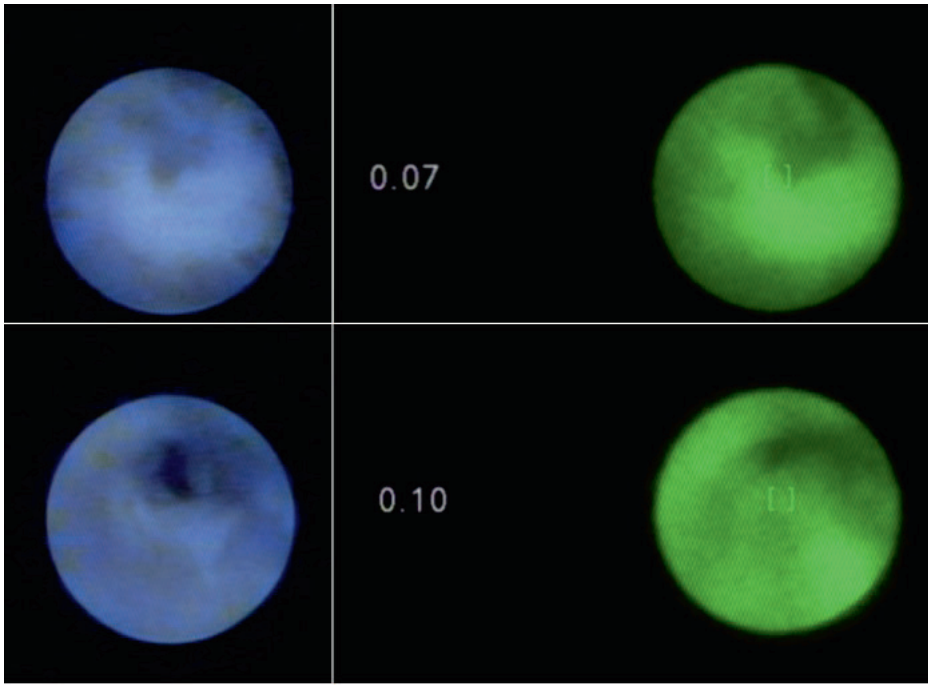


Figure 2a | Marking of the ductoscopic visualized duct in a patient undergoing prophylactic mastectomy for BRCA1. At (white light and autofluorescence) ductoscopy no abnormalities were found. Intraductal marking with Black Eye Endoscopic marker™ showed macroscopically clear localization for embedding.

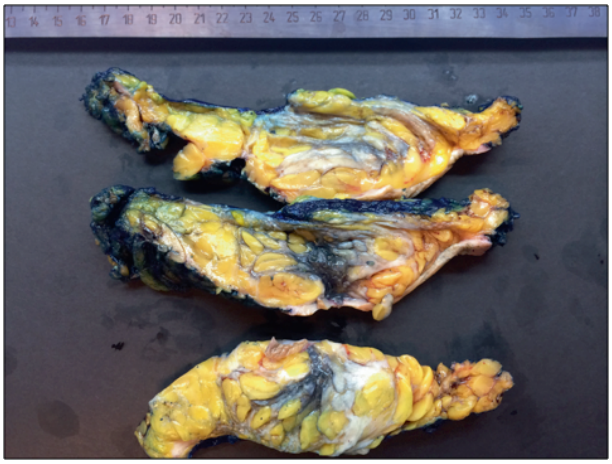


Figure 2b | Microscopically, the intraductal marking is easily traceable and the extravastion is not visible. The ductal marking shows the histological changes previously reported to be caused by ductoscopy: periductal clefts, epithelial detachment and epithelial loss[55].

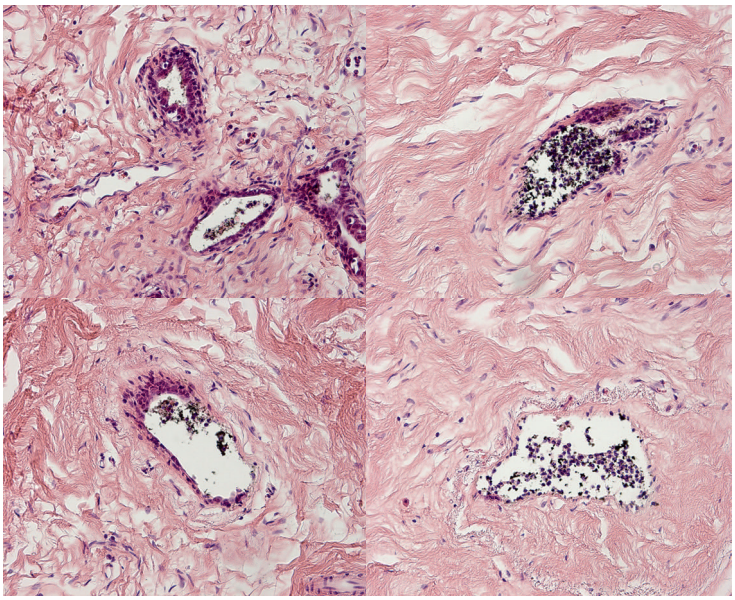


Figure 3 | Flat epithelial lesion in white light. On middle and right: showing the same lesion in autofluorescence mode, showing a red-coloured contrast with the green ductal lining and showing increased NCVs (0.43).

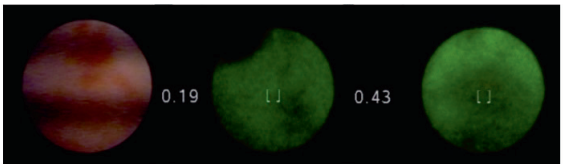
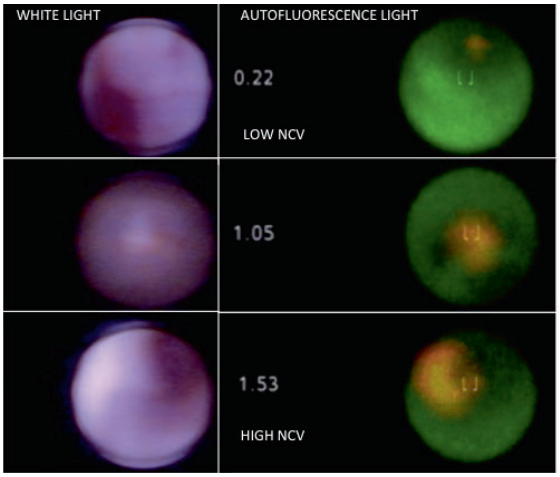


Figure 4a | Ductoscopic images taken immediately prior to therapeutic mastectomy for multicentric DCIS grade 3.



