Changing patterns in diagnosis and treatment of ductal carcinoma in situ of the breast and consequences for clinical outcome

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Changing patterns in diagnosis and treatment of ductal carcinoma in situ of the breast and consequences for clinical outcome

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Philip Meijnen

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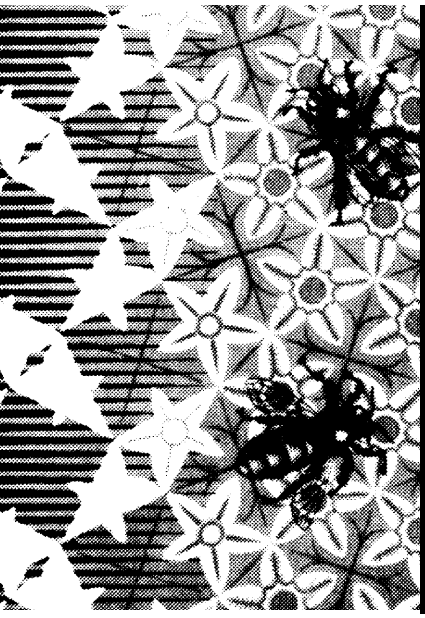

Chapter

1

Introduction and outline of the thesis

Introduction

Ductal carcinoma in situ (DCIS) of the breast refers to a proliferation of abnormal epithelial cells within the basement membrane of the mammary ductal system, without the presence of stromal invasion. It is a non-obligate precursor of invasive carcinoma and does not fully express the malignant phenotype of unlimited growth, invasiveness, angiogenesis, and metastatic potential. The diagnosis of in situ carcinoma was first recognized as an entity in 1893 by Bloodgood[1] and was characterized by the exudation of many grayish-white, granular cylinders, so called comedos, after the lesion had been cut into and pressed on. The concept of preinvasive carcinoma was initially postulated by MacCarty in 1911[2] and was firmly established in the early 1930s. By that time the term carcinoma in situ was introduced.[3] The first time that this lesion was referred to as ductal carcinoma in situ was in 1950,[4] although the concept of DCIS being a noninvasive lesion was hampered by the presence of invasive carcinoma in mastectomy specimens after initial biopsies showing only DCIS.[5,6] These findings were sufficient to indicate a modified radical mastectomy for patients diagnosed with DCIS. This procedure consists of the removal of the entire breast including an axillary lymph node dissection. It was not until the early 1980s that Lagios et al. observed less risk of associated occult invasive carcinoma in small DCIS lesions compared to large DCIS lesions.[7] These findings justified the potential role of breast-conserving treatment for DCIS, a treatment, which became established for invasive breast cancer from 1985.[8] From that period several randomized clinical trials were initiated to investigate the effect of radiotherapy in breast-conserving treatment for DCIS including the European Organisation for Research and Treatment of Cancer (EORTC) 10853 study.[9] Since lymphatic dissemination and lymph node involvement should not occur in DCIS by its noninvasive nature, axillary staging and treatment are not considered to be an integral part of the treatment of DCIS. However, in large DCIS, invasive foci cannot be excluded. This notion has brought surgeons to perform (partial) axillary dissection together with mastectomy in larger DCIS or, more recently, a sentinel node procedure together with breast-conserving treatment or simple mastectomy in DCIS at risk for invasion.

The implementation of the nation-wide breast cancer screening program in the Netherlands around 1990 led to a strong increase in the diagnosis of in situ carcinomas.[10] The crude incidence rate of DCIS increased from 4.9 per 100.000 women in 1989 to 13.6 per 100.000 women in 2003. In 2003, 12,801 women were diagnosed with breast cancer including 1,114 women with DCIS.[11] The clinical presentation of DCIS changed from a symptomatic finding of a palpable mass or nipple discharge to non-palpable lesions detected by the presence of microcalcifications on mammographic screening. Further, minimal invasive core biopsy replaced surgical excision biopsy as method of diagnosis to confirm DCIS.[12]

