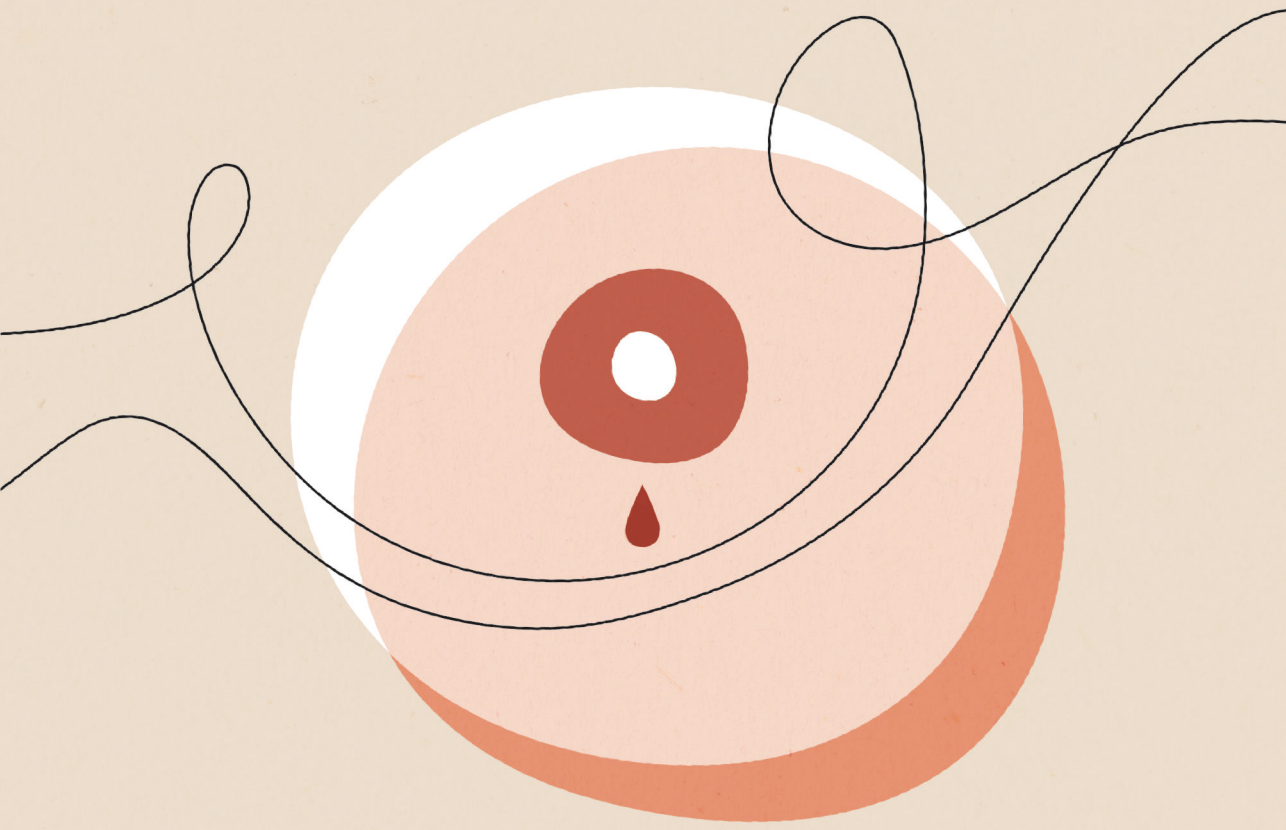
DE-ESCALATING BREAST SURGERY

Intraductal techniques and refining lumpectomy



Seher Makineli

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Seher Makineli

De-escalating breast surgery: intraductal techniques and refining lumpectomy
Thesis, University of Utrecht, The Netherlands

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DE-ESCALATING BREAST SURGERY:

intraductal techniques and refining lumpectomy

De-escalatie van borstoperaties: intraductale technieken en het verbeteren van lumpectomie

(met een samenvatting in het Nederlands)

Meme cerrahisinde yenilikler: intraduktal teknikler ve lumpektomi gelişimi

(Türkçe özetle)

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Voor mijn ouders

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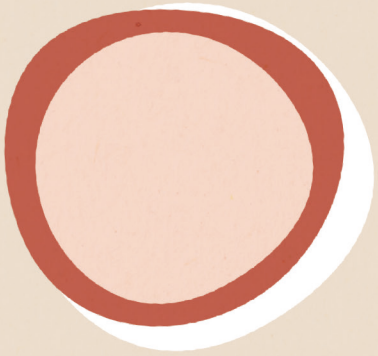
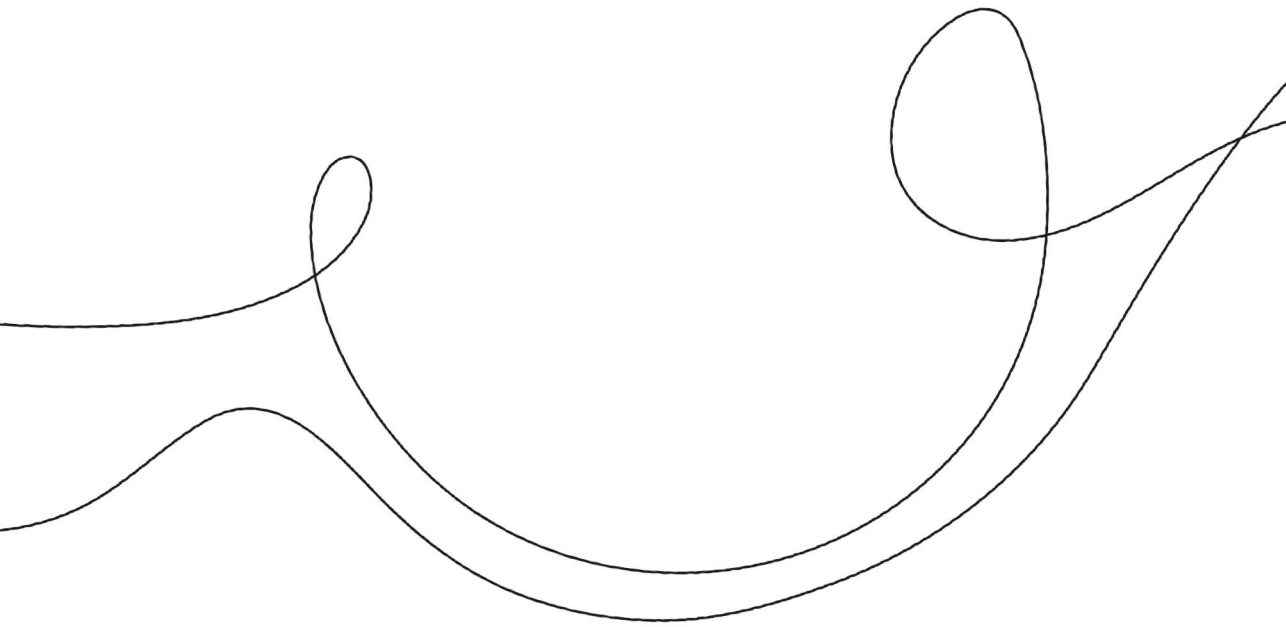


CHAPTER 1

General introduction and thesis outline



Breast cancer, the most frequently diagnosed cancer among women worldwide, was first documented over 3,500 years ago on an ancient Egyptian papyrus [1], [2]. This manuscript reported eight cases of breast tumors, which were treated but, unfortunately, without a cure. Since then, decades of medical milestones have brought us to our current position where successful treatments and long term survival rates >90% for breast cancer are possible.

However, despite these medical breakthroughs, approximately 1 in 6.6 women are still diagnosed with breast cancer during their lifetime [3]. Additionally, breast cancer is the leading cause of cancer-related deaths in women worldwide [4]. According to the latest data, there has been an increasing trend in incidence in The Netherlands. In 2023, 17,775 women were diagnosed with primary ductal carcinoma *in situ* (DCIS) or invasive breast cancer [5]. This trend is expected to continue, resulting in a surge of 15% in new cases between 2018 and 2040 [6].

The vast majority of patients with newly diagnosed breast cancer in developed countries present without evidence of metastatic disease. For these patients, the optimal treatment strategy depends on the stage at presentation. Mastectomy is indicated for patients who are unsuitable candidates for breast-conserving surgery or prefer mastectomy. **Breast-conserving surgery** (BCS), also referred to as lumpectomy or partial mastectomy, is a standard treatment option for early-stage breast cancer and locally advanced tumors after neoadjuvant chemotherapy [7], [8]. BCS offers the potential for better cosmetic outcomes and preserved breast function [9], [10]. However, one issue is the presence of positive margins after surgery, which is an important risk factor for tumor recurrence, leading to the need for additional surgery, adjuvant therapy and a worse cosmetic and oncological outcome [11], [12]. Therefore, minimizing the occurrence of positive margins is an important goal in BCS. This can be achieved by addressing the **factors influencing a positive margin.**

Furthermore, oncoplastic reconstructive surgery (BCS in combination with reconstruction) leads to better aesthetic results, an increase in tumor-free margins, and a reduction of re-excision rates [13], [14]. However, oncologic resection is often more extensive than expected, sometimes resulting in the plastic surgeon

deviating from the predetermined plan. For optimal planning of the reconstruction, it is mandatory to estimate **lumpectomy size** as accurately as possible.

PATHOLOGICAL NIPPLE DISCHARGE

Palpable lumps, pain, and nipple discharge are the most common breast-related complaints [15]. When nipple discharge is unilateral, spontaneous, and bloody or serous arising from a single duct orifice of the nipple, it is defined as pathological nipple discharge (PND) [16]. The etiology of PND is diverse and ranges from benign conditions, such as lactation, duct ectasia, and papilloma, to malignant conditions, such as carcinoma *in situ* and invasive breast cancer [17], [18]. PND accounts for 3-5% of surgical breast clinic referrals [19]–[22]. PND is a clinical diagnosis confirmed through patient history and physical examination. In patients with confirmed PND, current guidelines advise further evaluation with mammography and breast ultrasound to rule out underlying malignancy. Both these techniques have a low sensitivity (22-50%) in detecting malignancy when PND is the only complaint [23], [24]. Moreover, magnetic resonance imaging (MRI) has a high sensitivity for detecting malignancy, but specificity is low in patients with PND which leads to "overdiagnosis" and additional invasive procedures as a consequence [25], [26]. Furthermore, detecting small lesions with MRI has proven to be difficult. Therefore, the value of MRI is limited in patients with PND, and core needle biopsy or surgical excision is still necessary when MRI shows a suspicious lesion [27], [28]. Therefore, current diagnostics often result in a series of negative results, leaving the physician with a diagnostic dilemma.

Other diagnostics, such as cytology of the nipple discharge, are used to determine the risk of malignancy in patients with PND. However, cytology is a poor indicator of malignancy, and the clinical relevance is contested [22], [29], [30]. Nowadays, **nipple fluid-based microRNA assessment** in the context of early breast cancer detection as a potential new class breast cancer biomarker is a rising research topic. This new technique could lead to identifying novel biomarkers in nipple discharge to differentiate between benign and malignant lesions [31], [32].

DUCT EXCISION SURGERY

In patients suffering from PND without radiological and clinical abnormalities, surgical excision is traditionally required to rule out malignancy. Two widely adopted techniques of surgical excision are microdochectomy and major duct excision. These procedures are performed "blindly" and are not completely without risks. In 5-18% of cases, there is an underlying (pre)malignancy, but in most cases it is caused by benign intraductal abnormalities such as papillomas located directly behind the nipple, resulting in the majority of the surgical procedures being performed for benign causes [33]–[35]. Besides its diagnostic value, duct excision surgery is also thought to have a therapeutic effect on PND complaints. However, **recurrence of complaints** can occur in the operated patients. Figure 1 illustrates the work-up of PND.

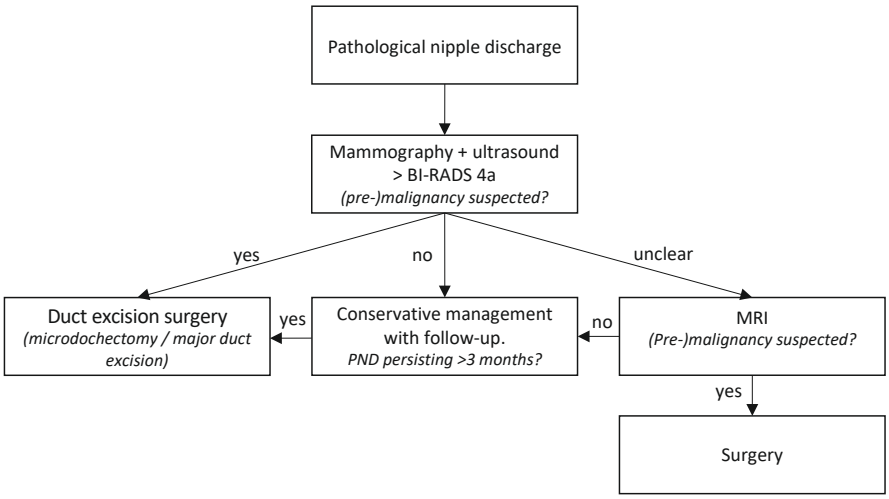


Figure 1: Work-up of PND without the implementation of interventional ductoscopy.

INTERVENTIONAL DUCTOSCOPY

Most breast diseases, both malignant and benign, are thought to derive from the epithelial lining of the milk ducts of the breast [36], [37]. This makes the intraductal system ("ductal tree") of the breast ideal for investigating the first signs of the development of breast lesions and early local treatment. The inability of current imaging techniques to detect these early lesions is the rationale behind the development of methods that would allow a direct approach to the ductal system of the breast. Ductoscopy is a minimally invasive micro-endoscopic technique that allows direct visualization of the milk ducts of the breast through the nipple. It can be performed under local anesthesia in the daily routine at the outpatient clinic and has proven to be safe with only a very low risk of < 2% on (mild) and self-limiting complications [38], [39]. Currently, ductoscopy is used in the diagnostic work-up in patients suffering from PND [40]–[42]. Ductoscopy can avoid unnecessary diagnostic surgical procedures in about 2 out of 3 patients [43]. Figure 2 shows the work-up of PND after the implementation of ductoscopy.

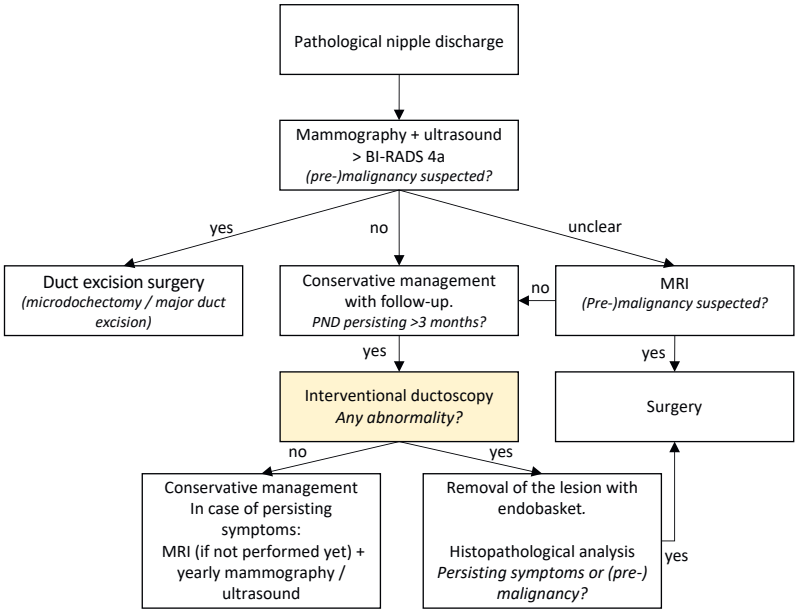


Figure 2: Work-up of PND after the implementation of interventional ductoscopy.

Ductoscopy is a developing diagnostic and interventional procedure as part of the guideline for the management of PND. However, it has not been widely adopted. The current biopsy tool is not sufficiently accurate, resulting in unreliable histopathological diagnosis in some patients. Additionally, a systematic review describing the diagnostic opportunities in PND shows that ductoscopy has an average sensitivity of 58% and specificity of 92% [24]. Therefore, there is a need for new techniques surrounding ductoscopy to increase the sensitivity for the detection of premalignant lesions.

To improve the interventional capabilities of ductoscopy, a cooperation with the department of Biomechanical Engineering of the Delft University of Technology was started. This cooperation has led to the development of new **intraductal biopsy tools** that can be used during ductoscopy.

Another way to improve the interventional possibilities of ductoscopy is by adding **laser ablation**. Laser ablation techniques are widely used in medicine and have proven to be safe and able to evaporate benign and (pre)malignant lesions [44], [45]. One *ex vivo* feasibility study of endoscopic intraductal laser ablation of the breast showed that laser ductoscopy is technically feasible and can serve as an adjuvant tool for the minimally invasive treatment of intraductal papillomas in patients with PND [46]. However, an in vivo feasibility study has yet to be performed to test the safety and effectiveness.

The University Medical Center (UMC) Utrecht is the only Dutch institution currently performing ductoscopy. Over the last decade, more than 500 women with PND were successfully treated. The research team in UMC Utrecht is one of the few worldwide that is actively pursuing innovative techniques to improve the interventional ductoscopy procedure. This doctoral thesis represents the third about the development of interventional ductoscopy by our research team.

THESIS OUTLINE

This thesis presents new intraductal techniques for the diagnosis and treatment of neoplastic breast diseases, alongside insights aimed at optimizing breast-conserving surgery in the context of breast cancer treatment.

Part I: New intraductal techniques in the diagnosis and treatment of *pathological nipple discharge*

The first part of the thesis focuses on new techniques for patients suffering from PND.

In **Chapter 2**, we systematically reviewed the role of duct excision surgery in the treatment of PND and the detection of breast carcinoma. In **Chapter 3,** the state of the art in performing ductoscopy in The Netherlands is described. **Chapter 4** shows the results of a second ductoscopy procedure in patients with recurrent and persistent pathological nipple discharge. In **Chapter 5**, we described the design and evaluation of novel miniature biopsy needles for ductoscopy. In **Chapter 6,** a protocol for an interventional study is reported for the feasibility of narrow-band imaging, intraductal biopsy, and laser ablation for intraductal breast papillomas. In **Chapter 7**, the results of in vivo intraductal laser ablation during ductoscopy in patients with PND are described. **Chapter 8** shows a case report of a rare complication (idiopathic granulomatous mastitis) after ductoscopy. **Chapter 9** focuses on the value of microRNA expression analysis in detection of intraductal papillomas in patients with PND.

Part II: Optimizing breast-conserving surgery in the treatment of breast cancer

The second part of the thesis focuses on new insights in breast-conserving surgery in the treatment of breast cancer.

Chapter 10 proposes predictors of postoperative lumpectomy size in optimizing breast-conserving surgery in breast cancer patients. In **Chapter 11**, we described

clinicopathological factors affecting positive margins after breast-conserving surgery.

In **Chapter 12** and **Chapter 13**, 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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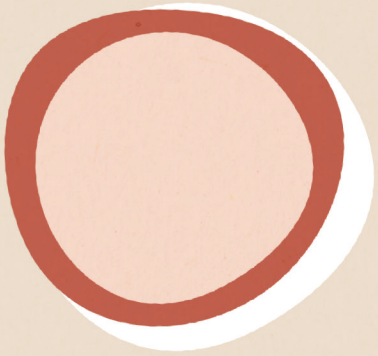
PART I

Intraductal techniques in the diagnosis and treatment of pathological nipple discharge



CHAPTER 2

The role of duct excision surgery in the treatment of pathological nipple discharge and detection of breast carcinoma: systematic review

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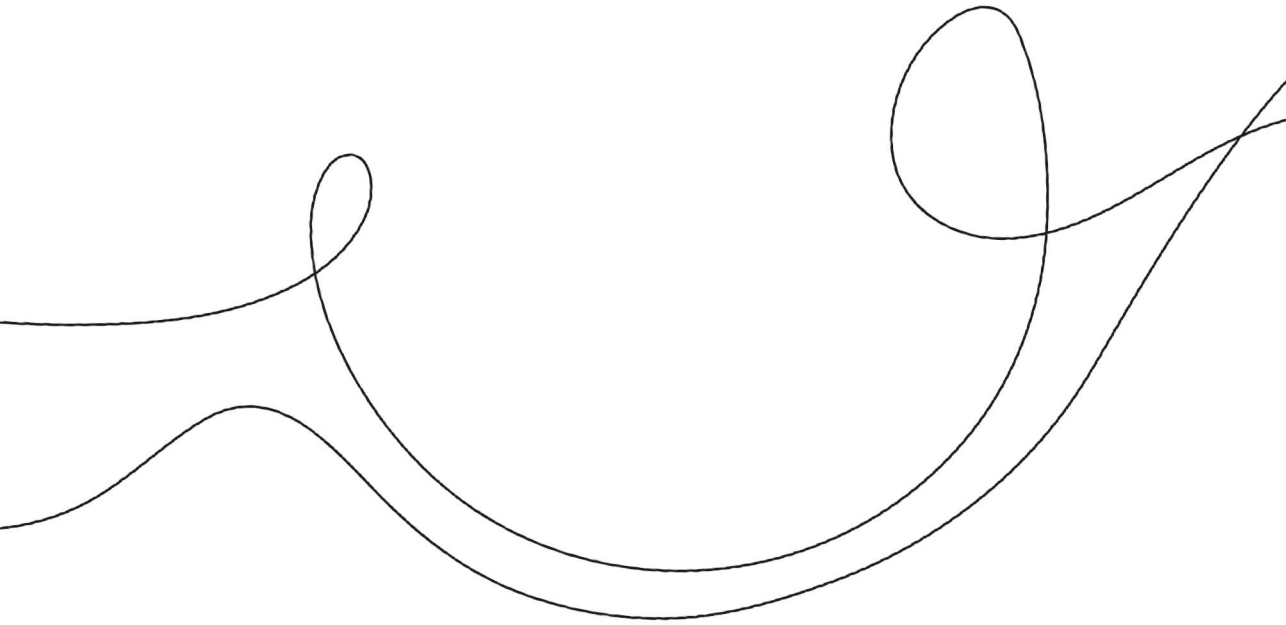

ABSTRACT

Background: The role of duct excision surgery is not clearly defined in patients with pathological nipple discharge without other clinical and radiological abnormalities. The primary aim of this systematic review was to determine the malignancy rate in patients with pathological nipple discharge after duct excision surgery (microdochectomy/major duct excision). The secondary aims were to determine the recurrence rate of pathological nipple discharge and to assess breast cancer development after surgery.

Methods: MEDLINE and Embase were searched from inception to March 2023, using search terms related to 'nipple discharge', 'nipple fluid', 'microdochectomy', 'duct excision' and 'minimally invasive surgical procedure'. Studies reporting data about women who underwent duct excision surgery for pathological nipple discharge without clinical and radiological suspicion of breast cancer, as well as reporting data on women diagnosed with breast cancer after duct excision surgery, were included.

Results: A total of 318 titles were identified, of which nine publications were included in the analysis. This resulted in 1108 patients with pathological nipple discharge who underwent a duct excision. The weighted mean rate of malignancy after duct excision surgery was 8.1 per cent (ranging from 2.3 to 13.5 per cent). Three studies described the recurrence rate of pathological nipple discharge (ranging from 0 to 12 per cent) and two studies reported breast cancer development in the follow-up in a total of three patients (less than 1 per cent).

Conclusion: The malignancy rate after duct excision surgery for pathological nipple discharge was low in patients with pathological nipple discharge without radiological and clinical abnormalities and approximately 9 of 10 patients undergo surgery for a benign cause. Improvement of the diagnostic and therapeutic workup is needed to prevent patients from undergoing (unnecessary) exploratory surgery.

INTRODUCTION

Nipple discharge is a common symptom, reported in 2-5 per cent of all women and in 8 per cent of women presenting with a breast complaint [1-3]. When nipple discharge is unilateral, spontaneous, bloody or serous, and arising from a single duct orifice of the nipple, it is defined as pathological nipple discharge (PND) [4]. The common causes of PND are benign (ductal ectasia and intraductal papillomas) [5,6]. However, it is also associated with breast cancer [7,8].

PND is a clinical diagnosis confirmed through patient history and physical examination. In patients with confirmed PND, current guidelines advise further evaluation with mammography and breast ultrasound to rule out underlying malignancy. These techniques both have a low sensitivity (22 and 50 per cent) in detecting malignancy when PND is the only complaint [9,10]. On the other hand, MRI has a high sensitivity in detecting malignancy at the cost of low specificity [10]. Furthermore, detecting small lesions with MRI has proven to be difficult [11,12]. Hence, surgical excision and histopathological examination are needed to confirm diagnoses made with MRI [13,14]. Therefore, MRI is of limited added value to patients with PND. Another available diagnostic technique is ductoscopy, which has not yet been widely adopted, despite advances and increasing interest in recent decades [15-17].

In patients suffering from PND without radiological and clinical abnormalities, surgical excision is traditionally required to rule out malignancy. Two widely adopted techniques of surgical excision are microdochectomy and major duct excision. Microdochectomy is the excision of a single duct, and major duct excision is the removal of all lactiferous ducts under the nipple. These procedures are performed 'blindly' and carry risks. Adverse cosmetic outcomes, as well as altered lactation and sensitivity of the nipple, have been reported [7,18,19]. Previous studies reported a malignancy rate of 9.3 – 37 per cent in patients suffering from PND, but these studies also included patients with radiological and/or clinical suspicion (palpable mass) of malignancy [19-22]. Therefore, the malignancy rate of patients suffering from PND without radiological and clinical suspicion is not yet accurately represented because the data are sparse and studied within small

populations. Moreover, the wide range of malignancy rates in previous studies makes interpretation of data difficult. A more representative malignancy rate in this population could be relevant to help better identify patients at risk and potentially prevent unnecessary exploratory surgery.

Besides its diagnostic value, duct excision surgery is also thought to have a therapeutic effect on PND complaints. Currently, there is no overview of the therapeutic effect and the recurrence rate of PND after surgery. Also, there are a lack of data about breast cancer development after duct excision surgery in patients suffering from PND.

The aim of this systematic review was to assess the rate of malignancy in patients with PND undergoing duct excision surgery, the recurrence rate of PND after surgery, and the development of breast carcinoma in the follow-up after surgery for PND in patients without other clinical or radiological abnormalities.

METHODS

This systematic review was designed and reported according to the principles of the PRISMA 2020 guidelines for reporting systematic reviews [23]. A checklist is presented in the supplementary material. The research was registered in the International Prospective Register of Systematic Reviews (PROSPERO 2022 CRD42022306622) [24].

Data sources and searches

With the help of an experienced librarian, a broad electronic search was conducted using index terms and free text words in MEDLINE and Embase from inception to March 2023, without language restrictions. Scopus was used to fine-tune the initial MEDLINE search. Also, forward citation analyses and backward bibliographic sampling of included articles were conducted. This search strategy included terms related to 'nipple discharge', 'nipple fluid', 'microdochectomy', 'duct excision', and 'minimally invasive surgical procedure'. The full search is shown in

the Supplementary Table 1. Reference lists from eligible articles were also examined to identify publications. The last search was conducted on 9 March 2023.

Study selection

Citations from all search results were downloaded and merged using Rayyan, an online program for systematic reviews [25]. According to the predefined inclusion and exclusion criteria, two authors (S.M. and J.W.) screened titles and abstracts independently. Then, full-text articles were reviewed for eligibility independently by the same authors. Disagreements were settled by consensus or a third author (A.J.W.) was consulted for adjudication.

Studies were included that reported data about women with PND without clinical and radiological suspicion of breast cancer and also reported data about women who were diagnosed with breast cancer after duct excision surgery. PND was defined as spontaneous, single-duct nipple discharge during a non-lactational interval, persisting for more than 3 months. This review used the definition of malignancy as described in the included studies.

Imaging criteria were the absence of abnormalities on radiological examination (lesions suspected of being indicative of breast cancer) and the use of diagnostic mammography and ultrasound in the work-up of PND, with or without biopsy.

Studies were excluded if they had any of the following characteristics: abstract-only publications, case reports, case series, papers from which the full-text was missing or unavailable, and conference abstracts; insufficient data or irrelevant research question for this review; studies written in languages other than English, Dutch, Turkish, German, or Spanish; use of interventional ductoscopy before intervention; data from patients with a palpable mass, and studies with a study population before 1995 because of discordant diagnostic possibilities compared with the current diagnostic workup.

Data extraction and analysis

The data extraction was performed independently by two authors (S.M. and J.W.), using an Excel-based spreadsheet (Microsoft®). Outcomes reported in any article

are summarized qualitatively in this systematic review. These include information on publication details, study design, number of eligible patients in each study, complication rate, follow-up, recurrence of complaints, and histological analysis. All data were tabulated and presented as percentages. A modified rating grade (from 1 to 5) from the Oxford Centre for Evidence-based Medicine was used to determine the quality of the evidence [26]. Re-excision surgery for malignancy was not noted as a complication.

RESULTS

Search outcome and study characteristics

The study selection process with reasons for exclusion is described in Fig. 1. The literature search resulted in 209 articles in MEDLINE and 109 articles in Embase, giving a total of 318 articles. After removing duplicate publications, 229 titles and abstracts were screened for eligibility, of which 184 articles were excluded for not meeting the inclusion criteria. Full-text articles were reviewed for the 45 studies identified as potentially eligible by one or both reviewers. Nine articles were selected for inclusion in the final analysis.

The study characteristics and results are summarized in Table 1. Of the selected nine studies, six were from Europe, two from North America, and one from Asia. The sample size of these studies ranged from 33 to 214, resulting in a total of 1108 patients included in this study. The age of patients ranged from 17 to 88 years (the median age ranged from 47.8 – 56.7 years). The quality rating score for the level of evidence ranged from 1 to 3.

Histopathological findings

The majority of the lesions at microdochectomy and major duct excision were benign (ranging from 86.5 to 97.7 per cent). Reported benign lesions were unspecified benign tissue, intraductal papilloma, hyperplasia, duct ectasia, atypia, inflammatory, fibroadenoma, fibrocystic changes, and sclerosing lesions. Reported malignant lesions were ductal carcinoma *in situ* (DCIS), invasive carcinoma, and in one study, lobular carcinoma *in situ* (LCIS). DCIS was the most common ma-

lignant lesion in the study population. Based on the selected studies, the weighted average rate of malignancy after duct excision surgery was 8.1 per cent (ranging from 2.3 to 13.5 per cent), as shown in Fig. 2.

Follow-up, breast cancer development, and recurrence of pathological nipple discharge

The length of follow-up after duct excision surgery was noted in three studies (ranging from 0.5 to 13 years) (Table 1). The other six studies did not report follow-up data. Two of the studies reported on the development of breast cancer; Gui et al. reported no breast cancer development during a follow-up of 3-9 years (zero of 66 patients) [27] and Wong Chung et al. reported three patients with breast cancer during a follow-up interval of 3-12 years (three of 184 patients, 1.6 per cent) [28]. These three patients had primary benign histology after surgery and developed a tumor in the ipsilateral breast, but at different locations. Those malignancies were considered as new and not related to the initial duct excision surgery.

Three studies described the recurrence rate of PND (ranging from 0 to 12 per cent). Çetin et al. reported a recurrence rate of 0 per cent; however, the follow-up period was not reported [29].

Complications

Complications were reported in 15 patients of the total study population of 1108 patients (1.4 per cent) after duct excision surgery (Supplementary Table 2): five patients had haematomas, five patients had postoperative surgical site infections, four patients had postoperative seromas, and one patient had partial necrosis of the areola, which healed with conservative treatment. The complication rate in the included studies ranged between 0 to 9 per cent; three studies did not report the complication rates.

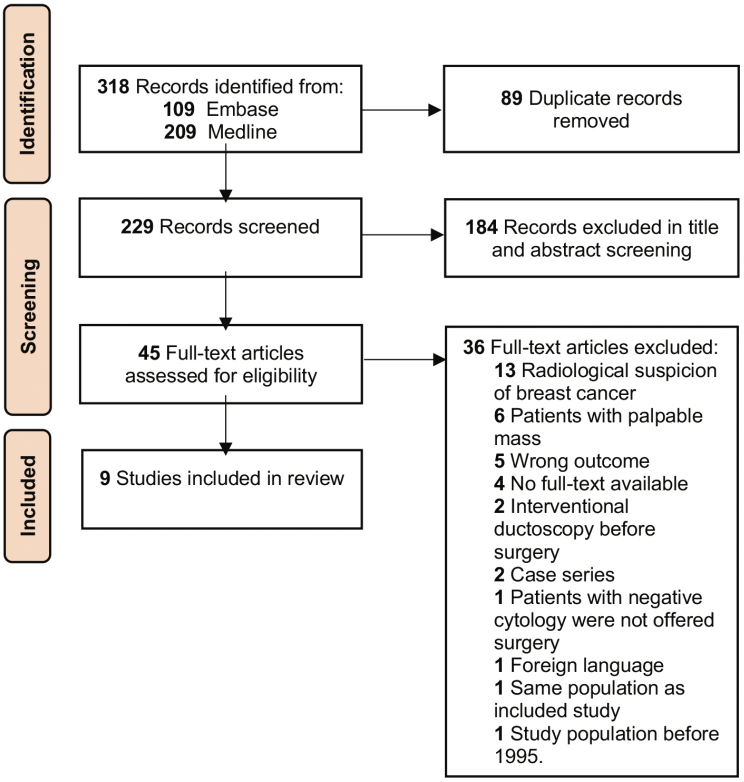

Fig. 1 PRISMA flow diagram of literature search and selection of studies

Table 1 Study characteristics and results

Study	Country	Study design	Quality rating score*	Sample size	Pathology: benign# (%)	Pathology: malignant (%)	Follow-up (years)	Follow-up: breast cancer development	Recurrence of PND (%)
Çetin et al, 2020 [29]	Turkey	Retrospective cohort	3	111	86.5	DCIS: 7.2 IC: 6.3	-	-	0
Foulkes et al, 2011 [30]	UK	Prospective cohort	2	194	94	DCIS: 4 IC: 2	-	-	12
Gui et al, 2018 [27]	UK	Randomized clinical trial	1	66	92.4	DCIS: 7.6 IC: 0	3 - 9	No	3
Hahn et al, 2009 [31]	Germany	Prospective cohort	3	33	93.9	DCIS: 3 IC: 3	0.5	-	-
Lustig et al, 2019 [32]	Canada	Retrospective cohort	3	155	87	DCIS: 10 IC: 3	-	-	-
Ohlinger et al, 2020 [33]	Germany	Prospective cohort	2	214	94.9	DCIS: 4.6 IC: 0.5	-	-	-
Richards et al, 2007 [34]	UK	Retrospective cohort	3	86	97.7	DCIS: 1.2 LCIS: 1.1 IC: 0	-	-	-
Simpson et al, 2009 [35]	Canada	Retrospective cohort	3	65	95.4	DCIS: 3.1 IC: 1.5	-	-	-
Wong Chung et al, 2016 [28]	The Netherlands	Retrospective cohort	3	184	89.2	DCIS: 7.6 IC: 3.3	3-13	Yes, in three patients	-

*= Quality rating score for studies and evidence. 1: Properly randomized clinical trial; systematic review with meta-analysis; 2: Well-designed controlled trial without randomization; prospective comparative cohort trial; 3: Case-control studies; retrospective cohort study; 4: Case series with or without intervention; cross-sectional study; 5: Opinion of respected authorities; case reports

#= benign tissue, intraductal papilloma, hyperplasia, duct ectasia, atypia, inflammatory, fibroadenoma, fibrocystic changes, sclerosing lesion

PND= pathological nipple discharge, DCIS= ductal carcinoma *in situ*, IC= invasive carcinoma, LCIS= lobular carcinoma *in situ*

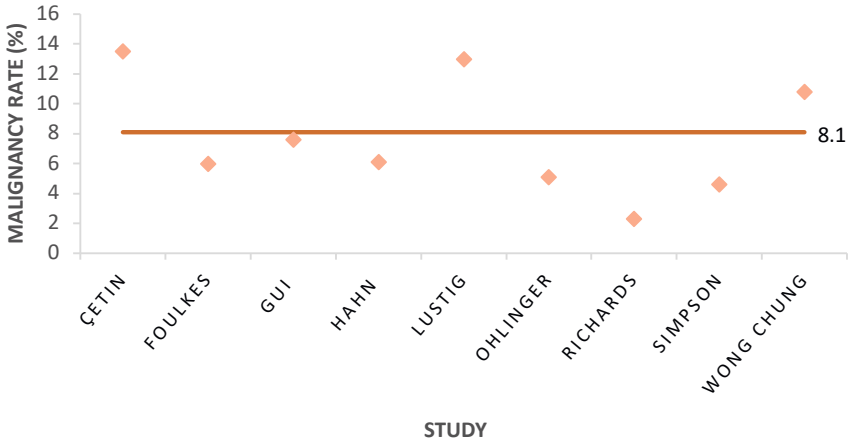

Fig. 2 Malignancy rates after duct excision surgery for pathological nipple discharge in the included studies. The histopathological results of malignancy after duct excision surgery in patients with pathological nipple discharge without radiological and clinical abnormalities from all included studies. The weighted average rate of malignancy was 8.1 per cent.

DISCUSSION

Here, the role of duct excision surgery in the detection of breast carcinoma in patients with PND is reported. Breast carcinoma was found in only 8.1 per cent of patients with PND without radiological and clinical abnormalities. This means that the majority of patients underwent surgery for benign lesions in this study. Thus, improvement of the diagnostic and therapeutic workup is needed to prevent patients from undergoing unnecessary exploratory surgery. Furthermore, recurrence of PND after duct excision surgery was reported in 0-12 per cent of patients, meaning that excision surgery cures PND complaints in more than 88 per cent of patients. Breast cancer development was poorly described in the included studies and could not be appropriately assessed.

Previous studies examining duct excision surgery did not differentiate between studies reporting cases with or without clinical abnormalities (palpable mass) and/or radiological abnormalities. These studies with clinical and/or radiological abnormalities reported a relatively high malignancy rate, ranging from 9.3

to 37 per cent [5,19-21,36], compared with the malignancy rate in this review. This difference is attributed to the presence of a palpable mass and radiological abnormalities and the chance of finding malignancy upon histopathological examination [37-39].

The complications reported after surgery were concordant with previous results [7,18,19]. According to the author's study population, duct excision surgery is a safe intervention, with a low complication rate of 1.4 per cent in the total study population. Nevertheless, it is performed under general anesthesia, and re-excision is needed in patients with histopathological confirmation of malignancy. Therefore, opting for surgical excision should be carefully considered.

Nowadays, in the standard workup of PND in women above 40 years, mammography and ultrasound are the key diagnostic methods to rule out malignancy [14]. Studies that included patients before 1995 were excluded in this review because of discordant diagnostic possibilities compared with the current diagnostic workup. Therefore, the largest report on microdochectomy for PND, which showed a malignancy rate of 23.9 per cent in 915 patients, was excluded because no ultrasound was performed before microdochectomy [7]. Studies were also excluded when interventional ductoscopy was performed before surgical intervention. According to previous studies, the therapeutic value of ductoscopy makes it possible to remove intraductal lesions [15,40]. Also, according to a previous study by the authors, ductoscopy prevented surgery in two of three patients with PND [17]. These findings would distort the results of this review and could lead to a higher malignancy rate due to a preselection bias of the population.

Furthermore, there was a wide range of ages for the included patients. The effect of age is not clear regarding the decision for surgical treatment. According to one included study, the median age of patients with bloodstained discharge due to breast cancer was higher than that of the patients with benign disease. Moreover, it has been suggested that a conservative policy could be adopted for women under the age of 40 years [41]. According to this review, this statement cannot be supported because of a lack of information about the ages of the included patients.

Yet, this information could be crucial in the decision for the workup of patients with PND and needs further evaluation.

The role of surgery in the actual treatment of PND was described in just three studies that reported a recurrence rate of PND ranging from 0 to 12 per cent [27,29,30]. Gui et al. reported a follow-up interval of 3 – 9 years; in the other two studies, the follow-up interval was not specified [27]. These results show that duct excision surgery cures PND in more than 88 per cent of patients. This was in line with other studies; Chang et al. reported no recurrence [42], and Dillon et al. reported 9 per cent recurrence of PND in a median interval of 7 months [8].

Furthermore, the included studies poorly described breast cancer development in patients after duct excision surgery. One study reported no breast cancer development during a follow-up of 3 – 9 years [27] and, in one study, three patients developed breast cancer during a follow-up of 3 – 12 years [28]. These three patients had a tumor in the ipsilateral breast, but at different locations and after such a time interval that Wong Chung et al. considered these as 'de novo' malignancies [28]. Nevertheless, these malignancies may have developed after a false-negative microdochectomy. Dillon et al. reported three patients diagnosed with malignancy at 2, 8, and 9 years after the initial resection [8]. However, it was not clear if these malignancies were 'de novo' or a result of the initial complaints and operation. More research is needed about breast cancer development during follow-up after duct excision surgery to address the efficacy of the procedure and help in decision-making in the workup of patients with PND.

To prevent patients from undergoing unnecessary surgery, the focus should be on improving the diagnostic and therapeutic capabilities for intraductal breast lesions. Nowadays, a percutaneous core or vacuum-assisted biopsy is performed to obtain tissue for histopathological examination. However, when the diagnosis after biopsy remains unclear, or a benign intraductal lesion such as papilloma is found and the nipple discharge persists after biopsy, a duct excision surgery is recommended. An additional diagnostic and interventional procedure such as ductoscopy in the workup of PND without clinical or radiological abnormalities can improve the selection of patients for surgical procedures, as it is possible to

remove intraductal lesions and treat PND ductoscopically. When no or histologically proven benign lesions are found during ductoscopy, surgical procedures such as a (major) duct excision have, in the author's opinion, no additional diagnostic value.

This review has limitations resulting from the quality and scope of articles identified through the systematic review. There is only a limited number of studies investigating the development of breast cancer in patients with PND after duct excision surgery. Also, few studies describe the therapeutic effect of duct excision surgery and the recurrence of complaints. Due to the heterogeneous designs of the included studies and limited data, it was not possible to perform a meta-analysis, which limited the strength of the evidence. In addition, in most included articles, the findings on imaging were not defined using the breast imaging-reporting and data system (BI-RADS) classification, which is a widely accepted reporting system for imaging of the breast and applies to mammography, ultrasound, and MRI. Therefore, papers were only included when the imaging criteria were not suspicious of breast cancer and the duct excision surgery was performed because the nature of the discharge caused concern. The wide range of radiological abnormalities and differing inclusion criteria for patients represent limitations. Thus, further prospective research is required to follow-up patients with PND after duct excision surgery to generate accurate data about recurrence of PND and breast cancer development.