White light ductoscopy (left). Upper: showing an intraductal puffy aspect of the duct wall with ductal asymetry (oval shape). Middle: intraductal polypoid lesion, not clearly identifiable from the duct wall. Lower: close up of the lesion.
Autofluorescence ductoscopy (right). Upper: focusing on the normal, green coloured, duct wall. NCV shows corresponding low values (0.22). Middle: focusing on the intraductal red coloured intraductal abnormality, NCV shows corresponding high NCV values (1.05). Lower: Close-up of same lesion, high NCVs (1.53).

Figure 4b | Endoscopic view in the same ductal system. Autofluorescence and white light image of the high NCVs on a small area of duct lining. Friable lesions seen in white light, showing no clear red-colour but increased NCVs.

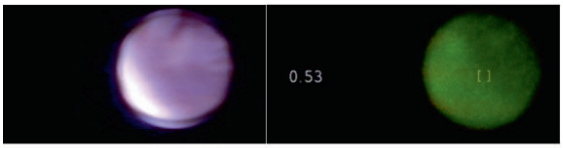
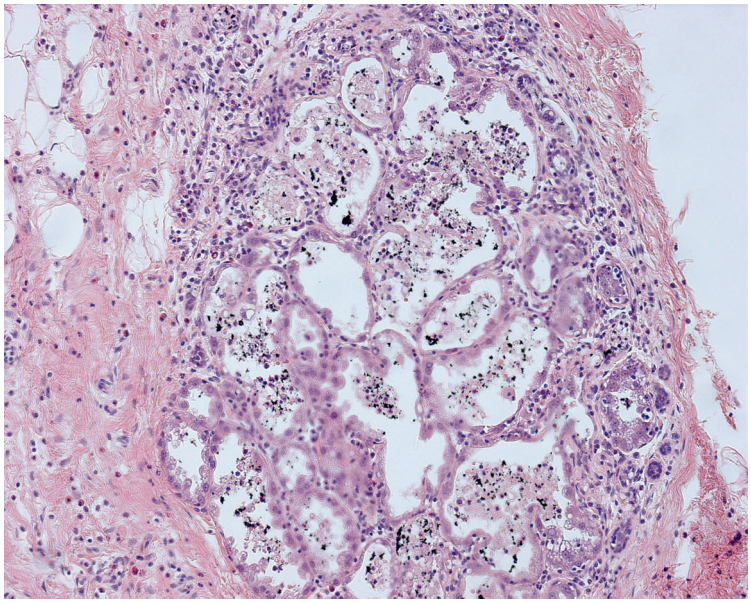
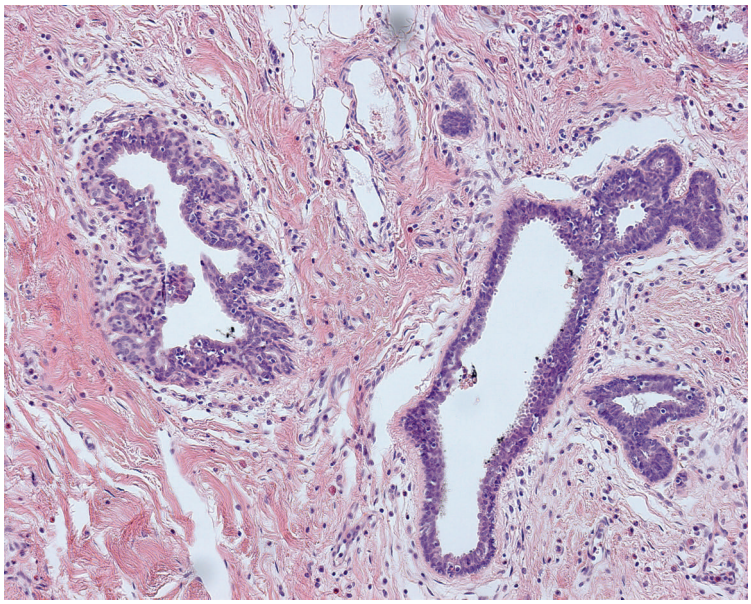


Figure 5 | Histology of the examined ductal system of figure A and B show the intraductal black dye marking and apocrine metaplasia and ductal hyperplasia. Since the dye-marker colours the complete examined ductal tree, precise correlation of the two separate intraductal abnormalities shown in A and B is not possible.



A



B

DISCUSSION

This exploratory study indicates that autofluorescence ductoscopy is technically feasible in both breast cancer patients and high-risk women, with successful cannulation in 7 of 8 (88%) women. Autofluorescence ductoscopy was capable of identifying ductal hyperplasia and apocrine metaplasia and showed to be correctly negative in histological normal ducts. The used technique of intraductal marking with endoscopic dye-marker resulted in accurate macro- and microscopic localization of the studied duct.

Previously, Douplik et al. have showed the *ex vivo* feasibility of autofluorescence ductoscopy [18]. Jacobs et al. performed ductoscopy with autofluorescence *in vivo* in three patients with pathologic nipple discharge without harvesting tissue for pathology [19]. To our best knowledge, ours is the first paper on *in vivo* autofluorescence in breast cancer and high-risk patients with pathologic correlation.

Although autofluorescence ductoscopy was positive in ductal hyperplasia and apocrine metaplasia, breast cancer precursor lesions were not found. The used autofluorescence settings are optimal for early-stage disease in other hollow organs such as the bronchus and gastro-intestinal tract, but further optimization of the imaging parameters may be necessary in order to increase specificity for premalignant lesions in breast. Intraductal debris seen in the lumen of the normal duct, which can be misinterpreted as cancer, appropriately appeared negative in autofluorescence mode, demonstrating the differentiating potential of autofluorescence ductoscopy in white light endoscopically suspect lesions.