DCIS comprises a heterogeneous group of lesions varying in (morphology and) malignant potential. Although DCIS is considered a precursor for invasive cancer, not all lesions will

progress to invasive malignant disease. Progression to invasive breast cancer was observed in 39% of low grade DCIS in women treated by biopsy alone.[13] If DCIS shows progression, it is most likely that low grade lesions transform into low grade invasive cancer and high grade lesions into high grade invasive cancer.[14] Unfortunately, we can not accurately predict which DCIS lesions will progress to invasive breast cancer and which not. Therefore, the optimal treatment for DCIS is controversial. Mastectomy is associated with the best control rates but might be considered over-treatment. Breast-conserving treatment followed by adjuvant radiotherapy has become an acceptable alternative for smaller lesions and provides survival rates comparable to that after mastectomy. Radiotherapy following breast conserving surgery achieved a significant reduction in the risk of DCIS and invasive local recurrence compared to local excision alone.[9] Nonetheless, approximately 50% of the patients with local failure have an invasive local recurrence and harbour the risk of developing distant metastasis. Identification of patients at high risk of recurrence is critical for improvement of treatment in patients diagnosed with DCIS.

Outline of the thesis

This thesis focuses on the changes in classification, diagnosis, and treatment of DCIS and on consequences of these changes on clinical outcome. Firstly, is there an alternative to the current, morphology-based classifications which would enable early adjustment and tailoring of the treatment strategy? And, in what way will new diagnostic tools have impact on the management of patients with DCIS; for instance can the risk of invasion on final pathology be predicted after initially diagnosing DCIS on core biopsy? Further, can we refine treatment advice to achieve better outcomes on the basis of long term results of randomized clinical trials combined with the experience of a single institute that is dedicated to the treatment of patients with breast cancer?

Chapter two of this thesis provides information on the pathology and molecular markers of DCIS, including different histological classification systems, genetic alterations and a model for breast carcinogenesis. In Chapter three the use of immunohistochemistry for improved classification of DCIS in comparison with the current histological classification system is addressed. The influence of new diagnostic and therapeutic tools like mammographic screening, core biopsy and reconstructive surgery on clinical management are described in Chapter four. Chapter five deals with the risk of invasion and axillary lymph node metastases in 172 patients with DCIS diagnosed on core biopsy and an attempt is made to select criteria in which patients sentinel node biopsy might be warranted. Chapter six describes the clinical outcome of 504 patients with DCIS who underwent final surgery at The Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital and outlines the current practice of clinical management for this disease. In addition, risk factors for local recurrence are identified. The

10 years results of the EORTC 10853 randomized trial investigating the role of radiotherapy after local excision of DCIS in 1010 patients are presented in Chapter seven. An overview of the randomized clinical trials undertaken in DCIS is given in Chapter eight. The thesis ends with concluding remarks and a description of future prospects in Chapter nine, while a summary of the presented results is given in Chapter ten.

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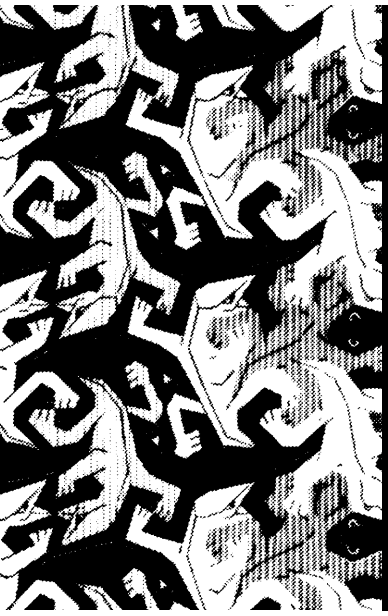