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Supplementary Table 1: Search strategy implemented for two databases

Database	Search	Results
PubMed	(nipple discharge*[Title/Abstract] OR nipple fluid*[Title/Abstract] OR "Nipple Discharge"[Mesh]) AND (minimally invasive surgical procedure*[Title/Abstract] OR microdochectom*[Title/Abstract] OR duct excision*[Title/Abstract] OR "Minimally Invasive Surgical Procedures"[Mesh])	209
Embase	('nipple discharge*':ti,ab,kw OR 'nipple fluid*':ti,ab,kw OR 'breast discharge'/exp) AND ('minimally invasive surgical procedure*':ti,ab,kw OR 'microdochectom*':ti,ab,kw OR 'duct excision*':ti,ab,kw OR 'minimally invasive procedure'/exp) AND [embase]/lim AND ('article'/it OR 'article in press'/it)	109

ti,ab,kw = terms in either title or abstract or keyword fields

Supplementary Table 2: Complications reported in the studies

Author, year	*Complications*
Çetin, 2020	1 patient surgical site infection
Foulkes, 2011	Not reported
Gui, 2018	1 patient hematoma
Hahn, 2009	1 patient with partial necrosis of areola
Lustig, 2019	Not reported
Ohlinger, 2020	No complications
Richards, 2007	Not reported
Simpson, 2009	4 patients seroma leak
Wong Chung, 2016	4 patients postoperative infection 4 patients minor hematoma

Supplementary table 3: The PRISMA guideline for reporting systematic reviews

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	4
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	5,6
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	5
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	5,6
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	5,6
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	5,6
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	6
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Na

Supplementary table 3: The PRISMA guideline for reporting systematic reviews *(continued)*

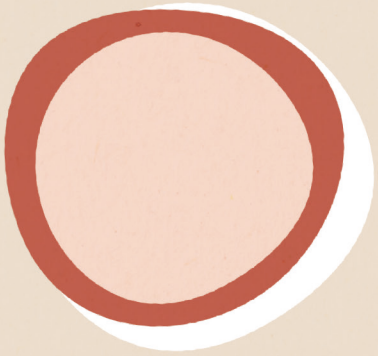
Section and Topic	Item #	Checklist item	Location where item is reported
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	6
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Na
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	6
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Na
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	5,6
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Na
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Na
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Na
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Na
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Na
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	7,8
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	7,8
Study characteristics	17	Cite each included study and present its characteristics.	7,8,9
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	7,8,9

Supplementary table 3: The PRISMA guideline for reporting systematic reviews *(continued)*

Section and Topic	Item #	Checklist item	Location where item is reported
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Na
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Na
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Na
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Na
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Na
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Na
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Na
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	9,10,11
	23b	Discuss any limitations of the evidence included in the review.	9,10,11
	23c	Discuss any limitations of the review processes used.	9,10,11
	23d	Discuss implications of the results for practice, policy, and future research.	9,10,11
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	5
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	5
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Na
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	12
Competing interests	26	Declare any competing interests of review authors.	12

Supplementary table 3: The PRISMA guideline for reporting systematic reviews *(continued)*

Section and Topic	Item #	Checklist item	Location where item is reported
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	13



CHAPTER 3

Has ductoscopy for pathological nipple discharge proved to be useful?

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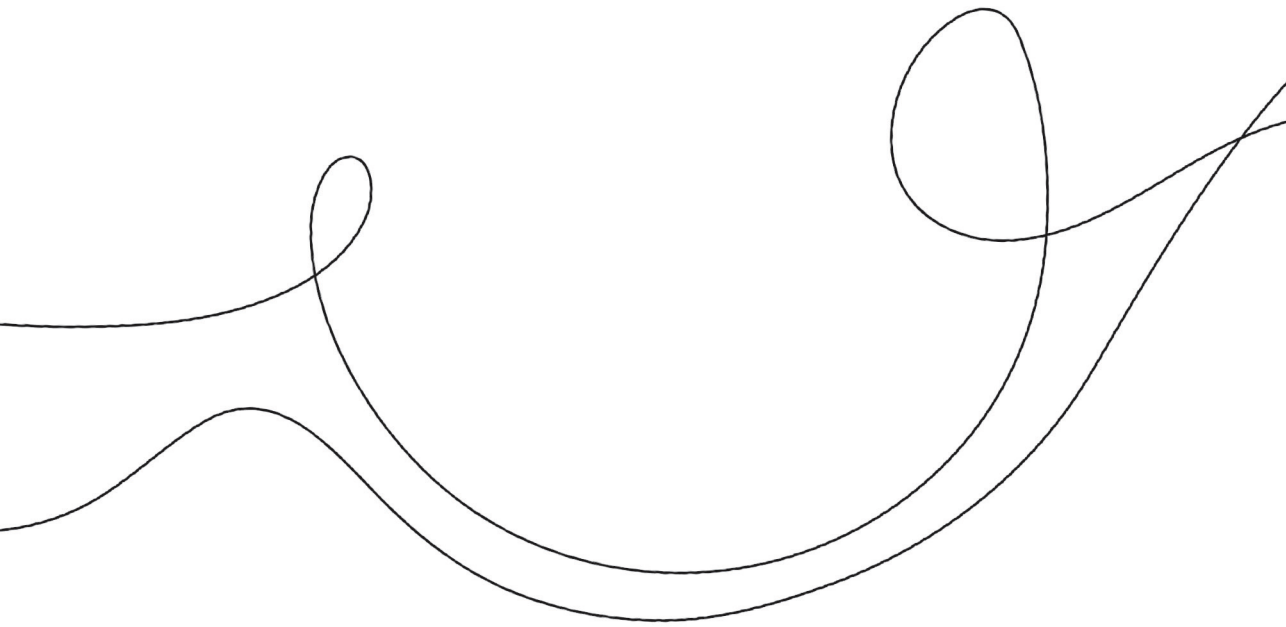

ABSTRACT

Pathological nipple discharge (PND) is a common complaint in women and is defined as spontaneous, unilateral (bloody or serous) nipple discharge from a single milk duct. In 5-10% of cases, there is an underlying (pre)malignancy, but in most cases, it is caused by benign intraductal abnormalities located directly behind the nipple. As a result, mammography and ultrasound often show no abnormalities, which necessitates a (diagnostic) surgical procedure. Ductoscopy is a minimally invasive, microendoscopic technique that allows the visualization of the milk ducts through the nipple orifice under local anesthetic. This technique was already described in 2013 in the NTvG as a new innovative technique, which makes it possible to diagnose and remove intraductal abnormalities and, therefore, is able to effectively select patients who will benefit from a classic operation under general anesthesia. Surgery can be prevented in 2/3 of patients by adding ductoscopy to the work-up in case of PND. Ductoscopy has now been included in the "breast cancer" guideline in The Netherlands.

Which technique?

Ductoscopy is a micro-endoscopic technique in which the milk ducts of the breast are visualized through the nipple [1]. In this way, intraductal abnormalities can be detected and, using the same approach, removed (Fig. 1). Ductoscopy is performed in the outpatient clinic on patients under local anesthesia. The procedure is performed by a surgeon, assisted by a nurse.

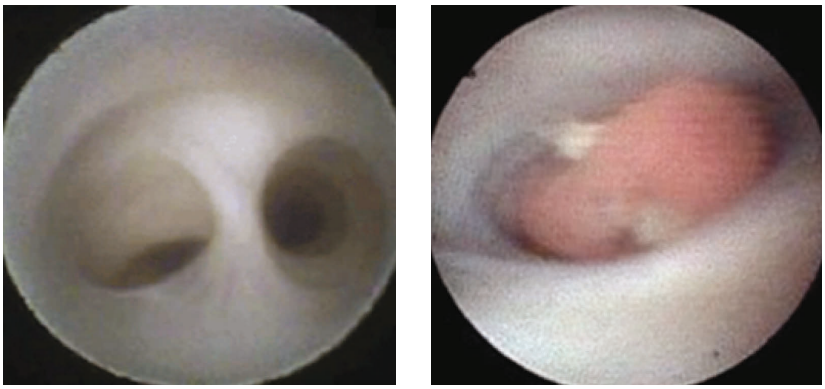

Fig. 1: Ductoscopic images of a normal milk duct (left) and an intraductal papilloma (right).

What is currently known about the effectiveness and side effects?

A meta-analysis of the diagnostic approach showed that ductoscopy can detect malignancies with a specificity of 92% and a sensitivity of 58%. Because the a priori risk of malignancy is low (5-8%) and the goal is to avoid unnecessary surgery, especially high specificity (indicating a low chance of a false positive result) is crucial. High sensitivity is less important due to the low a priori risk of malignancy. Furthermore, diagnostic surgery or long-term follow-up with imaging is still possible when ductoscopy does not provide a definitive diagnosis. The diagnostic accuracy of ductoscopy (how often a test is true positive and true negative compared to the total number of patients) is 95%, which is relatively high. For comparison, an MRI scan in patients with PND has an accuracy of 83%.

A study involving 215 patients in our center showed that after ductoscopy with intervention, approximately 66% of the patients no longer needed surgery. Therefore, ductoscopy reduces the number of patients undergoing surgery. Additionally,

ductoscopy is a safe procedure with minimal side effects; less than 1% of patients may experience mastitis, and 2.5% post-procedural pain lasting more than a day [2].

Have there been RCTs or large series published?

In 2018, a randomized study was published on the value of ductoscopy in patients with PND Patients in the intervention group underwent ductoscopy followed by microdochectomy or conus excision; the control group only underwent microdochectomy or conus excision. This study demonstrated that abnormalities causing PND can be detected just as effectively with ductoscopy as with the prior gold standard, conus excision [3].

Has the technique proven to be cost-effective?

Adding ductoscopy into the diagnostic process for women with PND enables more precise selection of candidates who would benefit from surgical treatment under anesthesia. The literature demonstrates that with the addition of ductoscopy, surgery can be avoided in approximately two-thirds of patients. This not only makes ductoscopy cost-effective but also highly efficient, particularly in times of staffing shortages. A retrospective analysis showed that for patients with PND without abnormalities on mammography and ultrasound, determining the necessity for surgery with ductoscopy proves to be more cost-effective compared to relying on MRI scans [4]. This is explained by the higher specificity of ductoscopy, leading to fewer false positive results.

What are the current indications?

Ductoscopy is used in patients experiencing PND lasting for more than 3 months, when mammographic and ultrasonographic examinations have shown no signs of malignancy. Nipple retraction and prior surgery to the nipple-areola complex are contraindications. Since 2020, ductoscopy has been included in the 'Breast Cancer' guideline as a treatment option for patients with PND.

Did the expectation come true?

Since 2010, nearly 500 patients with PND have been treated with ductoscopy. This has prevented surgery under anesthesia for a large portion of these patients.

To further improve the diagnostic and therapeutic possibilities of ductoscopy, UMC Utrecht, in collaboration with the TU Delft, is conducting research on new endoscopic biopsy techniques and the application of laser ablation for remaining intraductal abnormalities after biopsy.

Where in The Netherlands is the technique applied?

Currently, ductoscopy is only performed at UMC Utrecht. Patients are referred to our center from all across the country. In May 2022, the Dutch Healthcare Institute indicated in a preliminary position that ductoscopy meets "the state of science and practice." This potentially places the technique within the scope of "insurable services under the Health Insurance Act," making its application covered by insurance. It is expected that ductoscopy will be conditionally included in the basic insurance package by 2023. The intention is to initiate an implementation process in early 2023, allowing more hospitals to incorporate ductoscopy into their practices.

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2. Filipe MD, Waaijer L, van der Pol C, van Diest PJ, Witkamp AJ. Interventional Ductoscopy as an Alternative for Major Duct Excision or Microdochectomy in Women Suffering Pathologic Nipple Discharge: A Single-center Experience. Clin Breast Cancer. 2020;20:e334-43.
3. Gui G, Agusti A, Twelves D, et al. INTEND II randomized clinical trial of intraoperative duct endoscopy in pathological nipple discharge. Br J Surg. 2018;105:1583-90. doi:10.1002/bjs.10990.
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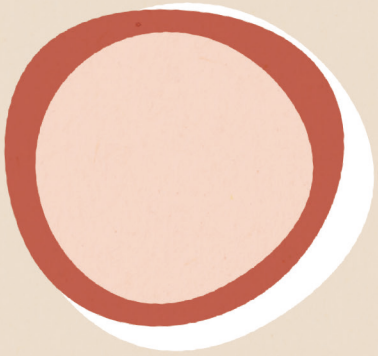

CHAPTER 4

A second ductoscopy procedure in patients with recurrent and persistent pathological nipple discharge

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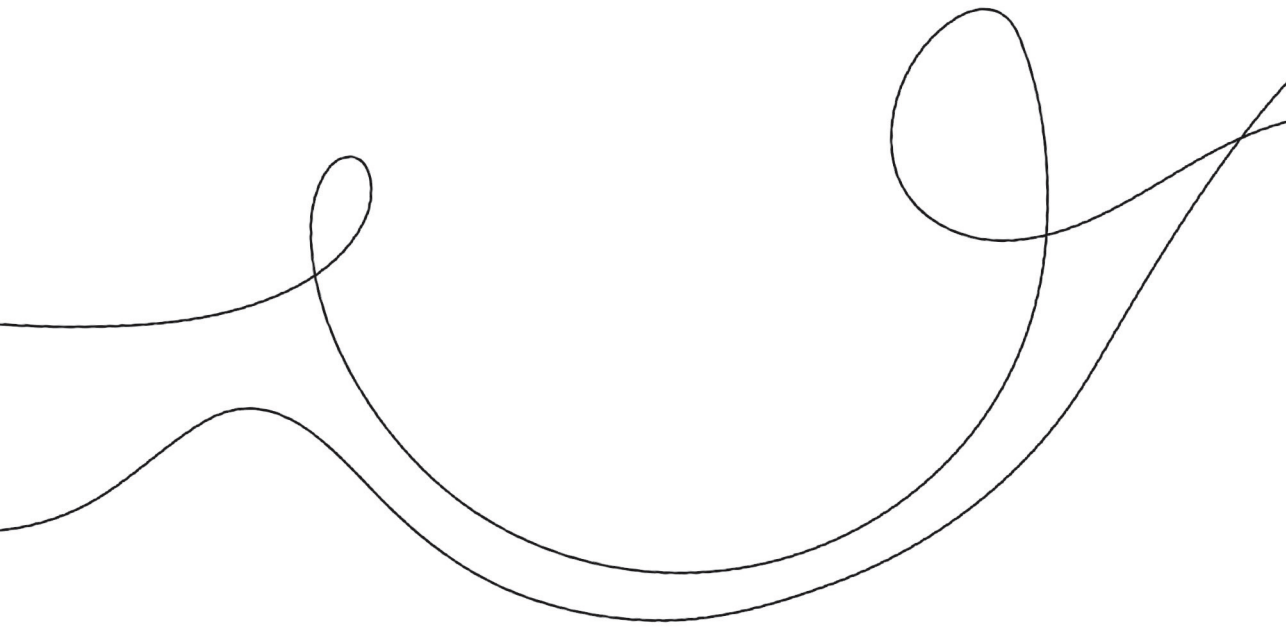

ABSTRACT

Background: Most patients suffering from pathological nipple discharge (PND) undergo local surgical procedures because standard radiologic imaging often fails to reveal the cause. Ductoscopy is a minimally invasive endoscopic technique that enables direct intraductal visualization and can avoid unnecessary diagnostic surgical procedures. However, patients with recurrent or persistent PND after an unsuccessful ductoscopy procedure still undergo unnecessary surgery. This study describes the experience of a second ductoscopy procedure in patients with recurrent or persistent PND without suspicious radiological findings.

Methods: Patients with recurrent or persistent PND who underwent two ductoscopy procedures between 2010 and 2017 were retrospectively analyzed. The second ductoscopy was performed when the first ductoscopic attempt was unsuccessful due to technical problems. The primary outcome was the number of prevented surgical procedures.

Results: A total of seventeen patients underwent two ductoscopy procedures. The first ductoscopy showed a polypoid lesion in ten patients (58.8%), no abnormalities in three patients (17.6%), and in four patients (23.5%), it was not possible to visualize the ductal tree. Post-procedure, all patients suffered from PND. After two ductoscopic attempts, PND stopped in ten patients (58.8%), and seven patients (41.2%) still suffered from PND and were operated on. Pathology of the resection specimens showed no abnormalities in one patient, a papilloma in five patients, and DCIS in one patient.

Conclusion: A second ductoscopy procedure can be considered in the diagnostic work-up of patients suffering from persistent or recurrent PND after an unsuccessful first ductoscopic attempt to avoid unnecessary surgery in about 59% of the cases.

INTRODUCTION

Nipple discharge is the third most common breast-related complaint among women after breast pain and a palpable lump [1]. It is often associated with breast cancer and accounts for up to 5 percent of surgical breast clinic referrals [2]–[4]. Pathological nipple discharge (PND) is defined as spontaneous, persistent, unilateral, and serous or bloody discharge, usually from a single duct opening [5]. The most common causes of PND are benign, namely an intraductal papilloma and duct ectasia [6]–[8]. In around 5-10% of the patients suffering from PND without radiological abnormalities, the cause of nipple discharge is an underlying malignancy (ductal carcinoma *in situ*, DCIS) or invasive carcinoma) [7]–[10].

Patients with PND usually undergo mammography and ultrasound to rule out cancer. However, when PND is the only complaint, mammography and ultrasound show limited sensitivity to detect malignancy (22% and 50% for mammography and ultrasound, respectively) [11]. Magnetic resonance imaging (MRI) has a higher sensitivity (83%), but specificity is low (76%) [11]. A low specificity with a low prevalence of malignancy means that, despite the high sensitivity, the positive predictive value is low. This means that MRI in this group of PND patients often leads to additional (invasive) procedures such as core needle biopsy and diagnostic surgical excision. [12]–[14]. Additionally, cytology of nipple discharge is used to determine malignancy. However, it is a poor indicator of malignancy, and the clinical relevance is contested [15], [16].

Ductoscopy is a minimally invasive micro-endoscopic approach for direct visualization and removal of intraductal lesions of the breast, performed under local anesthesia at the outpatient clinic. After mammography and ultrasound, ductoscopy can be performed in the diagnostic work-up in patients suffering from PND without radiological abnormalities. It is a safe and effective diagnostic and interventional procedure, with a specificity of 92% and a sensitivity of 58% [11], [17], [18].

The majority of patients suffering from PND still undergo local surgical procedures since standard radiologic imaging often fails to reveal the cause. These

surgical procedures may lead to undesirable side effects such as scar tissue, perioperative complications, decreased sensitivity of the nipple, and compromised breastfeeding in the future [18]–[23]. These side-effects are especially important to consider since the most common cause of PND is benign. Ductoscopy can avoid unnecessary diagnostic surgical procedures in around 2 out of 3 patients with PND [24]. However, patients can still suffer from PND after ductoscopy. In most cases, these patients eventually undergo a surgical procedure because of recurrent or persistent PND [18], [25], [26].

Imaging is often used as a follow-up tool for PND. However, there are currently no studies describing the value of a second ductoscopy procedure in patients suffering from PND after an unsuccessful first attempt. Consequently, this study describes the experience with a second ductoscopy procedure in patients with recurrent and persistent PND without suspicious radiological findings.

MATERIALS AND METHODS

Study design and population

This retrospective cohort study included patients with PND without radiological and/or pathological suspicion for malignancy who underwent two regular patient care indicated ductoscopy procedures between 2010 and 2017 at the University Medical Centre Utrecht (UMCU) in The Netherlands. PND was defined as persistent, unilateral, bloody or serous nipple discharge during a non-lactational period, persisting for at least three months. The study population was a part of a previously conducted cohort study by the same research team [24].

Standard clinical variables were collected, including age at presentation, characteristics of the nipple discharge (laterality and spontaneous versus expressed), physical exam findings (palpable breast mass and productive ducts), and follow-up period. In addition, diagnostic methods, findings from any imaging studies performed, operative procedures, and histopathological details were recorded for each case.

Before ductoscopy, standard diagnostic evaluation was performed in all patients with PND in the UMCU or from the referring hospital. This included a medical history, physical examination, and recent radiological imaging (mammography, ultrasonography, and/or MRI and/or core needle biopsy and/or cytology of nipple fluid within three months). Patients were eligible for a ductoscopy procedure when radiological and pathological findings were negative.

Ductoscopy procedure

Ductoscopy was performed by one of two specialized surgeons with experience in ductoscopy at the outpatient clinic under local anesthesia, as described before [24]. Patients without spontaneous PND on the day of the procedure received Oxytocin nose spray 30 minutes before ductoscopy. First, the surgeon identified the affected duct by pressing the nipple. After disinfection with 70% ethanol, the nipple was locally anesthetized with Lidocaine 1%. A salivary duct probe (size 0000 to 1; Karl Storz, Tuttlingen, Germany) and an obturator (Polydiagnost, Pfaffenhofen, Germany) were used for dilatation of the lactiferous duct orifice in the nipple to a diameter of 1.2 mm. After widening, a port (SoLex nipple expander; Polydiagnost) was placed in the affected duct to introduce the ductoscope. Ductoscopy was performed with a 6000-pixel 0.55 mm optic (LaDuScope T-flex; Polydiagnost) and a Polyshaft (1.15 mm outer diameter, PD-DS-1015; Polydiagnost). First, 4 ml of Lidocaine was infused into the affected duct. Collaps of the ducts was prevented by continuous saline irrigation into the ductal tree through the polyshaft. The surgeon explored the major ducts until the ducts became too narrow to pass. When necessary, additional intraductal anesthesia (Bupivacaine) was administered. In the presence of an intraductal polypoid lesion, a basket-shaped guidewire was used to extract the lesion, followed by a histologic examination for diagnosis.

Ductoscopy was regarded as successful when it was possible to visualize the ductal tree. Possible findings during ductoscopy were e.g. normal duct morphology, ductitis, polypoid lesions, epithelial lesions/damage.

Follow-up

All patients were at least followed two weeks and three months after ductoscopy to evaluate the effect of treatment on symptoms. Patients were offered a second ductoscopy procedure when persistent or recurrence of PND occurred in patients in which complete removal of the intraductal lesion was not possible during the first attempt (due to technical errors such as duct perforation or basket failure).

Patients underwent surgery (or not), depending on the findings at second ductoscopy (persistent or recurrent PND, suspicious findings, patients' preference).

Statistical analysis

SPSS (v25.0.0.2 Chicago, IL) was used to analyze the data for this study. For the normally distributed continuous data, the values were described using mean with standard deviation. If continuous data were not normally distributed, a median with an interquartile range of frequency were used.

RESULTS

Baseline characteristics

From 2010 to 2017, a total of 244 patients with PND underwent a ductoscopy procedure. Seventeen patients underwent a second ductoscopy procedures. This patient population had a mean age of 50.8 ±16.3 (range 21-75 years) and a mean follow-up period of 10.2 ±10 months. Clinical data of the patients are summarized in Table 1. Work-up began with a detailed history and physical examination. In 94% of the cases, the discharge was unilateral, and in 82%, it was spontaneous. The majority of patients suffered from single duct PND (82%), two patients from two ducts (12%), and one from four ducts (6%). A palpable abnormality was found in one patient (6%).

Ultrasound was performed as part of the standard evaluation in all patients. The ultrasound was normal or showed a benign lesion with a low suspicion of malignancy. Most patients had mammography performed (15 of 17 cases), two patients did not due to their young age. The mammogram was normal or showed a benign

lesion in 13 cases (76.5%) and a lesion with low suspicion for malignancy in two cases (11.8%). MRI was performed in 4 cases and was normal or showed a benign lesion in three cases and a lesion suspected of malignancy in one patient. Eight of the 17 patients had a biopsy before ductoscopy. Histology revealed that three cases had a papilloma, three cases had benign tissue, and two showed no abnormalities. Cytology of the nipple discharge before ductoscopy was performed in eleven patients. In most cases (63.6%), cytology showed no abnormalities or was benign.

Multiple ductoscopy procedures

Table 2 shows the results of the ductoscopy procedures. At first ductoscopy, it was possible to visualize the ductal tree in 14 patients (82.4%). The second ductoscopy procedure was successful in 13 patients (76.5%). The first ductoscopy showed a polypoid lesion in 10 patients (58.8%) and no abnormalities in 3 patients (17.6%). In 4 patients, it was not possible to visualize the ductal tree; in 3 patients, it was because of perforation of the duct and in one, it was because of narrow ducts. Nine basket extractions were suitable for histopathological examination. In 8 patients (47.1%), these lesions were papillomas without atypia, and in one patient normal tissue was found. After successful ductoscopy, PND stopped in 10 patients (58.8%) for a short period. After weeks to months, these patients suffered again from PND (recurrent symptoms). Seven patients (41.2%) suffered from persistent symptoms.

The interval between both ductoscopy procedures was 6.3 ± 7.9 months. The second ductoscopy showed a polypoid lesion in 9 patients (52.9%) and no abnormalities in 4 patients. In 5 patients (29.4%), it was possible to perform basket extraction. These lesions turned out to be papillomas on pathology. In three of these five patients, it was not possible to remove the lesion completely. The seven patients (41.2%) that still suffered from PND after 2 ductoscopic procedures were operated on.

After the first ductoscopy procedure, two of the 17 patients experienced post-procedural pain longer than one day, and two patients had minor complaints of pain. The remaining 13 patients (76.4%) did not experience post-procedural pain. There were no other complications reported after the first ductoscopy procedure. After the second ductoscopy procedure, two patients reported mild post-procedural

pain. Other complications after the second ductoscopy were a retracted nipple in one patient and less sensitivity of the nipple in one patient. Both complications were self-limiting and improved after weeks.

Surgery after ductoscopy

Radiologic, ductoscopic, and pathologic findings of seven patients who underwent surgery after two ductoscopy procedures are shown in Table 3. It was not possible to visualize the ductal tree during both ductoscopy procedures in two patients. Four patients were operated on because of recurrent PND and three because of persistent PND. Three patients underwent a major duct excision, two a microdochectomy, and two a lumpectomy. Pathology after surgery showed in five patients a papilloma and in one patient no abnormalities. One of 17 patients (5.9%) with PND and no suspicious radiologic findings was diagnosed with DCIS after surgery. During both ductoscopy procedures, it was not possible to visualize the ductal tree in this patient.

Table 1. Clinical data of 17 patients with pathological nipple discharge (PND) undergoing two ductoscopy procedures

Clinical findings	**No. N = 17**
Age - years (SD)	50.8 ±16.3
Follow-up - months (SD)	10.2 ±10
Affected breast - N (%)	
Left	6 (35.3)
Right	10 (58.8)
Both	1 (5.9)
Ultrasound findings - N (%)	
BI-RADS 1	4 (23.5)
BI-RADS 2	6 (35.3)
BI-RADS 3	5 (29.4)
BI-RADS 4a	2 (11.8)
Mammography findings - N (%)	
BI-RADS 1	2 (11.8)
BI-RADS 2	6 (35.3)
BI-RADS 3	5 (29.4)
BI-RADS 4a	2 (11.8)
Not performed	2 (11.8)

Table 1. Clinical data of 17 patients with pathological nipple discharge (PND) undergoing two ductoscopy procedures *(continued)*

MRI findings - N (%)	
BI-RADS 1	1 (5.9)
BI-RADS 2	0 (0)
BI-RADS 3	2 (11.8)
BI-RADS 4a	1 (5.9)
Not performed	13 (76.4)
Pathology before ductoscopy - N (%)	
No abnormalities	2 (11.8)
Papilloma	3 (17.6)
Benign	3 (17.6)
Not performed	9 (52.9)
Cytology PND - N (%)	
Normal/benign	7 (41.2)
Papilloma	2 (11.8)
Atypical cells	1 (5.9)
Other	1 (5.9)
Not performed	6 (35.3)
Abbreviations: SD= standard deviation; PND= pathological nipple discharge; MRI= magnetic resonance imaging	

Table 2. Evaluation of two ductoscopy procedures in patients with persistent and recurrent pathological nipple discharge (PND)

Findings at ductoscopy		**No. N=17**
Interval between ductoscopy procedures - months (SD)		6.3 (7.9)
Succeeded first ductoscopy - N (%)	Successful ductoscopy	14 (82.4)
	Unsuccessful ductoscopy	3 (17.6)
First ductoscopic diagnosis - N (%)	Polypoid lesion	10 (58.8)
	Normal	3 (17.6)
	No diagnosis, perforation	3 (17.6)
	No diagnosis, narrow ducts	1 (5.9)
Pathology after extraction during first ductoscopy - N (%)	Papilloma	8 (47.1)
	Normal	1 (5.9)
	No extraction (possible)	8 (47.1)
Reason for second ductoscopy	Recurrent symptoms	10 (58.8)
	Persistent symptoms	7 (41.2)

Table 2. Evaluation of two ductoscopy procedures in patients with persistent and recurrent pathological nipple discharge (PND) *(continued)*

Findings at ductoscopy		**No. N=17**
Succeeded second ductoscopy - N (%)	Successful ductoscopy	13 (76.5)
	Unsuccessful ductoscopy	4 (23.5)
Second ductoscopic diagnosis - N (%)	Polypoid lesion	9 (52.9)
	Normal	4 (23.5)
	No diagnosis, perforation	2 (11.8)
	No diagnosis, narrow ducts	2 (11.8)
Pathology after extraction during second ductoscopy - N (%)	Papilloma	5 (29.4)
	No extraction (possible)	12 (70.6)
Operated - N (%)	Not operated	10 (58.8)
	Operated	7 (41.2)

Table 3. Overview of seven patients with pathological nipple discharge (PND) who were operated on after two ductoscopy procedures

Patient	Age	Radiology	Ductoscopic findings 1	Ductoscopic findings 2	Reason for operation	Type operation	Pathology after surgery
1	49	US + MMG: BI-RADS 4a	Perforation, no visualization. No PA	Polypoid lesion visible, no successful PA	Persistent symptoms	Lumpectomy	Papilloma
2	24	US + MRI: BI-RADS 1	Polypoid lesion visible, no successful PA	Polypoid lesion visible, PA: papilloma	Recurrent symptoms	Microdochectomy	Papilloma
3	50	US + MMG: BI-RADS 2 MRI: BI-RADS 4a	Normal, no PA	Normal, no PA	Recurrent symptoms	Microdochectomy	Papilloma
4	68	US + MMG: BI-RADS 2	Perforation, no visualization. No PA	Perforation, no visualization. No PA	Persistent symptoms	Lumpectomy	DCIS grade 1
5	65	US + MMG: BI-RADS 2	Polypoid lesion visible, normal PA	Polypoid lesion, PA: papilloma	Recurrent symptoms	Conus excision	Papilloma
6	38	US + MMG: BI-RADS 3	Perforation, no visualization. No PA	No successful cannulation. No PA	Persistent symptoms	Conus excision	Benign
7	41	US + MMG: BI-RADS 3	Polypoid lesion visible, no successful PA	Polypoid lesion visible, no successful PA	Recurrent symptoms	Conus excision	Papilloma

Abbreviations: US= ultrasound; MMG= mammography; MRI= magnetic resonance imaging; PA= extraction and pathologic diagnosis

DISCUSSION

Here, we present the yield of a second ductoscopy procedure in patients with persistent or recurrent PND in which the first ductoscopic attempt was not successful. The results of this study, based on a cohort of 17 patients, indicate that a second ductoscopy procedure can successfully be performed in the vast majority after an unsuccessful first attempt due to technical problems, avoiding surgery in more than half of the cases.

The population of this study was based on a selected population consisting of patients with persisting or recurrent PND who underwent a second ductoscopy procedure after an unsuccessful first attempt. We performed a second ductoscopy on these patients because we considered an additional intervention might be effective to remove the (remaining) lesion that was visible during the radiological screening or first ductoscopy. Our results showed a positive effect of a second intervention in these patients. Hence, since the study population is a sub-selection of patients, the results were not representative of the entire patient population with PND in the outpatient clinic. In patients suffering from persisting or recurrent PND after ductoscopy, careful consideration has to be done whether another ductoscopic intervention can yet have an additional effect on treatment.

Currently, patients suffering from persistent or recurrent PND after a ductoscopy procedure are usually offered radiological follow-up, or undergo a surgical procedure for further diagnosis and treatment [18], [25], [26]. The focus of this study was to evaluate if unnecessary surgery for these patients in which the first ductoscopic attempt was unsuccessful for diagnosis and/or treatment can be prevented by a second ductoscopy. After the second ductoscopic attempts, PND stopped in ten patients (58.8%), and seven patients (41.2%) still suffered from PND and were eventually operated on. Thereby, a second ductoscopy procedure prevented surgery in 58.8% (10/17 patients). To the best of our knowledge, there are no other published studies on multiple ductoscopy procedures to compare our results with. In two of these seven operated patients, it was not possible to visualize the ducts during both ductoscopy procedures. In four of the seven operated patients, it was not possible to completely remove the benign intraductal lesion. Furthermore, in

two patients in which ductoscopy was not successful, surgery was not performed because PND disappeared. According to a previous study, in cases in which no obvious lesions were observed on ductoscopy, there was a 90% probability that the PND would disappear [27]. This indicates a self-limiting disease or a therapeutic effect of ductoscopic saline flushing alone.

On the operated patients, pathology showed a benign lesion in 6 of 7 patients after surgery. One patient was diagnosed with DCIS after surgery, leading to a malignancy rate in the study population of 5.9%. This is in line with previous studies in which the malignancy rate in patients with PND without radiological suspicion for malignancy was between 5 and 10 % [7]–[10]. This means that the vast majority of the patients who suffered from PND were operated on because of a benign lesion.

Besides the diagnostic nature, ductoscopy offers therapeutic options in patients with intraductal benign lesions like papillomas [28]–[30]. Hence, when in the first ductoscopy procedure a benign lesion is diagnosed but not (fully) removed by endobasket extraction leading to remaining or recurrent PND, a second ductoscopy procedure to attempt to remove the remainder of the lesion may be successful.

Limitations

To our knowledge, this is the first study to report on yield of a second ductoscopy procedure in patients with PND. The major limitation of this study is the small sample group size of included patients. Also, because of the retrospective nature of this study, we were unable to collect some baseline factors and long-term follow-up data in our center. A future prospective study can address the above limitations.

Conclusions

A second ductoscopy procedure in selected patients with remaining or recurrent PND after a first unsuccessful ductoscopy attempt can be useful to diagnose and/or treat the cause of PND, preventing about 59% of the patients from undergoing unnecessary surgery.

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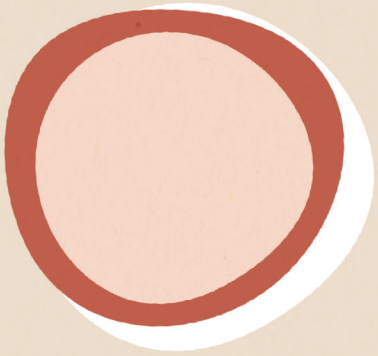

CHAPTER 5

Design and evaluation of a novel miniature biopsy needle for ductoscopy

Transactions on Biomedical Engineering, under review

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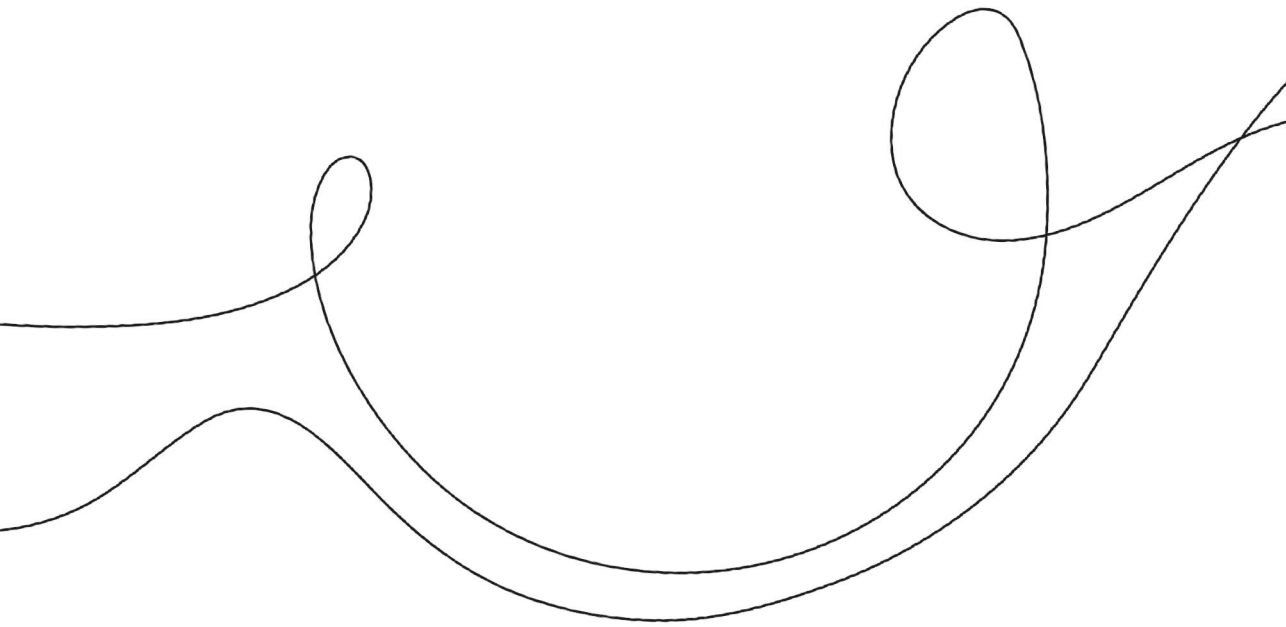

ABSTRACT

Early detection of breast cancer is critical for improving survival rates among women. Mammary Ductoscopy (MD), a minimally invasive micro-endoscopic technique, holds promise for detecting breast lesions at their earliest stages. However, challenges related to histological verification of findings and the need for more precise biopsy instruments have hindered its widespread adoption. In this study, we aimed to develop ultra-slender biopsy needles suitable for use during MD procedures. These needles are designed to enable early diagnosis of breast cancer precursors and facilitate histologic examination without the need for invasive surgical procedures.

Three biopsy needle designs were developed: the Dome, Pincer, and Cone biopsy needle, each offering unique features for tissue sampling. These designs were tested in gelatine tissue phantoms and mastectomy samples to assess their performance and practicality. While all biopsy needles successfully obtained tissue samples from gelatine tissue phantoms, the pincer biopsy needle was favoured due to its ability to collect significantly larger biopsy samples (3.0 mm^3) than the other two needles (1.2-1.5 mm^3). The mastectomy experiments revealed that the pincer and cone biopsy needles were easily controlled and successfully inserted into mastectomy samples. The dome biopsy needle proved unfeasible for MD procedures due to its limited visibility during insertion. The integration of these biopsy needles into the MD procedure offers a potential breakthrough in early breast cancer detection. With minimal modifications to the existing MD protocol, these needles can be seamlessly incorporated into clinical practice, enhancing the diagnostic capabilities of this promising technique.

Future research will focus on fine-tuning the needle designs, optimizing their sharpness, and exploring additional clinical applications beyond MD, such as bile duct biopsy. A clinical study is currently underway to further evaluate the developed biopsy needles in a real-world outpatient clinic setting. This study represents a significant step toward improving the early detection of breast cancer, ultimately leading to better outcomes for affected individuals.

1. INTRODUCTION

1.1 Ductoscopy in clinical practice

Early detection of breast cancer plays a vital role in increasing the survival rate of women. Current diagnostic methods for breast cancer, such as mammography and ultrasound, are essential for initial screening. Additionally, MRI is valuable for lesion diagnosis, treatment selection, progression monitoring, and detecting cancer recurrence. However, by the time a lump is felt by the patient themselves or with currently clinically available diagnostic tools, the lesion has been growing for approximately 8 years and is usually between Ø5–10 mm in size [1].

A minimally invasive micro-endoscopic technique called Mammary Ductoscopy (MD) holds promise for early breast cancer detection in the near future, as tools and continue to advance [2]. MD enables real-time visualization of some of the earliest lesions *in situ* (Ø0.1 mm) long before traditional imaging modalities, such as mammography, ultrasound, and MRI can detect them. MD has evolved from its initial development in Japan, and the most common indication nowadays is pathological nipple discharge (PND). PND is the third most common breast related symptom, accounting for 3–10% of all breast complaints and referrals to the surgery department [2, 3]. As many stage 0–2 breast cancers produce expressible nipple fluid, using MD for this indication may allow for early detection [2]. In patients suffering from PND without other radiological and clinical abnormalities, traditional surgical excision is typically performed to rule out malignancy [4, 5]. However, MD is a safe and effective diagnostic and interventional procedure, which can avoid unnecessary diagnostic surgical procedures in around two out of three patients when included in the diagnostic work-up of PND.

1.2 The ductoscope

In MD, a submillimetre Ø0.45–0.55 mm fibreoptic micro-endoscope is inserted through the ductal opening onto the nipple surface to provide direct access to the ductal epithelium [6]. This fibreoptic micro-endoscope consists of approximately 6.000–10.000 individual glass fibres, which collectively deliver a high-quality image and magnify the mammary duct up to 60 times its normal size [7-9]. The evolution of ductoscopy has progressed from directly inserting a fibreoptic mi-

cro-endoscope into the nipple orifice for visualization of the mammary ductal epithelium to its current capabilities, which include the ability to perform biopsy and analyse intraductal lesions histologically. Nowadays, the micro-endoscope is used in conjunction with a lumen extender, a cannula and a specialized handle that contains two or three lumens; one for the endoscope, one for irrigation, and one for an additional tool or treatment modality, such as insufflation, ductal lavage, and therapeutic interventions (Figure 1) [9]. The lumen extender has two main functions: 1) to provide access to the breast milk ducts through the nipple surface and 2) to extend the milk duct lumen. The cannula acts as a conduit for the micro-endoscope, therapeutic tool, and irrigation. Irrigation is used to gently dilate the milk duct of interest using saline and allows for advancement of the ductoscope into the duct under video guidance until further advancement is restricted by the size of the cannula, which typically has a diameter ranging from Ø1.15–1.4 mm [9]. An example of a therapeutic biopsy device used in conjunction with the ductoscope is the Endobasket, which is a 3-wire basket with a diameter of 380 µm (PD-TI-2104; Polydiagnost) [10].

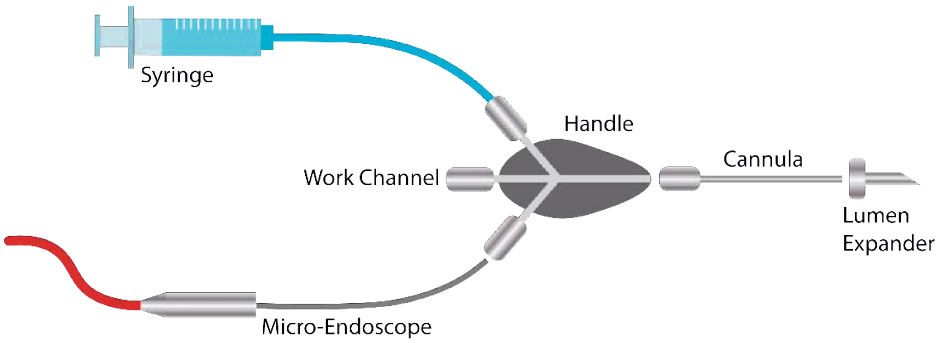

Figure 1: Schematic Illustration of the Ductoscope. The ductoscope consists of a handle with three working channels: 1) for the micro-endoscope for obtaining real-time images, 2) for providing irrigation by means of a syringe and saline, and 3) for inserting additional tools, such as biopsy baskets. Connected to the handle is the cannula that is inserted into the lumen expander that is placed in one of the orifices of the milk ducts in the nipple.