No lesions were found in women undergoing prophylactic mastectomy for high risk of breast cancer. These negative endoscopic findings were confirmed by histology. To evaluate the diagnostic accuracy of autofluorescence ductoscopy for screening of high-risk women, a larger cohort in whom a number of premalignant lesions are present needs to be studied and prospectively followed up with ductoscopy.

In previous studies using ductal lavage to guide risk-reducing strategies in high-risk women, (conventional) ductoscopy has already been suggested as a risk assessment tool for high-risk women[25,26]. Danforth et al. compared ductoscopic findings with lavage cytology in the contralateral high-risk breasts of breast cancer patients and visualised intraductal

lesions in 83% of the ducts with atypical cytology[27]. On contrary, in a study of ductal lavage cytology in asymptomatic, high-risk patients, poor concordance with histology was found and ductoscopy added little to this evaluation[28]. Although ductal lavage fails in yielding adequate specimens for reliable cytological diagnosis[29], diagnosis by the use proteomic biomarkers in serum or methylation in nipple aspiration fluid or ductal lavage forms a promising alternative[30–34]. Methylation of specific genes is known as an early hallmark of carcinogenesis and can be detected in only small amount of DNA, providing a potential method for early tumour detection. Addition of autofluorescence ductoscopy could possibly assist in visualising and locating early lesions. Together with the currently being studied novel intraductal treatment modalities e.g. via intraductal targeted therapy by RNA interference [35], intraductal radiotherapy [Sukumar, unpublished], intraductal chemotherapy [Groot, unpublished] or intraductal laser ablation [Waaijer, unpublished], this would form an appealing approach for early detection and treatment.

In a previous study performing ductoscopy prior to surgery for diagnosed ductal hyperplasia, DCIS or invasive breast cancer, cannulation was successful in 150 (74.6%) with the target lesion visualized in 146 (73%) of 201 patients[36]. Additional lesions outside the anticipated lumpectomy were found in 83 (41%) of all, resulting in wider resection and a lower rate of positive margins. Our study could not validate these observations. In none of the breast cancer patients the target lesion was found, although we did not extensively searched for it.

There are several more issues that need be addressed. Most breast cancer arises from the terminal ductolobular unit (TDLU), where the ducts are narrow[37]. For ductoscopic examination of these TDLUs further minimization of diameter is needed. More challenging could be the anatomy of the breast, with discrepancy between number of ducts and orifices in the nipple due to several ducts arising in the same cleft of the nipple[38]. Complete endoscopic examination will be difficult and sampling error could occur. Here, biomarker evaluation, e.g. methylation[34] in ductal lavage or nipple aspiration fluid could be of additional value.

Also, the current techniques of ductoscopic diagnostic tissue acquiring are far from optimal. The 'basket'-intervention device is only feasible in polypoid lesions[39–42], and intraductal biopsy devices are not commercially available[43,44]. For histologic correlation of

the endoscopic findings this study we were dependant of dye-marker injected through the working channel with subsequent surgical excision. This procedure caused marking of a complete single ductal system, precluding correlation of multiple lesions within one duct. Therefore, the development of a commercially available biopsy device suitable for superficial epithelial lesions remains much warranted.

Our results may have clinical implications for a different patient group. Due to the association with breast cancer, numerous women with pathologic nipple discharge and negative imaging undergo exploratory surgery to rule out malignancy and to treat symptoms, despite breast cancer being found in a minor 3-7% in this patient group[42,45–48]. Ductoscopy has been used as a diagnostic modality to rule out malignancy, but while some studies reported a significant correlation[49,50], others found no specific data except for gross morphological abnormalities such as papillomas[51,52,14,53]. Autofluorescence, possibly combined with hypermethylation biomarkers[54], may help increase specificity, improving risk assessment and correlated treatment decisions.

In conclusion, ductoscopy with the addition of autofluorescence is feasible in diagnosing intraductal breast lesions and could increase specificity for endoscopic morphologically suspicious findings. Possibly, autofluorescence settings may need optimization for use in breast tissue and the development of a commercially available biopsy device is needed to make tissue diagnosis possible.

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CHAPTER 09

Summary,

General discussion and

Future perspectives

The studies presented in this thesis focused on various features of minimally invasive diagnosis and treatment of neoplastic breast disease.

In the first part of the thesis we focused on minimally invasive treatment of breast cancer and its prerequisites. Radiofrequency ablation (RFA) is being developed as an alternative to surgery. With these ablative techniques, the core needle biopsy (CNB) specimen will be the only tissue available for assessment of tumour characteristics. As a result, clinical risk assessment and patient selection for adjuvant systemic treatment has to be made based on preoperative evaluation of the CNB.

Therefore we first evaluated the clinical impact of potential discordance of tumour grade on preoperative CNB on clinical decision-making in patients diagnosed with invasive ductal carcinoma. After *ex vivo* dosimetry, a clinical trial was set up to assess the safety and efficacy of a novel bipolar RFA device for ablative treatment of small breast cancers.

The second part of the thesis focused on the current and future applications of breast duct endoscopy.

First, we gave a description of the technique of ductoscopy and its introduction in The Netherlands. Then, we assessed the diagnostic accuracy of conventional ductoscopy in patients with symptoms of pathologic nipple discharge (PND) in a systematic review and meta-analysis. Subsequently, in a clinical cohort study, the therapeutic efficacy of interventional ductoscopy was evaluated. In order to increase the therapeutic efficacy of ductoscopy, we conducted a first-in-man experimental study to assess safety and feasibility of a novel treatment method for intraductal breast neoplasia by endoscopic laser ablation. Additionally, we evaluated the feasibility of autofluorescence ductoscopy for the detection of breast cancer precursor lesions.

In this chapter the major findings of Part I and Part II of this thesis are summarized. Clinical implications and limitations of these findings are discussed and future perspectives are given.

PART I
MINIMALLY INVASIVE TREATMENT OF BREAST CANCER

Surgical excision is considered the primary treatment for breast cancer. The amount of resected breast tissue appears to be an important factor influencing cosmetic outcome[1]. Potentially less deforming approaches such as in situ ablative treatments aim to preserve the greatest amount of normal breast tissue.