Chapter

2

Pathology and molecular markers

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CHAPTER 2

Introduction

Ductal carcinoma in situ (DCIS) represents a proliferation of malignant epithelial cells within the ducts of the breast, without invasion through the basement membrane. It is assumed that all invasive carcinomas of the breast are preceded by DCIS; however, it is not known what proportion of DCIS, if left untreated, will progress to invasive carcinoma. Before the introduction of population-based mammographic screening, most cases of DCIS were detected by clinical symptoms, including palpable mass, nipple discharge, or Paget's disease of the nipple. At mammography, DCIS is usually detected by typical patterns of microcalcifications.[1] The incidence of carcinoma in situ (including DCIS and lobular carcinoma in situ, LCIS) of the breast accounts for approximately 20% of screen-detected breast cancers, compared to 3-5% of all symptomatic cancers before the period of population-based mammographic screening.[2] The spread of DCIS through the ductal system is segmental, continuous and often extensive at the time of diagnosis.[3] When symptomatic disease led to the diagnosis of DCIS, the treatment of choice was usually mastectomy. Mastectomy often represents overtreatment for nonsymptomatic screen-detected DCIS, especially since breast-conserving treatment has become a generally accepted alternative for mastectomy in early invasive breast cancer. Moreover, not all cases of DCIS progress to invasive carcinoma within the lifetime of the patient. It is not possible, however, to reliably predict the biological behavior of DCIS.

DCIS is a heterogeneous spectrum of lesions, varying in morphology, extent and clinical presentation and it is evident that the degree of cytonuclear differentiation of DCIS corresponds with the malignancy grade of its invasive recurrence.[4] The risk of recurrence, however, does not differ much between well-differentiated DCIS and poorly differentiated DCIS.[5] Assessment of risk factors associated with histopathologic characteristics, and, more recently, genetic alterations in DCIS have become an important research area in recent years. Table 1 (randomized clinical trials in DCIS) depicts the results from four randomized clinical trials demonstrating that breast-conserving treatment followed by radiotherapy is a good alternative to mastectomy.[6-8] Although not designed to define subgroups with varying risks for recurrence, most of these studies reported that young age (less than 40 years), involved margins, decreasing width of tumor-free margins, and poorly differentiated DCIS were associated with increased risk. Still, a reliable assessment of risk in individual cases is not possible.

The impact of treatment of DCIS on breast-cancer-specific survival is not clear yet. Thus far, randomized studies suggest an equal survival after local surgery alone or surgery followed by radiotherapy, although recurrence rates differ. The incidence of metastatic disease and death after breast-conserving therapy is comparable with that after mastectomy, in general less than 2%. Taking the risk of a delayed (salvage) mastectomy for recurrent tumor may therefore be an acceptable alternative for immediate mastectomy. However, it will be of great clinical benefit if histological or genetic factors can be identified that accurately predict which cases of DCIS are likely to progress to metastasizing invasive breast cancer in order to use these markers to tailor treatment.

Table 1. Randomized clinical trials in ductal carcinoma in situ (DCIS)

Study and reference	No. of pts.	FU (yrs)	Number of ipsilateral recurrences (invasive and noninvasive)			
			Excision alone	Excision plus adjuvant RT	Excision plus tamoxifen	Excision plus RT plus tamoxifen
NSABP B-17[7]	818	12	32%	16%	-	-
NSABP B-24[7]	1804	7	-	11%	-	8%
EORTC 10853[6]	1010	10	26%	15%	-	-
UKCCCR DCIS trial[8]	1694	4	14%	6%	13%	15%

FU: follow-up; RT: radiotherapy; NSABP: National Surgical Adjuvant Breast and Bowel Project; EORTC: European Organization for Research and Treatment of Cancer; UKCCCR: United Kingdom Coordinating Committee on Cancer Research

It should be remembered that it is difficult for the surgeon to identify the resection margins for DCIS; evaluation of the margins by the pathologist requires sampling guided by the microcalcifications. In one study of 469 patients with DCIS it was demonstrated that radiation therapy did not lower the recurrence rate when the DCIS was excised with margins of 10 mm or more. In addition, among patients with margin widths of 1 to <10 mm there was no statistically significant benefit from postoperative radiation therapy. There was a statistically significant benefit from radiation among patients in whom margin widths were less than 1 mm.[9] It has been commented, however, that this study lacked a multivariate analysis and that longer follow-up and confirmation in independent patient series is required.[10,11]

The management of DCIS is currently directed mainly by histological classification, which is discussed in the next section of this chapter. The third section summarizes what is known about the genetic alterations in DCIS. Based on current knowledge, we propose a multi-step model for the progression of breast cancer, which may provide insight into the molecular mechanisms underlying breast carcinogenesis. And finally, future directions for the genetic research of DCIS are discussed.