1.3 Diagnosis in ductoscopy

Ductoscopy is an evolving diagnostic and interventional procedure that has become a routine part of the diagnostic process for patients with PND. Moreover, MD holds tremendous potential for breast cancer precursors. However, for MD to be widely adopted as an effective tool for early breast cancer detection, several limitations need to be overcome. One primary limitation in MD pertains to the histological verification of the findings. During MD, diagnosing lesions can be achieved through visual inspection, ductal lavage cytology, or by using a variety of biopsy instruments. Unfortunately, the diagnostic accuracy based solely on visual appearances alone ranges from 39% to 97% depending on the type and visibility of the lesion [6, 9]. Consequently, it is not always possible to make a conclusive diagnosis based on visual appearance alone.

MD has also been combined with ductal lavage cytology, in which a double-lumen catheter is used to introduce saline into the milk duct, and subsequently retrieve this using suction. Ductal lavage allows for obtaining cells from milk ducts that cannot be reached with the ductoscope but has a relatively low cellular yield and is also unable to obtain a tissue sample for histological examination [9]. The use of biopsy instruments, such as biopsy baskets, cytological brushes, and biopsy forceps offers a greater cellular yield than ductal lavage. Unfortunately, these instruments are often challenging to control and unreliable [9]. Furthermore, the current biopsy baskets lack the necessary accuracy and can only extract selected larger intraductal polypoid lesions. Consequently, the inability to obtain intraductal tissue sample remains a persistent problem during MD [2].

Due to the unreliability of visual diagnostics and intraductal biopsy instruments, lesions identified during ductoscopy are frequently removed via surgical excision for a definitive diagnosis [8, 9]. However, excisional biopsy is unnecessarily invasive, costly and carries potential risks. Therefore, there is a pressing need for the development of a reliable intraductal biopsy instrument [9], new techniques to enhance the sensitivity for detecting premalignant lesions and to expand the possibilities for intervention.

1.4 Goal of this study

The development of intraductal biopsy instruments has been hampered by the diameter restriction, imposed by the small diameter of the milk ducts, and working channel of the cannula. This severely limits the available space to integrate a biopsy instrument [7]. The main goal of this study is to develop a miniature intraductal biopsy needle suitable for use in MD. This needle is intended to enable early diagnosis of breast cancer precursors and facilitate histologic diagnosis without necessitating surgical intervention. The biopsy instrument will be designed with further miniaturization in mind to allow for examination closer to the of the Terminal Ductal Lobular Units (TDLUs) in the near future. This will enable the examination of a more extensive area within the milk ducts, significantly improving the diagnostic capabilities of MD.

1.5 Layout of this study

In the ensuing section we will discuss the design process of the ductoscopy biopsy needles. We will discuss the intended use, design directions, resection principles, and the developed designs. The developed designs will, subsequently, be tested on mastectomy samples, in order to determine the most feasible design. How to control and connects the biopsy needles to the currently available technology for ductoscopy, will be discussed in Section 3. Finally, we will discuss our findings and give recommendations for future research.

2. BIOPSY NEEDLE TIP DESIGN

2.1 Biopsy needle tip intended use

In the pursuit of developing an effective biopsy instrument for use during MD, it is essential to closely consider the clinical context in which this instrument will operate. Ductoscopy is currently performed under local anaesthesia at the outpatient clinic. This technique is primarily intended as an additional diagnostic tool in the work-up of women suffering from PND in the absence of suspicious radiological findings [10]. During MD, the ductoscope is inserted through one of the orifices on the nipple surface to access the milk ducts. There is significant variability in the number of milk ducts per patient, and little is known about the

spatial distribution of these ducts, their sizes, and their relationship to the orifices on the surface. Recent studies have found that the number of milk ducts at the nipple surface typically ranges between 5–50 ducts, with an average of 24 ducts per patient. The number of orifices typically ranges between 5 to 9 [11]. The diameter of the milk duct varies with depth. At 1–1.5 mm beneath the surface, the average duct diameter is approximately 0.06 mm, whereas at a depth of 3 mm, an average diameter of 0.7 mm is observed [12]. Smaller breast ducts (with a diameter of less than 0.5 mm) make up nearly 50% of the nipple ducts and can present challenges for ductoscopy technology [13].

Breast cancers most commonly arise from the ductal epithelium [14, 15]. An appealing approach is to target breast cancer precursors, such as ductal carcinoma *in situ* (DCIS) and other benign lesions originating from the epithelial lining of the breast ducts through ductoscopy. Real-time visualisation of the milk ducts in the breast enables the detection of these lesions. Depending on the extent of the lesion, various scenarios may be encountered (Figure 2). In the early stages of cancer development, the lesion remains confined within the milk duct epithelium. As the condition progresses, the lesion may protrude into the milk duct lumen, partially obstructing the duct. Further progression of the lesion can lead to a complete blockage of the milk duct lumen.

The biopsy needle must have the capability to extract tissue samples at various developmental stages of the lesion. To achieve this, when the lesion completely obstructs the milk duct lumen, the instrument should be able to obtain a tissue sample at the distal end (end-cut). In the case of a partially obstructed milk duct, a mechanism that can collect tissue samples from the circumference (side-cut) is preferred. To ensure reliable diagnosis of different lesions in distinct stages of development, the biopsy instrument should incorporate both end-cut and side-cut capabilities. Moreover, it is advisable to use biopsy needle tips that can excise the lesion in both the axial and radial directions rather than methods that can only cut in the axial or tangential direction. This approach allows for a more controlled resection of the lesion, even in a completely blocked milk duct.

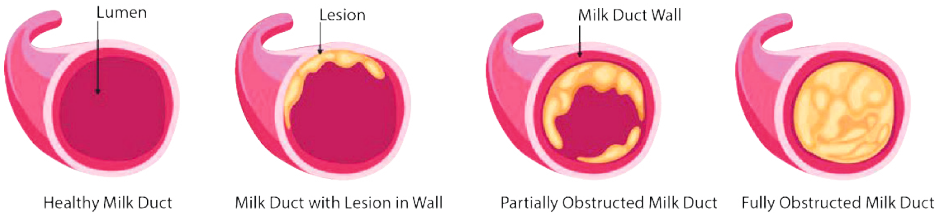

Figure 2: Schematic representation of the milk duct with an unidentified lesion. Left to right: healthy milk duct, milk duct with lesion in the milk duct wall, partially occluded milk duct, completely occluded milk duct. Pink = the milk duct wall, Orange = suspicious lesion.

2.2 Biopsy needle tip design directions

In order to incorporate a biopsy needle tip in the current ductoscope, three main options are explored.

1. We can insert the biopsy needle **into the existing cannula**, which possesses an inner diameter of approximately ∅1.05 mm and an outer diameter ranging from ∅1.15 to ∅1.40 mm. This cannula accommodates a micro-endoscope with a diameter of ∅0.45 to ∅0.55 mm and allows for the passage of irrigation fluid. Since there is no need to guide additional tools through the biopsy needle, it can be designed as either a solid rod or a tube. Consequently, the maximum outer diameter of our biopsy needle can reach ∅1.0 mm with a maximum wall thickness of 0.25 mm in the case of a cylindrical design, or ∅0.45 mm in the case of a solid design.

2. Another option is to **replace the cannula**. By replacing the cannula, we can use the full ∅1.2 mm as our biopsy needle diameter. However, as we need to guide the micro- endoscope and irrigation through the biopsy needle, the biopsy needle needs to be hollow and thin walled. Therefore, the biopsy needle can maximally consist of two to three capillary tubes (wall thickness of 0.1–0.2 mm) with a minimum inner diameter of ∅0.60 mm to leave room for the micro-endoscope and irrigation channel. Furthermore, as one of the functions of the cannula is to provide access to the milk ducts, it needs to be sufficiently stiff to allow for advancement, as well as contain a blunt edge to prevent milk duct trauma during the procedure.

3. The biopsy needle can combine option 1) and 2).

Taking further miniaturization into account, Option 1, in which the biopsy needle is placed inside the cannula, is unfeasible due to size constraints. Furthermore, in Option 3, the former cannula would lose its main functionality unless the outer part of the biopsy needle is inserted after a target lesion has been identified. Therefore, Option 2 is the most feasible option that will be explored further.

2.3 Biopsy needle tip resection principle

The biopsy needle tip must seamlessly integrate into current MD devices and procedures without major modifications. the intraductal needle's geometry should resemble that of the cannula it replaces, enabling the guidance of the micro-endoscope and irrigation fluid through its lumen.

Additionally, the biopsy needle should be easily connectable to a handle. The primary function of the biopsy needle is to obtain a tissue sample for examination by a pathologist. Therefore, it must collect a sufficiently large tissue sample, measuring equal to or greater than 0.1 mm^3, which is suitable for histopathological examination. This is comparable to the sample size achieved with the Endobasket (PD-TI-2104; Polydiagnost). It is crucial that the biopsy needle enables the precise extraction of the tissue sample from its surrounding environment, preventing unwanted damage to the surrounding structures.

2.4 Biopsy needle tip developed designs

Based on the design direction and requirements, three different needle tips were developed (see Figure 3). All the biopsy needle tips consist of two concentric capillary tubes and allow for both end- and side-cutting. All outer capillary tubes have a diameter of ∅1.2 mm.

1 Dome Biopsy Needle

The dome biopsy needle consists of an inner capillary tube with four pre-bend prongs at its distal end and a blunt outer capillary tube. By translating the outer tube over the inner tube, the pre- bend prongs can be opened and closed. Additionally, the inner capillary tube can be rotated. Due to the shape of the

prongs and the ability to both rotate and translate both tubes, it is possible to take a biopsy at the distal end and the circumference.

2 Pincer Biopsy Needle

The pincer biopsy needle consists of an outer capillary tube with a pre-bend pincer at its distal end and an inner capillary tube with a bevel tip and rectangular cut-out. This design allows for taking a biopsy at the distal end and circumference of the biopsy needle by translating the pre-bent pincer forward over the bevelled tip or by rotating the pre-bend pincer over the rectangular cut-out, respectively. The cutting blade was designed in such a way as to reduce the force needed for resection by using a slightly v-shaped cutting edge.

3 Cone Biopsy Needle

The cone biopsy needle consists of an inner capillary tube with three sharp "arrow-shaped" prongs and an outer capillary tube with a tapered distal end. By translating the inner tube forward and backwards, the arrow-shaped prongs move forward and close, or move backwards and open, respectively. A biopsy can be obtained by placing the tapered outer tube on the lesion and, subsequently, translating the inner tube forward, allowing the inner arrow-shaped prongs to resect and obtain the biopsy.

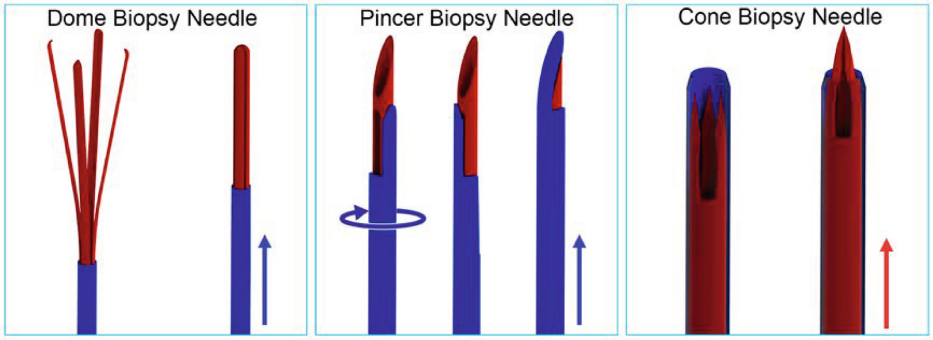

Figure 3: Biopsy Needle Tip Designs

Left: *dome biopsy needle* design consisting of four pre-bend prongs (red) that can open and close by axially translating the outer tube (blue) up and down. Middle: *pincer biopsy needle* design consisting of a bevelled inner needle with a rectangular cut-out (red) and an outer tube with a pre-bend pincer (blue). Right: *cone biopsy needle* design consisting of three arrow-shaped elastic blades (red) inside a tapered outer tube (blue). The arrows indicate the motion of the biopsy needles while taking a biopsy.

Figure 4: Biopsy Needle Tip Prototypes
Top: *cone biopsy needle*. Middle: *pincer biopsy needle*. Bottom: *dome biopsy needle*. Match indicated for scale purposes.

2.5 Biopsy needle tip developed prototypes

The three biopsy needle tip designs were manufactured from stainless steel and Nitinol using Electric Discharge Machining (EDM) and conventional CNC machines (Figure 4) at DEMO (TU Delft, Delft, The Netherlands). Nitinol was selected for the compliant capillary tubes due to its superelastic properties, which prevent plastic deformation of the flexible elements. The outer diameter of the designs was set to ∅1.2 mm and the inner diameter to ∅0.7 mm. After the initial manufacturing, all cutting edges were sharpened using a whetstone. The developed prototype biopsy needles were fitted with a 3D-printed handle and a Luer-lock. The cylindrical 3D-printed handle allowed for easy control of the biopsy needle, and the Luer-lock facilitated a simple and fast connection to the ductoscope handle. Furthermore, to allow for easy insertion of the micro- endoscope in the biopsy needle, a funnel was created inside the Luer-lock.

3. BIOPSY NEEDLE TIP EVALUATION

3.1 Experimental goal

The prototype intraductal biopsy needles were assessed using mastectomy samples and gelatine tissue phantoms. The goal of this experiment was twofold: 1) to evaluate the ability of the developed needles to perform biopsies (using gelatine tissue phantoms) and 2) to assess the practicality of the four biopsy needles for potential use during MD. Mastectomy samples were obtained from patients undergoing preventive or curative mastectomies. No patient-specific information was utilized in the study, obviating the need for informed consent, as determined by the ethical board of the University Medical Centre Utrecht.

3.2 Experimental set-up

3.2.1 Gelatine Tissue Phantom Experiment

The experimental set-up consisted of the developed intraductal biopsy needles connected to the ductoscope (∅0.55 mm, 6000 pixels, Laduscope T-Flex, Polydiagnost, Hallbergmoos, Germany) and two different gelatine tissue phantoms, each representing 1) a partly obstructed milk duct and 2) a completely obstructed milk duct (Figure 5). The ductoscope was clamped to a dovetail slider (RLA075/M, Thorlabs) using a component clamp (VC1/M, Thorlabs), which in turn was connected to a breadboard (MB3030/M, Thorlabs). The insertion depth for each experiment was set at 50 mm, regulated by a physical stop on the slider. The gelatine tissue phantoms were poured into rectangular containers (50x45x50 mm) laser-cut out of PMMA. Directly after pouring the gelatine in the containers, a 3D-printed insert (Formlabs 3B) resembling the milk duct lumen was placed into these containers. The model simulated an artificial milk duct lumen of ∅2.0 mm and a length of 50 mm. In the partly obstructed tissue phantom, the last 10 mm of the lumen was obstructed, reducing the cross-sectional area by 50%. In the completely obstructed milk duct, the last 10 mm of the lumen was fully obstructed. To mimic the properties of ductal carcinoma and papilloma, a mixture of 25wt% gelatin powder and water was utilized, which represents a Young's modulus of approximately 150 kPA, which is similar to that of breast cancer tumors [16]. The gelatine tissue containers were placed on a square clamp (BSH2/M,

Thorlabs) on the set-up which in turn was connected to the breadboard via three tableclamps (CL6, Thorlabs).

3.2.2. Mastectomy experiment

The experimental set-up consisted of the mastectomy sample, the developed intraductal biopsy needles, and the ductoscope (Laduscope T-Flex, Polydiagnost, see Figure 6) with 60 times magnification on mastectomy specimens connected to irrigation. The MD procedure was performed by an experienced ductoscopist and an assistant.

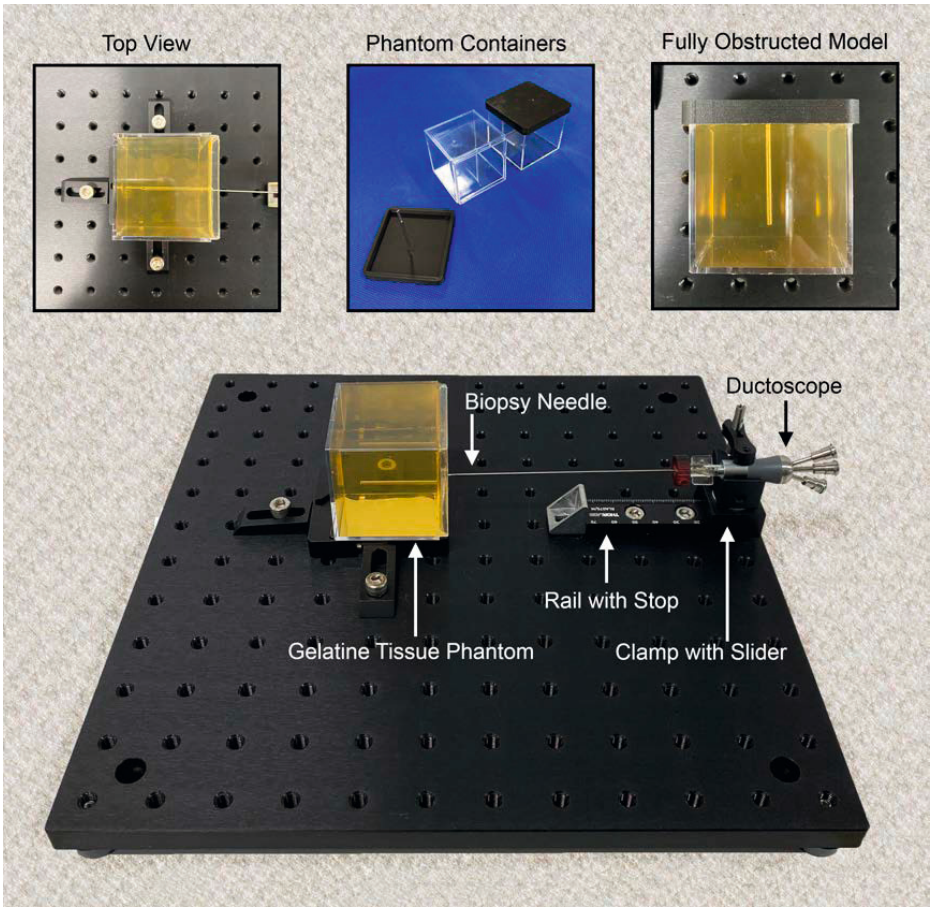

Figure 5: Experimental Set-Up Gelatine Tissue Phantom Experiment.
The experimental set-up consisted of the gelatine tissue phantoms connected to a rig and the developed biopsy needles connected to the ductoscope handle (Laduscope, Polydiagnost, Hallbergmoos, Germany) and slider.

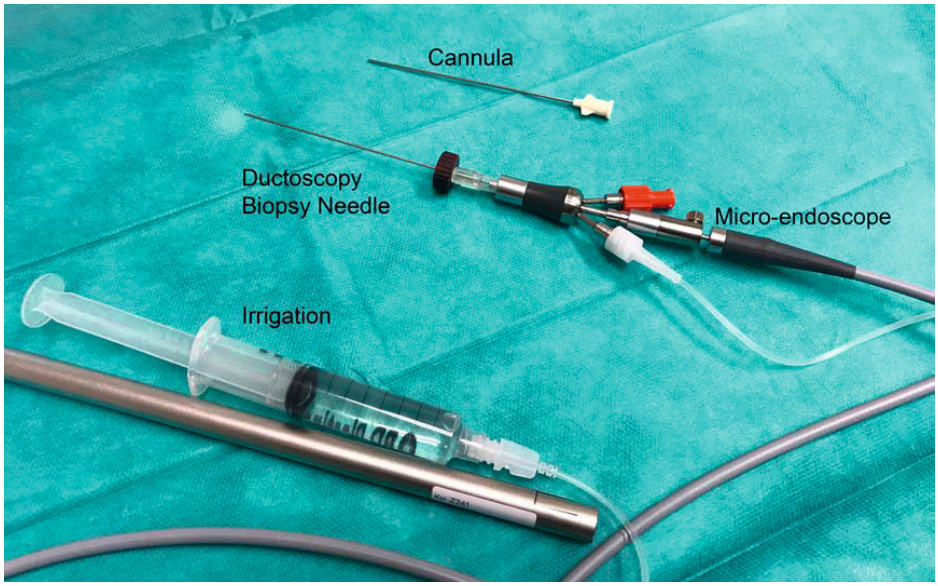

Figure 6: Experimental Set-Up Mastectomy Experiment.
The experimental set-up consisted of the developed ductoscopy biopsy needles connected to the ductoscope (Laduscope, Polydiagnost, Hallbergmoos, Germany) using a Luer- lock. The cannula is indicated for scale purposes. The cannula was not used during the experiment.

3.3 Experimental protocol

3.3.1 Gelatine tissue phantom experiment

In the gelatine tissue phantom experiment, the developed biopsy needles were first connected to the ductoscope. Subsequently, each of the developed biopsy needle was manually inserted in the fully and partially obstructed gelatine tissue phantoms at random. One attempt was made with each phantom to obtain a biopsy sample. Following the biopsy attempt, the biopsy needle was manually retracted. The evaluation of the three prototype biopsy needles focused on the presence of a tissue sample inside the lumen (Y/N), which was determined using a digital microscope (VHX- 7000N, Keyence). Subsequently, the obtained tissue samples were removed under microscope guidance using a small rod and needle. The removed tissue samples mass was measured using a high-precision scale (0.001g, PFB 200-3, Kern) to give an estimation on the tissue volume obtained using a density of 1.1×10^{-3} g/mm^3 (experimentally determined). Each experiment was

repeated 3 times, and after each experiment, the biopsy needles were cleaned using a small cylindrical brush.

3.3.2. Mastectomy experiment

Prior to biopsy needle insertion, the mastectomy sample was prepared. First, the tissue around nipple was injected with a saline solution fluid to make the orifices in the nipple more visible. Secondly, a salivary duct probe (size 0000 to 1; Karl Storz, Tuttlingen, Germany) and an obturator (Polydiagnost, Pfaffenhofen, Germany) widened duct of the nipple. After successfully identifying a milk duct orifice, the lumen extender was inserted. At this time, the ductoscope connected to one of the four developed biopsy needles was inserted in the milk duct. The biopsy needle was slowly advanced in the milk duct under constant irrigation to prevent the ducts from collapsing. During the procedure, the ability to insert the biopsy needles in the milk duct, the occurrence of perforations, the presence of potential biopsy material, the visibility from the biopsy needle, and the occurrence of adverse events were registered. After the procedure, the surgeon performing the ductoscopy was asked for his experiences with the three different biopsy needles.

3.4 Experimental results

3.4.1 Gelatine tissue phantom experiment

The results of the biopsy procedure attempts are schematically illustrated in Table 1. As can be seen from Table 1, all the needles were able to successfully collect a tissue sample from the gelatine tissue phantoms. However, upon closer examination with the digital microscope, it was found that the *dome biopsy needle* yielded the lowest tissue volume, followed by the *cone biopsy needle,* see Table 1 and Figure 7.

Table 1: Results of the biopsy procedures with the developed biopsy needles in gelatine tissue phantoms.

	Completely obstructed duct (n = 3)			Partially obstructed duct (n = 3)		
Biopsy needle type	**Number of successful biopsies**	**Biopsy Sample Mass**	**Approximated Biopsy Volume**	**Number of successful biopsies**	**Biopsy Sample Mass**	**Approximated Biopsy Volume**
Dome biopsy needle	3 (100%)	1.3 ± 0.6 mg	1.2 ± 0.5 mm^3	3 (100%)	1.0 ± 0 mg	0.9 mm^3
Pincer biopsy needle	3 (100%)	3.3 ± 0.6 mg	3.0 ± 0.5 mm^3	3 (100%)	1.7 ± 0.6 mg	1.5 ± 0.5 mm^3
Cone biopsy needle	3 (100%)	1.7 ± 0.6 mg	1.5 ± 0.5 mm^3	3 (100%)	1.0 ± 0 mg	0.9 mm^3

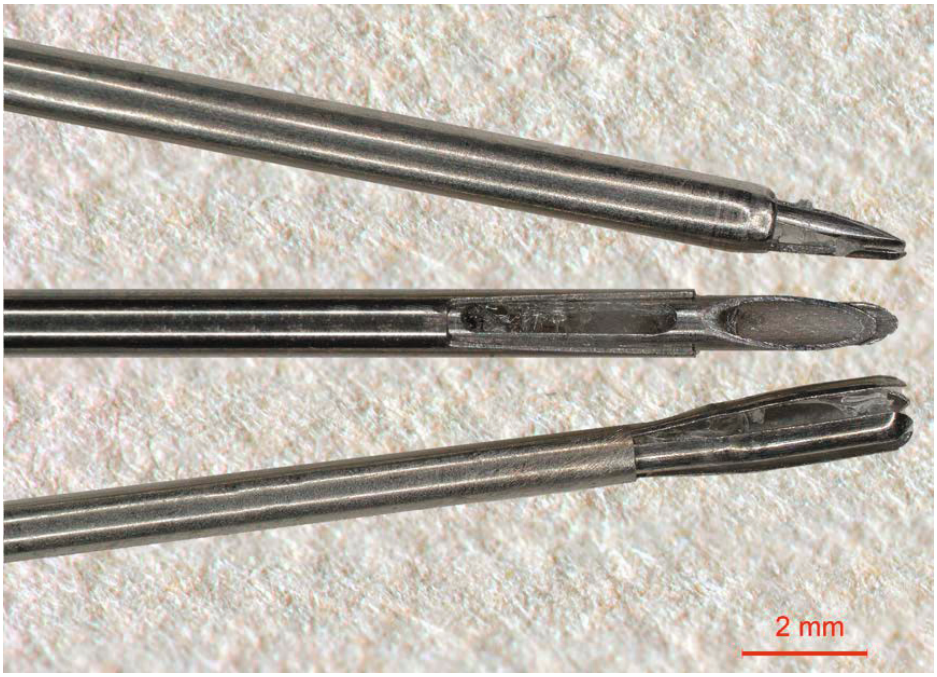

Figure 7: Tissue Samples obtained during the Gelatine Tissue Phantom Experiment.
Top: Tissue sample obtained using the *cone biopsy needle*. Middle: Tissue sample obtained using the *pincer biopsy needle*. Bottom: Tissue sample obtained using the *dome biopsy needle*.

3.4.2 Mastectomy experiment

The results of the mastectomy experiment are summarised in Table 2. An example image obtained from the ductoscope is given in Figure 8. Controlling all the biopsy needles was considered easy and no issues were encountered. All needles were successfully inserted into the mastectomy samples. The *cone biopsy needle* was inserted successfully in 5/8 tries (62.5%). The *pincer biopsy needle* was successfully inserted in 10/16 tries (62.5%). The dome cutter was inserted successfully one time (100%). However, due to the inability to open the dome cutter and visualise the milk duct, no further experiments were performed with this needle. The visibility with the *cone* and *pincer biopsy needle* was considered sufficient during the entire procedure. The ability to resect tissues along the ductal wall and directly in front of the tip in the *pincer biopsy needle* was considered advantageous. No perforation occurred during advancement of the biopsy needles except for 3 cases (12%). In these specific cases, the ductoscopist attempted to take a biopsy from

a healthy milk duct surface. Unfortunately, due to the lack of obstructions in the milk ducts of the mastectomy samples, no biopsy procedures could be performed.

3.5 Final biopsy needle tip

Based on the findings in the mastectomy and gelatine tissue phantom experiments, it was found that the cone and pincer biopsy needles were preferred over the dome biopsy needle. Both needles allowed for good visibility during insertion and performed equally well during the experiments. However, there were a few alterations that were made to improve the ease of obtaining a biopsy sample in both biopsy needles. For the cone biopsy needle, the distal end was modified by enlarging the opening, which reduces the visibility of the arrow-shaped blades at the tapered tip when it is inserted. For the pincer biopsy needle, a chamber with sharp side edges was added to the outer shaft to improve side cutting capabilities. Future steps to determine the "most" preferred design will be analysed in a clinical study that is currently ongoing. Both designs have been patented and are currently being commercialised, see Figure 9. Videos of the movement of the final prototype needles can be found in the Supplementary Materials.

Table 2: Results of the Mastectomy Experiments.

Type of Biopsy Needle Used	Successful Insertion [Y/N]	Perforation after Cannulation [Y/N/NA]	Biopsy Material Present [Y/N]	Visibility	Adverse Events [Y/N]
Dome	Y	N	NA	None	N
Cone	N	NA	NA	NA	NA
Cone	Y	N	N	Sufficient	N
Cone	N	NA	NA	NA	NA
Cone	Y	N	N	Good	N
Cone	Y	N	N	Good	N
Cone	N	NA	NA	NA	NA
Cone	Y	N	N	Good	N
Cone	Y	Y*	N	Good	N
Pincer	N	NA	NA	NA	NA
Pincer	N	NA	NA	NA	NA
Pincer	Y	N	N	Sufficient	N
Pincer	Y	N	N	Sufficient	N
Pincer	N	NA	NA	NA	NA

Table 2: Results of the Mastectomy Experiments. *(continued)*

Type of Biopsy Needle Used	Successful Insertion [Y/N]	Perforation after Cannulation [Y/N/NA]	Biopsy Material Present [Y/N]	Visibility	Adverse Events [Y/N]
Pincer	N	NA	NA	NA	NA
Pincer	Y	N	N	Good	N
Pincer	Y	N	N	Good	N
Pincer	Y	N	N	Good	N
Pincer	Y	N	N	Good	N
Pincer	N	NA	NA	NA	NA
Pincer	N	NA	NA	NA	NA
Pincer	Y	N	N	Good	N
Pincer	Y	N	N	Good	N
Pincer	Y	Y*	N	Good	N
Pincer	Y	Y*	N	Good	N

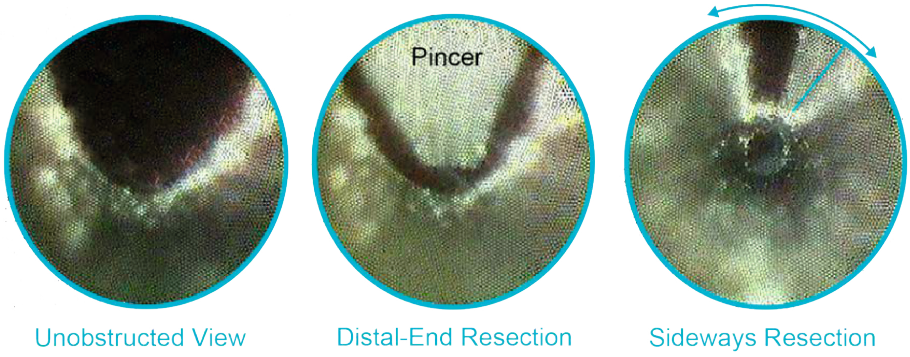

Figure 8: View from the Ductoscope with the *Pincer Biopsy Needle.*

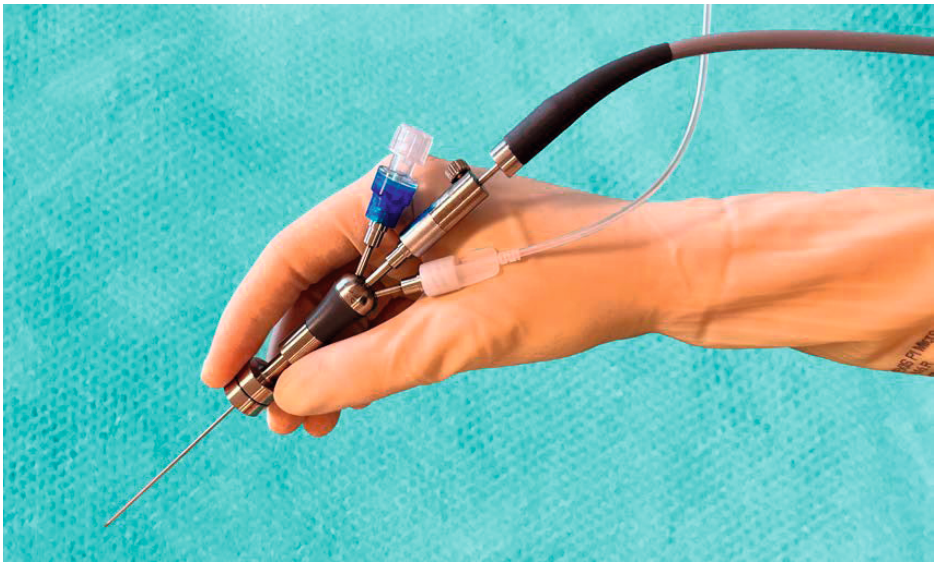

Figure 9: Final *Pincer Biopsy Needle* attached to the Ductoscope.

4. DISCUSSION

4.1 Summary of main findings

In this study we have developed a new type of ultra-slender biopsy needle that can be used during MD. We tested three different needle designs. All the biopsy needles were successfully combined with a ductoscope and tested in gelatine tissue phantoms and mastectomy samples. It was found that the biopsy volume was largest in the *pincer biopsy* needle, followed by the *cone biopsy needle* with an average of 3.0 and 1.5 mm^3, respectively in the full obstructed tissue phantoms, which is sufficient for histopathological examination. Unfortunately, it was difficult to determine the exact tissue volume due to the inability to remove the sample in one piece and the precision of the scale used during the experiments. Furthermore, it was found that taking a biopsy sample from a partly obstructed lesion was more challenging in the *cone* and *dome biopsy needles* due to their design.

Due to the lack of lesions inside the ducts, we were unable to perform biopsy procedures in the mastectomy samples. Furthermore, we also encountered some

trouble inserting the biopsy needles in the milk ducts, due to lack of natural ductal flow in approximately 37.5% of the cases with the *cone* and *pincer biopsy needle*. We have illustrated, however, that the biopsy needles were able to perform a biopsy procedure in gelatine tissue phantoms. All needles were able to obtain tissue samples in both partially and completely occluded milk duct phantoms. However, it was found that the tissue sample volume of the *pincer biopsy needle* was significantly larger than that of the c*one* and *dome biopsy needle,* giving a slight preference to the former needle. Furthermore, from the mastectomy experiment, it became clear that the *dome biopsy needle* was unfeasible for MD procedures, due to its inability to visualize the milk duct during insertion and advancement. Therefore, a clear preference to the *pincer* and *cone biopsy needle* was given, which are currently being used in a clinical study at the University Medical Centre Utrecht (UMCU).

By integrating the biopsy functionality into the ductoscope, we hope that MD will become a useful part of the diagnostic tool set for early breast cancer detection in future. Due to the integration of the biopsy needle with the ductoscope, only minimal alterations to the original MD procedure have to be made, which makes it easy to introduce the developed biopsy needles in clinical practice.

Based on the experience with the mastectomy experiment, the following MD procedure with the developed biopsy needle is proposed:

1. Connect the micro-endoscope and irrigation to the handle of the biopsy needle.
2. Insert the biopsy needle in the milk duct with the needle tip exposed and the pincer pulled back.
3. Inspect the milk duct for abnormalities.
4. Once an abnormality is detected the biopsy needle can be actuated by:
    a. Moving the handle forward: in case of lesions blocking the milk duct.
    b. Moving the handle sideways: in case of lesions in the milk duct wall.
5. After taking the biopsy, the needle is retracted with the pincer covering the needle tip.
6. The tissue sample is manually removed from the biopsy needle under irrigation.

4.2 Recommendations for future research

In future, the biopsy needles will be developed further into fully commercial prototypes that can be used during MD procedures. We will investigate techniques to minimize the outer diameter to simplify insertion and enable reaching the peripheral small branches of the ducts and TDLU, which will allow us to investigate close to 100% of the milk ducts. We will also investigate adding a spring-loaded mechanism in the handle to achieve high-speed needle movement and resection during the biopsy procedure. The addition of such a high-speed system may improve the effectiveness, precision, and quality of the biopsy procedure. Next to the main application in MD, we will also explore other application areas in which tissue samples need to be taken through small incisions, such as in bile duct biopsy. In October 2022, we initiated a clinical feasibility study to evaluate the efficacy of the developed biopsy needles in the outpatient clinic setting for patients with PND [17].

5. CONCLUSION

MD shows significant potential as a diagnostic tool for early breast cancer detection, but successful adoption relies on histological verification of its findings. Currently, there's a lack of reliable biopsy instruments for MD. In this study, we have developed innovative ultrathin biopsy needles for use in MD, which are now patented. We created three distinct biopsy needles: *the dome biopsy needle, pincer biopsy needle,* and *cone biopsy needle*. These needles were tested on gelatine tissue phantoms and mastectomy samples. The results demonstrate that performing intraductal biopsies in a phantom model closely resembling the clinical situation is feasible. Notably, the *pincer* and *cone biopsy needles* outperformed the dome biopsy needle in terms of sample size and ease of integration into the MD procedure. These initial tests highlight the substantial potential for diagnosing and treating intraductal lesions. Ongoing development and refinement of the *cone* and *pincer biopsy needles* are underway for use in a clinical study.

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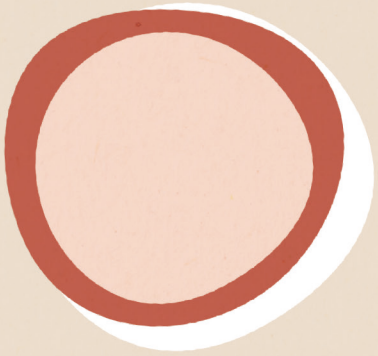

CHAPTER 6

Feasibility of narrow-band imaging, intraductal biopsy, and laser ablation during mammary ductoscopy: protocol for an interventional study

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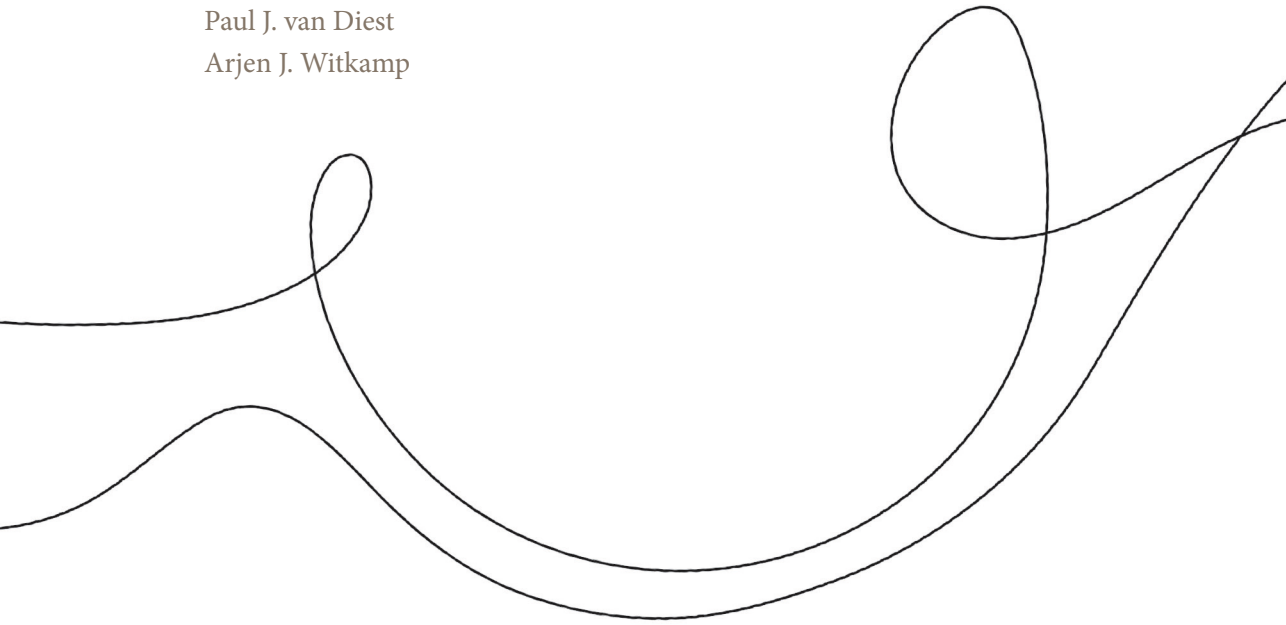

ABSTRACT

Introduction: Ductoscopy is a minimally invasive micro-endoscopic approach for direct visualization of intraductal lesions of the breast. Challenges of ductoscopy are low sensitivity for detecting malignancy, the lack of a proper intraductal biopsy device, and adequate treatment of intraductal lesions. This study will analyze three new approaches to enhance the effectiveness of interventional ductoscopy in patients with (premalignant) intraductal lesions: narrow-band imaging (NBI), new intraductal biopsy tools, and intraductal laser ablation. The main aims of the present study are to improve diagnostic accuracy and therapeutic efficacy of interventional ductoscopy in patients with pathological nipple discharge (PND) and to explore the feasibility of the new approaches in diagnosing and removing intraductal precursor lesions.

Methods and analysis: This prospective, single-center, diagnostic feasibility study will include two patient groups. Group A: women with PND with no radiological suspicion for malignancy. Group B: women undergoing mastectomy (preventive or therapeutic). The primary endpoints for both groups are the technical feasibility of NBI ductoscopy, intraductal biopsy, and laser ablation, and as secondary endpoint the number of diagnosed and successfully treated intraductal lesions.

Discussion: Enhanced ductoscopy with NBI, intraductal biopsy, and laser ablation could prevent unnecessary surgery in patients with PND.

Ethics and dissemination: This study was approved by the Medical Research Ethics Committee UMC Utrecht in The Netherlands (METC protocol number 21-688/H-D). The results of this study will be published in peer-reviewed journals and presented at national and international conferences.

INTRODUCTION

Pathological nipple discharge (PND) is one of the most common breast-related complaint [1]. PND is defined as unilateral, spontaneous, and bloody or serous discharge, usually arising from a single duct orifice of the nipple. It is often associated with breast cancer, while the most common causes of PND, ductal ectasia and intraductal papillomas, are benign [2], [3]. Mammography and breast ultrasound are important imaging techniques for the detection of breast cancer. However, when PND is the only complaint, they both have limited sensitivity [4]. Magnetic resonance imaging (MRI) has shown to be a sensitive imaging technique for detecting malignancy, but specificity is low. [5], [6]. Therefore, the value of MRI is limited in patients with PND, and core needle biopsy or surgical excision is still necessary when MRI shows a suspicious lesion [7], [8]. Because PND is regarded as a possible sign of breast cancer and standard radiologic imaging often fails to reveal the cause, most patients suffering from PND still undergo local surgical procedures (microdochectomy or major duct excision). This can lead to undesirable side effects such as scar tissue, perioperative complications, decreased sensitivity of the nipple, and compromised breastfeeding in the future [9]–[13]. Further, persistent or recurrent PND after local surgery is reported in 3 to 12% of patients [14], [15]. Malignancy is found in only 5% to 8% of these operated patients [16]–[18]. This means that around 90% to 95% of the surgical procedures were performed for benign causes.