As a consequence of these novel treatment techniques, the CNB specimen will be the only tissue available for assessment of tumour characteristics and clinical risk assessment. Therefore, patient selection for adjuvant systemic treatment will need to be made based on preoperative evaluation of the CNB. In **Chapter 2** we evaluated the impact of preoperative evaluation by analysis of CNB and resection specimens of 213 invasive ductal carcinomas in 199 patients. Comparison showed substantial discordance in tumour grading between CNB and resection specimens from the same breast cancer. This discordance, however, affected the indication for adjuvant therapy in only a small minority of patients with invasive ductal carcinoma. A decision on adjuvant treatment based on CNB would have resulted in overtreatment in seven (3.5%) and undertreatment in three (1.5%) of 199 patients. In the undertreated patients, incorrect omission of adjuvant systemic treatment would have increased the predicted 10-year mortality rate by only 2.6–5.2% and 10-year recurrence rate by 8.2–15.3%. We therefore concluded that the indication for systemic treatment in patients no longer undergoing surgical excision after minimally invasive therapies could safely be set using the CNB grade.

We continued our pursuit of minimally invasive treatment of breast cancer by evaluating treatment with bipolar application of radiofrequency ablation in **Chapter 3**. Previous studies found that RFA might be useful for the local control of small breast cancers[2], but reported relatively high percentages of skin and muscle burns due to monopolar electrodes[3]. By using a bipolar application device, with the two electrodes situated on the tip of the device, the electrical current can better be applied locally. Dosimetry for this novel application device was assessed *ex vivo* in bovine mammary tissue. Subsequently, ultrasound-guided RFA was performed in fifteen patients with invasive breast carcinoma, followed by immediate resection. Histopathology revealed complete cell death in 77% of patients, without causing any burns. Partial ablation was due to inaccurate probe positioning. Based

on this early evaluation, we concluded that ultrasound-guided RFA with a bipolar needle-electrode appears to be a safe local treatment technique for invasive breast cancers up to 2 cm.

Discussion and future perspectives

Although results of the studies in Part I are a step towards the minimally invasive treatment of breast cancer, more issues need to be addressed before it can be introduced for clinical treatment.

In order to accurately identify patients with good prognosis who might be safely spared chemotherapy, new prognostic markers have been developed[4]. Molecular multigene assays, such as Oncotype DX®, or MammaPrint® are currently used in tissue derived from resection specimens of early-stage, node-negative, ER-positive invasive breast cancer. These assays provide an individualized prediction of the 10-year risk of local recurrence and thus to help guide treatment decision-making[5]. In **Chapter 2** the assessment of tumour grade on CNB would have led to incorrect omission of systemic adjuvant treatment in 1.5% of the patients. All of these patients would have been candidates for molecular multigene assays that could have had an impact on decision-making. Preoperative evaluation of these molecular multigene assays on material derived from CNB might aid preoperative decision-making, but is unfortunately costly.

Although in **Chapter 3** bipolar RFA has shown its feasibility for complete tumour ablation without causing any burns, 23% of patients achieved only partial ablation of the invasive tumour was achieved. As partial ablation was mostly due to the shape of the tumour, shape needs to be reliably assessed preoperatively. Furthermore, the extent of the tumour needs to be assessed, since findings of invasive carcinoma, DCIS and lymphatic tumour foci beyond 1-cm from the edge of the dominant mass have been reported in up to nearly 50% of the tumours[6]. Selection of the tumours that are suitable for treatment by RFA, based on tumour size and shape, tumour extent and compliance, remains a challenge. Pre-treatment assessment of mammography findings, magnetic resonance (MRI) washout kinetics, ER status and quantity of DCIS in the index tumour has the potential to accurately identify breast cancer of limited extent, but up until now the best tool to select these tumours has not been identified[7].

When complete tissue ablation cannot yet be performed reliably, monitoring of the completeness of ablation needs to be performed. To guide the ablation, to monitor the therapeutic procedure and to assess treatment response, different imaging modalities have been assessed including fluoroscopy, ultrasonography, and MRI. **Chapter 3** showed that ultrasound does not provide an accurate measurement of the histological zone of complete coagulative necrosis. CNB of the ablation zone to assess the completeness of ablation has been proposed, but carries a risk of sampling error. With MRI being one of the most accurate imaging modalities for visualization of breast tumours, delineation of margins and its ability to provide temperature mapping, it may be used in monitoring of ablation techniques, although its guidance of RFA has already shown drawbacks[8]. Another approach for reliable tumor mapping could be optical imaging using targeted probes[9], but this technique does not allow temperature mapping. In what way guidance, monitoring and follow-up after RFA will come about is a subject that needs to be addressed in future research.

Due to the incidence of unexpected foci of carcinoma outside the index tumour, adjuvant radiation treatment might be required for local control in patients treated with RFA as well as in conventional breast conserving therapy[10]. Whether RFA leaves a permanent scar or induces fat necrosis, which has been reported after adjuvant radiotherapy on the ablated zone[11], has not been clarified. Both results could be more or less pronounced than the cosmetic result of standard lumpectomy. Since cosmetic outcome is one of the major reasons for development of this novel treatment technology, it needs to be addressed in future research.

Currently available minimally invasive image-guided thermotherapy techniques besides RFA, include laser irradiation and microwave irradiation. Cryotherapy and irreversible electroporation are also being studied for the treatment of breast cancer[12]. A completely non-invasive ablation technique is magnetic resonance-guided high intensity focused ultrasound (MR-HIFU), which was first reported in 2001[13]. Focused ultrasound waves induce a temperature increase with treatment guidance by MRI for target visualization and real-time thermometry. Over the last decade its safety and accuracy has been demonstrated in several clinical trials, mostly according to a treat-and-resect protocol[14]. Its effects on cosmetic outcome are yet unknown.

In conclusion, minimally invasive treatment strategies have shown their feasibility, but a fair number of prerequisites need to be addressed before it can replace surgery as the standard of care.