Table 2. Classification systems for ductal carcinoma in situ

Authors	Reference	Defining features			No. of categories
		Cytonuclear	Architectural	Necrosis	
Lagios et al.	14	yes	yes	yes	3
Ottesen et al.	15	no	yes	yes	3
Bellamy et al.	16	no	yes	yes	4
Poller et al.	17	no	no	yes	3
Holland et al.	18	yes	yes	no	3
Silverstein et al.	19	yes	no	yes	3
Scott et al.	20	yes	yes	yes	3
Tavassoli	21	yes	no	yes	3
Sloane et al.	22	yes	yes	no	3
Sloane et al.	22	yes	yes	no	2
Warnberg et al.*	23	yes	no	yes	2

*: including molecular markers as defining features

Histological classification

Ductal carcinoma in situ

For a long time, the textbook classification has been based on its architectural growth pattern, dividing it into solid, comedo, cribriform, (micro)papillary and clinging variants.[12] In recent years it has become clear that cytonuclear differentiation of tumor cells is more important than architectural growth patterns, and various novel classifications of DCIS have been proposed (Table 2).

Almost all modern classifications separate DCIS into three categories, but differ in the choice of features that are used for categorization. As DCIS type and the grade of coexisting or successive invasive carcinoma are related, it seems reasonable to use similar criteria for grading DCIS as those that are used to grade invasive carcinoma. We therefore prefer to classify DCIS based on cytonuclear features, architectural differentiation (polarization of cells on lumens, comparable with tubule formation), and mitotic activity (Table 3 and Figure 1), similar to the features used to assess histological grade of invasive carcinomas (Ellis-Elston).[13]

Unfortunately, there is marked interobserver variability for the assessment of histological type in DCIS, especially for lesions in the intermediately differentiated group. When testing the Holland classification of DCIS an overall κ value of 0.37 was found. The κ statistics for the three categories were 0.45 (poorly differentiated), 0.19 (intermediately differentiated), and 0.49 (well differentiated). Interobserver variability was similarly marked for the other