Ductoscopy is a minimally invasive micro-endoscopic technique, which allows direct visualization of the breast ducts and the possible intraductal lesions within. It can be performed under local anesthesia in the daily routine at the outpatient clinic and has proven to be safe with only a low risk of < 3% on (mild) and self-limiting complications [19], [20]. Nowadays, ductoscopy is routinely used in the diagnostic work-up in patients suffering from PND [20], [21]. In a previous study, ductoscopy avoided surgery in around 2 out of 3 patients with PND [22]. Ductoscopy is a developing diagnostic and interventional procedure. It has a pooled sensitivity of 58% and a pooled specificity of 92% for the diagnosis of malignancy in patients with PND with negative conventional imaging [23]. However, the current tissue extraction tool (in the form of an expandable basket) is

not sufficiently accurate and only allows extraction of selected larger intraductal polypoid lesions [19], [24]. Therefore, there is a need for new techniques to increase the sensitivity for the detection of premalignant lesions (especially the flat ones) and the interventional possibilities.

Firstly, to enhance the diagnostic accuracy of ductoscopy, narrow-band imaging (NBI) can be added to the procedure. NBI is an imaging technique for endoscopic diagnostic medical tests that uses a different light spectrum to mark suspicious lesions [25]. It can be electronically activated by a switch in the endoscope, leading to a peak light absorption of hemoglobin occurring at these wavelengths. Blood vessels will appear dark, allowing for improved visibility and identification of other surface structures. In gastrointestinal endoscopy, NBI has found use in identifying Barrett's esophagus [26]. NBI is also used to identify pit patterns to classify colorectal polyps and tumors [27] and atypical dysplastic cells in the colon of patients with ulcerative colitis [28]. However, no studies have yet been conducted in which NBI is applied during ductoscopy. NBI may be useful since (pre)malignancy is known to show different patterns of vascularization (including neovascularization and angiogenesis) compared to healthy breast tissue [29]–[33].

Secondly, to improve the interventional possibilities of a ductoscopy procedure, a cooperation between University Medical Center Utrecht (UMC Utrecht) and Biomechanical Engineering of the Delft University of Technology, The Netherlands, was started some years ago to develop new intraductal biopsy tools. These newly developed tools will be tested during ductoscopy for their suitability to take biopsies and accurately remove lesions. Also, adding intraductal laser ablation can be useful to enhance the interventional capacity of ductoscopy by vaporising smaller flat lesions or ablating lesion remnants after intraductal excision. Laser ablation techniques are widely used in medicine (neurosurgery, ophthalmology, head and neck surgery, and surgical urology) and have proven to be safe and able to evaporate (pre)malignant lesions [34], [35]. One ex vivo feasibility study of endoscopic intraductal laser ablation of the breast concluded that laser ductoscopy is technically feasible and useful for intraductal interventions [36].

This study will analyze three new approaches to enhance interventional ductoscopy of the breast: NBI, new intraductal biopsy tools, and intraductal laser ablation in patients with (premalignant) intraductal lesions. The main aims of the present study are: 1) to improve diagnostic accuracy and therapeutic efficacy of interventional ductoscopy in patients with PND, and 2) to explore the feasibility of NBI, biopsy tools and laser ablation in diagnosing and treating intraductal breast cancer precursor lesions. We hypothesize that NBI will improve the diagnostic accuracy of ductoscopy as it is effective in multiple other endoscopic procedures. Also, we propose that the newly developed biopsy tools will enhance the biopsy technique, which can lead to more specific histological diagnosis and thus improvement of specificity of the ductoscopy procedure. Finally, laser ablation may improve the removal of (premalignant) intraductal lesions (or their remnants after biopsy) more precisely to enhance therapeutic efficacy.

METHODS AND ANALYSIS

Study design

This study is a phase II prospective, single-center, diagnostic feasibility trial performed in the UMC Utrechtin The Netherlands. The duration of the study will be 6-8 months of inclusion of patients. This trial starts in September 2022.

Study population

Study subjects are selected from a clinical population from the UMC Utrecht on a consecutive basis for both cohorts. This study will consist of two groups: Group A: patients with PND with no radiological suspicion for malignancy referred to the UMC Utrecht for ductoscopy, and Group B: patients undergoing mastectomy.

Participants selection

Inclusion criteria

Group A: All adult women (≥18 years old) with unilateral PND and no radiological suspicion for malignancy referred to the UMC Utrecht for ductoscopy will be included.

Group B: All adult women (≥18 years old) undergoing mastectomy (preventive or therapeutic) will be included.

Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study for both groups:

- Pregnancy
- History of breast surgery at the affected breast wherefore ductoscopy is technically impossible
- History of radiotherapy of the breast or thorax
- Nipple retraction
- Not being able to provide written informed consent

Sample size

No sample size calculation is performed, since this is a feasibility study. There is no comparison of outcomes. In group A, the new implementations (NBI + biopsy + laser ablation) will be performed in a maximum of 20 patients in which cannulation is possible. In group B, the new implementations will be performed in 5 patients in which cannulation is possible.

Intervention

PND will be defined as spontaneous, single-duct nipple discharge during a non-lactating period, persisting for more than three months. Before ductoscopy, a standard diagnostic evaluation will be performed in all patients, including a complete history and physical examination and imaging (mammography, ultrasonography, and/or MRI and/or core needle biopsy) if indicated, to rule out malignancy.

Patients in both groups will undergo white-light ductoscopy followed by NBI ductoscopy, as shown in the flowchart of the study design (Fig. 1). Patients with intraductal lesion(s) will also undergo an intraductal biopsy with the newly designed ductoscopy tools, followed by laser ablation if indicated. Patients without

intraductal lesion(s) will not undergo an intraductal biopsy or laser ablation and will be followed according to the guideline.

In group A, patients will undergo surgery depending on the outcome of ductoscopy (intraductal lesion suspicious for malignancy, persistent PND, and/or patient preference).

In group B, patients will undergo a therapeutic mastectomy (when recently diagnosed with breast cancer or DCIS) or preventive mastectomy (patients with a largely increased risk of breast cancer because of hereditary mutations in breast cancer suppression genes BRCA1 and BRCA2).

Postoperatively, the surgical specimen will be histologically analyzed. The correlation between the pathological characteristics of an observed intraductal lesion in the surgical specimen and its projection in white-light / NBI / intraductal biopsy will be evaluated. Postoperative care will be according to local protocols.

Outcome measures

Primary objectives

- Determine the feasibility and added value of NBI ductoscopy in diagnosing (premalignant) intraductal lesions.
- Determine the feasibility of intraductal biopsy tools for harvesting tissue of intraductal lesions.
- Determine the in vivo feasibility of intraductal laser ablation in patients with intraductal lesions.

Secondary objectives

- To compare the number of intraductal lesions found by NBI and the number found by white-light ductoscopy and intraductal biopsy (group A and B).
- Treatment success of interventional ductoscopy (biopsy and laser ablation) in treating PND (i.e., reducing the number of surgical procedures needed) (group A).

- To analyze the efficacy of intraductal biopsy and laser ablation in completely removing (premalignant) intraductal lesion(s) (group B).
- To determine the effect of interventional ductoscopy on quality of life in patients with PND (group A).

Patient enrolment and follow-up

Study subjects will be selected from a clinical population from the UMC Utrecht on a consecutive basis for both cohorts. No additional methods of recruitment of patients for inclusion will be needed.

In both groups, all study subjects eligible to be included will be asked by the treating physician if they are interested in being approached by the study coordinator for this study. The aims of the study will be explained to the patient. If subjects confirm to enter the study, informed consent will be obtained according to Good Clinical Practice guidelines.

In group A: The procedure will be performed in a daily routine at the outpatient clinic. The ductoscopy procedure will take 10 minutes more than usual. Lidocaine 1% will be used for local anaesthesia of the nipple. We will also ask patients to fill out questionnaires (Breast-Q, EQ-5D-5L, Ductoscopy) to analyze the effect of treatment on quality of life.

In group B: The ductoscopy procedure will take an additional 20 minutes and will be performed under general anesthesia directly before surgery. Postoperative care will be according to local protocols.

There will be a follow-up after the procedures, as shown in Figure 2.

Endpoints

The primary endpoints were to determine the number of intraductal lesions diagnosed by using NBI ductoscopy and treated successfully by using laser ablation. Also, to determine the ability of the new intraductal biopsy tools by performing a successful intraductal biopsy.

Secondary endpoints include the treatment success of interventional ductoscopy (biopsy and laser ablation) in treating PND. Treatment success will be achieved when symptoms disappear and nipple discharge does not return at follow-up. Furthermore, in patients who undergo surgery, to determine the accuracy of findings of NBI ductoscopy, biopsy and laser ablation during ductoscopy. Additionally, the quality of life (QOL) will be examined in patients with PND after ductoscopy.

Definition of a successful procedure

Successful NBI is when the ductal tree is visible during NBI ductoscopy. Successful biopsy is when it is possible to perform a biopsy with the new tools and when this tissue is sufficient to establish a correct diagnosis. Successful laser ablation is when the intraductal abnormality is no longer visible after the laser treatment. In patients who undergo surgery, the accuracy of biopsy will be determined when the biopted tissue is sufficient to establish a correct diagnosis after the final histology finding of the surgical specimen. The accuracy of laser ablation will be determined by analyzing the surgical specimen for remaining intraductal lesion.

Statistical analysis

Descriptive statistics will be used to describe the patient and treatment characteristics of the study population. Depending on the distribution, continuous data will be described with mean and standard deviation (SD) or median and interquartile range (IQR). Differences between populations will be tested by appropriate parametric or non-parametric tests.

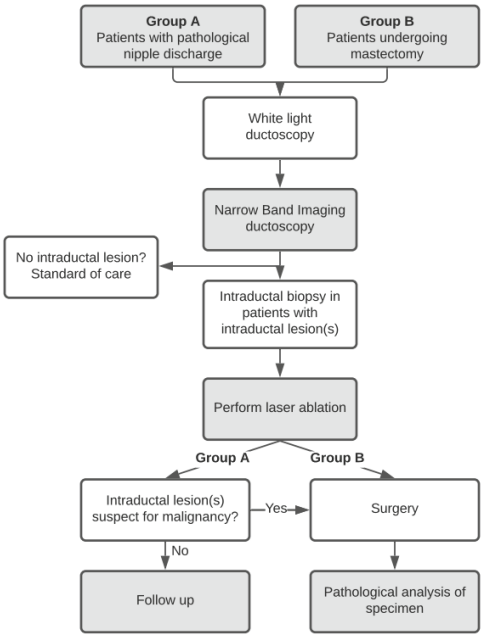

Fig. 1: Flowchart of the study design with three new approaches added to interventional ductoscopy of the breast: narrow band imaging, tissue biopsy and laser ablation.

	Group A		Group B
1	**Telephonic consultation**	Patients who are referred and meet the inclusion criteria will be informed about the study and will be asked if they want to join.	**Outpatient clinic**
2	**Telephonic consultation**	After 1 week, subjects will be called to ask if they join the study and if they have questions.	**Telephonic consultation**
3	**Outpatient clinic**	Fill in informed consent. White-light ductoscopy→ NBI → intraductal biopsy → laser ablation → mastectomy (B), questionnaires (A)	**Operating room**
4	**Pathology**	Extensive histopathological analysis of the biopsy and surgical specimen.	**Pathology**
5	**Follow-up: Telefonic consultation**	Group A: Telefonic consultation and questionnaires: 2 weeks and 3 months post- ductoscopy. Group B: 10 days post-surgery	**Follow-up: Outpatient clinic**

Fig. 2: An overview of the study procedures of interventional ductoscopy enhanced by narrow band imaging (NBI), intraductal biopsy and laser ablation in patients with pathological nipple discharge (Group A) or therapeutic/preventive mastectomy (Group B).

DISCUSSION

In this study, patients in both groups will undergo white-light ductoscopy, NBI ductoscopy, intraductal biopsy, and intraductal laser ablation. We defined enhanced ductoscopy as regular ductoscopy combined with NBI, improved biopsy tool, and intraductal laser ablation.

In patients with PND without radiological signs of malignancy, ductoscopy shows a sensitivity of 58% and specificity of 92% for the detection of breast cancer [23]. Additionally, ductoscopy detects (pre)cancerous lesions that were missed during regular imaging [14], [37]. At the same time, MRI has a sensitivity ranging from 46 to 86% and specificity from 76 to 98% in the same patient population [38]–[40]. Nevertheless, enhanced ductoscopy might increase the diagnostic performance (with NBI and/or intraductal biopsy). The first step is to analyze the feasibility of these new approaches within this study.

Ductoscopy has already been shown to prevent unnecessary surgery in patients with PND without radiological suspicion for malignancy [22], [37], [41]. Papillomas are the most common cause of PND and are difficult to remove completely with current extraction tools. Therefore, we expect that enhanced ductoscopy might improve the extraction of intraductal lesions, thereby alleviating symptoms of PND and preventing even more unnecessary surgery.

ETHICS AND DISSEMINATION

The Medical Research Ethics Committee UMC Utrecht in The Netherlands approved this study protocol (METC protocol number 21-688/H-D). To ensure patients' safety and rights, the investigators will ensure that the study will be conducted according to the ethical principles of the declaration of Helsinki [42]. Patients will be fully informed of the purpose and procedures of the study. Written informed consent will be obtained from each study participant in agreement with the General Data Protection Regulation. The results of this study will be published in peer-reviewed journals, national and international conferences on

corresponding fields of interest. The authorship for written publications has to be confirmed by all lead investigators.

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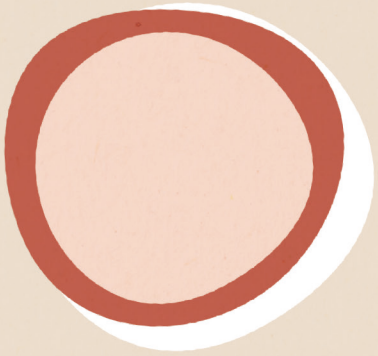

CHAPTER 7

Intraductal laser ablation during ductoscopy in patients with pathological nipple discharge

Breast Cancer Research and Treatment, under review

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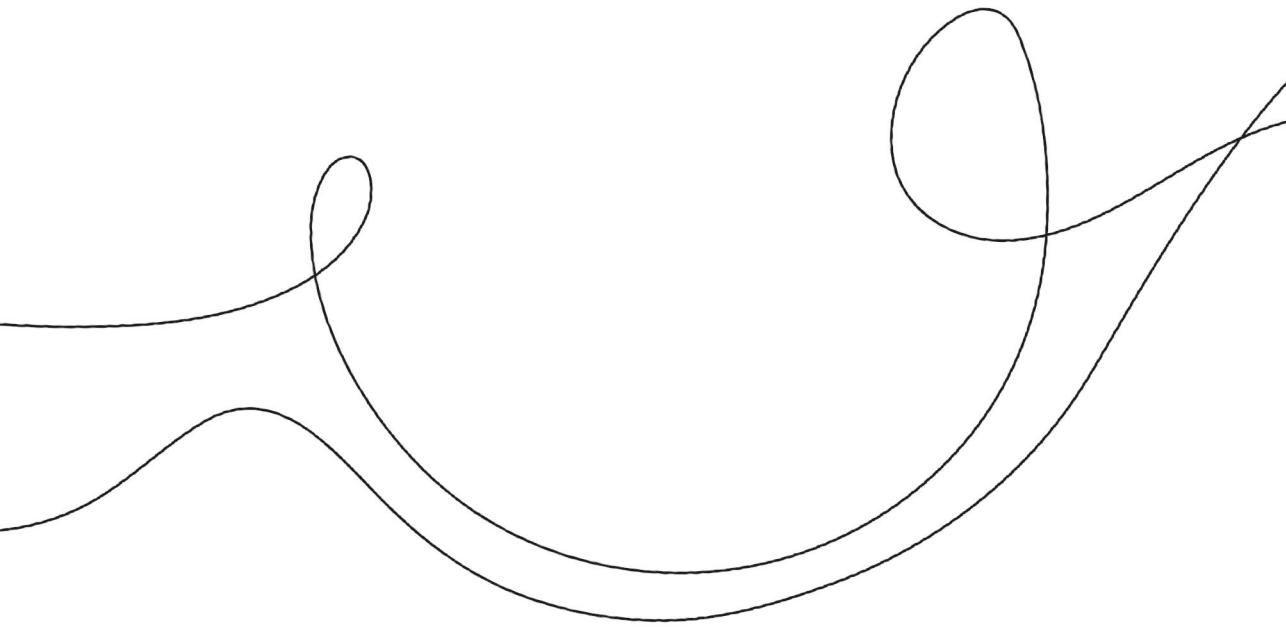

ABSTRACT

Background: Ductoscopy is a minimally invasive micro-endoscopic approach for direct visualization and removal of intraductal lesions of the breast. A challenge of ductoscopy is an adequate treatment of intraductal lesions by complete removal to prevent exploratory duct excision surgery. This study aimed to determine the in vivo feasibility of intraductal laser ablation during ductoscopy to remove intraductal lesions in patients suffering from pathological nipple discharge (PND).

Methods: A prospective, single-center diagnostic feasibility trial was conducted between October 2022 and November 2023, enrolling adult women with unilateral PND and no radiological suspicion of malignancy. Intraductal laser ablation was performed after incomplete intraductal biopsy using a Thulium laser.

Results: Duct cannulation and subsequent ductoscopic exploration were successful in 21 patients revealing an intraductal lesion in 13 patients (61.9%). From these 13 patients, 9 patients (69.2%) underwent intraductal laser ablation due to a residual lesion after biopsy. Pathology of the removed intraductal lesions showed a papilloma in eight (88.9%) patients and a papilloma/DCIS combination in one patient (11.1%). Post-procedure, PND stopped in 77.8% of the patients (7/9). Two patients had recurrent PND complaints caused by a residual lesion.

Conclusion: Intraductal laser ablation during ductoscopy in patients with papillary lesions seems to be feasible and safe. The Thulium laser enables ablation of residual lesions and is therefore suitable for an immediate second intervention after ductoscopic removal of intraductal lesions. Further refinement and validation in a follow-up clinical trial are necessary to further assess its therapeutic efficacy.

INTRODUCTION

Pathological nipple discharge (PND) is a common breast-related condition characterized by unilateral, spontaneous, and bloody or serous discharge arising from a single duct orifice of the nipple [1]. PND is often viewed as a breast cancer sign, but the most common causes of PND by far are benign (duct ectasias and intraductal papillomas) [2]-[4]. Surgical duct excision is traditionally required to rule out malignancy in patients with PND without radiological and clinical abnormalities [5]-[7]. However, the malignancy rate after duct excision surgery is only 8.1%, meaning that the majority of these surgical procedures (microdochectomy or major duct excision) are performed for benign causes. This can lead to surgery-related complications (1.4%) such as hematomas, surgical site infections, and seromas [8]. Other adverse effects of duct excision surgery include higher costs and the need for more medical personnel [9]. In times of staff shortages and rising healthcare expenses, gains can be achieved with a better selection of patients that actually will benefit from duct excision surgery. Ductoscopy is a minimally invasive micro-endoscopic approach for direct visualization and removal of intraductal lesions of the breast [10]. After mammography and ultrasound, ductoscopy can be performed in the diagnostic work-up for PND patients without radiological abnormalities [11], [12]. A randomized controlled trial found that ductoscopy is as accurate as conus excision in identifying the causative lesion of PND [13]. Additionally, PND patients with non-suspicious conventional imaging and negative ductoscopy have a low malignancy rate, making subsequent microdochectomy unnecessary in 2 out of 3 patients [14], [15]. However, some patients still suffer from PND after ductoscopy, and in most cases, these patients eventually undergo a surgical procedure or a second ductoscopy due to recurrent or persistent PND [16]-[19].

Current endoscopic interventional methods for PND remain suboptimal. Therefore, there is a need for more effective interventional possibilities of ductoscopy to remove intraductal lesions completely. One promising new intervention is adding laser ablation to the ductoscopy procedure to remove intraductal lesions completely. Laser ablation techniques have been widely used in various medical fields and have been proven to be safe and effective in evaporating lesions

[20]-[23] Consequently, our research team previously conducted an ex vivo feasibility study of endoscopic intraductal laser ablation of and found that laser ductoscopy is technically feasible and can serve as an adjuvant tool for minimally invasive treatment of (residual) intraductal papillomas in PND patients [24].

As a result, we have conducted an in vivo feasibility study with intraductal laser ablation during ductoscopy in PND patients. The main goal of this study was to determine the in vivo feasibility of intraductal laser ablation in patients with intraductal lesions. To the best of our knowledge, this is the first study to perform intraductal laser ablation during ductoscopy in patients.

METHODS

This study was approved by the Medical Research Ethics Committee of the University Medical Center Utrecht in The Netherlands (METC protocol number 21-688/H-D). All participants provided written informed consent. The study protocol was published in September 2022 [25].

Study design and population

This phase II, prospective, single-center, diagnostic feasibility trial was conducted at the University Medical Center (UMC) Utrecht in The Netherlands between October 2022 and November 2023. The study population included adult women (≥18 years) with unilateral PND and no radiological suspicion of malignancy, who underwent a ductoscopy procedure at UMC Utrecht. Laser ablation was performed when there was an intraductal lesion visible which was incompletely removed using the basket or biopsy tools while tissue for pathology was obtained. PND was defined as unilateral, bloody or serous nipple discharge during a non-lactational period, persisting for at least three months. The exclusion criteria were: pregnancy, previous breast surgery at the affected breast that would make ductoscopy technically impossible, radiotherapy of the breast or thorax, nipple retraction and the impossibility of obtaining tissue sampling from the lesion.

Data collection

Standard clinical variables were collected, including age at presentation, characteristics of the nipple discharge (laterality and spontaneous versus expressed), physical exam findings (palpable breast mass and productive ducts), and follow-up period. In addition, details of diagnostic methods, imaging studies, and histopathological findings were recorded for each case.

Work-up

Before ductoscopy, all patients underwent a standard diagnostic evaluation, including a complete medical history and physical examination and imaging (mammography, ultrasonography, and/or MRI) and core needle biopsy when indicated to rule out malignancy.

Ductoscopy procedure

Ductoscopy was performed in the daily routine at the outpatient clinic. Lidocaine 1% was used for local anaesthesia of the nipple. A salivary duct probe (size 0000 to 1; Karl Storz, Tuttlingen, Germany) and an obturator (Polydiagnost, Pfaffenhofen, Germany) were used to widen the lactiferous duct of the nipple to a diameter of 1.2 mm. The SoLex nipple expander® (Polydiagnost), was then inserted through the port into the affected duct. The 6000-pixel 0.55-mm optic (LaDuScope T-flex; Polydiagnost) and the Polyshaft® (1.15 mm outer diameter, PD-DS-1015; Polydiagnost) were used for ductoscopy. The Polyshaft® system has three channels: one for the endoscope, one for saline irrigation or additional intraductal anesthetic infusion, and one for the biopsy tool and laser fiber. The surgeon explored the major ducts until they became too narrow to pass. Intraductal biopsies were performed when lesions were identified. The final step of the procedure was intraductal laser ablation, which was performed when the lesion was incompletely removed using the biopsy tools. A MED-fiber (Tobrix, Waalre, The Netherlands) with a core diameter of 200 μm and an outer diameter of 375 μm was introduced through the working channel. Laser energy was delivered using 2013 nm thulium laser generator (Revolix Junior; LISA Laser Products, Katlenburg, Germany) at power settings of 1–4 W with single pulses of 100–1000 ms. Laser ablation was applied until no visible vital tissue of the lesion to be treated remained.

All patients were followed at least after two weeks and three months after ductoscopy to evaluate the effect of treatment on symptoms.

Statistical analysis

Prevalence and means with standard deviation (SD) were calculated with SPSS v29.0 to describe the study population.

RESULTS

Baseline characteristics

From October 2022 to November 2023, a total of 24 patients met the inclusion criteria. Duct cannulation and subsequent ductoscopic exploration were successful in 21 patients (87.5%), revealing an intraductal lesion in 13 patients (61.9%). Biopsy samples were successfully obtained from all patients with intraductal lesions using a biopsy tool or basket. Finally, 9 patients (42.9%) underwent intraductal laser ablation due to a remaining lesion after biopsy (Fig. 1).

The mean age of the patient population at the time of ductoscopy procedure was 53.4 ±10.7 years. Clinical data of the patients are presented in Table 1. Unilateral discharge was noted in all cases, and spontaneous discharge was observed in 8/9 cases. All patients presented with single duct PND with bloody, yellow/brown or a clear color.

Ultrasound was conducted as part of the standard evaluation in all patients. The results revealed normal findings or lesion(s) with a low suspicion of malignancy. Mammography was performed in 8/9 cases. Furthermore, MRI was performed in 5 cases, with two cases indicating normal findings, two cases with duct ectasia and one patient suspected of an intraductal papilloma. 6 out of 9 patients underwent core needle biopsy prior to ductoscopy.

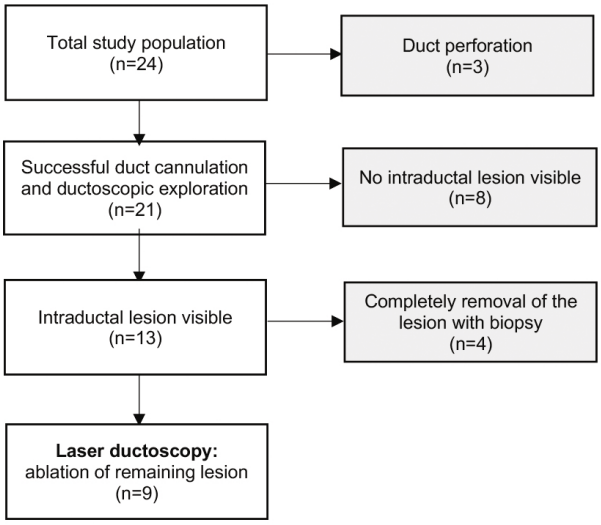

Fig. 1: Flowchart of the study population

Table 1. Clinical data of 9 patients with pathological nipple discharge undergoing laser ablation

Clinical findings	**No. N=9**
Age, mean ± SD, years	53.4 ±10.7
Affected breast, N (%)	
Left	5 (55.6)
Right	4 (44.4)
Palpable abnormality – N (%)	3 (33.3)
Color nipple discharge	1 (11.1)
Clear	2 (22.2)
Yellow/brown	6 (66.7)
Bloody	
Ultrasound findings - N (%)	
BI-RADS 1	1 (11.1)
BI-RADS 2	4 (44.4)
BI-RADS 3	3 (33.3)
BI-RADS 4a	1 (11.1)

Table 1. Clinical data of 9 patients with pathological nipple discharge undergoing laser ablation *(continued)*

Mammographic findings, N (%)	
BI-RADS 2	3 (33.3)
BI-RADS 3	5 (55.6)
Not performed	1 (11.1)
MRI findings, N (%)	
BI-RADS 2	3 (33.3)
BI-RADS 3	2 (22.2)
Not performed	4 (44.4)
Pathology before ductoscopy, N (%)	
No abnormalities	2 (22.2)
Papilloma	1 (11.1)
Ductectesia	3 (33.3)
Not performed	3 (33.3)
Cytology PND, N (%)	
No abnormalities	4 (44.4)
Papilloma	1 (11.1)
Cystic cells	1 (11.1)
Not performed	3 (33.3)

Abbreviations: SD= standard deviation; MRI= magnetic resonance imaging; PND=pathological nipple discharge

Power settings

Table 2 presents an overview of the outcomes derived from laser ductoscopy. Laser ablation was carried out using single pulses of 100–1000ms. Throughout the process of laser ablation, an endoscopic perspective was consistently maintained, utilizing power levels ranging from 1 to 4 Watts (W). Notably, at 1W, a mild impact was observable on the intraductal papilloma. Upon escalation to 3W, shrinkage of the intraductal papilloma was achieved. Increasing power to 4W resulted in a more pronounced reduction, although this power was not needed for the majority of procedures. The total energy used by removal of the intraductal papilloma ranged from 31 to 226 Joules (J). The duration of laser ablation ranged from 0.21 to 1.12 min.

Table 2. Overview of 9 patients with pathological nipple discharge that underwent intraductal laser ablation

Patient	Age	Nipple discharge	Radiology (BI-RADS)	Ductoscopic findings	Intraductal extraction of lesion	Laser setting	Pathology	Follow-up after 3 months
1	53	bloody	US + MG + MRI: 2	Polypoid lesion	basket	2.0 W / 133 J 1.09 min	Intraductal papilloma	Remaining lesion: recurrence of PND. Duct-excision surgery
2	37	bloody	US + MG: 3	Polypoid lesion	basket	2.0 W / 31 J 0.21 min	Intraductal papilloma with foci ADH/DCIS	Successful treatment: Follow-up with mammogram
3	50	clear	US + MG: 3	Polypoid lesion	biopsy tool	3.0 W / 120 J 0.50 min	Intraductal papilloma	Successful treatment
4	67	bloody	US + MG + MRI: 3	Polypoid lesion	basket	3.0 W / 80 J 0.32 min	Intraductal papilloma	Successful treatment
5	51	bloody	US + MRI: 4a	Polypoid lesion	basket	4.0 W / 226 J 1.12 min	Intraductal papilloma	Successful treatment
6	67	Yellow / brown	US + MMG + MRI: 3	Polypoid lesion	biopsy tools	3.0 W / 80 J 0.31 min	Intraductal papilloma	Successful treatment
7	45	bloody	US + MG: 3	Polypoid lesion	basket	3.0 W / 101J 0.45 min	Intraductal papilloma	Remaining lesion: recurrence of PND. Re-laser
8	65	bloody	US + MG + MRI: 2	Polypoid lesion	basket	3.0 W / 53J 0.22 min	Intraductal papilloma	Successful treatment
9	45	yellow	US + MG: 3	Polypoid lesion	basket	4.0 W / 205 J 1.07 min	Intraductal papilloma	Successful treatment

Abbreviations: BI-RADS= breast imaging reporting and data system; US= ultrasound; MG= mammography; MRI= magnetic resonance imaging; W= watt, J=joule; ADH= atypical ductal hyperplasia; DCIS= ductal carcinoma *in situ*

Histopathological findings

Pathology of the biopted tissue showed an intraductal papilloma in 8 patients. One patient (11.1%) experienced PND due to an intraductal papilloma with a focus of ADH/DCIS (Fig. 2). Post-procedure, there was no visibly remaining lesion due to complete laser ablation. In this case follow-up with mammography will be carried out.

Fig. 2: The removed intraductal lesion in patient 2 with a focus of ADH/DCIS. The remaining lesion in the milk duct was ablated with the Thulium laser.

Follow-up

Follow-up data were available for all included patients. After successful laser ductoscopy, PND stopped in 7/9 (77.9%) patients. Two patients had recurrent PND complaints caused by a remaining lesion. One patient was planned for a second ductoscopy with laser ablation and the other patient choose for duct excision surgery. Duct excision surgery (microdochectomy / major duct excision) could thereby be avoided in 8/9 (88.9%) patients.

One patient experienced post procedural pain in the nipple for 1 week. Post-procedure, an MRI was performed without any abnormalities. The remaining patients did not experience any post-procedural pain or other side effects. No other complications were reported.

DISCUSSION

The aim of this study was to assess the feasibility of laser treatment for intraductal papillomas causing PND. This interventional study demonstrated that intraductal laser ablation during ductoscopy was technically feasible in patients with intraductal lesions. The Thulium laser was capable of evaporating intraductal papillary lesions in cases with remaining lesions after biopsy resulting in discontinuation of PND complaints in 77.8% after treatment in the follow-up period of three months. There were no complications, and only 1 patient complained of post-procedural nipple pain, which can also generally be seen after ductoscopy so this cannot with certainty be attributed to the laser ablation. Laser ductoscopy thereby has potential to safely improve the therapeutic intervention capability of ductoscopy in patients with benign intraductal lesions and successfully prevent unnecessary exploratory surgery. However, further refinement and validation in follow up clinical trials are necessary.

Ductoscopy enables the detection of malignancies with a specificity of 92% and a sensitivity of 58% [26]. Although current intraductal biopsy tools can remove lesions during ductoscopy, their removal often remains incomplete[16], [27]. According to a prior study conducted by our research team, removal of the lesion was possible in only 36.8% of the study population [14]. In these cases, in which tissue sampling from the lesion can be obtained, laser ablation serves as a promising addition to the therapeutic capabilities of ductoscopy while retaining histological confirmation. In the present study, laser ductoscopy made it possible to remove intraductal lesions in 77.8% of patients with remaining intraductal lesions after basket removal. After undergoing regular ductoscopy, patients can still suffer from PND and therefore undergo a surgical procedure or a second ductoscopy [16]-[18]. According to a cohort study, persistent or recurrent PND after a first

ductoscopy procedure was primarily caused by a remaining intraductal papilloma in the majority of patients (95%) [19]. In such cases, if laser ductoscopy was performed during the primary ductoscopy procedure, complete removal of the intraductal lesion may have been possible in a greater number of patients, thereby potentially avoiding a second (surgical) intervention.

Laser ductoscopy can improve the patient selection process for surgical procedures in the workup of PND without clinical or radiological abnormalities, because successful (laser)ablation prevents the necessity for further invasive procedures [14]. However, the presence of an intraductal mass is a possible predictor for malignancy, so definitive histological diagnosis is mandatory before performing laser ablation [15]. Consequently, laser ductoscopy can lead to a reduction of the need for additional surgery and fewer surgery related complications such as hematomas, surgical site infections and seromas [8].

However, the role of laser ductoscopy in cases of PND caused by intraductal DCIS or invasive cancer is uncertain. In this study, one patient experienced PND due to an intraductal papilloma with a focus of ADH/DCIS grade 1. Following an intraductal biopsy during ductoscopy, laser ductoscopy was performed. Post-procedure, the localization of the tumor site for surgical resection by wide local excision was not possible because there was no remaining lesion on imaging due to complete removal with laser ablation. In this case, follow-up with mammography will be carried out. In view of the fact that observation for low grade DCIS is becoming more common and that the natural cause of ADH and DCIS in a papilloma is not known, this may be an acceptable risk under proper clinical and imaging surveillance. Whether laser ductoscopy may turn out to be a regular intervention for premalignant breast lesions is yet speculation.

According to our findings, laser ductoscopy can be safely integrated into the diagnostic and therapeutical approach for pathological nipple discharge to remove intraductal lesions in patients with remaining intraductal lesions after basket removal and subsequent histological biopsy. This procedure can be incorporated into the initial ductoscopy procedure in the presence of a visible residual lesion. Additionally, it can also be performed during a second ductoscopy procedure

in patients with recurrence of complaints due to a remaining lesion. Laser ductoscopy can be implemented in medical centers already performing ductoscopy procedures for pathological nipple discharge. The widespread adoption of this technique into the work-up of PND, particularly in centers performing duct excision surgery, holds promise for the future.

To our knowledge, this is the first study to report on the application of intraductal laser ablation within a ductoscopy procedure. However, certain limitations do warrant consideration. Given the design of this study as a feasibility study, it features a relatively small sample group size of included patients. This study clearly showed the feasibility of intraductal laser ablation during ductoscopy using a Thulium laser. Nevertheless, to comprehensively evaluate both diagnostic accuracy and therapeutic efficacy, further refinement and validation in a clinical trial is necessary. Additionally, the identification of optimal power settings for achieving adequate removal, as well as an examination of the effects of using different types of lasers (e.g. Holmium vs. Thulium laser) on intraductal papillomas, have to be studied [31], [32].

To conclude, laser ablation during ductoscopy is safe and feasible for evaporating residual intraductal breast lesions. This technique holds the potential to enhance the minimally invasive therapeutic intervention capabilities of ductoscopy procedures for patients suffering from PND without other clinical or radiological abnormalities.

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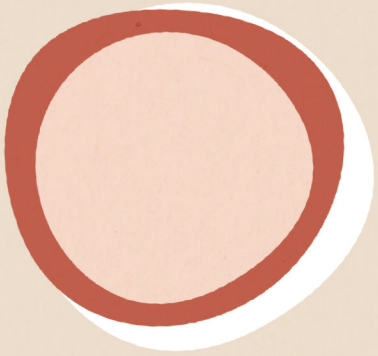

CHAPTER 8

Idiopathic granulomatous mastitis after ductoscopy: a case report

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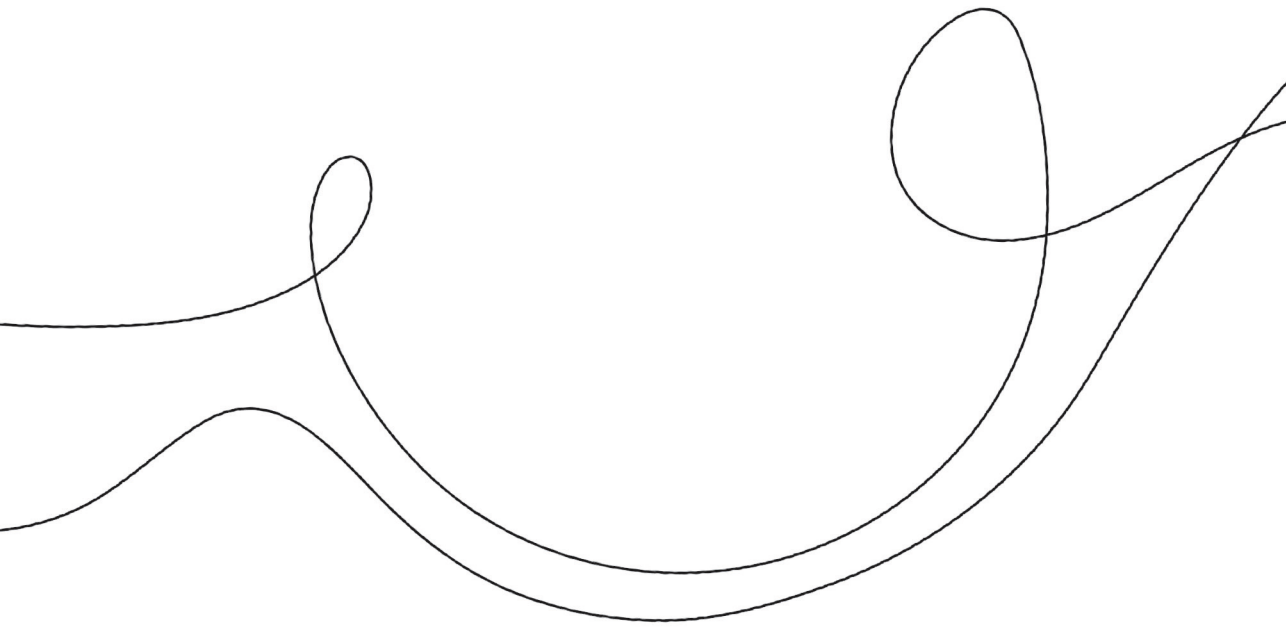

ABSTRACT

Introduction and importance: Idiopathic granulomatous mastitis (IGM) is an uncommon, benign, chronic inflammatory breast disease of unknown etiology, unpredictable duration, and unclear therapy.

Presentation of case: A 41-year-old woman presented with pathological nipple discharge for which ductoscopy was performed. Post-ductoscopy, the patient developed abscesses in her breast with histopathological confirmation of granulomatous mastitis (GM).

Clinical discussion and conclusion: IGM has an unknown etiology and atypical presentation. This is the only case described in which IGM occurred after ductoscopy. This can be related to trauma-induced GM or underlying IGM aggravated by ductoscopy.

INTRODUCTION

Idiopathic granulomatous mastitis (IGM) is an uncommon, benign, chronic inflammatory breast disease of unknown etiology and unpredictable duration. Symptoms of IGM are a mass, pain, erythema, swelling, fistula, areolar retraction, ulceration, and abscesses [1], [2]. The rarity of IGM causes a lack of data. Therefore, the best therapy remains unclear.

We describe a case of a woman who developed abscesses after ductoscopy, diagnosed as IGM on biopsy. To the best of our knowledge, IGM after ductoscopy has not been previously described. This report was written in line with the SCARE criteria [3].

PRESENTATION OF CASE

A 41-year-old gravida 3, para 3 woman presented to the outpatient clinic with a 2-year history of right-sided pathological nipple discharge (PND) with unremarkable medical history and drug history. She had milky/brown nipple discharge, which started days before her menstruation and persisted during her menstruation. Physical examination of the breast was normal. Mammography suggested a retroareolar cyst in the right breast (shown in Fig. 1a). Ultrasound showed dilated retroareolar ducts, some solid without vascularization (shown in Fig. 1b). Needle biopsy of this solid part and cytology of nipple discharge showed no abnormalities. We decided to perform a ductoscopy to diagnose and treat possible intraductal lesion(s). The ductoscopy procedure was performed by an experienced breast surgeon. Two major ducts were explored. They were filled with yellow debris, which was collected for pathology. Ductoscopy showed no lesions suspect for malignancy, infection, or benign lesions. After the procedure, the patient went home in good condition.

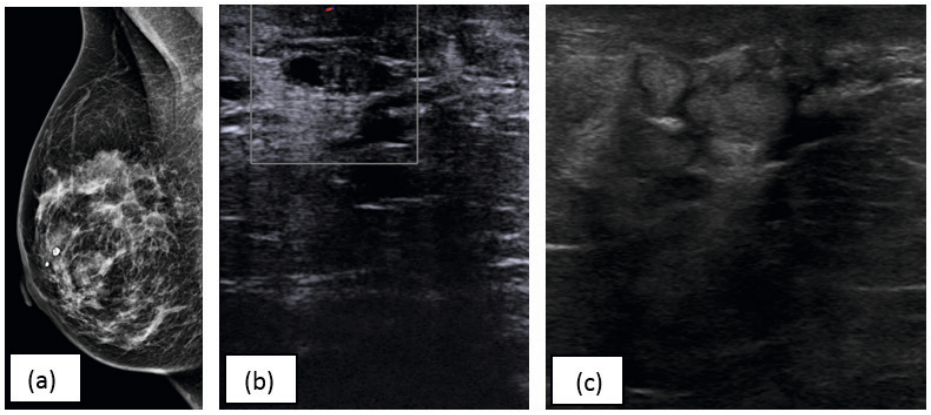

Fig. 1
(a) Mammography suggests a retroareolar cyst
(b) Ultrasound before ductoscopy: dilated retroareolar ducts
(c) Ultrasound after ductoscopy: duct ectasia, not suspicious for an infection or malignancy

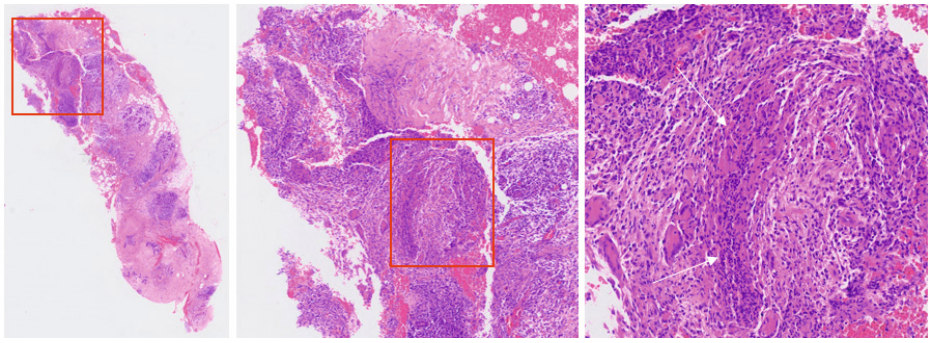

Fig. 2
Core needle biopsy showing GM with extensive infiltration of the breast parenchyma by epithelioid histiocytes, multinucleated giant cells and lymphocytes / (H&E)

Four days after ductoscopy, she developed pain and a palpable mass of 4-5 cm in the lower-outer quadrant. Ultrasound showed duct ectasia, not suspicious for infection or malignancy (shown in Fig. 1c). Histological analysis of the debris from ductoscopy showed no abnormalities. Antibiotics were started, and the patient initially reacted well but presented ten days later with abscesses. Surgical incision and drainage were performed. One week after surgery, she slowly recovered. A few days later, a new red palpable mass developed in the outer upper quadrant of

the breast. An ultrasound-guided biopsy was performed. Histopathology of this biopsy showed granulomatous mastitis (shown in Fig. 2), for which the patient was referred to an infectious diseases specialist. There was no evidence of other underlying or related illnesses, and it was decided to watch-and-wait. In the follow-up period (one year), she recovered slowly from the remaining abscesses as a self-limiting process.