PART II
BREAST DUCT ENDOSCOPY

As conventional diagnostic procedures are not sensitive in patients with pathologic nipple discharge (PND), they often pose the physician with a diagnostic dilemma. There is no consensus about the diagnostic approach to PND, but surgery, either by selective duct excision (microdochectomy) or major duct excision, is considered the diagnostic gold standard[15]. However, since malignancy is the underlying diagnosis in a minority, most women undergo the invasive surgical procedure for a benign cause. As an alternative to surgery, patients can be scheduled for follow-up. There is no scientific base for the duration or method of follow-up. Furthermore, follow-up imaging and visits to the outpatient clinic cause unnecessary investigations and feelings of anxiety, with accompanied costs and impact on quality of life.

Ductoscopy is a minimally invasive procedure that visualizes the ductal epithelium of the breast via the nipple. It can be performed under local anesthesia in the outpatient clinic. **Chapter 4** gives a description of the technique and its introduction as a novel technique in The Netherlands.

Although ductoscopy is worldwide used mostly for evaluating PND, the accuracy of ductoscopy in patients with symptoms of PND remains a matter of debate. In **Chapter 5** a systematic review and meta-analysis evaluating diagnostic accuracy of ductoscopy in patients with PND was performed. Twenty studies with a total of 3189 ductoscopy cases in 3144 patients with PND were included in the review. Successful introduction of the ductoscope into the breast ducts (cannulation) was achieved in 81 to 100%. Malignancy ranged from 0 to 27%. Meta-analysis, classifying any intraductal lesion visualized by ductoscopy as a positive finding (DS_{any}), was possible in 12 studies. These studies included a total of 1994 cases. Malignancy was diagnosed in 151 (7.6%). Ductoscopy had a pooled sensitivity of 94% and specificity of 47%. So, although ductoscopy visualizes 94% of all underlying malignancies,

discrimination based on endoscopic morphology is not possible. These results imply that histological diagnosis, acquired either endoscopically or surgically, remains inevitable in order to exclude malignancy in patients with PND. Due to the high sensitivity and the low incidence of malignancy in patients with PND, surgery can be waived in case of negative ductoscopy.

Besides visualizing intraductal breast lesions causing symptoms of PND, ductoscopy can also be used as a therapeutic modality[16]. The therapeutic efficacy of interventional ductoscopy was assessed in **Chapter 6** in a prospective study conducted in 82 consecutive patients with PND and routine diagnostic workup not suspect for malignancy. Intraductal lesions were removed by ductoscopic extraction. Surgery was performed in case of suspect ductoscopic findings or upon patient's request. Intraductal abnormalities were visualized in 65% of patients. Most lesions are polypoid type. In 79% of the 34 attempted ductoscopic extractions, the lesion could be removed. In 68% of all 82 patients surgery was avoided. After a median follow-up of 17 months, symptom resolvement without surgery was effectuated in 49% of all 82 patients. This showed the feasibility of interventional ductoscopy for acquiring diagnosis and effectuation of symptom resolvement and aiding in reduction of surgery for a benign cause.

To increase therapeutic efficacy, a novel endoscopic treatment method was assessed in **Chapter 7**. First, in an *ex vivo* study the safety and feasibility of intraductal thulium laser ablation was examined. Subsequently, the feasibility and efficacy of the technique *in vivo* was examined. In the clinical study laser ablation of the intraductal papilloma was feasible in all patients without complications. Symptom resolvement could be effectuated. This first-in-man study showed that laser ductoscopy is technically feasible and can be performed under local anaesthesia. Thulium laser ductoscopy is feasible in ablation of intraductal papillomas and can increase therapeutic effectiveness.

Most breast cancers are thought to arise from the ductal epithelium[17,18]. Therefore an appealing approach would be to target breast cancer precursors originating from the epithelial lining of the breast ducts through ductoscopy. In **Chapter 8** ductoscopy was equipped with an autofluorescence image enhancement technique in order to assess the diagnostic accuracy for detection of precursor lesions of breast cancer in patients affected by breast cancer or with a mutation in BRCA1 or BRCA2. The results from this study are

first in showing *in vivo* feasibility of autofluorescence ductoscopy to detect, pathologically confirmed, intraductal abnormalities that were occult under white light.

Discussion and future perspectives

Part II of this thesis shows that ductoscopy deserves a place in the routine work up of patients with pathologic nipple discharge without suspicion of malignancy (BIRADS ≤ 3) on conventional imaging. Ductoscopy can be used to triage patients for surgery. Surgery may be safely avoided in case of negative ductoscopy or endoscopically acquired benign histology. Removal of lesions by basket extraction cannot only acquire histological tissue for diagnosis, but can also lead to symptom resolvement.

Morphological characteristics of intraductal lesions are, unfortunately, not reliable for diagnosis of malignancy. Endoscopic removal of intraductal lesions by the currently available devices is not always possible. When endoscopically-assisted tissue acquisition is not possible, surgery remains inevitable to exclude malignancy in patients with positive findings at ductoscopy. Future research and investment should focus on additional techniques, such as micro-endoscopic biopsy devices, to improve diagnostic accuracy. In patients with benign endoscopic tissue diagnosis and in patients with negative ductoscopy, surgery could be avoided. Focus of further research should be the optimization of currently used ductoscopic extraction instruments and the development of a micro-endoscopic biopsy device to acquire histological tissue. With further increase in minimally invasive diagnostics, the therapeutic arsenal of ductoscopy can be broadened. Thulium laser ablation has shown its feasibility in treatment of intraductal papillomas causing PND but needs further, long-term evaluation. In the future, complete duct coagulation may also be used for symptom relieve in patients with negative ductoscopy, comparable with surgical microdochectomy.

Our results indicate the need for alertness in patients with PND, but with a systematic approach the risk of underlying carcinoma can likely be further defined, whereas benign pathology can be diagnosed and treated without surgery. Cost-effectiveness of addition of ductoscopy into the diagnostic work up remains to be established. Nevertheless, even a minimal reduction of surgeries by this non-invasive procedure, will likely outweigh the relatively high costs of surgery, hospital admission, and morbidity.