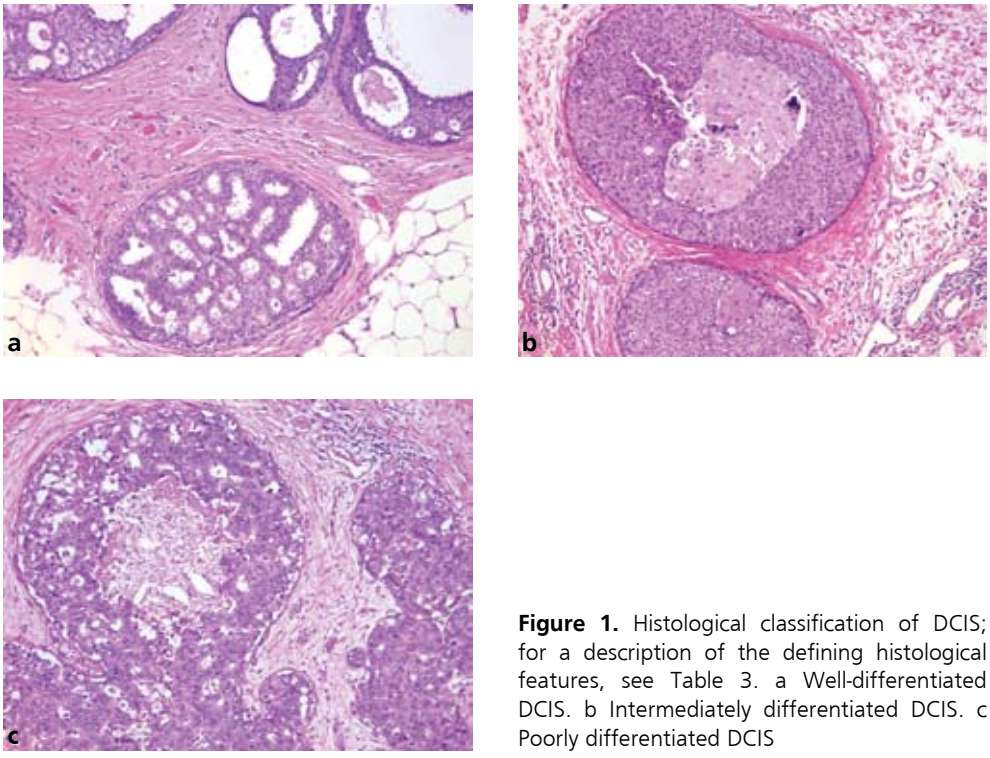

Figure 1. Histological classification of DCIS; for a description of the defining histological features, see Table 3. a Well-differentiated DCIS. b Intermediately differentiated DCIS. c Poorly differentiated DCIS

Table 3. Histological classification of DCIS according to the Holland classification (adapted from Holland et al.[18])

	Poorly differentiated	Intermediately differentiated	Well-differentiated
Defining features			
Primary			
Nuclei	Pleomorphic +++ Variation in size, irregular outline and spacing	Pleomorphic + Some variation in size, outline and spacing	Monomorphic Uniform size, regular outline and spacing
Chromatin	Coarse, clumped	Fine to coarse	Uniform, fine
Nucleoli	Prominent	Evident	Insignificant
Mitoses	Often present	Occasionally present	Rare
Secondary			
Architectural differentiation	Absent or minimal	Present	Marked
Frequently associated features			
Central necrosis	Usually present often prominent	Variable	Absent or minimal
Individual cell necrosis and autophagocytosis	Usually present	May be focally present	Absent
Growth pattern	Solid, clinging or pseudo-micropapillary/ cribriform	All patterns	Clinging, micropapillary cribriform or rarely solid
Calcification	Amorphous	Amorphous or laminated	Laminated, psammomalike or rarely amorphous

histological classification systems for DCIS.[22] It is unlikely that the interobserver reproducibility in the histological classification of DCIS can be much improved. Therefore, histological classification alone is probably insufficient to guide therapy in individual patients.

Intraductal epithelial proliferations

If we want to discuss the molecular alterations in DCIS, we should have a uniformly agreed definition on what DCIS is. In addition to the lesions described in the previous section, there are various intraductal epithelial proliferations that may have similarities to DCIS, which are considered by some to be precursors to DCIS, or are even diagnosed as DCIS in some instances. The relationship between these intraductal proliferations with DCIS is discussed in this section. It is a matter of debate to what extent intraductal epithelial hyperplasias of the usual type (usual ductal hyperplasia, UDH) and DCIS are related. The majority of intraductal proliferations can be reliably categorized as either (benign) epithelial hyperplasia or carcinoma in situ. In rare cases, it may be difficult or even impossible to distinguish a small focus of well-differentiated (cribriform) DCIS and UDH, and the term atypical ductal hyperplasia (ADH) is often used for such lesions. For lesions categorized as ADH there is an extremely high

interobserver variability even among expert breast pathologists.[24] This is probably due in part to different conceptual ideas on intraductal proliferations. Some consider UDH, ADH and DCIS as steps in the development of cancer; in their opinion ADH is a borderline lesion with features of both UDH and DCIS. We, and others, believe that DCIS is the only recognisable precursor of invasive carcinoma, a clonal proliferation sharing many genetic changes with invasive carcinoma. UDH is a multiclonal proliferation; although correlated with increased risk to develop breast cancer, UDH is not, in our view, a precursor lesion of invasive carcinoma. In this concept, there is no place for ADH as a borderline lesion between UDH and DCIS. Theoretically, genetic studies could help to resolve this issue, but they are hampered by lack of a gold standard in classification and the small number of lesions studied.