Retrospective analysis of radiological images

A breast radiologist (AF) retrospectively analyzed the ultrasounds and mammography. The mammography showed more asymmetry in the retromamillar region right than left. The ultrasound images before ductoscopy showed duct ectasia, some with thickened duct wall. This can be related to granulomatous mastitis, but it was very limited in these images and not immediately suspect. The ultrasounds images after ductoscopy showed again duct ectasia, now filled with echogenic material. Therefore, the thickening of the wall was less clear. Because there was no vascularization, the images were not immediately suspect for granulomatous mastitis.

DISCUSSION

IGM is an uncommon benign chronic inflammatory breast disease first described by Kessler and Wolloch in 1972 [4]. Although the pathogenesis of IGM remains unclear, factors that have been related to the disease include a reaction to trauma, autoimmunity, hormonal or metabolic processes, and infection with Corynebacterium Kroppenstedtii [2].

This case study is the first to report the unusual presentation and complicated diagnosis of a patient with IGM after ductoscopy. In our case, we hypothesize that the patient may have suffered from PND, with GM as an underlying cause, and the ductoscopy procedure aggravated the chronic inflammatory disease, which led to the formation of abscesses in the breast. Another hypothesis is that the patient was suffering from PND from another cause, and the ductoscopy was a traumatic event that led to ”trauma-induced GM”. In three previously reported

cases, a possible ''trauma-induced GM" was also described: in one case induced by a foreign body [5] and in two cases by a blunt trauma[6], [7].

Ductoscopy is a minimally invasive procedure with mild and rare complications. Only mild nipple pain, bacterial mastitis, and perforation of the ductal lining have been reported [8]. No other case of IGM after ductoscopy has been described before.

The diagnosis and treatment of IGM are challenging because of a lack of valid data. Currently, the application of corticosteroids and the use of surgery in cases with insufficient response to conservative treatment is the most common approach [1],[2],[9]. In this presented case, IGM was treated by surgery without steroids. The patient developed abscesses, but these were self-limiting. The treatment was difficult and prolonged. Therefore, good communication about disease management is crucial.

CONCLUSION

IGM has an unknown etiology and atypical presentation. Radiological findings are non-specific in diagnosing IGM. This is the only case described in which IGM occurred after ductoscopy. Whether this concerns trauma-induced GM or underlying IGM aggravated by ductoscopy remains unknown. With this case report, we want to create awareness of the rare presentation of this condition after ductoscopy.

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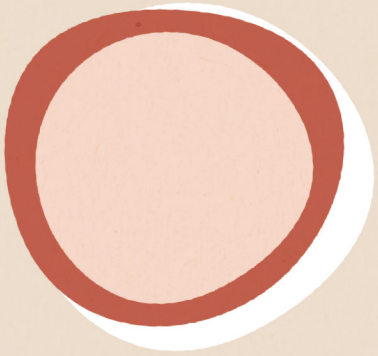

CHAPTER 9

The diagnostic value of miRNA expression analysis in detecting intraductal papillomas in patients with pathological nipple discharge

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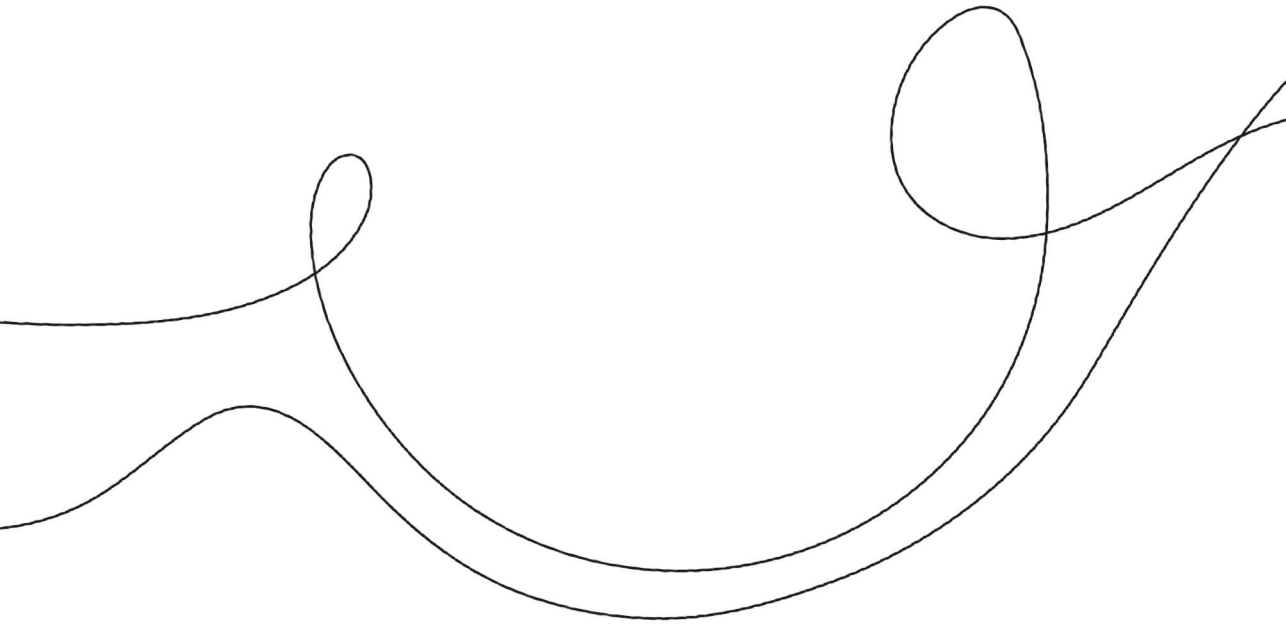

ABSTRACT

Background: Patients with pathological nipple discharge (PND) often undergo local surgical procedures because standard radiologic imaging fails to identify the underlying cause. MicroRNA (miRNA) expression analysis of nipple fluid holds potential in distinguishing between breast diseases. This study aimed to compare miRNA expression levels between nipple fluids from patients with PND to identify possible relevant miRNAs that could differentiate between intraductal papillomas and no abnormalities in the breast tissue.

Methods: Nipple fluid samples from patients with PND without radiological and pathological suspicion for malignancy who underwent a ductoscopy procedure between May 2019 and August 2020 were analyzed. We used univariate and multivariate regression analyses with odds ratios (OR) and 95% confidence intervals to identify nipple fluid miRNAs differing between pathologically confirmed papillomas and breast tissue without abnormalities. The diagnostic accuracy of differentially expressed genes was determined by analyzing receiver operating characteristics.

Results: A total of 27 nipple fluid samples from patients with PND were included for miRNA expression analysis. Out of the 22 miRNAs examined, only miR-145-5p was significantly differentially expressed (upregulated) in nipple fluid from patients with an intraductal papilloma compared to patients showing no breast abnormalities (OR 4.76, $p = 0.046$) with a diagnostic accuracy of 92%.

Conclusion: miR-145-5p expression in nipple fluid differs for intraductal papillomas and breast tissue without abnormalities, and has therefore potential as a diagnostic marker to signal presence of papillomas in PND patients. However, further refinement and validation in clinical trials are necessary to establish its clinical applicability.

INTRODUCTION

Nipple discharge is a common symptom, reported in 4.8-7.4% of women presenting with a breast complaint [1]. When nipple discharge is unilateral, spontaneous, and bloody or serous from a single duct, it is defined as pathological nipple discharge (PND) [2]. The underlying causes of PND are diverse and range from benign conditions, such as lactation, duct ectasia, and papilloma, to malignant conditions [3].

In patients with PND, evaluation with mammography and breast ultrasound is performed to rule out malignancy. However, when PND is the only complaint, they both have limited sensitivity [4]. Magnetic resonance imaging (MRI) is a sensitive imaging technique for detecting malignancy, but specificity is low in patients with PND with small lesions [5], [6]. Therefore, surgical excision is still required to rule out malignancy in patients with PND without radiological and clinical abnormalities. Nevertheless, the malignancy rate after duct excision surgery is only 8%, meaning the majority of the surgical procedures are performed for benign causes [7]. Ductoscopy is a promising diagnostic and interventional tool used in the workup of PND [8], [9]. However, it is not widely used. Also, nipple discharge cytology is limited due to low sensitivity [5], [10]. Therefore, the identification of biomarkers in nipple discharge to differentiate between benign lesions and no abnormalities could be essential for improving diagnostics.

In previous studies, biomarkers for breast cancer derived from nipple aspirate fluid (NAF) have been described as a promising tool for detecting the initiation of carcinogenesis [11]–[13]. NAF can be defined as a physiological fluid in the breast ductal system that does not spontaneously leave the breast and can be acquired via the nipple by a suction device [14]. PND differs from NAF since PND is spontaneous nipple fluid. In the context of breast disease development, the rationale is that both malignant and benign breast diseases are thought to derive from the epithelial lining of the milk ducts of the breast [15], [16]. This makes NAF, as biofluid secreted by the intraductal system of the breast, ideal for investigating the first signs of the development of breast diseases [17]. Many biomarker classes have been investigated in nipple fluid. However, nowadays,

NAF-based miRNA assessment in the context of early breast cancer detection is an increasingly investigated research topic because of its high stability in biofluids and its reported association with carcinogenesis [18]. MiRNAs play a critical role in gene regulation, controlling many cellular processes including cell growth, differentiation, proliferation, and apoptosis [19]. Moreover, specific miRNAs can function as either oncogenes or tumor suppressors [20], [21]. MiRNA analysis has not only demonstrated its impact on the pathogenesis of various cancer types [22]–[25], but also on a range of non-malignant conditions such as colon polyps, nasal polyps, benign prostatic hyperplasia, e.g. [26]–[28]. Data concerning miRNA involvement in certain benign conditions are limited and derived from a few studies. There have been no investigations into the role of miRNA analysis in detecting frequently occurring intraductal papillomas in patients with PND.

The aim of this study was to compare miRNA expression levels in nipple fluid of patients suffering from PND without radiological suspicion of breast cancer, in order to identify possible relevant miRNAs that can discriminate intraductal papillomas from breast tissue with no abnormalities.

MATERIALS AND METHODS

Study design and population

This prospective cohort study included all consecutive female adult patients with PND without radiological and pathological suspicion for malignancy who underwent a ductoscopy procedure between May 2019 and August 2020 at the University Medical Center Utrecht (UMC Utrecht). This study was approved by the Institutional Review Board and the UMC Utrecht Biobank Research Ethics Committee (nr 14-373). All participants provided written informed consent.

The inclusion criteria were adult women (≥18 years old) with unilateral PND and no radiological suspicion for malignancy referred to UMC Utrecht for ductoscopy. PND was defined as persistent, unilateral, bloody, or serous nipple discharge persisting for at least three months during a non-lactational period. Exclusion criteria

were: any malignancy in the past 5 years and histopathological confirmation of DCIS or invasive cancer in the biopted tissue during ductoscopy.

A standard diagnostic evaluation was performed on all patients. This included a medical history, physical examination, and recent radiological imaging (mammography, ultrasonography, and/or MRI and/or core needle/vacuum-assisted biopsy and/or cytology of nipple fluid within three months). All examinations were reviewed and reported by specialized radiologists.

Patients were eligible for a ductoscopy procedure when radiological and pathological findings were negative. Standard clinical variables were collected, including age at presentation, characteristics of the nipple discharge (laterality and color) and physical exam findings (palpable breast mass and productive ducts). In addition, diagnostic methods, findings from any imaging studies performed, and histopathological details were recorded for each case. Specialized breast pathologists assessed the biopsies.

Nipple fluid collection and processing

Intranasal oxytocin administration was performed before nipple fluid collection [14]. Since patients suffered from spontaneous nipple discharge, no vacuum device was needed for nipple fluid collection. The collected fluid was conserved in a buffer solution (RLT buffer (Qiagen) supplemented with 1:100 v/v β-mercaptoethanol) at -80 ºC until required for analysis. Details concerning sample collection success, sample volume, and sample color were registered.

RNA Isolation, Reverse Transcription, and Pre-Amplification

To study associations between NAF miRNA expression levels and the presence of intraductal papillomas, the expression of 22 human mature miRNAs were evaluated using Taqman Advanced miRNA assays (ThermoFisher Scientific, Catalog number A25576) and Taqman Fast Advanced mastermix (ThermoFisher Scientific) on a ViiA7 real-time PCR system (ThermoFisher Scientific): miR-25a-5p, miR-145-5p, miR-148a-3p, miR-151a-5p, miR-153-3p, miR-155-5p, miR-16-5p, miR-181a-5p, miR-18a-5p, miR-19a-3p, miR-205-5p, miR-21-5p, miR-221-3p, miR-222-3p, miR-29c-5p, miR-30b-5p, miR-320a, miR-339-5p, miR-374b-5p,

miR-425-5p, miR-92a-3p and miR-99b-5p. These oncogenic and tumor suppressor microRNAs were selected for evaluation due to previous investigations and our own pilot study identifying them as potential biomarkers for breast cancer. A search in EMBASE was conducted using search terms related to 'microRNA', 'breast', 'intraductal papilloma', and 'benign disease' to identify studies about miRNAs predictive for intraductal papillomas in the breast. Reference lists from articles were also examined to determine related publications. However, since there are no known microRNAs in the literature related to intraductal papillomas in nipple fluid, we opted for the well-known oncogenic miRNAs associated with breast cancer that were reliably measurable in nipple fluid, since a history of benign breast disease can be associated with increased risk of subsequent breast cancer. Tumor suppressor miRNAs, i.e. miR22 and miR-7, were not selected. miR-99b-5p was used as endogenous control miRNA for NAF as it demonstrated high expression stability in previous miRNA profiling studies [29], [30].

First, total RNA was extracted from 10 µL of NAF if available, according to the manufacturer's instructions using the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen, Hilden, Germany). Non-human synthetic ath-miR-159a (with a 5′ phosphate) was spiked in as procedural control by pre-mixing with RLT plus lysis buffer. Total RNA was eluted in 30 µL RNAse-free water. RNA concentrations were determined with the Qubit RNA HS Assay Kit (Invitrogen, Q32852, Waltham, MA, USA) measured by Qubit 3.0 (ThermoFisher Scientific, Waltham, MA, USA) fluorometric quantification. Evaporation was performed on a subset of the samples after RNA isolation in order to enhance the concentration. For reverse transcription, the undiluted total RNA was poly-A tailed. After adaptor ligation and reverse transcription, cDNA was pre-amplified for nineteen cycles using the TaqMan Advanced miRNA cDNA Synthesis Kit (ThermoFisher Scientific, Waltham, MA, USA) on a Veriti 96-well thermal cycler (ThermoFisher Scientific, Waltham, MA, USA). The pre-amplification product was subsequently diluted 10× in 0.1× Tris buffer, pH 8.0 and stored at −20 °C until quantitative PCR. The data was transferred to the Thermo Fisher ConnectTM system (Thermo Fisher Cloud) where miRNA-specific thresholds and baselines were set. The delta Ct value was used for PCR data analysis. Delta Ct was calculated as a difference

of the Ct value of the target miRNA and the Ct of the reference miRNA (miRNA-99b-5p): ΔCT = Ct(target miRNA) − Ct(miR-99b-5p).

Statistical analysis

No sample size analysis was performed beforehand, due to the limited number of ductoscopy procedures conducted at our clinic. Prevalence and means with standard deviation (SD) were calculated to describe the study population. Depending on distributions, comparisons of continuous variables were performed using the Student t test or the Mann–Whitney U test. Chi-square ($\chi 2$) test was used to compare categorical variables between patients with intraductal papillomas and no abnormalities. Univariate linear regression analysis was performed for all analyzed miRNAs in intraductal papillomas compared to no breast abnormalities, including the variables age, evaporation and nipple discharge color. Multivariable logistic regression analysis estimating odds ratios (OR) and 95% confidence intervals (CI) was performed to identify factors associated with intraductal papillomas in patients with PND. Variables with an estimated p-value of <0.05 in the univariable logistic regression were included in the multivariable logistic regression model. Also, age, nipple discharge color and evaporation were included in the regression model because previous in-house data showed a significant association between these parameters and miRNA expression in breast cancer. The color of discharge was separated into five groups: colorless, white, yellow, beige and orange/pink/bloody/green/brown. P-values ≤ 0.05 were considered to be statistically significant. The diagnostic accuracy of differentially expressed genes was determined by analyzing receiver operating characteristics (ROC-curve). SPSS v29.0 was used to analyze the data for this study.

RESULTS

From May 2019 to August 2020, a total of 35 patients underwent a ductoscopy procedure at our clinic. Of these patients, seven were excluded due to no discharge at time of the procedure (20%) and one patient was excluded because of the presence of DCIS in final histological analysis (2.9%). To evaluate miRNA expression levels, a total of 27 women with PND without radiological and histo-

logical suspicion of breast cancer that underwent a ductoscopy procedure were included for analysis: 16 patients with intraductal papillomas and 11 patients with normal breast tissue (no abnormalities). None of the patients had any malignancy in the past 10 years in their medical history. Also, not other disease that may be associated with altered expression of the selected miRNAs was reported.

Baseline characteristics of both cohorts are shown in Table 1. The patient population had a mean age of 50 ± 11.7 years in the intraductal papilloma samples group and a mean age of 42 ± 10.4 years in the no abnormalities group (p=0.09). There were also no significant differences between the other baseline patient characteristics in both groups. The volume of obtained nipple fluid ranged from 10 to 40 μL in both groups. In the intraductal papilloma group, 15 patients (94%) had one productive duct and 1 patient (6%) 2 productive ducts. In the normal breast tissue group, 9 patients (81.8%) had one productive duct, one patient (9.1%) had 2 productive ducts and one patient (9.1%) had three productive ducts.

Of the 22 target miRNAs, only miR-145-5p was significantly differentially expressed (upregulated) between NAF samples from patients with an intraductal papilloma and NAF samples from patients with no intraductal abnormalities (p = 0.050) (Table 2). Furthermore, in univariate linear regression analysis, the presence of a papilloma predicted a significant upregulation of miR-145-5p (p=0.012) with a mean fold change of 7.563 (fold changes per interrogated miRNA depicted in Figure 1).

A multivariable logistic regression analysis was performed including the factors age, evaporation, discharge color and miR-145-5p normalized expression level. Upregulated miR-145-5p was the only factor significantly and independently predicting the presence of intraductal papillomas, with an OR of 4.76 (CI 1.03 - 20; p=0.046; n=27).

The area under the curve of the ROC- curve showed an diagnostic accuracy rate of 0.920 (CI 0.801 – 1.000, p=0.002) (supplementary Table S1 and Figure S1). The delta CT mean expression levels for the other miRNAs were not significantly different between both groups.

Table 1: Clinical data and discharge analysis of patients with pathological nipple discharge (PND) undergoing a ductoscopy procedure (n=27)

Patient characteristics	Intraductal papilloma (n=16)	No abnormalities (n=11)	p-value
Age (years), mean ± SD	50 ± 11.7	42 ± 10.4	0.09
Affected breast, left – n (%)	9 (56)	6 (55)	0.93
Volume of collected discharge (µL), mean ± SD	17.2 ± 7.7	15 ± 4.5	0.41
Color of discharge – n (%)			
Colorless	5 (31.3)	3 (27.3)	
White	5 (31.3)	2 (18.2)	
Yellow	3 (18.7)	1 (9.1)	
Orange/pink/bloody/green/brown	1 (6.3)	3 (27.3)	
Beige	2 (12.5)	2 (18.2)	0.30
Viscosity – n (%)			
Watery	13 (81.3)	10 (90.9)	
Viscous	3 (18.7)	1 (9.1)	0.49
Cloudiness – n (%)			
Clear	11 (68.7)	9 (81.8)	
Cloudy	5 (31.3)	2 (18.2)	0.45

Table 2 Differential miRNA analysis in patients with pathological nipple discharge (PND), subclassified into patients with intraductal papilloma and patients with no breast abnormalities

miRNA	Intraductal papilloma (n=16)	No abnormalities (n=11)	p-value
miR-125a-5p			
Analyzed (%)	15 (94)	11 (100)	
Mean expression levels (DCT)	-0.446	-1.667	0.264*
miR-145-5p			
Analyzed (%)	16 (100)	7 (64)	
Mean expression levels (DCT)	-3.222	-0.779	0.050
miR-148a-3p			
Analyzed (%)	16 (100)	11 (100)	
Mean expression levels (DCT)	-1.188	-2.077	0.276
miR-151a-5p			
Analyzed (%)	12 (75)	7 (64)	
Mean expression levels (DCT)	-1.266	-1.3421	0.946
miR-153-3p			
Analyzed (%)	16 (100)	11 (100)	
Mean expression levels (DCT)	2.512	2.582	0.941

Table 2 Differential miRNA analysis in patients with pathological nipple discharge (PND), subclassified into patients with intraductal papilloma and patients with no breast abnormalities *(continued)*

miRNA	Intraductal papilloma (n=16)	No abnormalities (n=11)	p-value
miR-155-5p			
Analyzed (%)	15 (94)	9 (82)	
Mean expression levels (DCT)	1.725	-0.307	0.068
miR-16-5p			
Analyzed (%)	16 (100)	11 (100)	
Mean expression levels (DCT)	-4.354	-3.906	0.754
miR-181a-5p			
Analyzed (%)	16 (100)	11 (100)	
Mean expression levels (DCT)	-1.642	-1.740	0.805*
miR-18a-5p			
Analyzed (%)	15 (94)	9 (82)	
Mean expression levels (DCT)	0.293	0.135	0.900
miR-19a-3p			
Analyzed (%)	16 (100)	10 (91)	
Mean expression levels (DCT)	-2.648	-2.559	0.874*
miR-205-5p			
Analyzed (%)	16 (100)	11 (100)	
Mean expression levels (DCT)	-5.780	-4.993	0.193
miR-21-5p			
Analyzed (%)	16 (100)	11 (100)	
Mean expression levels (DCT)	-5.574	-6.673	0.204
miR-221-3p			
Analyzed (%)	16 (100)	10 (91)	
Mean expression levels (DCT)	-3.549	-2.865	0.560
miR-222-3p			
Analyzed (%)	16 (100)	10 (91)	
Mean expression levels (DCT)	0.168	0.270	0.673*
miR-29c-5p			
Analyzed (%)	14 (88)	8 (73)	
Mean expression levels (DCT)	4.749	3.042	0.181
miR-30b-5p			
Analyzed (%)	13 (81)	11 (100)	
Mean expression levels (DCT)	-3.163	-3.102	0.961
miR-320a			
Analyzed (%)	16 (100)	11 (100)	
Mean expression levels (DCT)	-3.3957	-3.608	0.402*

Table 2 Differential miRNA analysis in patients with pathological nipple discharge (PND), subclassified into patients with intraductal papilloma and patients with no breast abnormalities *(continued)*

miRNA	Intraductal papilloma (n=16)	No abnormalities (n=11)	p-value
miR-339-5p			
Analyzed (%)	14 (88)	9 (82)	
Mean expression levels (DCT)	1.472	-0.346	0.159
miR-374b-5p			
Analyzed (%)	16 (100)	11 (100)	
Mean expression levels (DCT)	0.828	0.117	0.395
miR-425-5p			
Analyzed (%)	16 (100)	7 (64)	
Mean expression levels (DCT)	1.380	0.998	0.640*
miR-92a-3p			
Analyzed (%)	16 (100)	11 (100)	
Mean expression levels (DCT)	-3.864	-3.484	0.961*

DCT = Delta CT; *p-values based on Mann-Whitney U test.

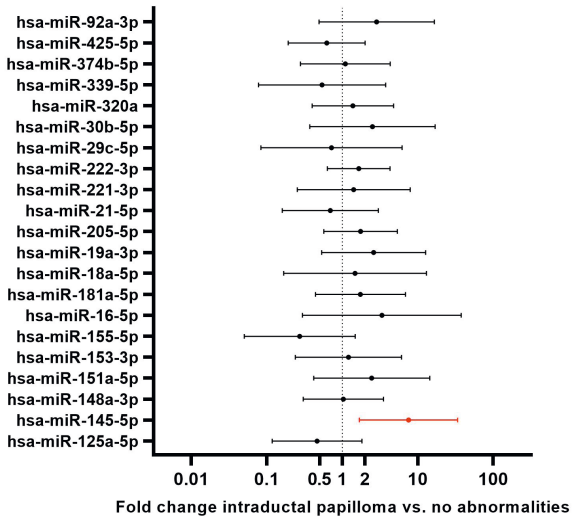

Figure 1. Univariate linear regression-based fold changes and 95% confidence-intervals of all analyzed miRNAs nipple discharge in intraductal papillomas compared to no breast abnormalities. MiRNAs with p-values <0.05 were considered of interest for subsequent multivariate analysis. miRNA 145-5p (p=0.012) was upregulated in nipple discharge of patients with intraductal papillomas, with a fold change of 7.56 (CI 1.69 – 33.90).

DISCUSSION

To identify potentially relevant miRNAs that can discriminate intraductal papillomas from normal breast tissue, expression levels of 22 human mature miRNAs were evaluated in nipple fluid samples collected from PND patients with and without an intraductal papilloma. This study demonstrated that elevated miR-145-5p levels in nipple fluid predicted the presence of an intraductal papilloma. This suggests that miR-145-5p could help distinguish intraductal papillomas from normal breast tissue in patients without clinical and radiological abnormalities suffering from PND.

In breast cancer, a variety of miRNAs are known to be down- and upregulated and therefore have potential as new biological therapeutic agents, targets, or biomarkers for patient-tailored breast cancer treatment [31], [32]. However, there is no data about miRNA expression differences in breast-specific liquid biopsies such as NAF between patients with intraductal papillomas and patients with no histological breast abnormalities. This study showed an upregulated expression of miR-145-5p in patients with intraductal papillomas compared to patients with no abnormalities. miR-145-5p is encoded by the MIR145 gene which is located on Chromosome 5 [33]. This miRNA is mainly considered as a tumor suppressor miRNA in diverse types of cancers and reported downregulated in breast cancer [34], bladder cancer [35], [36], cervical cancer, renal cancer and gastrointestinal cancers. However, a few studies reported upregulation of this miRNA in esophageal cancer [37], breast cancer [38], [39] and lung cancer [40], and one study reported no difference in expression between normal breast tissue and breast cancer tissue [41]. Moreover, miR-145-5p has been shown to affect the pathogenesis of a number of non-malignant conditions such as aplastic anemia (downregulation), asthma (upregulation), cerebral ischemia/reperfusion injury (upregulation), diabetic nephropathy, and rheumatoid arthritis (upregulation) [42]. Interestingly, according to one study, high expression levels of miR-145–5p were associated with clinical features in breast cancer such as early menarche, HER2 positivity and poorly differentiated tumors [39].

Previous research results on the relationship between miRNAs and the development/ progression of breast diseases have been inconsistent. The exact changes in miR-145-5p expression during the normal-benign-malignant sequence of breast cancer are currently unknown. This study suggests that miR-145-5p may impact the pathogenesis of benign breast disorders such as intraductal papilloma, where it is upregulated, differently than the pathogenesis of malignant breast disorders. While miR-145-5p is generally recognized as a tumor suppressor miRNA that is often downregulated in cancer, two studies have observed upregulated levels of miR-145-5p in the NAF of breast cancer patients [38], [39]. The authors related this to the differences (in ethnic backgrounds) of the studied populations, highlighting the need to validate the applicability of this miRNA marker in diverse groups.

A hypothesis that could explain the upregulation of miR-145-5p in PND samples from patients with intraductal papillomas of the breast is its association with the level of macrophage infiltration and polarization towards M2 and tumor-associated macrophages [43], [44]. Macrophages are components of the immune infiltrate within the tumor microenvironment, and they can produce diverse phenotypes in different microenvironments, including alternately activated (M2) macrophages [45]. M2-like cells facilitate tissue remodeling and anti-inflammatory processes, and are associated with tumor progression [46]. In the adult non-pregnant, non-lactating breast, fluid is secreted into the ducts, which may contain exfoliated ductal epithelial cells as well as foam cells, lymphocytes, and neutrophils. Foam cells, thought to be of macrophage lineage, are the most abundant cells found within ductal fluid [47], [48]. In the case of nipple discharge, foam cells are also prominent in most samples [49], [50]. This is because the presence of an intraductal papilloma can incite an inflammatory reaction within the duct, leading to bloody nipple discharge [51]. Therefore, the upregulation of miR-145-5p in PND samples from intraductal papillomas may be correlated with the level of macrophage infiltration, particularly when compared to PND samples from breast tissue without abnormalities. Nevertheless, further research is needed to validate this hypothesis.

In this study, within the out-patient clinic's patient population, 20% were excluded due to the absence of discharge at time of the procedure. However, for the majority of the patients (80%) it was possible to obtain nipple fluid at the outpatient clinic. Previous studies involving NAF collection utilized other non-invasive methods such as vacuum devices, manual palpation, or hand pumps, and reported successful sample collection ranging from 38 to 90% [52]–[55]. In our study population, nasal oxytocin administration was performed before nipple fluid collection. Since these patients suffered from spontaneous nipple discharge, no vacuum device was required for nipple fluid collection. However, considering that in 20% of our study population nipple fluid collection was not possible, incorporating a vacuum device in these cases may enhance the overall sample collection success rate. Although miRNA expression analysis may still not be suitable for all PND patients, it shows potential as a minimally invasive diagnostic tool in patients with intraductal papillomas with spontaneous discharge and may prevent unnecessary duct excision surgery in patients with benign disease.

Limitations

Major limitations of this study were the relatively small sample size and small volume of the analyzed nipple fluid [56]. Larger sample volumes would allow to obtain a greater number of circulating miRNA, useful for more efficient analysis. Furthermore, we selected 22 human mature miRNAs for evaluation selected from previous investigations identifying them as potential biomarkers for breast cancer. Therefore, other potentially relevant miRNAs may thus not have been discovered in this study. Future prospective research should strive a careful investigation of a broader range of candidate miRNAs using a non-targeted or multi-targeted approach, and to collect a larger number of patient samples categorized into three groups: breast tissue without abnormalities, intraductal papillomas, and breast cancer (divided into *in situ* and invasive cancer). This setup will help identify possible relevant miRNAs that might, in combination, tell apart benign from malignant breast diseases with high diagnostic accuracy. Ultimately, this would permit research to advance a step closer to risk prediction in patients with PND. Also, validation of the discriminative value of the miRNAs between benign and normal conditions should be analyzed. Furthermore, additional inflammatory and immune markers can be examined to correlate the miRNA findings.

Conclusions

In conclusion, the results of this study suggest that miR-145-5p levels in nipple fluid can differentiate between intraductal papilloma and breast tissue without abnormalities in PND patients. This shows the potential of miR-145-5p in nipple fluid analysis as a diagnostic tool in the work-up of patients suffering from PND. Adding nipple fluid analysis with miRNA expression profiling to the diagnostic work-up may help patients with no radiological suspicion of disease to a final diagnosis. However, further refinement and validation in clinical trials are necessary to establish its clinical applicability. In the future, microRNA expression profiling holds promise as a useful tool for optimizing the diagnosis and treatment of PND patients.

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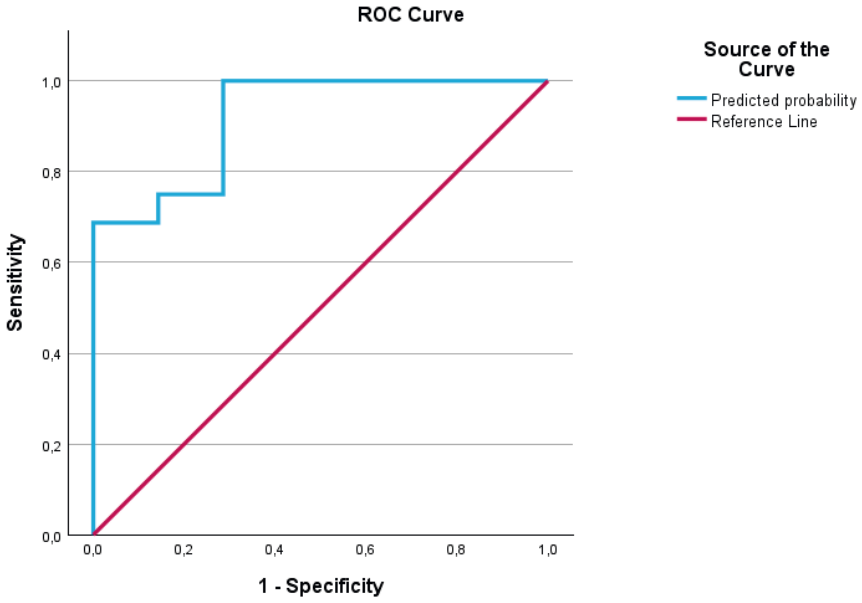
Supplementary Table S1: The coordinates of the ROC curve using the predicted probabilities

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
,0000000	1,000	1,000
,0133635	1,000	,857
,0330240	1,000	,714
,1018290	1,000	,571
,1794431	1,000	,429
,3284698	1,000	,286
,5064091	,938	,286
,6159065	,875	,286
,6968089	,813	,286
,7436575	,750	,286
,7952032	,750	,143
,8234184	,688	,143
,8305200	,688	,000
,9045949	,625	,000
,9792028	,563	,000
,9849318	,500	,000
,9859012	,438	,000
,9871177	,375	,000
,9908372	,313	,000
,9967484	,250	,000
,9996004	,188	,000
,9999713	,125	,000
,9999824	,063	,000
1,0000000	,000	,000

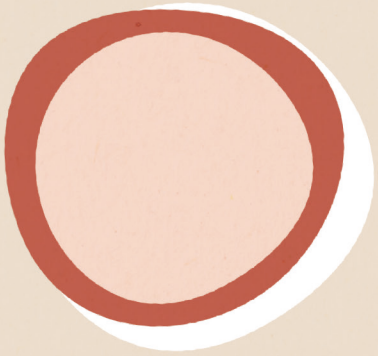
[a.] The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.



Supplementary Figure S1: Receiver operating characteristic (ROC) curve of microRNA miR-145-5p is shown demonstrating its accuracy rate of 0.920 (CI 0.801 – 1.000, p=0.002) in distinguishing benign from intraductal papillomas in pathological nipple discharge samples.

PART II

Optimizing breast-conserving surgery in the treatment of breast cancer



CHAPTER 10

Predictors of postoperative lumpectomy size in breast-conserving surgery in breast cancer patients: a retrospective cohort study

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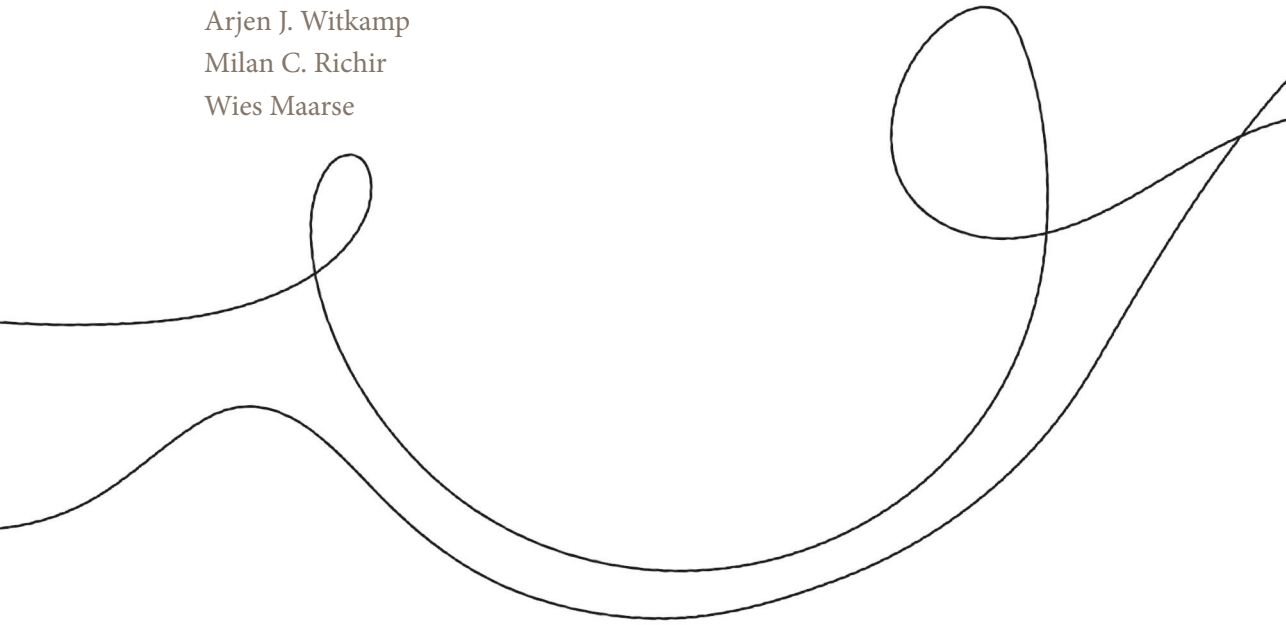

ABSTRACT

Background: Oncoplastic reconstructive surgery as an extension of breast-conserving surgery leads to better aesthetic results, an increase in tumor-free margins, and a reduction of re-excision rates. However, oncologic resection is often more extensive than expected, sometimes resulting in the plastic surgeon deviating from the predetermined plan. For optimal planning of the reconstruction, it is mandatory to estimate volume defects after lumpectomy as accurately as possible. This study aims to find preoperative predictors of lumpectomy resection size.

Methods: All consecutive patients diagnosed with invasive breast carcinoma or carcinoma *in situ* and treated primarily with breast-conserving surgery between 2018 and 2020 at the University Medical Center Utrecht and Alexander Monro Hospital were included. Variables measured were patient characteristics and tumor characteristics. Data were analysed in a multiple linear regression analysis.

Results: A total of 423 cases (410 patients) were included, with a median age of 58 (range 32-84) and a mean BMI of 25.0 (SD=9.3). The mean maximum radiological tumor diameter was 18.0 mm (SD=13.2), and the mean maximum lumpectomy diameter was 58.8 mm (SD=19.2). Multiple linear regression analysis found an explained variance of R2 = 0.60 (p = < .00), corrected for operating surgeon. Significant predictors for postoperative lumpectomy size were BMI, breast size, and maximum preoperative radiological tumor diameter. Moreover, a predictive tool for lumpectomy size was developed and a web-based application was created to facilitate the use of our tool in a clinical setting.

Conclusion: Postoperative lumpectomy size can be predicted with BMI, breast size and radiological tumor size. This model could be beneficial for (plastic) breast surgeons in planning reconstructions and to prepare and inform their patients more accurately.

INTRODUCTION

Every year 15,000 women are diagnosed with invasive breast cancer (BC) and around 2,300 with carcinoma *in situ* in The Netherlands [1], making it the most common cancer following skin cancer. In 2017, 60.1% of BC patients who underwent surgery had breast-conserving surgery (BCS) [2]. Several studies have already shown that BCS combined with adjuvant radiotherapy is an adequate treatment for early-stage BC with comparable oncological results to mastectomy and better quality of life [3-7].

The survival rate for BC has improved significantly over the last years [8]. The 5- and 10-year relative survival increased from respectively 76.8% and 55.9% in 1989-1999 to 91.0% and 82.9% in 2010-2016 [2]. Due to this improvement, the focus of treatment has expanded from solely survival to quality of life. In recent years, oncoplastic surgery (OPS), meaning BCS in combination with reconstruction, has improved both oncologic and reconstructive outcomes and has expanded indications for breast conservation [9]. Research shows it is a safe alternative to BCS, both for small and large tumors [10,11]. OPS has several advantages compared to BCS only and mastectomy. A more extensive resection can be performed without aesthetic limitations, the incidence of tumor-free margins has increased, and the need for re-excision has decreased. Therefore, this leads to a decrease in conversion to a mastectomy, in which the complete breast tissue is fully removed [12,13].

For plastic surgeons, it is more relevant to estimate the actual lumpectomy size than the tumor size, because for reconstruction purposes, the estimated volume defect is most important. In general, oncologic surgeons try to maintain 1 cm of macroscopic free margin around the tumor during BCS [14]. Nonetheless, research shows that the oncologic resection is often more extensive than expected, especially for smaller tumors [15]. As a result, the plastic surgeon sometimes must deviate from the predetermined plan. Reconstruction techniques or extra scarring may be necessary that have not been properly discussed with the patient preoperatively or for which additional examination is desirable (e.g., vascular examination for a pedicled flap). A better estimate of the size of the lumpectomy could help the plastic surgeon to make a more reliable reconstructive plan. No

prior research has been conducted comparing preoperative tumor size to the actual size of the excised lumpectomy. This study aims to identify which factors influence lumpectomy size, in addition to radiological tumor size, and provide a model to pre-operatively calculate the lumpectomy size.

METHODS

Study design

This retrospective cohort study was performed in the University Medical Center Utrecht (UMCU) and the Alexander Monro Hospital (AMH) in Bilthoven, a hospital specialized in breast (cancer) care in The Netherlands. These hospitals work closely together in breast cancer care. All female adult patients were included if clinically diagnosed with invasive breast carcinoma or carcinoma *in situ* and had primary BCS between 1 January 2018 and 31 December 2020. Cases were filtered from the electronic health records (EHR) by using the diagnosis treatment combination code [16]. Patients undergoing surgery for benign lesions or malignant and benign lesions combined in the same breast were excluded. Other exclusion criteria were previous oncological surgery to the same breast, previous breast augmentation, surgery resulting in two separate lumpectomies in one breast, surgery for Paget's disease, or removing the tumor 'en bloc' with the breast reduction tissue.

Patient characteristics

Patient-, tumor- and surgery characteristics were obtained from the EHRs. Height, weight, body mass index (BMI), smoking status, and breast size were reported. Smoking status was registered as 'no' if the patient had not smoked for at least ten years. Breast size was requested from the patient and reported by using European Union standards [17].

Preoperative diagnostics

A stereotactic vacuum-assisted breast biopsy was performed to examine suspect lesions. They were reported as invasive ductal-, ductolobular- or lobular carci-

noma (IDC/IDLC/LC), ductal/lobular carcinoma *in situ* (DCIS/LCIS), or others (e.g., papillary-, tubular- or mucinous carcinoma).

All patients received radiological breast examinations consisting of magnetic resonance imaging (MRI), ultrasound, mammography, and/or tomography. All studies were reviewed and reported by specialized radiologists. The maximum diameter of the area to be excised was recorded in the database for each radiological modality if present. The modality with the largest measurement was used in the analysis. In case of neoadjuvant therapy, the measurements were determined before and after therapy.