Nowadays, conventional surgery, by either microdochectomy or major duct excision, is considered the gold standard for diagnosis of underlying malignancy in patients with PND. However, due to lesions in the periphery of the breast being missed, conventional surgery may not be the perfect standard[19,20]. A retrospective study by Moncrief et al. diagnosed a slightly greater proportion of women with intraductal neoplasia in the group undergoing ductoscopy-directed microdochectomy than in the conventional microdochectomy group and concluded that ductoscopy-guided surgery may be more sensitive than conventional 'blindly' performed surgery[21]. Also, since malignancy in patients appears to be more frequently situated in the periphery of the breast, conventional surgery in which only the proximal ducts are removed is likely to miss these lesions[22]. To evaluate the accuracy of conventional surgery as a gold standard, a prospective study is required, comparing ductoscopy-guided and conventional surgery.

As most breast cancers are thought to arise from ductal epithelium, direct evaluation of this location forms a potential means of identifying early markers of malignant development. Together with diagnosis by the use of proteomic biomarkers in serum or methylation in nipple aspiration fluid or ductal lavage, autofluorescence ductoscopy provides a potential method for early tumour detection. The intraductal approach may assist in for example, risk-stratification in high-risk patients. Together with the currently being studied novel intraductal treatment modalities e.g. via intraductal targeted therapy by RNA interference, intraductal radiotherapy, intraductal chemotherapy or intraductal laser ablation, the intraductal approach would form an appealing approach for early detection and treatment. In the near future autofluorescence can be evaluated for diagnosis of PND in an attempt to increase endoscopic diagnostic accuracy while optimizing the image enhancement technique for use in the breast ducts. More issues need to be addressed before autofluorescence ductoscopy could possibly assist in risk-stratification in high-risk patients. As most breast cancers arise from the narrow endings of the ducts (terminal ductolobular unit), further minimization of diameter is needed. Discrepancy between number of ducts and orifices in the nipple due to several ducts arising in the same cleft of the nipple will make complete endoscopic examination of the breast difficult, leading to sampling error[23]. Biomarker evaluation, e.g. methylation in serum, ductal lavage or nipple aspiration fluid could be of additional value in selecting patients and/or ducts at risk[24].

In conclusion, ductoscopy is able to reduce the number of surgical procedures for a benign cause and claims its role in the diagnostic and therapeutic approach of patients with PND. Aim of future research should be the optimization of diagnostic and therapeutic instruments. The potential future role of ductoscopy in the intraductal approach of breast cancer and its precursor lesions needs further evaluation.

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CHAPTER 10

Summary in Dutch

Samenvatting in het Nederlands

MINIMAAL INVASIEVE BENADERING VAN NIEUWVORMINGEN VAN DE BORST

Dit proefschrift gaat over minimaal invasieve benadering van nieuwvormingen in de borst. "Minimaal invasief" is de term die wordt toegepast voor ingrepen waarbij een veel kleinere incisie (snede in de huid) gemaakt wordt dan bij een traditionele ingreep. Nieuwvormingen van de borst kunnen goedaardig zijn (zoals een fibroadenoom, een goedaardig bindweefsel knobbeltje), potentieel kwaadaardig (bijvoorbeeld ductaal carcinoom in situ, kwaadaardige cellen die niet in staat zijn om het weefsel in de omgeving in te groeien) of kwaadaardig (borstkanker).

Het eerste deel van dit proefschrift richt zich op de minimaal invasieve behandeling van borstkanker. In Nederland is borstkanker de meest voorkomende vorm van kanker bij vrouwen. Jaarlijks worden ongeveer 14.000 vrouwen gediagnosticeerd met borstkanker. Een operatie is vaak de eerste stap in de behandeling. De besluitvorming voor aanvullende behandeling met bijvoorbeeld chemotherapie en radiotherapie is voornamelijk gebaseerd op het weefsel dat bij die operatie wordt verkregen.

De hoeveelheid weggenomen borstweefsel is een belangrijke factor voor het cosmetische resultaat van de operatie. Indien mogelijk wordt daarom een borstsparende operatie verricht, waarbij zo min mogelijk gezond borstweefsel wordt weggenomen. Echter, om volledige verwijdering van de tumor te bereiken, wordt de tumor met een marge rondom verwijderd en wordt na de operatie radiotherapie gegeven. Het cosmetische resultaat van een borstsparende behandeling is hierdoor vaak teleurstellend. Bovendien kan een operatie gepaard gaan met complicaties en postoperatieve klachten als pijn en oedeem. Een operatieve ingreep kan zo leiden tot een verminderde kwaliteit van leven. Wanneer de behandeling van borstkanker zonder operatie kan, zal dit mogelijk leiden tot betere cosmetische resultaten en een hogere kwaliteit van leven.

Adjuvante systemische therapie (behandeling met medicamenten) is een aanvullende behandeling die gegeven wordt in aansluiting op de operatie om eventuele niet waarneembare uitzaaiingen te bestrijden. Zo wordt de kans op overleving vergroot. De keuze voor adjuvante systemische therapie is afhankelijk van de klinische kenmerken zoals de graad van de tumor, de tumor grootte en de aanwezigheid van eventuele lymfklier

metastasen in de oksel, de leeftijd van de patiënt, de verdere gezondheid van de patiënt en een aantal andere tumorkenmerken. De tumorgraad wordt bepaald onder de microscoop en geeft aan in hoeverre de tumorcellen nog op normale cellen lijken. Hoe minder de tumor op gezond borstweefsel lijkt, hoe slechter gedifferentieerd de tumor is en hoe hoger de gradering. Adjuvante systemische therapie kan bestaan uit chemotherapie, hormoontherapie, immunotherapie of een combinatie hiervan. Bij de meeste patiënten wordt de diagnose borstkanker gesteld door middel van biopsie, waarbij met een naald door de huid een kleine hoeveelheid weefsel uit de tumor wordt verkregen. Wanneer borstkanker behandeld zal worden met technieken waarbij de tumor niet meer wordt weggesneden, is er geen operatieweefsel voor de beoordeling. De indicatie voor adjuvante systemische therapie zal dan moeten worden gesteld op het weefsel van de biopsie.