More recently, the term ADH has also been applied to - often extensive - lesions that are considered as DCIS (well-differentiated clinging type) by some, and benign (columnar cell alterations, and many other names) by others.[25] In view of the coincidence of this lesion with micropapillary DCIS and tubular carcinoma, it appears to be an early stage of DCIS. As follow-up studies do not show an increased risk of breast cancer when left untreated, some investigators have advocated avoidance of the term in situ carcinoma for these lesions.[5,26] In the World Health Organization classification, these lesions are defined as flat epithelial atypia.[27] As these lesions appear to be part of the spectrum of well-differentiated DCIS, we believe that they can best be classified as well-differentiated DCIS with a clinging/ micropapillary growth pattern. For clinical management of these lesions, watchful waiting with yearly mammography can be considered. In the interpretation of genetic studies of DCIS, these lesions should be considered as part of the spectrum of DCIS and not as ADH or columnar alterations with apical snouts.

Genetic alterations

The transformation of normal cells to invasive and metastatic cancer cells is a multistep process that may take many years. As discussed in the previous sections, one of the models of breast carcinogenesis proposes that normal epithelium becomes proliferative and subsequently atypical, and eventually evolves into carcinoma in situ and than to invasive carcinoma. Although proliferation is a risk factor for breast cancer, in our view, the only intraductal proliferation that can be considered as obligate precursor to every invasive breast cancer is carcinoma in situ.

The genetic alterations found in breast cancer are amplification of oncogenes and inactivation of tumor suppressor genes. For invasive breast cancer, there is extensive research to link knowledge of genetic alterations with clinical outcome. An important reason for this is that the assessment of prognosis affects treatment of breast cancer patients. This will also be true for DCIS: if the risk of progression to invasive carcinoma can be assessed more reliably, patient-tailored treatment for DCIS will greatly improve.

If it can be predicted which cases of carcinoma in situ will progress to invasive breast cancer and how long this progression will take, it can, for example, be decided which patients need a mastectomy, which patients need excisional biopsy followed by radiotherapy, which patients do not require radiotherapy, and which patients can be left untreated after diagnosis (preferably by image-guided core biopsy). Specific genetic alterations in DCIS may be associated with outcome, and study of these alterations holds hope for improved diagnostic tools in DCIS.

Different methods have been used for the assessment of genetic alterations, including immunohistochemistry (IHC), fluorescent in situ hybridization (FISH), loss of heterozygosity (LOH), comparative genomic hybridization (CGH), and microarray analysis. Studies of DCIS have been hampered by the very limited availability of frozen tumor material: DCIS is usually not (easily) visible at pathologic examination, and DCIS, unlike invasive breast cancer, is rarely frozen. However, a recent study demonstrated that gene expression profiling can be performed from formalin-fixed, paraffin-embedded tissue of invasive breast cancer.[28] Here, we describe the oncogenes and tumor suppressor genes that are known to be altered during breast cancer development, focusing on their role in DCIS.

Oncogenes

A single mutation, translocation, or amplification can give rise to the activation of oncogenes. Translocations and point mutations are very rare and do not seem to play an important role in breast cancer development, but amplification of several specific chromosomal regions do.[29] For many of these frequently amplified regions one or more oncogenes have been identified, but for some it is not yet clear which oncogene is driving the amplification. Each of the amplified regions is identified in a subset of approximately 10-25% of invasive breast carcinomas. There have not been as many studies of DCIS as there are for invasive breast cancer, but it appears that the same chromosomal regions are found amplified in DCIS and with comparable frequencies as found in invasive breast cancer. This underscores the notion that DCIS should, from a biological point of view, already be considered a late stage in the development of metastasizing invasive breast cancer. Of course the biologically important result of oncogene amplification is overexpression of the relevant oncoprotein. This has proven useful in studies of DCIS, because the IHC detection of these overexpressed proteins is often much easier than study of the gene amplification event. This is especially true for DCIS, where it is difficult to isolate DNA from the tumor tissue as this often required microdissection.