Primary tumor, lymph node, and metastasis (TNM) classification was determined and recorded during multidisciplinary consultation in the presence of an oncologic surgeon, plastic surgeon, oncologist, radiologist, and pathologist, following the American Joint Committee on Cancer Eighth Edition Cancer Staging Manual [18].

Surgery

BCS was performed by an oncologic surgeon, and in some cases, in combination with a plastic surgeon. Surgery characteristics were obtained from the surgery reports. Perioperative tumor localization was performed by palpation, wire localization, ultrasonography, and/or radio-guided occult lesion localization with iodine-125 seeds. The used reconstructive technique was reported and divided into four categories: 1= primary closure (including local transposition), 2= volume re-arrangement and skin correction (for example round block, batwing, B-plasty), 3= volume/breast reduction, and 4= volume replacement. Additional surgery, due to an incomplete resection, was also reported.

Pathology

All removed tissue were evaluated in the UMCU by specialized pathologists. Reports were reviewed for the maximum diameter of the excised lump, maximum diameter of the tumor, radicality of the resection, and the presence of a shave. Resection was considered incomplete if the margins were more than focally positive.

Statistical analysis

Data was analyzed using R statistical computing, version 4.0.4 (R Core Team, 2017, Vienna, Austria). Continuous data were described using mean with standard deviation for normally distributed data and the χ^2 test was used for categorical values to assess differences between groups.

Analysis of variance and Pearson correlations were used to determine whether interaction effects between predictor variables existed. No strong interactions between variables were found (Pearson correlation higher than 0.15). Therefore, no interaction terms were added to the multiple regression analysis. The outcome was corrected for the surgeon and the hospital in the regression analysis. Missing data was handled using imputation with Multiple Imputation by Chained Equations (MICE) [19]. P-values below 0.05 were considered to be statistically significant.

RESULTS

A total of 529 cases (508 patients) were identified from the EHRs of the AMH and UMCU. We excluded 106 cases (20.0%) for several reasons (Figure 1). Ten patients (1.9%) objected to participating in pseudonymized research. Forty patients (7.6%) were excluded because of surgery for benign lesions or malignant and benign lesions combined, and two patients (0.4%) were due to surgery for Paget's disease. Twenty-four patients (4.5%) had already had previous oncological breast surgery, and nine patients (1.7%) had received breast augmentation. Fourteen patients (2.6%) had two separate lumpectomies in one breast, and in seven cases (1.3%), the tumor was removed 'en bloc' with the reduction tissue.

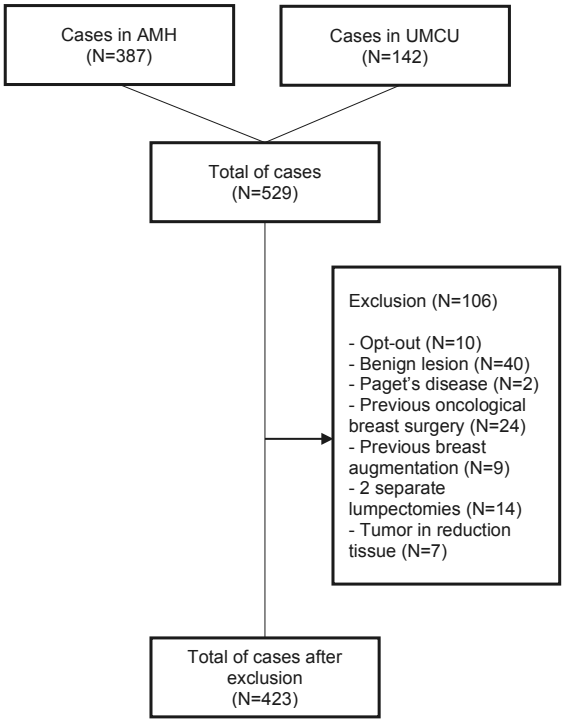

Figure 1: Flowchart of the study
Abbreviations: AMH, Alexander Monro Hospital; UMCU, University Medical Center Utrecht

In total, 423 cases were eligible for analysis. Patient characteristics (Table 1) showed that most cases were treated in the AMH (76.1%). OPS was more common in the AMH than in the UMCU (81.1% versus 13.9% of operations per hospital). The mean BMI was 25.0 (SD=9.3), the median age during surgery was 58 (range 32-84), and 13.5% smoked. Common breast sizes were C (25.1%), B (24.1%) and D (23.9%). Tumor and surgery characteristics (Table 2) showed that most tumors were in the upper outer quadrant (43.7%) and were diagnosed as IDC (53.7%). Most were categorized as cT1c (>10mm, ≤20mm) (35.9%) and cT2 (>20, ≤50mm) (27.9%). The majority of cases did not have lymph node metastases (cN0: 91.3%). The mean radiological maximum diameter of the area to be excised was 18.0 mm (SD=13.2). A quarter of all cases (25.8%) received neoadjuvant therapy before surgery. Primary closure was the most common reconstruction technique (56.0%), followed by volume reduction (24.6%). The mean maximum lumpectomy diameter was 58.8 mm (SD=19.2), with no significant difference between the groups

with and without a plastic surgeon involved (two-sample t-test p = .559). Results showed no significant difference in incomplete resections if a plastic surgeon was involved (12.8% versus 6.9%, χ^2 test: p = .065).

Table 3 shows the results of the multiple linear regression analysis for predictors for postoperative lumpectomy size. After correction for different surgeons, significant predictors for maximum postoperative lumpectomy size were BMI, age, breast size, maximum preoperative radiological diameter and involvement of plastic surgeon (table 3, model 1).

After performing the analysis without 'plastic surgeon', age became an insignificant predictor and was removed from the analysis. BMI (β = 1.127, p < .001) and preoperative tumor size (β = 0.597, p < .001) were positively correlated with postoperative lumpectomy size. Breast size showed a variable significance in positive correlation, depending on the actual size (Table 3). By using the significant beta coefficients, we could make a prediction model in which the relationship between the observed and predicted lumpectomy size were stated (supplemental figure 1 and 2) of the following formula:

To be excised lump diameter (mm) = 32.710 + BMI*1.127 + breast size (#) + radiological tumor size (mm) *0.597

#= breast size: A=1, B=2, C=3, D=4, E=5, F=6, G=7, H=8, I=9

For example, a patient with breast cancer with the following clinical features:

BMI: 25
Breast size: C
Radiological tumor size: 17 mm

To be excised lump diameter= 32.710 + (25*1.127) + 3 + (17*0.597) = 74 mm

The underlying statistical formula was also implemented in a web-based calculator, accessible at www.evidencio.com under the title '' Predicted lumpectomy size".

Table 1: Patient characteristics

Characteristic	Total (n=423)
Hospital, n (%)	
AMH	**322 (76.1)**
Plastic surgeon involved	*261 (81.1)*
UMCU	101 (23.9)
Plastic surgeon involved	*14 (13.9)*
Age (years), median (range)	58 (32-84)
Height (m), mean ± SD	1.69 ± 6.6
Weight (kg), mean ± SD	71.4 ± 11.9
BMI (kg/m^2), mean ± SD	25.0 ± 4.0
Smoking, n (%)	57 (13.5)
Breast size, n (%)	
A	21 (5.0)
B	102 (24.1)
C	106 (25.1)
D	101 (23.9)
E	50 (11.8)
F	20 (4.7)
G	8 (1.9)
H	3 (0.7)
I	2 (0.5)
Unknown	10 (2.4)

Table 2: Tumor and surgical characteristics

Characteristic	Total (n=423)	Plastic surgeon involved		
		No (n=148)	Yes (n=275)	p-value
Tumor left side, n (%)	215 (50.8)	82 (55.4)	133 (48.4)	0.167
Tumor localization, n (%)				0.122
Upper inner quadrant	67 (15.8)	17 (11.5)	50 (18.2)	
Lower inner quadrant	44 (10.4)	17 (11.5)	27 (9.8)	
Upper outer quadrant	185 (43.7)	71 (48.0)	114 (41.5)	
Lower outer quadrant	72 (17.0)	22 (14.9)	50 (18.2)	
Central	13 (3.1)	3 (2.0)	10 (3.6)	
Overlapping	42 (9.9)	18 (12.2)	24 (8.7)	
Histological finding, n (%)				0.347
IDC	227 (53.7)	74 (50.0)	153 (55.6)	
IDLC	68 (16.1)	25 (16.9)	43 (15.6)	

Table 2: Tumor and surgical characteristics *(continued)*

Characteristic	Total (n=423)	Plastic surgeon involved		
		No (n=148)	Yes (n=275)	p-value
ILC	34 (8.0)	10 (6.8)	24 (8.7)	
DCIS	68 (16.1)	28 (19.9)	40 (14.5)	
LCIS	5 (1.2)	4 (2.7)	1 (0.4)	
Other	21 (5.0)	7 (4.7)	14 (5.1)	
cT category†, n (%)				0.001
is	74 (17.5)	32 (21.6)	42 (15.3)	
1a	9 (2.1)	6 (4.1)	3 (1.1)	
1b	63 (14.9)	32 (21.6)	31 (11.3)	
1c	152 (35.9)	49 (33.1)	103 (37.5)	
2	118 (27.9)	27 (18.2)	91 (33.1)	
3	6 (1.4)	2 (1.4)	4 (1.5)	
4	1 (0.2)	0 (0.0)	1 (0.4)	
cN category†, n (%)				0.864
0	386 (91.3)	137 (92.6)	249 (90.5)	
1	29 (6.9)	9 (6.1)	20 (7.3)	
2	3 (0.7)	1 (0.7)	2 (0.7)	
3	5 (1.2)	1 (0.7)	4 (1.5)	
Neoadjuvant therapy, n (%)	109 (25.8)	32 (21.6)	77 (28.0)	0.153
Max. radiological diameter of disease‡ (mm), mean ± SD	18.0 ± 13.2	13.1 ± 8.9	20.7 ± 14.4	0.001
Localization technique†, n (%)				0.001
Palpation	64 (15.1)	12 (8.1)	52 (18.9)	
Wire	258 (61.0)	51 (34.5)	207 (75.3)	
Ultrasound	4 (0.9)	0 (0.0)	4 (1.5)	
Iodine-125	98 (23.2)	85 (57.4)	13 (4.7)	
Category of reconstruction technique§, n (%)				0.001
1	237 (56.0)	147 (99.3)	90 (32.7)	
2	68 (16.1)	1 (0.7)	67 (24.4)	
3	104 (24.6)	0 (0.0)	104 (37.8)	
4	14 (3.3)	0 (0.0)	14 (5.1)	
Shave, n (%)	84 (19.9)	25 (16.9)	59 (21.5)	0.262
Max. pathological lumpectomy diameter (mm), mean ± SD	58.8 ± 19.2	59.6 ± 19.7	58.4 ± 18.8	0.559
Incomplete resection, n (%)	38 (9.0%)	19 (12.8%)	19 (6.9%)	0.065

Table 2: Tumor and surgical characteristics *(continued)*

Characteristic	Total (n=423)	Plastic surgeon involved		
		No (n=148)	Yes (n=275)	p-value
Additional surgery, n (%)	39 (9.2%)	19 (12.8%)	20 (7.3%)	0.077

Abbreviations: IDC/IDLC/ILC, invasive ductal/ductolobular/lobular carcinoma; DCIS/LCIS, ductal/lobular carcinoma *in situ*; cT, clinical primary tumor size; cN, clinical lymph node status.

† TNM classification following the American Joint Committee on Cancer Eighth Edition Cancer Staging Manual [18].

‡ Maximum diameter of total area to be excised, including all foci of pathologically proven invasive tumor and carcinoma *in situ* and non-mass enhancement.

§ 1: primary closure, 2: volume re-arrangement, 3: volume reduction, 4: volume replacement.

Table 3 Multiple linear regression analysis, predictors for postoperative lumpectomy size

	Model 1 (R^2 = 0.616)			Model 2 (R^2 = 0.598)		
Predictors	**β coefficient**	**95% CI**	**p-value**	**β coefficient**	**95% CI**	**p-value**
(Intercept)	**31.868**	**18.034, 45.703**	**< .001**	**32.710**	**20.394, 45.026**	**< .001**
BMI	1.133	0.819, 1.446	< .001	1.127	0.808, 1.446	< .001
Age	0.140	0.013, 0.268	.031	-	-	-
Breast size						
B	1.560	-3.499, 6.619	.545	1.852	-3.316, 7.020	.482
C	4.774	-0.278, 9.826	.064	4.962	-0.187, 10.110	.059
D	9.736	4.534, 14.938	< .001	9.733	4.418, 15.047	< .001
E	11.423	5.643, 17.204	< .001	12.322	6.423, 18.221	< .001
F	13.007	6.031, 19.983	< .001	12.916	5.792, 20.039	< .001
G	16.760	7.313, 26.206	< .001	15.969	6.327, 25.610	.001
H	34.983	20.234, 49.732	< .001	34.710	19.632, 49.788	< .001
I	27.611	10.118, 45.105	.002	31.376	13.562, 49.190	< .001
Max. rad. diameter*	0.540	0.446, 0.634	< .001	0.597	0.504, 0.690	< .001
No plastic surgeon	-7.095	-10.361, -3.829	< .001	-	-	-

Abbreviations: 95% CI, 95% confidence interval

* Maximum diameter of total area to be excised on magnetic resonance imaging, ultrasound or mammography, including all foci of pathologically proven invasive tumor and carcinoma *in situ* and non-mass enhancement.

DISCUSSION

In this study, we found a significant correlation between postoperative lumpectomy size and the variables BMI, breast size, and preoperative radiological tumor size, corrected for the operating surgeon. Using multiple linear regression analysis, we constructed a formula that can be useful for surgeons during clinical practice. This study shows that postoperative lumpectomy size is often larger than just adding 1 centimeter margin to the radiological tumor size, a common practice to estimate lumpectomy size.

Although our study shows a significantly larger mean radiological tumor diameter in the OPS group, no significant difference was found in mean postoperative lumpectomy size. It seems that tumors in the OPS group were removed with smaller margins. However, this is a biased result due to differences in the incidence of OPS between hospitals. Per hospital individually, both preoperative tumor size and postoperative lumpectomy size were larger in the OPS group. There were more incomplete resections in the BCS group compared to OPS. However, this difference was just not significant. Previous research stated that incomplete resections were less common in OPS [9,12,13].

No previous research on predictors of postoperative lumpectomy size has been published. A partially comparable study investigated the relationship between tumor and lumpectomy volume [15]. The outcome is consistent with our finding that the lumpectomy is often larger than the tumor plus 1 cm margin. However, they did not examine other predictors of lumpectomy size or volume. Besides, they assumed that the tumor and the lumpectomy had the shape of a perfect sphere and ellipsoid, respectively, to calculate volumes. This may not be a reliable estimation because research shows that only 19% of all breast tumors are spherical [21].

Another retrospective cohort study did not find a significant correlation between intraoperative localization technique and lumpectomy size either. They also used an estimated lumpectomy volume as an outcome [22]. A multicenter, randomized controlled trial found that ultrasound-guided surgery gave smaller excision vol-

umes than palpation-guided surgery without an increase of incomplete resections [23]. They measured the volume directly after excision in the operating theatre by using the fluid displacement technique.

A strength of this study is the extensive database. Many patients were included, and there was a minimum number of missing data in the variables used for statistical analysis, which could be completed with MICE. Besides, this is the first study on predictors of postoperative lumpectomy size. Nonetheless, there are several limitations. Due to the retrospective design, no additional tests or measurements could be done to complete the database. It was not possible to analyze the influence of breast ptosis or underbust circumference. There were differences in used radiological modality and intraoperative tumor localization. Therefore, it was not possible to investigate the influence of these factors on the outcomes. Another limitation is the use of breast cup size as volume estimation. Although many patients and plastic surgeons use "bra cup", there is no uniform sizing standard. It is an arbitrary scale with interobserver variability and differences between countries and bra brands [24]. In the absence of more reliable measurements and the retrospective design of this study, we used this way to estimate breast volume. In future studies, more reliable techniques such as volumetry CT or MRI, water displacement or 3D photography could be used.

To validate the accuracy of the lumpectomy size tool in estimating lumpectomy size before surgery, we are currently in the preparation phase of a prospective validation study. Given the prospective nature of the study, physicians can precisely assess and record ptosis, underbust circumference, cup size/volume measurement, weight, and height to collect current and reliable data. Furthermore, it will be interesting to investigate the correlation between lumpectomy size, radiological modality, and intraoperative localization methods.

Conclusion

In conclusion, our study showed that postoperative lumpectomy size can be predicted in a simple model with BMI, breast size, and radiological tumor size. This could help plastic surgeons during clinical practice to make reconstruction plans and to inform their patients better.

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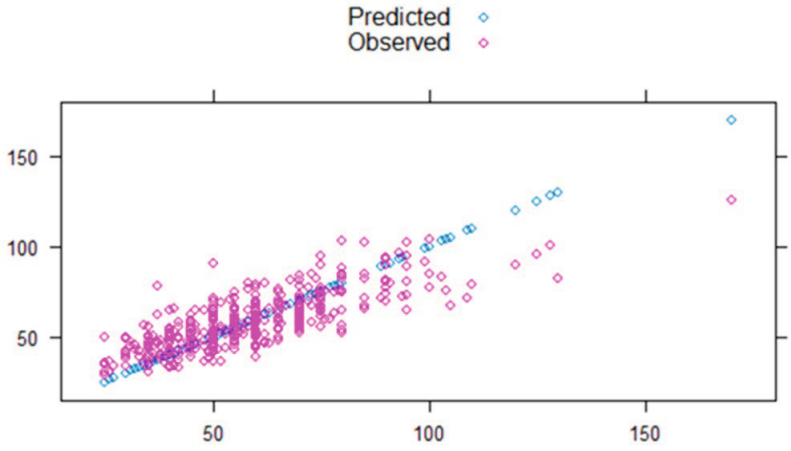
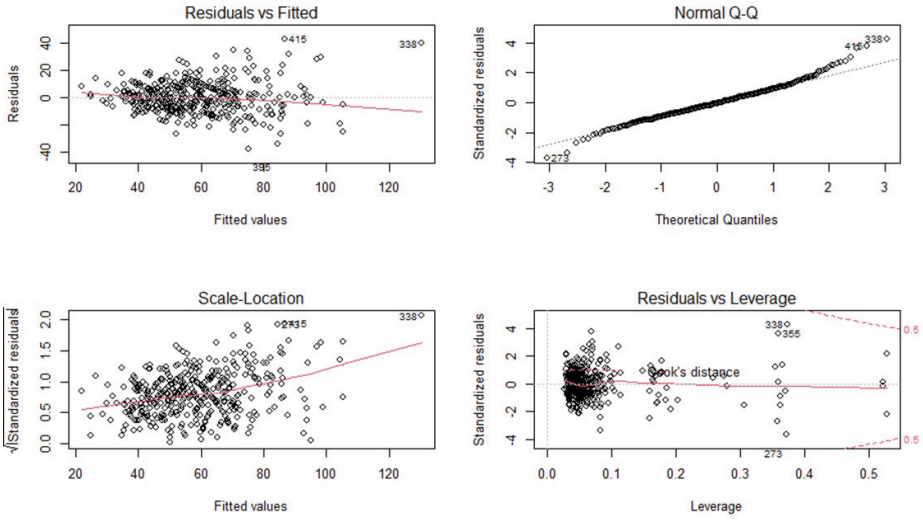
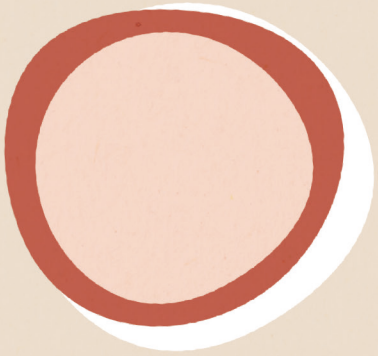


Supplemental Figure 1: Visualization of model 1: the relationship between the observed and predicted lumpectomy size. X/y-axis: postoperative lumpectomy size. Observed (blue) versus predicted (pink)



Supplemental Figure 2: Goodness of fit analyses of the predicted vs. observed lumpectomy size models.



CHAPTER 11

Clinicopathological factors affecting positive margins after breast-conserving surgery

Breast Care, under review

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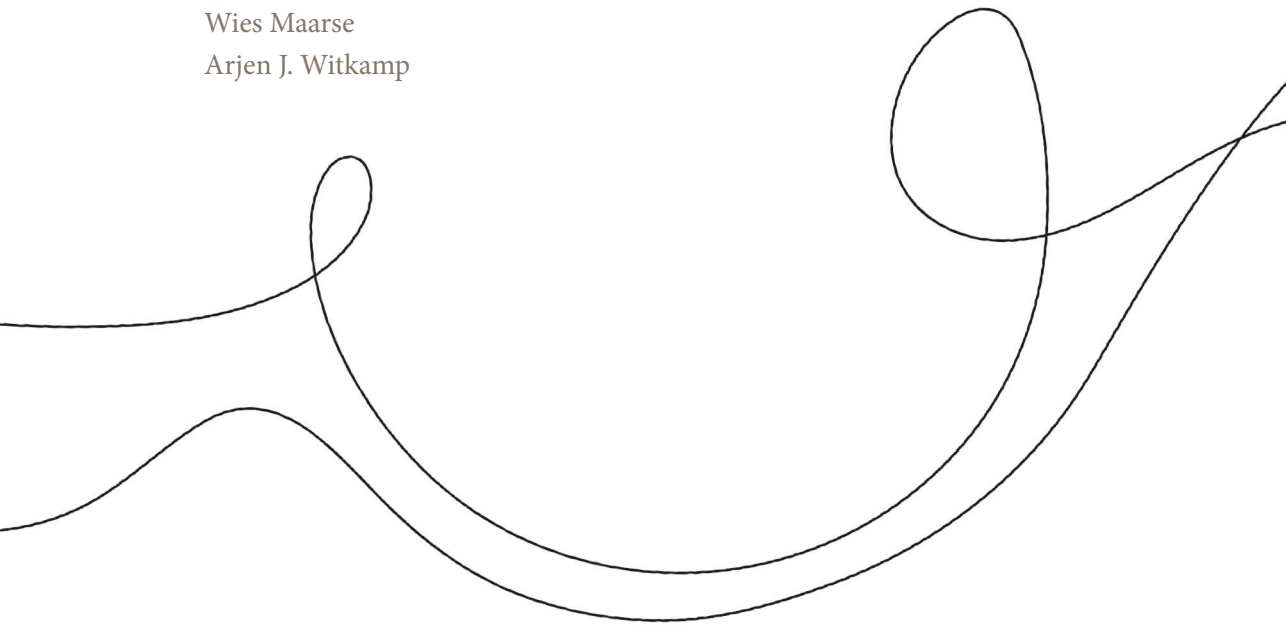

ABSTRACT

Purpose: Positive margins after breast-conserving surgery (BCS) are an important risk factor for local tumor recurrence and the need for re-excision in women with breast cancer. It remains unclear which factors collectively influence positive margins after BCS and whether the outcomes vary among hospitals. This study investigated the occurrence and risk factors of positive margins after BCS in women with breast cancer in two Dutch hospitals.

Methods: Data was collected from medical records of women who were diagnosed with newly invasive breast carcinoma or carcinoma *in situ* and underwent primary BCS between January 1, 2018 and December 31, 2020 in two Dutch hospitals.

Results: A total of 423 cases (410 patients) were included, with a median age of 58 years (IQR 51-66). On average the positive margin rate after BCS was 8.0%, which was significantly higher in the low-volume hospital (14.9%) than in the high-volume hospital (5.9%). Invasive lobular carcinoma (OR= 4.97, CI= 1.91 – 12.94), postoperative tumor size (OR= 1.07, CI= 1.03 – 1.11), low hospital volume (OR= 3.90, CI= 1.58 – 9.66), and lumpectomy size (OR= 0.97, CI= 0.94 – 1.00) were significantly associated with positive margins after BCS.

Conclusion: Positive margin rate after BCS was low with 8.0% among all cases and varied significantly between the hospitals. Patient-, tumor-, radiological- and surgery-related factors may contribute to these variations. An optimized collaborative multidisciplinary approach to breast cancer care, particularly between radiologists and surgeons, should be strived to achieve improved oncological outcomes in women after BCS.

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer and leading cause of cancer-related mortality amongst women worldwide [1]. Breast-conserving surgery (BCS), also known as lumpectomy or partial mastectomy, is a standard treatment option for early-stage breast cancer and locally advanced tumors after neoadjuvant chemotherapy [2], [3]. While BCS is associated with good cosmetic outcomes and preserved breast function, it is not without challenges [4], [5].

The presence of positive margins after surgery, is an important risk factor for tumor recurrence, leading to the need for additional surgery or radiotherapy [6], [7]. Approximately one-fourth of patients who undergo initial BCS for breast cancer additionally undergo a subsequent surgical intervention [8]. Therefore, minimizing the occurrence of positive margins is an important goal in BCS, as it reduces the risk of cancer recurrence and the need for additional treatment. Several risk factors for positive margins after BCS have been identified in previous studies, including size and location of the tumor, type of cancer, patient factors (i.e. age and comorbidities), and the experience of the surgeon [9]–[12].

Breast cancer treatment follows a multidisciplinary approach that aims to deliver personalized and effective care to patients, involving coordinated care among different specialties [13]. Decisions made by multidisciplinary teams have been shown to improve clinical decisions, evidence-based practice, and better treatment outcomes [13], [14]. In The Netherlands, breast cancer is treated in hospitals by multidisciplinary teams in accordance with the established breast cancer guidelines [15]. However, the composition and level of experience of the multidisciplinary teams may vary across hospitals and there may be (minor) differences in the work-up process and hospital volume. It is unclear whether those differences between hospitals are associated with positive margin rate after BCS.

To address this, we compared data from BCS in women with invasive or *in situ* breast cancer from two hospitals in search of factors related to positive margin status.

METHODS

Study design and population

This study included all consecutive female adult patients clinically diagnosed with invasive breast carcinoma or carcinoma *in situ* who underwent BCS between January 1, 2018 and December 31, 2020 in two Dutch hospitals, an academic hospital (University Medical Center Utrecht) and a breast cancer clinic (Alexander Monro Hospital Bilthoven). The study population was a subset of a previously conducted cohort study [16]. Exclusion criteria were: patients undergoing surgery for presumed malignant lesion in whom final pathology was benign, malignant and benign lesions combined in the same breast, previous oncological surgery to the same breast, previous breast augmentation, surgery for Paget's disease, and surgery requiring two separate lumpectomies in one breast. The study was approved by the hospital's institutional review board (nr 21/327).

Data collection

Standard clinical variables were retrospectively collected from the electronic medical health records by two data collectors, including age, weight, height, smoking status, physical exam findings, cup size, tumor location, pathological TNM staging, findings from any imaging studies performed, resection margin status, and histopathological details including tumor type.

Clinical work-up

Before surgery, a standard diagnostic evaluation was performed in all patients, including medical history, physical examination, and recent radiological imaging (mammography, ultrasonography, and/or MRI and/or core needle biopsy). All radiological images were reviewed and reported by specialized radiologists as part of routine clinical care. The maximum diameter of the tumor in the breast was reported for each radiological modality, and the largest measurement was used in the analysis. All cases were discussed during a multidisciplinary meeting. In the case of neoadjuvant therapy, sizes were determined before and after therapy. Surgery was performed by an oncologic surgeon regularly in collaboration with a plastic surgeon. Perioperative tumor localization was performed by palpation, wire localization, and/or radio-guided with iodine-125 seeds. Additional surgery

due to incomplete resection was also reported. All removed tissues were processed according to standard procedures at grossing, applying ink to the resection margins, and evaluated by specialized breast pathologists. Reports were reviewed for the maximum diameter of the excised lump, maximum diameter of the tumor, tumor type (ductal versus lobular) and the margin status.

The radiological estimation was calculated as the difference between the maximal pre-operative radiological diameter of the tumor and the post-operative maximum pathological diameter of the tumor. Radiological underestimation was reported for analysis.

Definition margin status

The closest distance between the inked lumpectomy edge and any cancerous tissue was analyzed. Resection was considered incomplete (positive margin) if the margins were more than focally positive (tumor touching the inked margin over a length of more than 2 mm or multiple focally positive foci). In most of these cases, re-excision surgery was performed. However, for focally positive margins, defined as tumor touching the inked margin over a length of 2 mm or less in one focus, in most cases adjuvant radiotherapy was administered, but in some cases secondary surgery was performed. Suspicion of multifocality was defined as the presence of two or more tumor foci within the same quadrant of the ipsilateral breast.

Previous studies

A search in EMBASE was conducted using search terms related to 'breast conserving surgery', 'lumpectomy', 'positive margins', and 'incomplete resection' to identify studies about the proportions of positive margins after BCS and factors associated with non-radical resections. Reference lists from these articles were also examined to determine publications. This resulted in an overview of data from 14 studies about factors associated with positive margins in patients with invasive breast cancer or carcinoma *in situ* to compare to our results.

Statistical analysis

Prevalence and means with standard deviation (SD) or medians with interquartile range (IQR) were calculated to describe the study population. Chi-square (χ^2) test

was used to compare categorical variables between women with positive and clear resection margins after BCS. Multivariable logistic regression analysis estimating odds ratios (OR) and 95% confidence intervals (CI) were performed to identify factors associated with positive margins after BCS. In this multivariable logistic regression model, all variables were adjusted for all other variables except for the one of interest. Variables with an estimated p-value of <0.05 in the univariable logistic regression, i.e. presence of invasive lobular carcinoma, postoperative pathological tumor size and hospital type, were included in the multivariable logistic regression model. Also, variables that have been identified in the literature study as potential risk factors, i.e. age [9], [11], node positivity [12], [17], cup size [18], radiological underestimation [19], [20], and pathological lumpectomy size [21], [22], were included in the model. P-values <0.05 were considered to be statistically significant. SPSS v29.0 was used to analyze the data for this study.

RESULTS

Baseline characteristics

From 2018 to 2020, a total of 529 cases (508 patients) clinically diagnosed with invasive breast carcinoma and/or carcinoma *in situ* underwent primary BCS.

We excluded 106 cases (20%) for the following reasons: 40 cases (7.6%) due to surgery for benign lesions or malignant and benign lesions combined, two cases (0.4%) due to surgery for Paget's disease, 24 cases (4.5%) due to previous oncological breast surgery to the same breast, and nine cases (1.7%) had received breast augmentation. Fourteen patients (2.6%) had two separate lumpectomies in one breast, and in seven cases (1.3%), the tumor was removed 'en bloc' with the reduction tissue. Ten patients (1.9%) objected to participating in pseudonymized research. The remaining 423 cases (410 patients) were eligible for analysis. 13 patients were included with tumors in both breasts, which were considered as two separate cases eligible for analysis.

Tumor characteristics and radiological findings

The median age of the total study population was 58 years at the time of surgery (IQR 51 - 66 years) and a mean BMI of 25.0 kg/m^2 (SD 4). Of the 423 cases, 34 (8.0%) had positive margins and 389 cases (92.0%) had clear margins after BCS. Of the 34 cases with positive margins, 29 (85.3%) had invasive breast cancer and 5 (14.7%) DCIS.

Tumor and surgery characteristics (Table 1) showed that most tumors were in the upper outer quadrant (43.7%) and were diagnosed as infiltrating ductal cancer (IDC) (53.7%). Patients with positive margins were significantly more frequently diagnosed with invasive lobular carcinomas (ILC) (32.4%), compared to patients with clear margins (5.9%, p=0.00). Moreover, a significant difference in tumor classification between the groups (p=0.01) was observed, in which cT2 (41.2% vs. 26.7%) and cT3-4 (8.8% vs. 1.0%) tumors were more likely to present in the positive margin group. Additionally, cases with positive margins had significantly a higher median radiological underestimation of the tumor size compared to cases with clear margins (12 vs. 4 mm, p=0.00). No significant differences were found in the nodal status and neo-adjuvant therapy rates between both groups.

Surgical and pathological outcomes

The median postoperative pathological tumor size showed a significant difference between both groups: 25 mm in positive margins vs. 15 mm in clear margins. (p=0.00). However, no significant difference was observed in the maximal pathological lumpectomy diameter (60 mm in positive margins vs. 55 mm in clear margins, p=0.55). Among the 34 patients with positive margins, 79.4% (27/34) underwent additional surgery, 5.9% (2/34) had clear margins after reduction surgery, 11.8% (4/34) received adjuvant radiotherapy and 2.9% (1/34) no additional therapy due to her underlying medical condition.

After surgery, histological analysis showed 40 patients (9.5%) with focally positive margins after BCS. These margins were considered as clear margins after adjuvant radiotherapy or surgery when indicated. No difference was observed in DCIS involvement in the resection between positive and clear margins.

Factors associated with non-radical resection

The multivariable logistic regression analysis showed that invasive lobular carcinoma (ILC) (OR= 4.97, p= 0.001) and larger postoperative tumor size (OR= 1.07, p= 0.001) were the strongest factors associated with positive margins, followed by low hospital volume (OR= 3.90, p= 0.003), and smaller lumpectomy size (OR= 0.97, p= 0.022) (Table 2). Age at time of surgery, cup size, node status, and radiological underestimation of the tumor size were not independently associated with positive margins.

Differences between hospitals

In the high-volume hospital (322 cases), surgical procedures were performed by 4 breast surgeons and 6 plastic surgeons. In the low-volume hospital (101 cases), surgical procedures were conducted by a team comprising 2 breast surgeons, 2 fellow-surgeons, surgeons in training, and 2 plastic surgeons.

Table 3 shows the differences of tumor and surgical characteristics between both hospitals. Patients in the low-volume hospital were significantly older (59 years, p=0.01), had more often positive margins after BCS (14.9% vs. 5.9%, p=0.00) and a larger maximal pathological lumpectomy diameter (70 mm vs. 53 mm, p=0.00). The postoperative maximal pathological tumor size was slightly higher in the high-volume hospital (16 mm vs. 18 mm, p=0.13), as was the median maximal radiological tumor diameter (16 vs. 12 mm, p=0.00). The high-volume hospital had a lower median of the radiological underestimation of the tumor size (4, vs. 7 mm, p=0.04) and had more often an MRI before surgery (75.2% vs. 62.4%, p=0.04). In the high-volume hospital, localization was performed by palpation or a wire and in the low-volume hospital by palpation and Iodine-125.

Table 1. Tumor and surgical characteristics of 423 cases with breast cancer with clear or positive resection margins after breast conserving surgery in two hospitals

Variables	Total cases (n=423)	Number of cases with positive margins (n=34, 8%)	Number of cases with clear margins (n=389, 92%)	p-value
Age at time of surgery (years), median (IQR)	58 (51 – 66)	60 (50 – 69)	58 (51 – 65)	0.21
BMI (kg/m²), mean ± SD	25 ± 4.0	25.2 ± 4.8	25 ± 3.9	0.74
Smoker, yes (%)	57 (13.5)	5 (14.7)	52 (13.4)	0.83
Cup size, n (%)				0.56
A - B	123 (29.1)	8 (23.5)	115 (29.6)	
C - D	207 (49)	20 (58.8)	187 (48.1)	
E - I	83 (19.6)	6 (17.6)	77 (19.8)	
Missing	10 (2.3)	0 (0.0)	10 (2.5)	
Tumor localization, n (%)				0.60
Upper inner quadrant	67 (15.8)	2 (5.9)	65 (16.7)	
Lower inner quadrant	44 (10.4)	5 (14.7)	39 (10.0)	
Upper outer quadrant	185 (43.7)	15 (44.1)	170 (43.7)	
Lower outer quadrant	72 (17.0)	6 (17.6)	66 (17.0)	
Central	44 (10.4)	5 (14.7)	39 (10.0)	
Overlapping	11 (2.6)	1 (2.9)	10 (2.6)	
Histological finding, n (%)				0.00
DCIS	69 (16.3)	5 (14.7)	64 (16.5)	
IDC	227 (53.7)	13 (38.2)	214 (55.0)	
IDLC	68 (15.7)	5 (14.7)	63 (16.2)	
ILC	34 (8.0)	11 (32.4)	23 (5.9)	
Other[1]	25 (5.9)	0 (0.0)	25 (6.4)	
cT category[#], n (%)				0.01
1a-c	224 (53)	12 (35.3)	212 (54.5)	
2	118 (27.9)	14 (41.2)	104 (26.7)	
3-4	7 (1.6)	3 (8.8)	4 (1.0)	
is	74 (17.5)	5 (14.7)	69 (17.7)	
cN status, n (%)				0.20
Positive	37 (8.7)	5 (14.7)	32 (8.2)	
Negative	386 (91.3)	29 (85.3)	357 (91.8)	
Neo-adjuvant therapy, n (%)	109 (25.8)	11 (32.4)	98 (25.2)	0.36
MRI before surgery, n (%)	305 (72.1)	28 (82.4)	277 (71.2)	0.32
Maximal radiological diameter of disease (mm), median (IQR)	15.5 (9-23)	19.5 (7-40)	15 (9-23)	0.01
Radiological underestimation of tumor size (mm), median (IQR)	5 (2-12)	12 (6-24)	4 (2-11)	0.00
Maximal pathological lumpectomy diameter (mm), median (IQR)	55 (45-70)	60 (49-73)	55 (45-70)	0.55

Table 1. Tumor and surgical characteristics of 423 cases with breast cancer with clear or positive resection margins after breast conserving surgery in two hospitals *(Continued)*

Variables	Total cases (n=423)	Number of cases with positive margins (n=34, 8%)	Number of cases with clear margins (n=389, 92%)	p-value
Postoperative maximal pathological tumor size (mm), median (IQR)	16 (9-25)	25 (18-45)	15 (9-24)	0.00
Presence of DCIS component in resection, n (%)	261 (61.7)	22 (64.7)	239 (61.4)	0.73
Hospital, n (%)				0.00
High-volume	322 (76.1)	19 (55.8)	303 (77.9)	
Low-volume	101 (23.9)	15 (44.2)	86 (22.1)	

Abbreviations: IQR, interquartile range; BMI, body mass index; SD, standard deviation; IDC, invasive ductal carcinoma; IDLC, invasive ductolobular carcinoma; ILC, invasive lobular carcinoma; DCIS, ductal carcinoma *in situ*; cT, clinical primary tumor size; cN, clinical lymph node status; MRI, magnetic resonance imaging

[1] = lobular carcinoma *in situ*, mucinous carcinoma, metaplastic carcinoma, papillary carcinoma and tubular carcinoma

[#] = TNM classification following the American Joint Committee on Cancer Eighth Edition Cancer Staging

Chi-square test was used to compare categorical covariates between cases with clear and positive resection margins after BCS.

Table 2. Multivariable logistic regression analysis results of factors associated with positive margins after breast conserving surgery in 423 cases with breast cancer or carcinoma *in situ*.

Variables	Adjusted OR	95% CI	p
Age at time of surgery (years)	1.01	0.97 – 1.05	0.685
Cup size			
A-B	0.85		
C-D	0.97	0.22 – 3.19	0.804
>E	1 (reference)	0.31 – 3.02	0.958
Node status			
Positive	1.13		
Negative	1 (reference)	0.27 – 4.77	0.865
Invasive lobular carcinoma			
Yes	4.97		
No	1 (reference)	1.91 – 12.94	0.001
Radiological underestimation (mm)	0.99	0.95 – 1.04	0.663
Lumpectomy size (mm)	0.97	0.94 – 1.00	0.022
Postoperative pathological tumor size (mm)	1.07	1.03 – 1.11	0.001
Hospital type			
Low-volume	3.90		
High-volume	1 (reference)	1.58 – 9.66	0.003

Abbreviations: OR = odds ratio, CI = confidence interval. In this multivariable logistic regression model, all variables were adjusted for all other variables except for the one of interest.

Table 3. Tumor and surgical characteristics for 423 cases with breast cancer or carcinoma *in situ* undergoing breast conserving surgery in two hospitals

Variables	Number of cases treated in high-volume hospital (n=322)	Number of cases treated in low-volume hospital (n=101)	p-value
Age at time of surgery (years), median (IQR)	57 (51-65)	59 (53-69)	0.01
BMI (kg/m²), mean ± SD	24.8 ± 3.6	25.7 ± 5.0	0.06
Smoker, yes n (%)	44 (13.7)	13 (12.9)	0.84
Cup size, n%			0.73
A - B	94 (29.2)	29 (28.7)	
C - D	159 (49.4)	48 (47.5)	
E – I	67 (20.8)	16 (15.8)	
Missing	2 (0.6)	8 (7.9)	
Tumor localization, n (%)			0.56
Upper inner quadrant	51 (15.8)	16 (15.8)	
Lower inner quadrant	32 (9.9)	12 (11.9)	
Upper outer quadrant	141 (43.8)	44 (43.6)	
Lower outer quadrant	58 (18.0)	14 (13.9)	
Central	34 (10.6)	10 (9.9)	
Overlapping	6 (1.9)	5 (5.0)	
Histological finding, n (%)			0.06
DCIS	48 (14.9)	21 (20.8)	
IDC	185 (57.5)	42 (41.6)	
IDLC	51 (15.8)	17 (16.8)	
ILC	22 (6.8)	12 (11.9)	
Other[1]	16 (4.3)	9 (6.2)	
cT category#, n (%)			0.01
1a-c	174 (54)	50 (49.5)	
2	94 (29.2)	24 (23.7)	
3-4	4 (1.2)	3 (3.0)	
is	50 (15.5)	24 (23.8)	
cN status, n (%)			0.62
Positive	31 (9.6)	6 (5.9)	
Negative	291 (90.4)	95 (94.1)	
Neo-adjuvant therapy, n (%)	89 (27.6)	20 (19.8)	0.12
MRI before surgery, n (%)	242 (75.2)	63 (62.4)	0.04
Maximal radiological diameter of disease (mm), median (IQR)	16 (10 – 24)	12 (0 – 57)	0.00
Radiological underestimation of tumor size (mm), median (IQR)	4 (2 – 11)	7 (3 – 15)	0.04
Maximal pathological lumpectomy diameter (mm), median IQR)	53 (45 – 65)	70 (53 – 85)	0.00

Table 3. Tumor and surgical characteristics for 423 cases with breast cancer or carcinoma *in situ* undergoing breast conserving surgery in two hospitals *(Continued)*

Variables	Number of cases treated in high-volume hospital (n=322)	Number of cases treated in low-volume hospital (n=101)	p-value
Postoperative maximal pathological tumor size (mm), median (IQR)	16 (9-24)	18 (10 – 31)	0.13
Focally positive margins, n (%)	33 (10.2)	7 (6.9)	0.32
Non-radical resection (positive margins), n (%)	19 (5.9)	15 (14.9)	0.00
Positive margins: DCIS, n (%)	4 (21.1)	1 (6.7)	
Positive margins: invasive tumor, n (%)	15 (78.9)	14 (93.3)	
Presence of DCIS component in resection, n (%)	208 (64.3)	53 (52.5)	0.02

Abbreviations: IQR, interquartile range; BMI, body mass index; SD, standard deviation; IDC, invasive ductal carcinoma; IDLC, invasive ductolobular carcinoma; ILC, invasive lobular carcinoma; DCIS, ductal carcinoma *in situ*; cT, clinical primary tumor size; cN, clinical lymph node status; MRI, magnetic resonance imaging

[1] = lobular carcinoma *in situ*, mucinous carcinoma, metaplastic carcinoma, papillary carcinoma and tubular carcinoma

[#] = TNM classification following the American Joint Committee on Cancer Eighth Edition Cancer Staging

[*] = Resection was considered incomplete if the margins were more than focally positive. Focally positive margins were considered as negative margins.