Radiofrequentie ablatie (RFA) is ontwikkeld als alternatief voor een operatie. Hierbij wordt met behulp van echografie een naald door de huid heen in de tumor gebracht en aangesloten op een generator. Deze generator laat de cellen trillen. Met de warmte die door de trilling ontstaat, worden de kankercellen verbrand, waarna ze in de borst aanwezig blijven. Deze nieuwe techniek heeft als doel de grootste hoeveelheid normaal borstweefsel te behouden en is daardoor in potentie minder deformerend.

Eerdere studies hebben aangetoond dat de beoordeling van de tumorgraad minder betrouwbaar is in biopsie weefsel dan in het weefsel dat bij de operatie wordt verkregen. Op dit moment wordt de beoordeling van de tumorgraad daarom routinematig uitgevoerd op het operatieweefsel.

In **hoofdstuk 2** werd de tumorgraad op het biopt en op het operatieweefsel bekeken voor 213 borsttumoren in 199 patiënten. Vervolgens werd de impact van preoperatieve indicatiestelling voor het geven van adjuvante systemische behandeling bepaald. Er was een aanzienlijke discrepantie tussen de bepaling van de tumorgraad op het biopt en op het operatieweefsel. Ondanks dit aanzienlijke verschil beïnvloedde dit de indicatie voor adjuvante systemische behandeling slechts bij een minderheid van de patiënten. Een besluit met betrekking tot adjuvante systemische behandeling op basis van het biopt zou hebben geleid tot overbehandeling in zeven van de 199 patiënten (3,5%) en onderbehandeling in drie van de 199 patiënten (1,5%).

Bij de drie onderbehandelde patiënten, zou het onterecht weglaten van adjuvante systemische therapie de kans om binnen 10 jaar te overlijden doen stijgen met 2,6 tot 5,2% en de kans op een recidief binnen 10 jaar met 8,2 tot 15,3%. Onze studie laat zien dat de indicatie voor systemische behandeling na minimaal invasieve behandeling van borstkanker veilig kan worden bepaald op basis van het biopt.

In **hoofdstuk 3** werd een nieuwe minimaal invasieve techniek voor de behandeling van borstkanker onderzocht, te weten bipolaire radiofrequentie ablatie. Eerdere studies toonden al aan dat RFA uitvoerbaar is voor de behandeling van kleine borstkanker tumoren. Deze studies gebruikten echter *monopolaire* elektroden waarbij, naast een elektrode in de naald, een elektrode op de huid nodig is om een gesloten elektrisch circuit te creëren. Dit veroorzaakte een stroom door de patiënt en kon zo leiden tot ongewenste complicaties van brandwonden. Bij *bipolaire* RFA liggen de beide elektroden echter op één naald applicator. Daardoor wordt de radiofrequente energie alleen lokaal toegevoerd aan het doelweefsel. Dit bipolaire systeem werd nog niet eerder toegepast in de borst.

In deze studie naar bipolaire RFA werden daarom eerst de optimale instellingen voor bipolaire RFA bepaald. Vervolgens werd de veiligheid en werkzaamheid van deze techniek voor de lokale behandeling van borstkanker onderzocht in vijftien patiënten met een borstkanker tumor tot 2 cm in diameter. Onder algehele narcose werd, onder echogeleiding, de naald in de tumor geplaatst. Daarna werd de tumor operatief verwijderd, zodat het effect van RFA kon worden gecontroleerd. In het weefsel werd bij 77% van alle patiënten geen levende kankercel meer aangetroffen. Er waren geen complicaties van brandwonden. In de tumoren waar slechts gedeeltelijk resultaat was opgetreden, was dit te wijten aan onnauwkeurige positionering van de naald. Bij één patiënt had foutieve naaldpositionering een klaplong tot gevolg. Op basis van deze studie hebben wij geconcludeerd dat echogeleide RFA met een bipolaire naald electrode een veilige lokale behandelingstechniek is voor invasieve borstkanker tot 2 cm.

Het tweede deel van dit proefschrift richt zich op minimaal invasieve kijkonderzoek van de melkgangen van de borst.

Ductoscopie is een micro-endoscopische techniek waarbij via de uitmonding in de tepel, met een minuscule camera de melkgangen kunnen worden geïnspecteerd. Hiermee is

het mogelijk om onder lokale verdoving afwijkingen in de melkgangen op te sporen. Via de ductoscoop kan ook weefsel voor onderzoek worden verkregen en een afwijking in de melkgang worden verwijderd.

Pathologische tepeluitvloed wordt gedefinieerd als aanhoudende, spontane, helder tot bloederige uitvloed van vocht uit een van de openingen van de melkgangen. Het is een relatief veel voorkomende klacht van vrouwen die verwezen worden naar de mammapolikliniek. Meestal wordt de klacht veroorzaakt door een goedaardige afwijking in de melkgangen. Dit is meestal een papilloom (goedaardige poliep) in de melkgang. Slechts in een minderheid van de patiënten is een kwaadaardige afwijking de oorzaak van de klachten. Gebruikelijke diagnostische technieken, bestaande uit een mammogram (röntgenfoto van de borst), echo en onderzoek van het tepelvocht (cytologie), zijn echter niet gevoelig genoeg voor het opsporen van eventuele onderliggende kwaadaardige oorzaken. Deze onderzoeken resulteren dan ook vaak in een reeks van negatieve (niet-afwijkende) resultaten en plaatsen de arts hiermee voor een diagnostisch dilemma. Er is geen consensus over de diagnostische aanpak van vrouwen met pathologische tepeluitvloed.

Een operatie wordt beschouwd als de gouden standaard (de diagnostische methode die de grootste zekerheid geeft) om onderscheid te maken tussen goedaardige en kwaadaardige oorzaken van pathologische tepeluitvloed. Bij deze ingreep onder narcose verwijdert de chirurg het weefsel achter de tepel. Dit gebeurt veelal 'blind', omdat de exacte locatie van de afwijking niet bekend is. Slechts een minderheid van patiënten heeft kanker. Daarom zullen de meeste vrouwen deze invasieve ingreep met bijbehorende complicaties en gevolgen voor cosmetiek, borstvoeding en de gevoeligheid van de tepel ondergaan voor een onschuldige afwijking.