The three oncogenes that have been studied most extensively in DCIS (HER-2, cyclin D1, and C-MYC) are discussed in the following sections. There are also several other chromosomal regions that have been found to be amplified in DCIS and these are discussed as well.

Amplification of the HER-2 gene is frequent in DCIS

The HER-2 gene, also known as c-erbB2 or neu, is located on chromosome 17q12, and encodes a cell-membrane-located growth factor receptor. The gene is amplified in 15-25% of invasive carcinomas.[30-32] Overexpression of the amplified gene can be detected by IHC, being available as a standard technique in all pathology laboratories.[33-36]

Several studies have shown that HER-2 overexpression in invasive breast cancer is correlated with poor prognosis, shorter overall survival,[30,37-40] and also altered response to hormonal therapy and chemotherapy.[32,41-46] In DCIS, overexpression of HER-2 is found in over 50% of the cases (Figure 2) and is predominantly associated with the poorly differentiated type.[35,47-51] FISH and Southern Blot analysis have shown that the overexpression of HER-2 in DCIS is also the result of HER-2 gene amplification.[32,50,52,53]

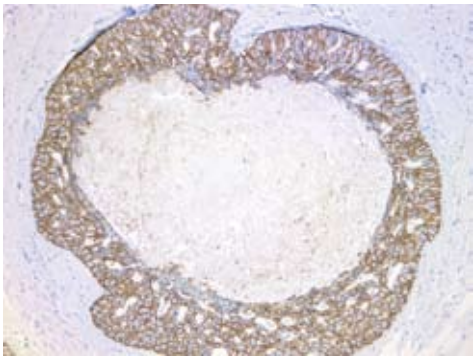

Figure 2. Poorly differentiated DCIS, stained immunohistochemically with antibodies directed against HER-2. The HER-2 protein, residing in the cell membrane, is overexpressed as a result of HER-2 gene amplification

The absence of HER-2 overexpression in normal ducts and ADH, and the frequent HER-2 amplification found in DCIS suggests that HER-2 alterations are an important event in early malignant transformation.[54-56] However, an apparent paradox is the higher frequency of HER-2 gene amplification in DCIS (50%) compared to invasive breast cancer (15-25%). The most likely explanation for this finding is that DCIS containing HER-2 gene amplification is a specific entity with a relatively low prospensity to become invasive.[57] Finally, HER-2 is very rarely overexpressed in LCIS[58,59] while Paget's disease of the nipple shows overexpression of HER-2 in most, if not all cases.[60,61] The hypothesis that HER-2-amplified DCIS has a lower likelihood of becoming invasive compared to other types of DCIS should not be confused with the notion that these are innocent lesions: when HER-2 amplified DCIS becomes invasive, the HER-2-amplified invasive carcinoma is associated with relatively poor outcome.

Recent data have demonstrated that HER-2 positivity is associated with an upregulation of cyclooxygenase type-2 (COX-2) expression in DCIS.[62] COX-2 has been linked to the process of tumorigenesis in mice.[63] Further reports have demonstrated a higher COX-2 expression level in DCIS than in invasive ductal carcinomas.[64-66] These findings would suggest that the HER-2 pathway plays a role in the upregulation of COX-2 at the preinvasive stage of breast cancer tumorigenesis.

Cyclin D1 protein overexpression in the precursors of invasive breast cancer

The cyclin D1 (CCND1) gene on chromosome 11q13 encodes a nuclear protein that is important in regulation of the cell cycle. Amplification is observed in 10-15% of primary invasive breast cancers[39,67-72] and in 10-18% for DCIS,[73,74] predominantly in estrogen receptor positive tumours.[43,74-80] It has been suggested that stepwise increases in cyclin D1 expression play a key role in the transition to ADH, and from ADH to DCIS and invasive breast cancer,[81,82] but there have not been recent findings related to this theory. The frequency of cyclin D1 protein overexpression in DCIS exceeds the frequency of DNA amplification, as is also observed in invasive breast cancer[73,74,81-84] indicating that an alternative mechanism distinct from DNA amplification may cause upregulation of cyclin D1.