DISCUSSION

The present study shows that 8.0% of all breast cancer or carcinoma *in situ* cases treated with BCS had positive margins post-BCS. The multivariable adjusted logistic regression showed that the presence of invasive lobular carcinoma (ILC), a larger postoperative tumor, a smaller lumpectomy size and a low hospital volume were associated with a higher risk of a positive margin after BCS. There were significant differences in several (radiological and surgical) variables between both hospitals related to age at time of surgery, presence of ILC histological type, radiological underestimation of the tumor size, MRI rates, lumpectomy size, and positive margin rate.

The positive margin rates after BCS in this study, i.e. 5.9% and 14.9%, were lower than or similar to prior studies, which reported ranges between 7.3% and 52% [9]–[12], [17], [21], [23]–[29] (supplementary table 1). In our study, factors associated with a positive margin were both tumor-related and hospital-related.

Tumor-related factors, such as ILC histological type [10], [23], [24], [27] and larger postoperative tumor size [10], [12], [29] were consistent with prior studies. This study found that a larger lumpectomy size significantly reduced the risk of a positive margin after BSC. This association was verified by another cohort study and could be related to novel approaches in BCS to improve reconstructive outcomes [22]. BCS is associated with an average 2 to 4-fold removal of excessive tissue [30]. However, according to previous studies, with oncoplastic surgery (BCS in combination with reconstruction), more extensive resection can be performed without aesthetic limitations, the incidence of tumor-free margins increased, and the need for re-excision can be decreased [31], [32]. Therefore, it is crucial to thoroughly evaluate each case prior to surgery to make a resection plan aimed at achieving clear margins. According to this study, this plan should consider relevant factors such as tumor size and ILC type. Our study team has developed a tool to predict lumpectomy size with BMI, breast size, and radiological tumor size, however, further validation is needed [16].

Although many studies have looked at factors affecting positive margins after initial surgery, less is known about the influence of hospital characteristics on margin involvement in BCS. Previous studies have only shown that breast cancer surgery in specialized, teaching, and high-volume hospitals is associated with improved long-term survival [33], [34]. The results of this study indicate that patients treated in a high-volume specialized breast clinic less often had positive margins than in the low-volume center, likely due to several patient-related, tumor-related, radiological, surgeon- and hospital-related factors.

The age of patients at time of surgery was significantly different between both hospitals. In the low-volume hospital, more older patients with multimorbidity were treated. There is evidence that age [9], [11] and multimorbidity [12] may be related to higher non-radical resection rates. However, our multivariable logistic regression analysis did not show a significant association between age and positive margins. Tumor-related factors, such as clinical primary tumor size (cT), presence of ILC and presence of DCIS component in resection were also significantly different between the two hospitals. In the low-volume hospital, clinical primary tumor sizes were larger, there were more ILC, and less DCIS component in the resection

compared to the high-volume hospital. According to our multivariate analysis, the presence of ILC and larger tumor size lead to positive margins, which can at least partially explain the higher positive margin rate in the low-volume hospital.

Radiological factors were also found to play a role between both hospitals. The high-volume hospital performed significantly more often an MRI scan before surgery than the low-volume hospital. However, according to this study, the absence of MRI did not result in more positive margins. This is in line with a multicenter trial in which preoperative MRI was also not associated with positive margins [35]. In contrast with our findings, there are prior studies that have shown that the omitting MRI can lead to more positive margins after BCS [17], and that MRI is generally superior to ultrasound and mammography in tumor size measurement [36], [37]. However, MRI has the highest overestimation rate and might cause overtreatment by increasing the mastectomy rate [38]. Therefore, caution should be taken for the indication and interpretation of MRI influence. Moreover, the radiological diameter of the disease and underestimation rate were different between both hospitals.

The present study did not show an association between radiological underestimation and positive margins. However, in two other studies, the radiological underestimation of the tumor size showed an association with positive margins and re-excision rate after BCS [19], [20]. Radiological underestimation can lead to less accurate lumpectomy sizes performed by surgeons, resulting in more resections with positive margins. Furthermore, the difference in estimation of the radiological diameter of the disease can be related to the significant difference in performing an MRI for tumor size measurement between both hospitals. Nevertheless, the MRI utilization rate observed in this study (75.2% and 62.4% for the high- and low-volume hospitals, respectively) was far above the rate reported in a separate population-based cohort study involving 36,050 patients in The Netherlands (29.8%) [39]. Additionally, both hospitals performed different localization techniques: wire-marking in the high-volume hospital and Iodine-125 in the low-volume hospital. Literature shows that localization of non-palpable breast cancer using Iodine-125 seeds is, at least, as good as the standard wire localization procedure, while Iodine-125 is an easier and more comfortable method [40]. Also,

positive margin, re-excision, and mastectomy rates seem to be similar in both groups [41]. Therefore, we did not consider the localization technique a factor associated with positive margins in this study.

Furthermore, surgeon- and hospital-related factors such as hospital-volume and lumpectomy diameter were different between both hospitals. In the low-volume hospital, fewer BCS were performed compared to the high-volume hospital. An increasing body of literature demonstrates a positive correlation between volume (either surgeon or hospital) and oncological outcomes, resulting in centralization for procedures [42]. However, a previous multicenter study derived from 20 institutions in The Netherlands, including community-based and university-affiliated hospitals, showed no difference between positive surgical margin rates from university affiliated and community hospitals [17]. This was supported by two other studies investigating hospital volume [21], [43]. As a result, we should not consider hospital volume to be the major predictor of positive margins, since many other closely associated characteristics also contribute to BCS margin status like patient characteristics and radiological work-up.

Apart from hospital volume, surgeon volume has also been previously identified as a predictive factor for positive margins. Since the low-volume hospital is an academic teaching hospital, there were also fellow-surgeons and surgeons in training with less experience performing BCS. The available literature is inconsistent regarding the impact of surgeon volume on the occurrence of positive margins. A former cohort study indicated that surgeons with a higher case volume tended to inflict less often positive margins after the initial resection [21]. Also, another study analyzing the re-operation rates across breast surgeons in a single center stated that performing BCS demonstrated variability in re-operation rates and margin practices among the breast surgeons [44]. In contrast, another study found no correlation between surgeon volume and margin status [21]. Consequently, there is need for studies to analyze surgeon-specific factors that may affect re-operation rates.

Limitations

This study provides an important overview of factors that are associated with a positive margin and the influence of hospital characteristics on margin involvement in BCS. However, the retrospective data collection of this study did not allow for the collection of certain baseline factors, long-term follow-up data, and detailed information about the work-up in both centers. Furthermore, the relatively small sample size in the low-volume hospital may result in wider confidence intervals, potentially increasing the uncertainty of the results.

As a result, a future prospective study is needed to further investigate the multidisciplinary approach to breast cancer and its effect on margins status and re-operation rates in greater detail.

Conclusion

In summary, 8.0% of all cases with breast cancer or carcinoma *in situ* had positive margins after BCS. Several factors were associated with positive margins after BCS, including ILC histological type, higher postoperative tumor size, smaller lumpectomy size, and low hospital volume. Significant variations in hospital characteristics influenced radiological and surgical outcomes between both hospitals, emphasizing the importance of a multidisciplinary approach to breast cancer care that optimizes collaboration between radiologists and surgeons to minimize location-related differences and improve oncological outcomes for women undergoing BCS.

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Supplementary Table 1: Overview of previous studies on factors associated with positive margins after breast-conserving surgery.

Studies	Country	Hospital type	Study design	Sample size	Study inclusion	Population	Positive margins	Factors associated with positive margins
Moore et al., 2000 [23]	USA	Academic	Retrospective cohort	197	1975-1999	Invasive breast cancer, all grades	23%	- lobular histology
Chaghpar et al., 2004 [24]	USA	Clinic and academic	Prospective study	2658	1998-2003	stage T1 N0 and T2 N0 breast cancer	12%	- tumor size - lobular histology
Dzierzanowski et al., 2005 [25]	Canada	Academic	Retrospective cohort	95	2000-2002	invasive breast cancer, grade 1-3	19%	- presence of extensive intraduct component
Aziz et al., 2006 [9]	Canada	Academic	Retrospective cohort	1430	1987-1997	invasive breast cancer, grade 1-3	14%	- younger age - tumor size - presence of DCIS component - presence of lymphovascular invasion
Dillon et al., 2006 [26]	Ireland	Academic	Retrospective cohort	612	1999-2003	invasive breast cancer of stages cT1-T2	34%	- pathologic size - presence of extensive intraduct component - symptomatic patients (no screening) - lack of preoperative diagnosis
Smitt et al., 2007 [27]	USA	Academic	Retrospective cohort	395	1971-1996	breast cancer, stage 1-2	43%	- lobular histology - presence of lymphovascular invasion - lack of preoperative diagnosis

Supplementary Table 1: Overview of previous studies on factors associated with positive margins after breast-conserving surgery. *(continued)*

Studies	Country	Hospital type	Study design	Sample size	Study inclusion	Population	Positive margins	Factors associated with positive margins
Kurniawan et al., 2008 [10]	Australia	Clinic and academic	Prospective cohort	1,648	1994-2005	invasive breast cancer and DCIS, all stages	14%	- mammographic calcifications - tumor size (>3 cm) - multifocal disease - lobular histology - lack of preoperative diagnosis
Lovrics et al., 2009 [21]	Canada	Academic	Retrospective cohort	489	2000 - 2002	invasive breast cancer of stages cT1-T2	26%	- tumor factors: palpability, size, histology - lack of preoperative diagnosis - resected tissue volume
Saadai et al., 2011 [11]	USA	Clinic	Retrospective cohort	127	2000-2007	Patients with preoperative core biopsies positive for breast cancer	52%	- younger age - tumor size on imaging - calcifications on mammography - multifocal disease - DCIS or necrosis on core biopsy - close margins on specimen films

Supplementary Table 1: Overview of previous studies on factors associated with positive margins after breast-conserving surgery. *(continued)*

Studies	Country	Hospital type	Study design	Sample size	Study inclusion	Population	Positive margins	Factors associated with positive margins
Shin et al., 2012 [28]	Korea	Academic	Prospective cohort	1034	2008-2009	invasive breast cancer and DCIS, cT1-T2-T3	14.6%	- micro calcifications - grade 4 mammographic density - >0.5 cm difference in tumor size between MRI and ultrasonography - DCIS on needle biopsy - lobular component on needle biopsy

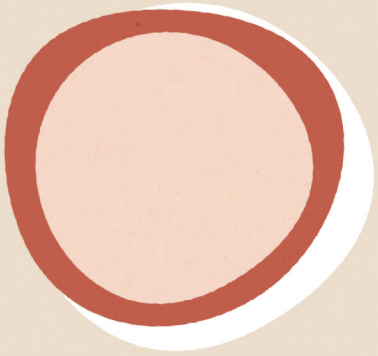
Supplementary Table 1: Overview of previous studies on factors associated with positive margins after breast-conserving surgery. *(continued)*

Studies	Country	Hospital type	Study design	Sample size	Study inclusion	Population	Positive margins	Factors associated with positive margins
Pleijhuis et al., 2013 [17]	The Netherlands	Clinic and academic	Retrospective cohort	1185	2008-2009	T1-2N0-1Mx-0 invasive breast carcinoma	19.7 – 24.5%	- suspicion of multifocal disease - preoperative MRI-scan absent - positive preoperative N-stage - non-palpable tumor - microcalcifications on mammogram - preoperative T2-stage - breast density on mammogram - presence of DCIS component - lobular histology - ER positive - Elston III grade
Barentsz et al., 2015 [29]	The Netherlands	Clinic and academic	Prospective cohort	576	2007-2011	Non-palpable invasive breast cancer	12%	- mammographic microcalcifications - tumor size - presence of DCIS - Bloom and Richardson grade 2/3 - caudal location of the lesion

Supplementary Table 1: Overview of previous studies on factors associated with positive margins after breast-conserving surgery. *(continued)*

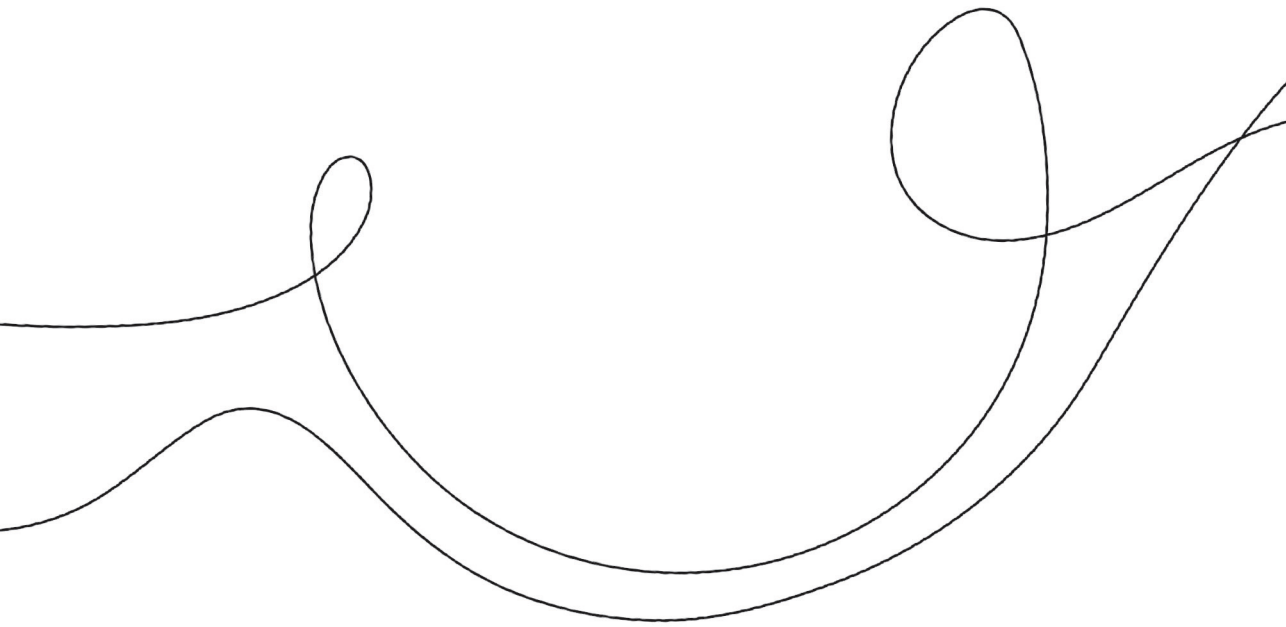
Studies	Country	Hospital type	Study design	Sample size	Study inclusion	Population	Positive margins	Factors associated with positive margins
Alves-Ribeiro et al., 2016 [22]	Portugal	Academic	Retrospective cohort	252	2013-2014	invasive breast cancer of stages cT1-T2	10.3%	- weight and volume of surgical specimen
Hanna et al., 2016 [12]	USA	Clinic and academic	Retrospective cohort	1,132,352	1998-2010	invasive breast cancer and DCIS, cT1-T2-T3	7,3%	- black people - multiple comorbidities - living in a Pacific state (USA) - tumor size - high grade, node positive tumors - no neoadjuvant chemotherapy - no adjuvant radiation therapy
Present study: Makineli et al.	The Netherlands	Clinic and academic	Retrospective cohort	423	2018-2020	invasive breast cancer and DCIS, all stages	8.0%	- invasive lobular carcinoma - post operative tumor size - lumpectomy size - hospital volume

DCIS = ductal carcinoma *in situ*, ILC =invasive lobular carcinoma



CHAPTER 12

Summary



In this thesis, the objective was to describe new techniques and insights for the diagnosis and treatment of neoplastic diseases of the breast. In the first part of the thesis, we aimed to focus on developing intraductal techniques in the diagnosis and treatment of pathological nipple discharge (PND). The second part involved new insights in optimizing breast-conserving surgery in the treatment of breast cancer. In this chapter, the major findings of Part I and Part II of this thesis are summarized.

Part I: New intraductal techniques in the diagnosis and treatment of pathological nipple discharge

In **Chapter 2**, a systematic overview of the role of duct excision surgery (microdochectomy / major duct excision) in the treatment of PND and detection of breast carcinoma is provided. Nine studies with a total of 1108 patients with PND without radiological and other clinical abnormalities after a duct excision were included in the review. The weighted average rate of malignancy after duct excision surgery was 8.1%. Three studies described the recurrence rate of PND, which varied from 0 to 12%, and two studies breast cancer development in a total of three patients (<1%). Complications were reported in 1.4% of the total study population. These results imply that the majority of the patients underwent surgery for a benign cause and therefore improvement of the diagnostic and therapeutic workup is needed to prevent patients from undergoing (unnecessary) exploratory surgery.

To improve the diagnostic and therapeutic workup of PND, interventional ductoscopy has been developed as a minimally invasive micro-endoscopic approach for the direct visualization and removal of intraductal lesions in the breast. In **Chapter 3**, the current state and the role of interventional ductoscopy in the diagnosis and treatment of PND are discussed. The efficacy of ductoscopy is outlined, demonstrating that the addition of ductoscopy to the work-up of PND prevents surgery in 2/3 of patients. Additionally, the challenges of ductoscopy and the implementation of this technique in and outside The Netherlands are described.

Chapter 4 describes our experience of a second ductoscopy procedure in patients with recurrent or persistent PND without suspicious radiological findings. A second ductoscopy was performed when the first ductoscopic attempt was unsuccessful due to technical problems. A total of seventeen patients underwent two ductoscopy procedures. After two ductoscopic attempts, PND stopped in ten patients (58.8%), and seven patients (41.2%) still suffered from PND and were operated on. Pathology of the resection specimens showed no abnormalities in one patient, a papilloma in five patients, and DCIS in one patient. These results indicate that a second ductoscopy procedure can be considered in the diagnostic work-up of patients suffering from persistent or recurrent PND after an unsuccessful first ductoscopic attempt to avoid unnecessary surgery in about 59% of the cases.

To enhance the interventional capabilities of ductoscopy, a collaboration was initiated with the Department of Biomechanical Engineering at Delft University of Technology. This collaboration has led to the development of new intraductal biopsy tools that can be used during ductoscopy. In **Chapter 5**, the development of thre enew biopsy needles is described. All the biopsy needles were successfully integrated with a ductoscope and tested on mastectomy samples and gelatin tissue samples. From these tests, two clear winners were identified: the cone and pincer biopsy needles. The developed ultrathin biopsy needles have illustrated that it is possible to perform intraductal biopsies during ductoscopy. These ex vivo results show great potential for diagnosis and treatment of intraductal lesions. However, a clinical feasibility study needs to be performed to evaluate the efficacy of the developed biopsy needles in the outpatient clinic setting for patients with PND.

In **Chapter 6**, a study protocol is presented for the use of three new techniques during interventional ductoscopy to enhance outcomes. These three novel techniques include Narrow-Band Imaging, two new biopsy needles developed by TU Delft, and intraductal laser ablation. The primary objectives of this research were to improve the diagnostic accuracy and therapeutic efficacy of interventional ductoscopy in patients with PND, and to investigate the feasibility of the new approaches for the diagnosis and removal of intraductal precursor lesions. An

enhanced ductoscopy technique has the potential to reduce unnecessary surgical interventions in patients with PND without radiological abnormalities.

In **Chapter 7**, the feasibility of laser ablation during ductoscopy was analyzed. In this study, nine patients underwent intraductal laser ablation due to a remaining lesion after biopsy. This study showed that intraductal laser ablation during ductoscopy in patients with papillary lesions is feasible and safe. The Thulium laser enables ablation and is suitable for intervention. In 77.8% of the patients with a remaining lesion after biopsy, laser ductoscopy was successful and exploratory surgery could be avoided in 88.9%. Laser ductoscopy has potential to improve the therapeutic intervention capacity of ductoscopy.

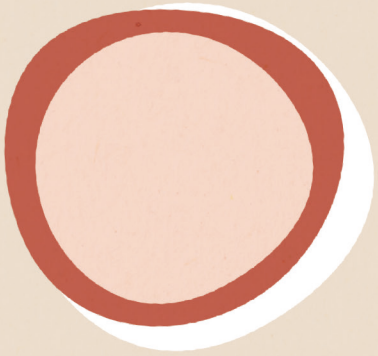
Chapter 8 includes a case report of a 41-year-old woman with PND who underwent a ductoscopy procedure. Post-ductoscopy, the patient developed breast abscesses with histopathological confirmation of granulomatous mastitis (GM). Idiopathic GM has an unknown etiology and atypical presentation. This was the only case described in which IGM occurred after ductoscopy. This can be associated with trauma-induced GM or underlying idiopathic GM exacerbated by the ductoscopy procedure.

In **Chapter 9**, we focused on microRNA (miRNA) expression analysis in patients with PND. This study aimed to compare miRNA expression levels between nipple fluids from patients with PND to identify possible relevant miRNAs that could differentiate between intraductal papillomas and no abnormalities in the breast tissue. A total of 27 nipple fluid samples from patients with PND were included for miRNA expression analysis. Out of the 22 miRNAs examined, only miR-145-5p was significantly differentially expressed (upregulated) in nipple fluid from patients with an intraductal papilloma compared to patients showing no breast abnormalities (OR 4.76, $p = 0.046$) with a diagnostic accuracy of 92%. This means that miR-145-5p has potential as a diagnostic marker to signal presence of papillomas in PND patients.

Part II: Optimizing breast-conserving surgery in the treatment of breast cancer

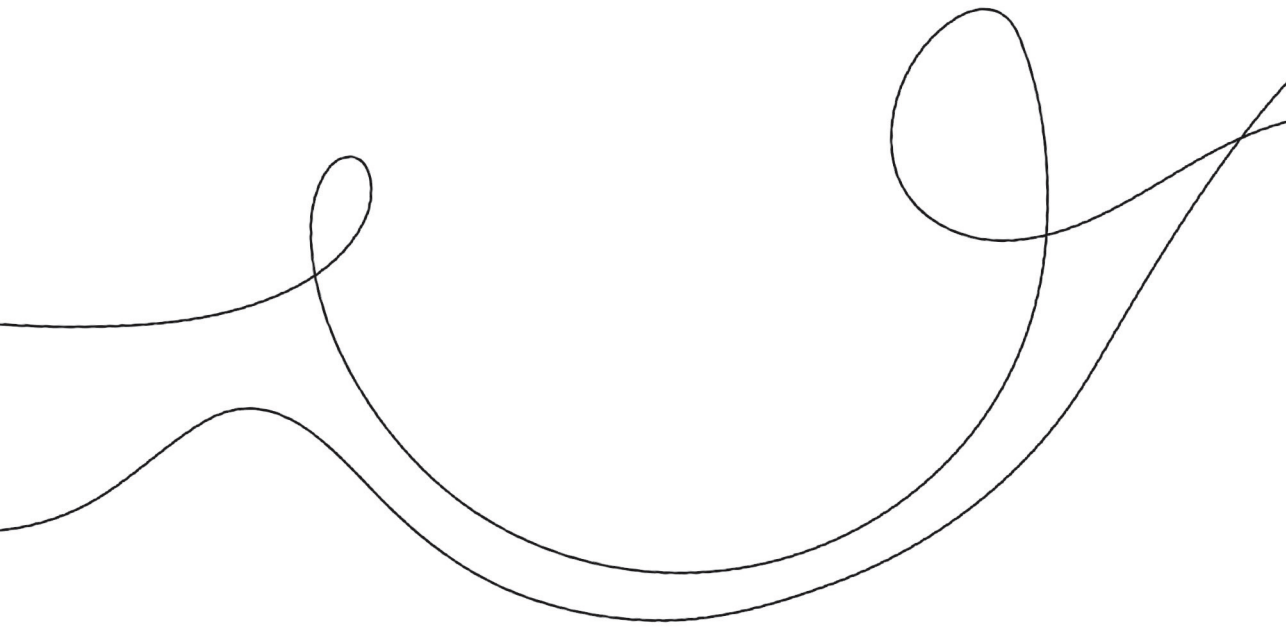
Oncoplastic reconstructive surgery in breast cancer offers several advantages that contribute to both aesthetic and functional outcomes. However, oncologic resection is often more extensive than expected, occasionally causing the plastic surgeon to deviate from the predetermined plan. For optimal planning of the reconstruction, **Chapter 10** evaluated predictors of postoperative lumpectomy size in breast-conserving surgery for breast cancer patients. A total of 423 cases (410 patients) were included, with a median age of 58 (range 32-84) and a mean BMI of 25.0 (SD=9.3). Significant predictors for postoperative lumpectomy size included BMI, breast size, and radiological tumor size. A predictive tool for lumpectomy size was developed, and a web-based application was created to facilitate the use of our tool in a clinical setting. This model could be beneficial for plastic surgeons in planning reconstructions and to prepare and inform their patients more accurately.

Chapter 11 investigated clinicopathological factors affecting positive margins after breast-conserving surgery. A total of 423 cases (410 patients) were included from two Dutch hospitals. This study showed that 8.0% of all breast cancer or carcinoma *in situ* cases treated with breast-conserving surgery had positive margins after surgery. The multivariable adjusted logistic regression showed that the presence of invasive lobular carcinoma (ILC), a larger postoperative tumor, a smaller lumpectomy size and a low hospital volume were associated with a higher risk of a positive margin after breast-conserving surgery. There were significant differences in several (radiological and surgical) variables between both hospitals related to age at time of surgery, presence of ILC histological type, radiological underestimation of the tumor size, MRI rates, lumpectomy size, and positive margin rate.



CHAPTER 13

General discussion and future perspectives



The aim of this thesis was to describe new intraductal techniques to improve the work-up of patients suffering from pathological nipple discharge (PND) and to address new insights to optimize breast-conserving surgery outcomes in the treatment of breast cancer. In this chapter, clinical implications and limitations of the major findings of this thesis are discussed and future perspectives are given.

Part I: New techniques in the diagnosis and treatment of pathological nipple discharge

In patients suffering from PND, current guidelines advise further evaluation with mammography and breast ultrasound to rule out underlying malignancy. Nevertheless, these radiological diagnostics often result in a series of negative results, leaving the surgeon with a diagnostic dilemma. In these patients suffering from PND without radiological and other clinical abnormalities, surgical excision is traditionally performed to rule out malignancy. According to our results, breast carcinoma (invasive or *in situ*) was found in only 8.1 per cent of patients with PND without radiological and other clinical abnormalities after surgery [1]. This means that the majority of patients undergo surgery for benign lesions. Opting for surgical excision should be carefully considered, since these procedures are performed 'blindly' and carry surgery-related complications, such as hematomas, surgical site infections, and seromas. Surgery also leads to higher costs and the need for more medical personnel [2]. In times of staff shortages and rising healthcare expenses, gains can be achieved with a better selection of patients that actually will benefit from duct excision surgery and potentially prevent unnecessary exploratory surgery.

Interventional ductoscopy

Interventional ductoscopy is an additional diagnostic and therapeutic procedure in the workup of PND in patients with nipple discharge lasting for more than 3 months, when mammographic and ultrasonographic examinations have shown no signs of malignancy. The implementation of ductoscopy to the workup of PND has proven effective in preventing surgery in approximately 2/3 of patients, enhancing the selection of patients for surgical procedures by enabling the detection and removal of intraductal lesions [3]. However, despite the evident benefits of

ductoscopy, some patients can still experience recurrent or persistent PND after ductoscopy, often due to the presence of a remaining lesion [4]. Currently, these patients are offered radiological follow-up or undergo a surgical procedure for further diagnosis and treatment. In cases where the initial ductoscopy identifies a benign lesion but fails to (fully) remove it through endobasket extraction, leading to persistent or recurrent PND, a second ductoscopy procedure may be considered. This approach has shown success in preventing surgery in more than half of the cases [5]. Nevertheless, careful consideration is necessary to determine whether an additional ductoscopic intervention can have an additional effect on treatment. A second intervention will not likely be a success in patients with small ducts or those who experienced duct perforation during the first procedure.

To further enhance the diagnostic and therapeutic capabilities of ductoscopy, UMC Utrecht and TU Delft conducted research on new endoscopic biopsy techniques and the application of laser ablation for intraductal residual abnormalities. With these new interventions, the aim was to remove intraductal lesions completely and prevent additional surgery or a second ductoscopic intervention [6]. *Ex vivo and in vitro* results of the biopsy needles demonstrated successful intraductal biopsies on mastectomy samples and gelatin tissue samples, showing great potential to improve the success of intraductal biopsies for histopathological analysis of the lesion. Currently, the clinical feasibility study for the newly developed intraductal biopsy needles is ongoing. Furthermore, a study on the clinical feasibility of intraductal laser ablation during ductoscopy has demonstrated that it was technically feasible and safe for evaporating residual intraductal breast lesions. As a result, laser ductoscopy has the potential to safely improve the therapeutic intervention capability of ductoscopy in patients with benign intraductal lesions and to successfully prevent unnecessary exploratory surgery. Moreover, laser ductoscopy can improve the patient selection process for surgical procedures in the workup of PND without clinical or radiological abnormalities, because successful (laser)ablation obviates further invasive procedures. However, the presence of an intraductal mass is a possible predictor for malignancy, so a definitive histological diagnosis is mandatory before performing laser ablation [7].

In conclusion, these new interventions during ductoscopy can lead to a reduction of the need for additional surgery and fewer surgery related complications such as hematomas, surgical site infections and seromas [1]. However, further refinement and validation in follow up clinical trials are necessary to further assess their therapeutic efficacy.

Micro RNA analysis of nipple discharge

In search of diagnostics to improve the diagnostic work-up of PND, new minimally invasive techniques were investigated. Cytological analysis of nipple discharge previously demonstrated a low sensitivity and therefore is not well able to distinguish between various intraductal lesions. However, identifying biomarkers in nipple discharge to differentiate between intraductal lesions could be essential for improving diagnostics. Therefore, nipple fluid analysis with miRNA expression profiling was conducted to identify potentially relevant miRNAs that can discriminate intraductal papillomas from normal breast tissue in patients with PND. Based on our findings, miR-145-5p levels in nipple fluid can differentiate between intraductal papilloma and breast tissue without abnormalities in PND patients, showing the potential of miR-145-5p in nipple fluid analysis as a diagnostic tool in the work-up of PND [8]. However, previous research on the relationship between miRNAs and the development progression of breast diseases has been inconsistent. The exact changes in miR-145-5p expression during the normal-benign-malignant sequence of breast cancer are currently unknown. This study suggests that miR-145-5p may impact the pathogenesis of benign breast disorders such as intraductal papilloma, differently than the pathogenesis of malignant breast disorders. Hence, the exact rational is not clear. Future prospective research should strive for a careful investigation of a broad range of candidate miRNAs using a non-targeted or multi-targeted approach, and to collect a larger number of patient samples categorized into three groups: breast tissue without abnormalities, intraductal papillomas, and breast cancer (divided into *in situ* and invasive cancer). This setup will help identify possible relevant miRNAs that might, in combination, differentiate benign from malignant breast diseases with high diagnostic accuracy.

In conclusion, adding nipple fluid analysis with miRNA expression profiling to the diagnostic work-up may help patients with no radiological suspicion of disease to a final diagnosis. Although miRNA expression analysis may still not be suitable for all PND patients, it shows potential as a minimally invasive diagnostic tool in patients with intraductal papillomas with spontaneous discharge and may prevent unnecessary duct excision surgery in patients with benign disease. However, further refinement and validation in clinical trials are necessary to establish its clinical applicability.

Figure 1 illustrates the diagnostic and therapeutic pathway of PND after the new implementations.

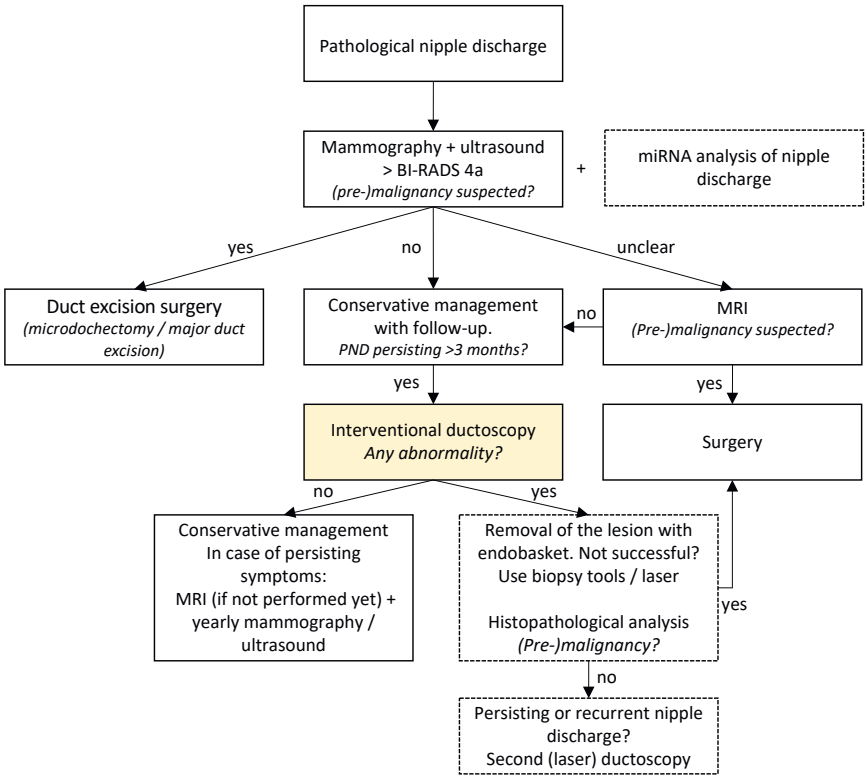


Figure 1: New techniques added to the work-up of PND. Interpretation of the results of this thesis.

How to interprate the pathway with interventional ductoscopy:

- Ductoscopy can be used as a triage instrument in the diagnostic process for patients with PND persisting for more than three month in patients without abnormalities in mammography or ultrasound. When the discharge is less than 3 months, it can be self-limiting and no additional diagnostics are needed.
- A percutaneous core or vacuum-assisted biopsy is performed to obtain tissue for histopathological examination in case of a BIRADS-4a lesion on imaging. However, when the diagnosis after biopsy remains unclear, or a benign intraductal lesion such as papilloma is found and the nipple discharge persists after biopsy, ductoscopy can remove the intraductal lesion.
- If no abnormalities are visible during ductoscopy, clinical follow-up is pursued. When no or histologically proven benign lesions are found during ductoscopy, duct excision surgery have no additional diagnostic value. If the pathological nipple discharge persists or recurs, an MRI can be performed (if not performed yet) and a microdochectomy may be considered. Diagnostic surgery or long-term follow-up with imaging are always possible when ductoscopy does not provide a definitive diagnosis.
- When an abnormality is visible through ductoscopy in the milk duct, an endo-basket is used to take a biopsy and remove the abnormality. After testing the feasibility of the newly designed biopsy tools, these can be used in patients with an abnormality that can not be removed with the basket. In case of a remaining lesion, laser ductoscopy can be performed (after a histological biopsy) until no visible vital tissue of the lesion to be treated remain.
- In cases with persistent or recurrent PND after a ductoscopy procedure due to a remaining lesion, a second ductoscopy procedure (with laser) can be performed to treat the remaining lesion.
- In cases with good interpretable mammography and ultrasound, no additional MRI has to be performed prior to ductoscopy, since ductoscopy has higher diagnostic accuracy, with lower chance of a false positive result, comparing to MRI. However, in cases of no diagnosis after ductoscopy, MRI can be performed.

- Ductoscopy does not replace microdochectomy, but obviates this procedure in a subset of patients. When ductoscopy turns out to be not suitable for the individual patient for whatever reason, microdochectomy can still be performed.
- After further refinement and validation in larger clinical trials, miRNA analysis might be added to the diagnostic pathway to differ between lesions.

FUTURE PERSPECTIVES

Based on the positive results of previous ductoscopy-related studies in the work-up of PND, ductoscopy has been incorporated into the Dutch National Breast Cancer Guideline [9]. In The Netherlands, UMC Utrecht is the only center performing ductoscopy so far, with patients referred from all across the country. Starting January 2024, full reimbursement for ductoscopy by health insurance companies in The Netherlands is expected to facilitate the widespread adoption of this technique [10]. The intention is to initiate an implementation process in 2024, allowing more hospitals to incorporate ductoscopy into their practices nationwide. Furthermore, following national implementation, an international course will be set to train surgeons worldwide, covering the new techniques involved. Successful reimbursement will facilitate the initiation of larger multicenter studies to evaluate the therapeutic effectiveness of the newly designed biopsy tools and laser ablation in the treatment of PND.

Laser ductoscopy can be safely implemented in medical centers already performing ductoscopy procedures for PND. The widespread adoption of this technique into the work-up of PND, particularly in centers performing duct excision surgery, holds promise for the future.

The main goal of the implementation of interventional ductoscopy in the work-up of PND was to have a better selection of patients that actually will benefit from duct excision surgery and potentially prevent unnecessary exploratory surgery. Fewer patients undergoing duct excision surgery means fewer surgery-related complications, reduced need for operating room personnel, shorter surgery times and lower overall costs. This thesis proposed potential ways to improve the diag-

nostics and therapeutic interventions of the work-up of PND to prevent unnecessary duct excision surgery.

Part II: Optimizing breast-conserving surgery in the treatment of breast cancer

Breast-conserving surgery (BCS) is a vital component of breast cancer treatment, aiming to preserve as much healthy breast tissue as possible while ensuring optimal oncological outcomes achieved by maintaining clear surgical margins. In this thesis, we aimed to improve the outcomes of BCS by investigating factors influencing lumpectomy size and positive margin rates in patients with *in situ* and invasive breast cancer.

Factors influencing lumpectomy size

According to the findings of our study, predictors for postoperative lumpectomy size included BMI, breast size, and radiological tumor size [11]. Moreover, this study showed that postoperative lumpectomy size is often larger than just adding 1 centimeter margin to the radiological tumor size, a common practice to estimate lumpectomy size. Following these results, we developed a predictive tool for lumpectomy size and a web-based application to facilitate its use in a clinical setting [12]. This tool could be beneficial for (plastic) breast surgeons in planning reconstructions and preparing patients more accurately before surgery, thereby improving cosmetic outcomes and the overall quality of care for breast cancer patients. Future research is needed to validate the accuracy of the lumpectomy size tool in a clinical setting in estimating lumpectomy size before surgery. Additionally, we underscored the limitations of using breast cup size for estimating breast size due to its lack of standardization, interobserver variability, and differences between countries and bra brands [13]. In future studies, utilization of more reliable techniques like volumetry CT or MRI, water displacement or 3D photography could be integrated into the lumpectomy size tool for enhanced precision. Furthermore, a prospective study design could enable physicians to precisely assess and record ptosis, underbust circumference, cup size/volume measurement, weight, and height to collect current and reliable data.

Factors influencing positive margins

The presence of positive margins after surgery poses a significant risk factor for tumor recurrence, leading to the need for additional surgery or radiotherapy, and potentially resulting in worse cosmetic and oncological outcomes. In search of factors related to positive margin status after BCS, we analyzed data from two hospitals. This study showed that 8.0% of all patients had positive margins post-BCS. Factors associated with a higher risk of positive margins included the presence of invasive lobular carcinoma (ILC), larger postoperative tumor size, smaller lumpectomy size and treatment in a low hospital volume. Significant differences in various (radiological and surgical) variables between both hospitals were observed, including age at the time of surgery, presence of ILC histological type, radiological underestimation of the tumor size, MRI rates, lumpectomy size, and positive margin rates.

While many studies have explored factors influencing positive margins after initial surgery, less attention has been given to the impact of hospital characteristics on margin involvement in BCS. Previous research has suggested that breast cancer surgery in specialized, teaching, and high-volume hospitals correlates with improved long-term survival [14], [15]. Moreover, an increasing body of literature demonstrates a positive correlation between volume (either surgeon or hospital) and oncological outcomes, resulting in centralization for procedures [16]. However, a previous multicenter study derived from 20 institutions in The Netherlands, including community-based and university-affiliated hospitals, showed no difference between positive surgical margin rates from university affiliated and community hospitals [17]. In our opinion, hospital volume should not considered to be the major predictor of positive margins, since many other closely associated characteristics also contribute to BCS margin status like patient characteristics and radiological work-up.

This study emphasizes the importance of a multidisciplinary approach to breast cancer care, which optimizes collaboration between radiologists and surgeons to minimize location-related differences and improve oncological outcomes for women undergoing BCS. In our opinion, the comparison between both hospitals once more depicts the importance of multidisciplinary teamwork when it comes

to the treatment of breast cancer. The quality of the team as a whole can determine the outcome. A future prospective study is needed to further investigate the multidisciplinary approach to breast cancer and its effect on margins status and re-operation rates in greater detail.

In conclusion, in this thesis, we provided an overview of factors affecting lumpectomy size and the margin status in BCS. In the future, it should be possible to determine the predicted lumpectomy size before surgery for improving the (cosmetic) outcomes. Also, awareness is needed of the factors influencing positive margins to strive for better oncological outcomes in BCS in patients with breast cancer.

Highlights of this thesis per chapter

Chapter 2 The majority of patients with intraductal papillomas undergo unnecessary duct excision surgery when suffering from pathological nipple discharge.

Chapter 3 Ductoscopy has been integrated into the Dutch National Breast Cancer Guideline, with full reimbursement by health insurance companies in the Netherlands starting from January 2024.

Chapter 4 A second ductoscopy procedure can be considered in the diagnostic work-up of patients suffering from persistent or recurrent PND after an unsuccessful first ductoscopic attempt to avoid unnecessary surgery.

Chapter 5 We have developed innovative ultrathin biopsy needles for use during ductoscopy, which are now patented.

Chapter 6 Enhanced ductoscopy with intraductal biopsy and laser ablation could prevent unnecessary surgery in patients with pathological nipple discharge.

Chapter 7 Intraductal laser ablation during ductoscopy in patients with papillary lesions seems to be feasible and safe and has potential to improve the therapeutic intervention capability of ductoscopy.

Chapter 8 Idiopathic granulomatous mastitis is a rare complication after ductoscopy.

Chapter 9 MiR-145-5p expression in nipple fluid has the potential to serve as a diagnostic marker to signal the presence of papilloma in patients suffering from pathological nipple discharge.

Chapter 10 Lumpectomy size can be calculated using BMI, cup size, and radiological tumor size prior to surgery.

Chapter 11 The factors affecting positive margins after breast-conserving surgery in patients with breast cancer of carcinoma in situ are the presence of invasive lobular carcinoma, a larger postoperative tumor, a smaller lumpectomy size, and a low hospital volume.