In **hoofstuk 4** werd de techniek van ductoscopie en de introductie ervan in Nederland beschreven. Hoewel wereldwijd ductoscopie voornamelijk ingezet wordt voor de evaluatie van pathologische tepeluitvloed staat de nauwkeurigheid ervan nog ter discussie.

In **hoofdstuk 5** hebben wij daarom een systematische literatuurstudie en meta-analyse uitgevoerd waarin de diagnostische nauwkeurigheid van ductoscopie voor patiënten met pathologische tepeluitvloed wordt beschreven en vergeleken. Twintig studies met

een totaal van 3.189 ductoscopie procedures bij 3.144 patiënten met pathologische tepeluitvloed werden opgenomen in de beoordeling. Het percentage kwaadaardige tumoren varieerde in de verschillende studies van 0 tot 27%. Meta-analyse, waarbij onderzoeken worden samengevoegd om één, nauwkeurigere, uitkomst te verkrijgen, was mogelijk bij twaalf studies met in totaal 1.994 gevallen, waarvan in 151 gevallen (7,6%) een kwaadaardige afwijking werd gevonden. In de studies die wij hebben geëvalueerd bleek dat ductoscopie gevoelig is voor het opsporen van de onderliggende afwijking, en 94% van alle onderliggende kwaadaardige afwijkingen (maligniteiten) in beeld brengt. Discriminatie op basis van enkel de endoscopische beelden is echter niet mogelijk. Het kijkonderzoek van de melkgangen kan de afwijking dus wel opsporen, maar kan niet onderscheiden of de gevonden afwijking goed- dan wel kwaadaardig is. Deze resultaten impliceren dat weefsel diagnose, hetzij endoscopisch of chirurgisch verkregen, noodzakelijk blijft ter uitsluiting van maligniteit. Door de hoge gevoeligheid en lage incidentie van maligniteiten bij patiënten met pathologische tepeluitvloed, kan in het geval van negatieve ductoscopie, afgezien worden van chirurgie.

Naast het visualiseren van afwijkingen in de melkgangen, kan ductoscopie ook worden gebruikt voor de behandeling van symptomen van pathologische tepeluitvloed. Ductoscopie waarbij ook verrichtingen in de melkgangen kunnen worden uitgevoerd, heet 'interventie ductoscopie'.

De therapeutische werkzaamheid van interventie ductoscopie werd beoordeeld in **hoofdstuk 6**. In deze studie werden 82 patiënten met pathologische tepeluitvloed bij wie routine-diagnostisch onderzoek niet verdacht was voor kanker onderzocht met ductoscopie. Gevonden afwijkingen in de melkgangen werden verwijderd met een speciaal 'vangnetje'. Bij verdachte ductoscopische bevindingen of op verzoek van de patiënt werd na ductoscopie een operatie verricht. Bij 65% van de 82 patiënten (te weten 53 patiënten) werd met ductoscopie een afwijking in de melkgang gevonden, het merendeel was een papilloom. Bij 34 van die patiënten werd geprobeerd de afwijking te verwijderen via ductoscopie. Bij 79% van de 34 pogingen tot verwijdering van de afwijking, slaagde deze. Bij 68% van de 82 patiënten werd een operatie vermeden. Zonder operatie verdwenen in 49% van de 82 patiënten de symptomen van pathologische tepeluitvloed. Deze studie toonde de haalbaarheid van interventie ductoscopie voor de diagnostiek en behandeling van pathologische tepeluitvloed. Het aantal operaties kan hiermee worden verminderd.

Om de behandeling van de symptomen middels ductoscopie te verbeteren werd een nieuwe behandelmethode onderzocht in **hoofdstuk 7**. Er werd een speciaal vervaardigde laser fiber gekoppeld aan de ductoscoop. Eerst werd de veiligheid en haalbaarheid onderzocht op reeds verwijderd borstweefsel van vrouwen die een borstamputatie hadden ondergaan. Vervolgens werden drie patiënten met een goedaardige poliep in de melkgang onderzocht met ductoscopie. De poliep werd met de laser weggebrand. Er was geen sprake van complicaties en het was mogelijk de symptomen effectief te behandelen. Met deze studie toonden we voor het eerst in patiënten aan dat laser ductoscopie technisch haalbaar is en onder plaatselijke verdoving kan worden uitgevoerd. Het wegbranden van poliepen in de melkgang is mogelijk en kan ervoor zorgen dat de hinderlijke symptomen van pathologische tepeluitvloed verdwijnen.

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In **hoofdstuk 9** bespreken wij de huidige situatie op het gebied van minimaal invasieve benadering van nieuwvormingen van de borst. Tevens delen wij onze toekomstperspectieven op dit vlak en worden er enkele mogelijkheden voor verder onderzoek aangegeven.

PART III

Addenda

CHAPTER 11

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List of publications

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ABOUT THE AUTHOR

Laurien Waaijer was born on the 29th of January 1987 in Waddinxveen, the Netherlands. At the age of eight, she was given a book on the human body, which immediately caught her interest and made her decide she wanted to become a doctor.

In 2005 she received her high school degree with honours at the Coornhert Gymnasium in Gouda and moved to Utrecht to study Medicine at the University of Utrecht. Her interest in surgery emerged during her junior internships at the Department of Surgery at the University Medical Center (UMC) Utrecht. During her studies she performed laboratory research at the University of Utrecht and worked as a junior anatomy-teaching assistant. After graduating from Medical School in 2012 she started working as a surgical resident (ANIOS) at the Department of Surgery at the Meander Medical Center in Amersfoort; a position which fortified her choice of General Surgery.

In March 2013 she was given the opportunity to work as a PhD-candidate at the UMC Utrecht under the supervision of prof. dr. P.J. van Diest, prof. dr. I.H.M. Borel Rinkes and dr. A.J. Witkamp. The results of her research are presented in this thesis.

Besides her work, Laurien enjoys spending time with her family and friends, travelling and sports. She is an avid runner and completed the Berlin Marathon in September 2014. She commenced her official surgical residency in July 2015 at the St. Antonius Hospital under the supervision of dr. D. Boerma. As of July 2018 she will continue her surgical training in the UMC Utrecht under the supervision of prof. dr. M.R. Vriens.