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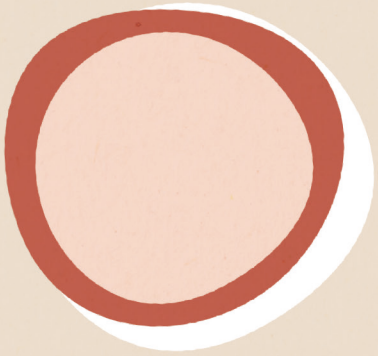
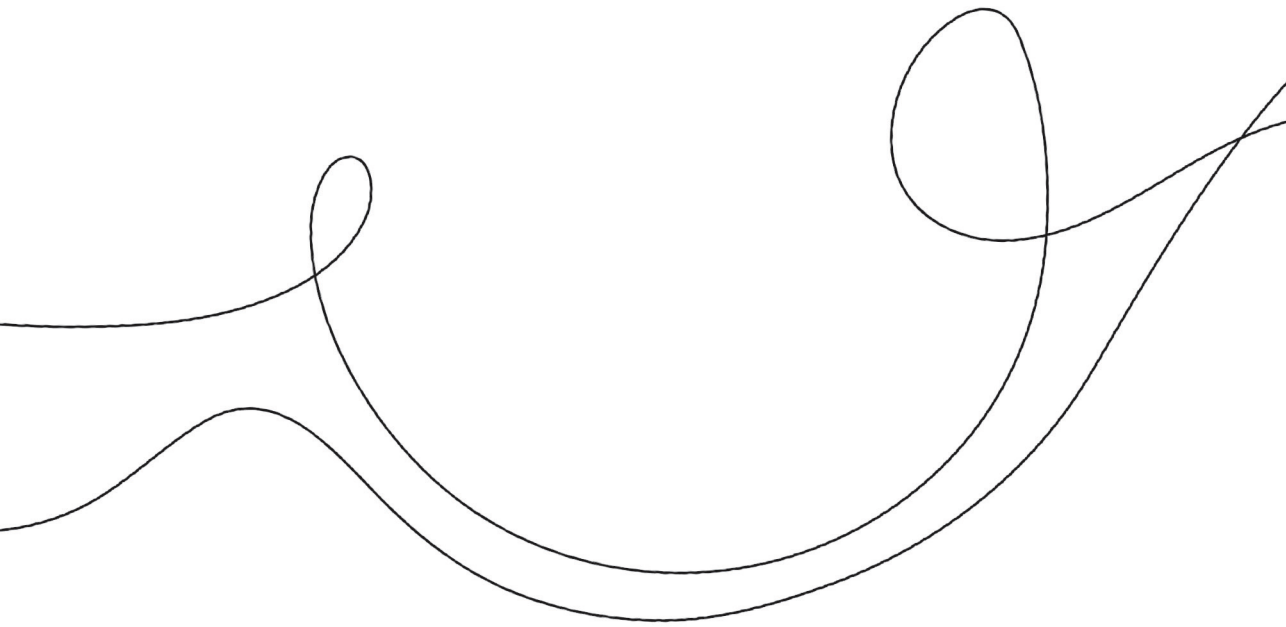
PART III

Addenda



CHAPTER 14

- Beslisboom pathologische tepeluitvloed
- List of abbreviations
- Contributing authors and affiliations
- Review committee
- Dutch summary | Nederlandse samenvatting
- Turkish summary | Türkçe özet
- List of publications
- Acknowledgements
- About the author



BESLISBOOM PATHOLOGISCHE TEPELUITVLOED

Beslisboom pathologische tepeluitvloed voor patiënten: ductoscopie of een operatie?

Als u last heeft van spontane, enkelzijdige (bloederige, bruine of troebele) tepeluitvloed uit een enkele melkgang wat langer dan drie maanden aanhoudt, dan heeft u last van pathologische tepeluitvloed. Dit kan wijzen op borstkanker en moet daarom onderzocht worden. Als er op de mammografie en echografie geen afwijkingen zichtbaar zijn, kunt u met uw arts bespreken wat u wilt: een ductoscopie of een operatie. Deze beslisboom helpt u bij het maken van deze keuze.

Behandelmogelijkheden:	**Ductoscopie**	**Operatie**
Wanneer is er indicatie voor een ductoscopie / operatie:	- Bij een negatieve testuitslag en aanhoudende klachten kan ductoscopie worden ingezet als een extra onderzoek om afwijkingen aan te tonen en verwijderen. - Als er een afwijking aanwezig is, kan een netje worden ingezet om de afwijking te verwijderen. Zo wordt een operatie voorkomen. - Ductoscopie lukt in gemiddeld 70% van de patiënten. <u>Contra-indicatie:</u> Afwijkingen op beeldvorming zonder dat daarbij borstkanker is uitgesloten, een ingetrokken tepel en een eerdere operatie aan de tepel waardoor ductoscopie niet mogelijk is.	- Bij een negatieve testuitslag en aanhoudende klachten kan op verzoek van de patiënt een operatie worden uitgevoerd. - Wanneer een afwijking wordt gevonden die niet kan worden gebiopteerd en de tepeluitvloed aanhoudt, dan wordt een operatie uitgevoerd. - Als een ductoscopisch onderzoek niet lukt en de tepeluitvloed aanhoudt, dan volgt een operatie.
Hoe werkt de behandeling?	- Ductoscopie wordt uitgevoerd op de polikliniek. - Het vindt plaats onder plaatselijke verdoving met 3 prikjes in de tepel. - Een dun kijkbuisje met een camera wordt vervolgens door de tepel naar binnen gebracht waarbij de melkgangen worden afgebeeld op een monitor. Afwijkingen kunnen met een kleine netje worden verwijderd.	- Tijdens de operatie worden er een of enkele melkgangen verwijderd (dit wordt een microdochectomie genoemd). - Het betreft een ingreep onder algehele narcose waarbij de chirurg zonder een diagnose “blind” weefsel wegsnijdt in de hoop daarmee de oorzaak van de tepeluitvloed te hebben verwijderd.
Wat is het resultaat van de behandeling?	- Met ductoscopie kunnen wij zien of u een afwijking heeft in de melkgang of niet. - Als u een afwijking heeft, kunnen wij deze verwijderen waarna uw tepeluitvloed stopt en u niet meer geopereerd hoeft te worden. Als we de afwijking kunnen biopteren kunnen we uw met zekerheid vertellen of de tepeluitvloed wordt veroorzaakt door borstkanker.	- Bij vrijwel alle vrouwen zal de tepeluitvloed stoppen na microdochectomie als de oorzaak inderdaad in de verwijderde melkgangen zit. - Na een operatie kunnen we u met zekerheid vertellen of de tepeluitvloed wordt veroorzaakt door borstkanker. Borstkanker komt in slechts 5-10% van de vrouwen voor, waardoor 90% van de patiënten voor een goedaardige afwijking “onnodig” worden geopereerd.
Wat zijn de risico’s en bijwerkingen?	- Complicaties na ductoscopie zijn pijnklachten en een borstontsteking (mastitis) in <2,5% van de vrouwen.	- Complicaties na microdochectomie zijn een wondinfectie, een hematoom <5% en een kleine kans op complicaties van de algehele narcose. - Tevens houdt de patiënt een litteken onder de tepel over.

Wat is de kans dat de klachten terugkomen?	- Na een succesvolle ductoscopie stopt de tepeluitvloed in 60% van de vrouwen. - Na een niet succesvolle ductoscopie stopt tepeluitvloed toch in 30% van de vrouwen. - Na een ductoscopie procedure moet 28% van de patiënten nog een operatie ondergaan.	- Als er een afwijking in de melkgang aanwezig is en wij die verwijderen met de operatie zal uw tepeluitvloed stoppen. Indien dit niet het geval is, kan de tepeluitvloed weer terugkomen omdat het dan wordt veroorzaakt buiten uw melkgangen.
Wat is de vervolgbehandeling?	- Wanneer bij ductoscopie geen afwijking zichtbaar is, volgt klinische follow-up bij aanhoudende tepeluitvloed: jaarlijkse echografie/ mammografie, maximaal voor 5 jaar. Ook kan microdochectomie alsnog overwogen worden. - Wanneer via de ductoscoop wel een afwijking zichtbaar is in de melkgang, vindt in principe microdochectomie plaats, behalve als de tepeluitvloed is gestopt.	- De meeste patiënten hebben na een operatie geen klachten meer en hoeven geen follow-up meer. - Indien u last blijft houden van tepeluitvloed wordt u vervolgd met een jaarlijkse echografie/mammografie. - Als de uitslag na de operatie borstkanker toont, wordt u doorbehandeld volgens de richtlijn Borstkanker.

LIST OF ABBREVIATIONS

AMH	Alexander Monro hospital
BCS	Breast-conserving surgery
BI-RADS	Breast imaging-reporting and data system
BMI	Body mass index
BRCA	Breast cancer gene
CI	Confidence interval
cN	clinical lymph node status
cT	clinical primary tumor size
CT scan	Computed tomography scan
DCIS	Ductal carcinoma *in situ*
DCT	Delta CT
EHR	Electronic health records
H&E	Haemotoxylin and eosin
IGM	Idiopathic granulomatous mastitis
ILC	Invasive lobular carcinoma
IQR	Interquartile range
LCIS	Lobular carcinoma *in situ*
MD	Mammary ductoscopy
METc	Medical research ethics committee
MICE	Multiple imputation by chained equations
miRNA	MicroRNA
MRI	Magnetic resonance imaging
NAF	Nipple aspirate fluid
NBI	Narrow-band imaging
OPS	Oncoplastic surgery
OR	Odds ratio
PCR	Polymerase chain reaction
PND	Pathological nipple discharge
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
PROSPERO	International prospective register of systematic reviews
ROC curve	Receiver operating characteristic curve

SCARE guidelines	Surgical case report guidelines
SD	Standard deviation
TDLU	Terminal duct lobular unit
TNM	Tumor, lymph node and metastasis
TU Delft	Technical University Delft
UMCU	University Medical Center Utrecht
$\chi 2$	Chi-square

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DUTCH SUMMARY | NEDERLANDSE SAMENVATTING

Dit proefschrift gaat over nieuwe technieken en inzichten in de behandeling van nieuwvormingen in de borst. Nieuwvormingen van de borst kunnen goedaardig zijn (zoals een poliep of fibroadenoom), potentieel kwaadaardig (zoals ductaal carcinoma *in situ*, ook wel voorloper borstkanker genoemd) of kwaadaardig (borstkanker).

Deel I: Nieuwe techniek in de behandeling van pathologische tepeluitvloed

Pathologische tepeluitvloed is spontane, enkelzijdige bloederige, bruine of sereuze tepeluitvloed uit een enkele melkgang wat langer dan drie maanden bestaat. In een minderheid van de gevallen is er sprake van een onderliggende kwaadaardige afwijking, echter in de meeste gevallen is een goedaardige afwijking (poliep) in de melkgang direct achter de tepel de oorzaak. Een mammografie en echografie tonen vaak geen afwijkingen, waardoor een operatieve ingreep nodig is waarbij de aangedane melkgang of het weefsel achter de tepel wordt verwijderd. Deze operatie wordt ook wel een conusexcisie genoemd waarbij het ''blind" wordt uitgevoerd, omdat de exacte locatie van de afwijking niet bekend is. De meeste vrouwen ondergaan deze ingreep met bijbehorende complicaties en gevolgen voor borstvoeding, cosmetiek en de gevoeligheid van de tepel voor een niet kwaadaardige, onschuldige afwijking.

In **hoofdstuk 2** hebben wij een systematische literatuurstudie uitgevoerd waarin we het percentage van kwaadaardige tumoren na conusexcisie hebben berekend bij patiënten met pathologische tepeluitvloed zonder radiologische en andere klinische afwijkingen. Negen studies met een totaal van 1108 patiënten met pathologische tepeluitvloed die een conusexcisie ondergingen werden opgenomen in de beoordeling. Het percentage van tumoren na een operatie was 8,1 % (variërend tussen 2,3% en 13,5%). Drie studies beschreven het recidiefpercentage van tepeluitvloed na een operatie, wat varieerde tussen 0% en 12%. Twee studies rapporteerden de ontwikkeling van borstkanker tijdens de follow-up bij in totaal drie patiënten (minder dan 1 procent). Deze resultaten tonen aan dat het percentage (voorloper) kwaadaardige tumoren na een conusexcisie laag was bij patiënten met pathologische tepeluitvloed en ongeveer 9 van de 10 patiënten een operatie

ondergaan vanwege een goedaardige oorzaak. Hierdoor is verbetering van de diagnostische en therapeutische behandelmogelijkheden nodig om te voorkomen dat patiënten onnodige operaties ondergaan.

Om het opsporen en verwijderen van afwijkingen bij pathologische tepeluitvloed te verbeteren, werd er in de jaren '90 een nieuwe techniek geïntroduceerd, genaamd ductoscopie. Ductoscopie is een micro-endoscopische techniek waarbij de melkgangen van de borst via de tepelopening in beeld worden gebracht. Zo kunnen afwijkingen in de melkgang direct worden opgespoord en vervolgens, via dezelfde weg, worden verwijderd. Ductoscopie wordt uitgevoerd op de polikliniek bij patiënten onder lokale anesthesie. Eerder onderzoek heeft aangetoond dat door de toevoeging van ductoscopie aan de behandeling van pathologische tepeluitvloed, bij twee derde van de patiënten een operatie kan worden voorkomen. Ondanks het succes in het voorkomen van een operatie bij een overgroot deel van de patiënten, is ductoscopie wereldwijd nog geen standaardbehandeling voor pathologische tepeluitvloed. Bovendien is het huidige biopteur niet altijd geschikt voor het (gedeeltelijk) verwijderen van afwijkingen, waardoor patiënten toch nog geopereerd moeten worden voor een diagnose en/of om van de klachten af te komen.

In **hoofdstuk 3** werd de stand van zaken en de rol van interventionele ductoscopie bij de diagnostiek en behandeling van pathologische tepeluitvloed beschreven. De effectiviteit van ductoscopie is beschreven, waarbij we hebben aangetoond dat dankzij ductoscopie een operatie in de meerderheid van de patiënten kan worden voorkomen. Tevens beschrijft dit artikel dat ductoscopie inmiddels is opgenomen in de richtlijn 'Borstkanker' en ook is geaccepteerd als verzekerde zorg in Nederland.

Een ductoscopie procedure is succesvol bij twee derde van de patiënten. Bij een derde van de patiënten is het echter niet mogelijk om uitsluitsel te hebben vanwege verschillende oorzaken, zoals te smalle melkgangen waardoor een perforatie van de melkgang kan optreden, technische problemen of het niet kunnen afnemen van een biopt. Hierdoor kunnen patiënten na een ductoscopie procedure nog steeds last hebben van tepeluitvloed, waardoor ze alsnog een operatie moeten ondergaan.

In **hoofdstuk 4** hebben we onderzocht of een tweede ductoscopie procedure een toegevoegde waarde heeft voor patiënten met terugkerende of aanhoudende pathologische tepeluitvloed. Het primaire doel was het analyseren van het aantal operaties dat kon worden voorkomen na een tweede ductoscopie procedure. In totaal hebben zeventien patiënten twee ductoscopie procedures ondergaan. De eerste ductoscopie toonde een poliepvormige laesie bij tien patiënten (58,8%), bij drie patiënten werden geen afwijkingen gevonden (17,6%) en bij vier patiënten (23,5%) was het niet mogelijk om het de melkgangen te visualiseren. Na de procedure hadden alle patiënten nog steeds last van tepeluitvloed. Na twee ductoscopische pogingen stopte de tepeluitvloed bij tien patiënten (58,8%), terwijl zeven patiënten (41,2%) nog steeds last hadden van tepeluitvloed en geopereerd moesten worden. Pathologie van de resectiepreparaten toonde geen afwijkingen aan bij één patiënt, een papilloom bij vijf patiënten en voorloper borstkanker bij één patiënt. Dit onderzoek suggereert op basis van deze bevindingen dat een tweede ductoscopie procedure kan worden overwogen in de diagnostiek van patienten die lijden aan aanhoudende of terugkerende pathologische tepeluitvloed na een onsuccesvolle eerste ductoscopie poging, om onnodige operaties in ongeveer 59% van de gevallen te voorkomen.

Om de diagnostische en therapeutische mogelijkheden van ductoscopie verder te verbeteren, hebben het UMC Utrecht en de TU Delft gezamenlijk onderzoek gedaan naar nieuwe endoscopische biopsietechnieken. In **hoofdstuk 5** beschrijven we de ontwikkeling van nieuwe biopsienaalden voor de ductoscopie procedure. We hebben drie verschillende naaldontwerpen getest tijdens ductoscopie op gelatine weefselmonsters en mastectomie preparaten (geopereerde borsten). Dit onderzoek toonde aan dat deze ultradunne biopsienaalden geschikt zijn voor het uitvoeren van intaductale biopsie tijdens ductoscopie. Twee van deze drie naalden zijn vervolgens verder ontwikkeld voor het testen in een klinische studie.

In **hoofdstuk 6** wordt het studieprotocol weergegeven voor het gebruik van drie nieuwe technieken tijdens interventionele ductoscopie om de uitkomsten te verbeteren. Deze drie nieuwe technieken omvatten narrow-band imaging (een lichttechniek), twee nieuwe biopsienaalden ontwikkeld door TU Delft en laserbehandeling. De voornaamste doelstellingen van dit onderzoek waren het ver-

beteren van de diagnostische nauwkeurigheid en therapeutische effectiviteit van interventionele ductoscopie bij patiënten met pathologische tepeluitvloed, en het onderzoeken van de haalbaarheid van de nieuwe benaderingen voor de diagnose en verwijdering van afwijkingen in de melkgangen. Een verbeterde ductoscopie techniek kan leiden tot minder onnodige chirurgische ingrepen bij patiënten met pathologische tepeluitvloed zonder radiologische afwijkingen.

De toepasbaarheid van laser behandeling tijdens ductoscopie wordt beschreven in **hoofdstuk 7.** Hoewel laser technieken al worden toegepast in behandelingen van andere ziektes, is dit nog niet het geval tijdens ductoscopie. Deze studie is de eerste die deze techniek beschrijft. Het haalbaarheidsonderzoek werd uitgevoerd tussen oktober 2022 en december 2023. Volwassen vrouwen met pathologische tepeluitvloed zonder radiologische verdenking van een kwaadaardige afwijking werden geïncludeerd. Intraductale laser therapie werd uitgevoerd nadat een biopsie was uitgevoerd tijdens de ductoscopie procedure. Er werd gebruik gemaakt van een Thulium laser met een vermogensinstelling van 1 tot 4 Watt. Deze therapie werd met succes toegepast bij negen patiënten tijdens ductoscopie. Na de ingreep stopte de tepeluitvloed bij zeven patiënten en werden geen complicaties geregistreerd. Dit onderzoek toont aan dat laser behandeling tijdens ductoscopie bij patiënten met afwijkingen in de melkgangen haalbaar en veilig is. Laser ductoscopie heeft daarmee potentie om de therapeutische interventiemogelijkheden van ductoscopie te verbeteren. Verdere validatie in een grotere klinische studie is noodzakelijk om de therapeutische effect van laser ductoscopie te beoordelen.

In **hoofdstuk 8** beschrijven we een casus met een zeldzame complicatie van ductoscopie. De casus betreft een 41-jarige vrouw met pathologische tepeluitvloed waarvoor zij een ductoscopie procedure onderging. Na de ductoscopie ontwikkelde de patiënt abcessen in haar borst, met histopathologische bevestiging van granulomateuze mastitis. Idiopathische granulomateuze mastitis is een zeldzame, goedaardige, chronische inflammatoire borstaandoening van onbekende oorzaak, met een atypische presentatie en onduidelijke behandeling. Deze casus waarin granulomateuze mastitis optrad, is de enige beschreven casus na ductoscopie.

Wanneer patiënten last hebben van pathologische tepeluitvloed, kunnen er ook andere diagnostische methoden ingezet worden om de oorzaak van de tepeluitvloed op te sporen. Zo is cytologie een methode om de cellen van de tepelafscheiding te onderzoeken en het risico op kwaadaardige tumoren te bepalen. Echter, cytologie is een slechte indicator voor het bepalen van kwaadaardigheid in tepeluitvloed en de klinische relevantie wordt betwijfeld. Tegenwoordig is het beoordelen van microRNA expressie analyse in tepelvocht als mogelijke nieuwe klasse biomarker voor borstkanker een opkomend topic. Deze techniek zou kunnen leiden tot de identificatie van nieuwe biomarkers in tepelafscheiding om een onderscheid te maken tussen afwijkingen die tepeluitvloed veroorzaken.

In **hoofdstuk 9** hebben we onderzocht of microRNA expressieanalyse potentie heeft om onderscheid te maken tussen borstaandoeningen bij patiënten met pathologische tepeluitvloed. Het doel van deze studie was om relevante microRNA's te identificeren die onderscheid kunnen maken tussen intraductale poliepen en geen afwijkingen in het borstweefsel. We hebben tepelvochtmonsters van 27 patienten verzameld en geanalyseerd van patiënten die last hadden van pathologische tepeluitvloed die een ductoscopie procedure zouden ondergaan. Er werden in totaal 22 microRNA's geanalyseerd, waarvan alleen miR-145-5p significant vaker voorkwam in tepelvocht van patiënten met poliepen in de melkgangen. De expressie van miR-145-5p in tepelvocht verschilt tussen poliepen en borstweefsel zonder afwijkingen en heeft daarom potentie als een diagnostische marker om onderscheid te maken tussen goedaardig borstweefsel bij patiënten met tepeluitvloed.

Deel II: Nieuwe inzichten in de chirurgische behandeling van borstkanker.

De overgrote meerderheid van patiënten met een recente diagnose van borstkanker in ontwikkelde landen presenteert zich zonder uitgezaaide ziekte. Voor deze patiënten is de optimale behandelstrategie afhankelijk van het stadium waarin de aandoening wordt vastgesteld. Mastectomie wordt aanbevolen voor patiënten die geen geschikte kandidaten zijn voor borstsparende chirurgie of degenen die de voorkeur geven aan mastectomie. Borstsparende chirurgie, ook bekend als lumpectomie, vormt een standaardbehandeling voor vroeg stadia borstkanker en voor lokaal gevorderde tumoren na neo-adjuvante chemotherapie. Borstsparende chirurgie biedt de mogelijkheid tot goede cosmetische resultaten en behoud van

de borstfunctie. Echter blijkt de oncologische resectie vaak uitgebreider te zijn dan verwacht, wat soms leidt tot afwijkingen van het vooraf bepaalde plan door de plastisch chirurg. Voor een optimale planning van de reconstructie is een nauwkeurige schatting van de omvang van de lumpectomie noodzakelijk.

In **hoofdstuk 10** hebben we onderzocht welke preoperatieve voorspellers er zijn voor de grootte van de lumpectomie. In dit onderzoek werden alle patiënten met (voorloper) borstkanker geïncludeerd tussen 2018 en 2020 in twee ziekenhuizen. In totaal werden 410 patiënten geïncludeerd, met een mediane leeftijd van 58 jaar. Uit de analyses bleek dat BMI, borstgrootte en de maximale preoperatieve radiologische tumordiameter de belangrijkste voorspellers waren voor de postoperatieve lumpectomiegrootte. Er is een tool ontwikkeld die de voorspelde lumpectomiegrootte kan berekenen. Dit model zou nuttig kunnen zijn voor plastisch chirurgen bij het plannen van reconstructies om hun patiënten nauwkeuriger voor te bereiden en te informeren. Dit model wordt nog verder onderzocht om te valideren in de klinische setting.

Bij borstsparende chirurgie vormen positieve snijranden na de operatie een uitdagend probleem. Het is een significante risicofactor voor tumorrecidief, wat leidt tot de noodzaak van aanvullende chirurgische ingrepen of adjuvante radiotherapie. Daarom is het van groot belang om het voorkomen van positieve snijranden te minimaliseren. Dit kan worden bereikt door de factoren the identificeren die van invloed zijn op positieve snijranden. In **hoofdstuk 11** hebben we onderzocht welke risicofactoren van invloed zijn op positieve snijranden bij patiënten die borstsparende chirurgie hebben ondergaan. In de populatie van 410 patiënten had 8% na de operatie positieve snijranden. Dit percentage was significant hoger in het laag-volume ziekenhuis (14,9%) vergeleken met het hoog-volume ziekenhuis (5,9%). Het type tumor (invasief lobulair carcinoom), de postoperatieve tumorgrootte, het ziekenhuisvolume en de lumpectomiegrootte waren significant geassocieerd met positieve snijranden na borstsparende chirurgie. Ook waren er significante (radiologische en chirurgische) verschillen in variabelen tussen beide ziekenhuizen, zoals leeftijd, aanwezigheid van invasief lobulair carcinoom, variaties in onderschatting van de tumorgrootte, het aantal MRI's, de lumpectomiegrootte en het percentage positieve snijranden. Concluderend toonde dit

onderzoek aan dat het percentage positieve snijranden laag was (8%). Factoren gerelateerd aan de patiënt, tumor, radiologie en chirurgie kunnen bijdragen aan deze variaties. Een geoptimaliseerde samenwerking tussen multidisciplinaire teams, met name tussen radiologen en chirurgen, moet worden nagestreefd om verbeterde oncologische resultaten te behalen bij vrouwen na borstsparende chirurgie.

In **hoofdstuk 12 & 13** bespreken we de huidige stand van zaken met betrekking tot nieuwe inzichten in de chirurgische benadering van nieuwvormingen in de borst. Tevens delen wij onze toekomstperspectieven op dit gebied en worden er enkele mogelijkheden voor verder onderzoek aangegeven.

TURKISH SUMMARY | TÜRKÇE ÖZET

Bu tez, meme tümörlerinin tedavisindeki bazı yeni teknikleri ve görüşleri incelemektedir. Meme tümörleri kabaca iyi huylu (örneğin, bir polip veya fibroadenom), muhtemel kötü huylu (örneğin, in situ duktal karsinom veya öncü meme kanseri) ve kötü huylu (meme kanseri) olarak 3 grupta incelenebilir.

Birinci Kısım: Meme başı akıntısı tedavisinde yeni bir teknoloji

Birinci Bölüm - Giriş

Meme başı akıntısı tek taraflı, kanlı veya kahverengi renkli bir akıntıdır ve genellikle üç aydan daha uzun bir süre boyunca spontan olarak bir tek süt kanalından gelir. Meme başı akıntısı çoğunlukla iyi huylu nedenlere (örneğin, polip) bağlı olarak ortaya çıkar. Mamografi ve ultrasonografi bulguları çoğu hastada normaldir. Bu nedenle kesin tanı için etkilenen süt kanalının veya meme başının arkasındaki dokunun patolojik incelenmesi için cerrahi bir müdahale ile biyopsi almak gereklidir. Bu operasyon "duktus ekzisyonu" olarak adlandırılır ve "körlemesine" yapılır, çünkü anormalliğin tam konumunu işlem öncesinde belirlemek mümkün değildir. Ameliyat edilen hastaların çok az bir kısmında kanser veya patolojik bir lezyon saptandığından, hastaların önemli bir bölümüne gereksiz bir cerrahi işlem uygulanmış olur. Hastaların bir kısmında, bu işlemden sonra meme beslenmesinin bozulması, kozmetik şikayetler ve meme başında his kaybı dahil olmak üzere komplikasyonlar ortaya çıkabilir.

İkinci bölümde, patolojik meme ucu akıntısı olan hastalarda duktal eksizyon sonrası meme kanseri yüzdesini araştırmak üzere gerçekleştirdiğimiz sistematik literatür incelemesinin sonuçlarını paylaştık. Toplamda 1108 patolojik meme ucu akıntısı olan hastanın verilerini içeren dokuz çalışmayı incelememize dahil ettik. Bu çalışmada, ameliyat sonrası biyopsi materyallerindeki tümör yüzdesini %8,1 olarak hesapladık. İncelemeye dahil edilen çalışmalarda bu oran %2,3 ile %13,5 arasında değişmekteydi. Üç çalışma, ameliyat sonrası meme ucu akıntısının nüks yüzdesini bildirmişti ve bu oran %0 ile %12 arasında değişmekteydi. İki çalışmada, toplamda üç hastada (yüzde 1'den az) işlem sonrasında patolojik bulgu

rastlanmadığı halde takip eden dönemde meme kanseri gelişimi rapor edilmişti. Bu çalışmadaki bulgular, patolojik meme ucu akıntısı olan hastalarda duktal eksizyon sonrası meme kanseri yüzdesinin düşük olduğunu göstermektedir. Meme ucu akıntısı olan 10 hastadan yaklaşık olarak 9'u iyi huylu nedenlerden dolayı ameliyat olmaktadır. Bu hasta grubunda gereksiz cerrahi işlemlerden kaçınmak için tanı yöntemlerinin geliştirilmesi gereklidir.

Duktoskopi, meme kanallarını meme başı açıklığından içeri gönderilen bir mikro-endoskopik kamera ile görüntüleme imkanı sağlayan bir yöntemdir. Bu teknik ilk olarak 1990'lı yıllarda meme ucu akıntısı tanısında alternatif bir tanısal yöntem olarak ortaya çıkmıştır. İşlem poliklinik koşullarında, lokal anestezi altında uygulanır. Bu görüntüleme yöntemi sayesinde meme kanalındaki anormallikler doğrudan görüntüleme ile tespit edilebilir, uygun lezyonlardan biyopsi alınabilir. Önceki araştırmalar, patolojik meme ucu akıntısının tanı ve tedavi sürecine duktoskopinin eklenmesinin hastaların üçte ikisinde ameliyat gereksinimini azaltabileceğini gösteriyor. Bununla birlikte, birçok ülkede duktoskopi patolojik meme ucu akıntısı tanı ve tedavisinde hala standart bir yöntem olarak kabul görmüş değil. Yönteme yönelik en büyük eleştiri bir grup hastada lezyonların bu yöntemle biyopsi almaya uygun olmamasından ötürü bu hastaların tanı veya tedavi için işlem sonrası hala ameliyata gereksinimi duymasıdır.

Üçüncü bölümde, patolojik meme ucu akıntısının tanı ve tedavisinde duktoskopinin yerini inceledik. Bu bölümde yer alan çalışmamızda duktoskopinin etkinliğini duktoskopi sayesinde azalan ameliyat gereksiniminden yola çıkarak tartıştık. Ayrıca, bu makalede duktoskopinin artık Hollanda'da meme kanseri tanı ve tedavi kılavuzlarında yer aldığını ve Hollanda'da sağlık sigortası kapsamına alındığını bildirdik.

Duktoskopi prosedürü, hastaların üçte ikisinde başarılı sonuç vermektedir. Ancak hastaların üçte birinde, farklı nedenlerle, örneğin dar meme kanalları nedeniyle kanalın delinme riski, teknik sorunlar veya biyopsi alınamaması gibi sebeplerle işlem bir sonuca varamadan sonlanabilmektedir. Bu nedenle, duktoskopi prosedürü sonrasında bu grup hastalarda meme ucu akıntısı şikayeti hala devam edebilir ve kesin tanı ve tedavi için yine de cerrahi müdahale gerekebilir.

Dördüncü bölümde, tekrarlayan patolojik meme ucu akıntısı yaşayan hastalarda ikinci bir duktoskopi prosedürünün faydasını araştırdık. Temel amaç, ikinci bir duktoskopi prosedürünün yapılması halinde kaç ameliyatın önlenebileceğini araştırmaktı. Toplamda, iki kez duktoskopi prosedürü geçiren 17 hastayı bu çalışmamıza dahil ettik. İlk duktoskopi sırasında, on hastada (%58,8) polip benzeri bir lezyon saptandı, üç hastada herhangi bir anormallik bulunamadı (%17,6). Dört hastada (%23,5) meme kanalları görüntülenemediği için işlem sonlandırıldı. İşlem sonrasında tüm hastalarda meme ucu akıntısı şikayeti devam etti. İkinci duktoskopik işlemden sonra on hastada (%58,8) meme ucu akıntısı sonlandı, ancak yedi hastada (%41,2) devam eden akıntı şikayeti ile cerrahi müdahalede bulunuldu. Patolojik inceleme sonucunda bir hastada herhangi bir anormallik görülmezken, beş hastada papillom ve bir hastada öncü meme kanseri saptandı. Bu araştırmanın bulguları, başarısız ilk duktoskopi denemesinin ardından tekrarlayan patolojik meme ucu akıntısı yaşayan hastaların tanı ve tedavisinde ikinci bir duktoskopi prosedürünün düşünülebileceğini ve bu işlemin vakaların yaklaşık %59'unda gereksiz cerrahi işlemleri önleyebileceğini desteklemektedir.

Duktoskopi teknolojisinin tanı ve tedavi etkinliğini geliştirmek amacıyla UMCU ve TU Delft yeni endoskopik biyopsi teknikleri üzerine bir işbirliği başlattı. **Beşinci bölümde**, bu işbirliği kapsamında duktoskopi için özel olarak tasarlanmış yeni biyopsi iğnelerinin geliştirilmesini ayrıntılı bir şekilde ele aldık. Üç farklı iğne tasarımı jelatin doku örnekleri ve mastektomi örnekleri (cerrahi ile çıkarılan meme dokusu) üzerinde test edildi. Bu çalışmanın bulguları tasarlanan ultra ince biyopsi iğnelerinin duktoskopi sırasında intraduktal biyopsi almak için uygun olduğunu gösterdi. Bu üç iğneden ikisi klinik araştırmalarda kullanılmak üzere optimize edilmek için daha detaylı geliştirilme sürecine alındı.

Altıncı bölüm, duktoskopi işlemi sırasında kullanılabilecek üç yeni tekniğin klinik çalışmalarda test edilmesi için hazırlanan protokolü içermektedir. Bahsi geçen üç yeni teknik sırasıyla, Narrow-Band görüntüleme, TU Delft tarafından geliştirilen iki yeni biyopsi iğnesi ve duktoskopi ile eş zamanlı lazer tedavisidir. Bu yeni tekniklerin araştırılmasının başlıca hedefi, patolojik meme ucu akıntısı olan hastalarda duktoskopinin tanısal doğruluğunu ve tedavi etkinliğini artırmak ve bu yeni yaklaşımların klinik uygulanabilirliğini incelemektir. Duktoskopi tekniğinin

geliştirilmesi, radyolojik anormallikleri olmayan patolojik meme ucu akıntısı olan hastalarda gereksiz cerrahi müdahalelerin azaltılmasına katkı sağlayabilir.

Önceki bölümde bahsi geçen yeni tekniklerden lazer duktoskopi tedavisi, **yedinci bölümde** daha detaylı ele alınmaktadır. Lazer halihazırda meme dışındaki pek çok organın hastalıklarının tedavisinde kullanılmaktadır, ancak bu tekniğin duktoskopi sırasında kullanımının klinik pratikte henüz yeri yoktur. Bu çalışma, bu tekniği ilk defa uygulayann bir çalışmadır ve Ekim 2022 ile Aralık 2023 tarihleri arasında gerçekleştirilmiştir. Radyolojik olarak kötü huylu bir anomali şüphesi olmayan patolojik meme ucu akıntısı yaşayan yetişkin kadınlar bu çalışmaya dahil edilmiştir. Çalışmaya dahil edilen hastaların şikayet olan memelerine duktoskopi prosedürü sırasında biyopsi alımını takiben intraduktal lazer tedavisi uygulanmıştır. Kullanılan lazer, gücü 1 ile 4 Watt arasında değişen Thulium lazerdir. Tedavi, 9 hastada başarıyla uygulanmıştır. Müdahale sonrasında, tüm hastalarda meme ucu akıntısı kesilmiş ve herhangi bir komplikasyon kaydedilmemiştir. Bu araştırma, meme kanal anormallikleri olan hastalarda duktoskopi sırasında lazer tedavisinin mümkün ve güvenli olduğunu göstermektedir. Bu nedenle, duktoskopi sırasında lazer tedavisi, duktoskopinin terapötik müdahale olanaklarını artırma potansiyeline sahiptir. Lazer duktoskopisinin terapötik etkisini değerlendirmek için bu çalışmanın sonuçlarının daha büyük bir klinik çalışma ile doğrulanması gereklidir.

Sekizinci bölümde, duktoskopinin nadir bir komplikasyonunu içeren bir vaka tanımlanmaktadır. Vakaya konu olan hasta, duktoskopi prosedürüne tabi tutulan patolojik meme ucu akıntısı yaşayan 41 yaşında kadın bir hastadır. Hastada duktoskopi işlemini takip eden süreçte abse gelişmiş, yapılan histopatolojik inceleme sonucu absenin granülomatöz mastit sonucu oluştuğu ortaya çıkmıştır. İdiyopatik granülomatöz mastit, nedeni bilinmeyen, nadir, iyi huylu, kronik bir iltihaplı meme hastalığıdır ve atipik bir prezantasyonla ortaya çıkar. Etkinliği kanıtlanmış bir tedavisi yoktur. Duktoskopiden sonra granülomatöz mastit gelişen bu vaka, literatürde duktoskopiden sonra granülomatöz mastit görülen tek vakadır.

Dokuzuncu bölümde, patolojik meme akıntısı yaşayan hastalarda meme akıntısının ayırıcı tanısı için mikroRNA analizi uyguladık. Bu çalışmanın hedefi, int-

raduktal polipler ile normal meme dokusu ayırımında kullanılabilecek mikroRNA'ları tanımlamaktı. Patolojik meme akıntısı yaşayan ve duktoskopi prosedürü uygulanacak 27 hastadan meme akıntısı örnekleri topladık ve inceledik. Alınan örneklerde toplamda 22 farklı mikroRNA'nın görülme sıklığını araştırdık. Test edilen mikroRNAlar içinde yalnızca miR-145-5p'nin poliplerde görülme sıklığında normal dokuya göre istatistiksel olarak anlamlı bir fark bulundu. MiR-145-5p ekspresyon farklılığı meme akıntısı yaşayan hastalarda polipleri normal meme dokusunu ayırt etmede potansiyel bir tanı belirteci olarak kullanılabilir. Bu tekniğin klinik uygulanabilirliğini test etmek için daha fazla klinik araştırma ve validasyon çalışmaları gereklidir.

Ikinci Kısım: Meme kanserinin cerrahi tedavisinde yeni bakış açıları

Günümüzde gelişmiş ülkelerde meme kanseri teşhisi konmuş hastaların büyük çoğunluğu tanı esnasında erken evre hastalık ile başvurur. Meme koruyucu cerrahi veya lumpektomi olarak da bilinen işlem, erken evre meme kanseri ve neoadjuvan kemoterapi sonrası lokal ilerlemiş tümörler için standart bir tedavi seçeneğidir. Meme koruyucu cerrahisi, iyi kozmetik sonuçlar sağlama ve meme fonksiyonunu koruma olanağı sunar. Bununla birlikte, onkolojik rezeksiyon genellikle beklenenden daha geniş olur, bu da bazen plastik cerrahın önceden belirlenen planlarından sapmasına neden olur. Rekonstrüksiyonun en iyi şekilde planlanabilmesi için lumpektomi boyutunun doğru bir şekilde tahmin edilmesi gereklidir.

Bölüm 10'da, lumpektomi boyutunu tahmin etmek için preoperatif belirleyicilerin neler olabileceğini araştırdık. Bu çalışma, 2018 ve 2020 yılları arasında iki hastanede gerçekleştirildi. Toplamda, yaş ortalaması 58 olan 410 hasta çalışmaya dahil edildi. Beden-kitle indeksi, sütyen ölçüsü ve preoperatif radyolojik tümör çapının, postoperatif lumpektomi boyutunu belirleyen ana faktörler olduğu belirlendi. Tahmini lumpektomi boyutunu hesaplayabilen bir model geliştirdik. Bu model, plastik cerrahların hastalarını daha hassas bir şekilde hazırlaması ve bilgilendirmesi açısından faydalı olabilir. Ancak, bu modelin klinik ortamda doğrulanabilmesi için daha fazla araştırma yapılması gerekmektedir.

Meme koruyucu cerrahi sonrası pozitif cerrahi sınırlar önemli bir sorun teşkil eder. Bu, tümör nüksü için önemli bir risk faktörüdür ve pozitif cerrahi sınır bulunan hastalarda ek cerrahi müdahaleler veya adjuvan radyoterapi verilmesi gerekebilir. Bu nedenle, pozitif cerrahi sınırların görülme sıklığını en aza indirmek büyük önem taşır.

11. bölümde, meme koruyucu cerrahi geçirmiş hastalarda pozitif cerrahi sınırları etkileyen risk faktörlerini araştırdık. 410 hastanın yer aldığı bu kohort çalışmasında, ameliyat sonrası pozitif cerrahi sınırlara sahip hastaların oranı %8 olarak belirlendi. Bu oran, daha düşük hacimli hastanede (%14,9) diğer hastaneye (%5,9) göre daha yüksekti. Tümör tipi (invaziv lobüler karsinom), ameliyat sonrası tümör boyutu, hastanede ameliyat edilen vaka sayısı ve lumpektomi boyutu meme koruyucu cerrahi sonrası pozitif cerrahi sınırlarla anlamlı derecede ilişkili faktörler olarak belirlendi. Ayrıca, iki hastane arasında ameliyat edilen hastaların yaş ortalaması, invaziv lobüler karsinomun varlığı, tümör boyutunun yanlış tahmin edilmesi, MRI sayısı, lumpektomi boyutu ve pozitif cerrahi sınırlarının yüzdesi gibi değişkenlerde farklılıklar gözlendi. Bu araştırma, meme koruyucu cerrahide pozitif cerrahi sınırların genel olarak düşük olduğunu (%8) gösterdi. Ancak, bu oran hastaya, tümör tipine, radyolojik görüntülemenin doğruluk derecesine ve cerrahi işleme bağlı olarak değişkenlik gösterebilir. Daha iyi onkolojik sonuçlar elde edebilmek için cerrahlar ve radyologlar arasındaki işbirliğinin optimize edilmesi hedeflenmelidir.

12. ve 13. bölümde, iyi veya kötü huylu meme lezyonlarının cerrahi yaklaşımına ilişkin güncel yaklaşımları tartıştık. Ayrıca, bu alandaki gelecek öngörülerimizi paylaştık ve ileride yapılabilecek araştırmalar için bazı önerilerde bulunduk.

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"And, when you want something, all the universe conspires in helping you to achieve it"

Paulo Coelho, The Alchemist

ABOUT THE AUTHOR

Seher Makineli was born on the 27th of March 1995 in Wageningen to Turkish parents. She grew up in Veenendaal with her mother, sister Tuğba, brother Yasin, and younger sister Beyza.

From a young age, Seher aspired to become a doctor. With this dream in mind, she completed her high school studies at Rembrandt College in Veenendaal. She then pursued her medical degree at the Vrije Universiteit in Amsterdam (2013 – 2019). During her studies, Seher worked as a teaching assistant in the Anatomy department, gave first aid lessons and suturing lessons. She also participated in several research groups. One of the highlights of her university years was co-founding a diversity commission at the Faculty of Medicine, aimed at making the curriculum more inclusive. Seher graduated as a medical doctor at the age of 24.

Seher is married to Selçuk Baran, and they live together in Amsterdam. Outside of work, Seher enjoys visiting film houses, exploring new restaurants and small cafes, and engaging in sports. As an ambitious and curious traveler, she has explored diverse destinations across Europe, Asia and the Americas. She also cherishes spending time and making memories with her family and friends.

As a medical doctor, Seher started her career in the Surgery department at Gelre Ziekenhuizen. After a year, she became a PhD candidate at the Department of Surgical Oncology at UMC Utrecht, where her research focused on improving surgical care for patients with breast diseases. The results of her research are presented in this thesis. During her career, Seher developed a strong interest in reconstructive and hand surgery. In March 2024, she started as a non-training resident in Plastic Surgery at the Haaglanden Medical Center. Seher aspires to become a caring and skilled Plastic Surgeon, with plans to continue her research in this field.