C-MYC gene amplification: involved in progression of DCIS to invasion?

Amplification of the C-MYC gene was the first identified genetic alteration associated with progression from the in situ to the invasive stage of breast carcinoma. CGH and FISH analysis of invasive breast carcinoma with a large associated in situ component revealed high-level amplification of C-MYC in the invasive component only.[85] Overexpression of C-MYC, either by gene amplification or other regulatory means, has been found in 6-32% of breast tumors and breast tumor cell-lines, and is associated with locally advanced disease and poor prognosis.[37,86,87] Although in DCIS no amplification was found of C-MYC[50,85] Watson et al. found, using reverse transcriptase-polymerase chain reaction analysis that C-MYC was amplified both in the invasive lesion and in the in situ component.[88]

Tumor suppressor genes

In breast cancer, like in other tumor types, inactivation of tumor suppressor genes plays an important role. Knudson postulated in his two-hit model the classical mechanism of inactivation as functional loss of both alleles of the tumor suppressor gene.[89] In general, one allele is mutated by a relatively subtle mutation (point mutation, small insertion, or deletion), while the wild-type allele is inactivated by LOH. The presence of these subtle mutations has made it possible to identify all of the currently known tumor suppressor genes. Inactivation of tumor suppressor genes by epigenetic mechanisms, most notably methylation, is emerging as yet another mechanism for their inactivation.[90] From a technical point of view, inactivation of tumor suppressor genes is more difficult to study than oncogene amplification, especially from lesions where DNA from frozen tumor cells is harder to come by, such as DCIS. To date, the most well characterized tumor suppressor genes in carcinoma in situ are p53 and E-cadherin. Frequent LOH points to the existence of a role for more as yet identified tumor suppressor genes. Since CGH detects loss of chromosomal regions (which may also point to the existence of tumor suppressor genes in the deleted regions), LOH and CGH are discussed together, including discussion of the chromosomal regions that can be found amplified with CGH.

Inactivation of the p53 gene in DCIS

The p53 gene, located on 17p13.1 is critical in inducing G1 arrest in response to DNA damage, allowing the activation of DNA repair mechanisms prior to the entry of the cell into the S-phase, or allows signaling for apoptotic death of the cell.[91] Abnormalities in p53 may result in unchecked cell proliferation and development of a malignant clone.

Inactivating p53 mutations are found in approximately 20% of invasive breast carcinomas,[92,93] and have been associated with poor prognosis and resistance to chemotherapy.[94-98] P53 mutations have been demonstrated in 40% of high-grade DCIS lesions, while its frequency is very rare in low- and intermediate-grade DCIS (0 and 5% respectively).[99,100] Approximately 20% of the p53 mutations lead to a truncated p53 protein, which cannot be detected by IHC; the majority of the mutations lead to the substitution of a single amino acid, resulting in p53 protein with an increased half-life, which can be detected by IHC (Figure 3). P53 mutations or p53 overexpression have not been demonstrated in ADH or other benign lesions.[32,51,101-104]

Table 4. Comparative genomic hybridization studies in DCIS

Author	No. of cases	Reference
Kuuksjarvi et al.	5	121
James et al.	9	120
Buerger et al.	38	119
Vos et al.	15	50
Aubele et al.	7	118
Waldman et al.	18	122
Boecker et al.	52	116
Aubele et al.	5	56

E-cadherin gene inactivation in LCIS but not in DCIS

The E-cadherin gene, located on 16q22.1, encodes a cell adhesion protein involved in cell-to-cell contact between epithelial cells. It has been shown that the majority of invasive lobular carcinomas exhibit an inactivating mutation in the E-cadherin gene, which is never observed in ductal carcinomas.[78,105,105,106,106,106-108] Inactivation of the E-cadherin gene results in the absence of E-cadherin protein, which can be demonstrated using IHC (Figure 4). LCIS always lacks E-cadherin, whereas DCIS is always positive for E-cadherin staining.[107,109] In invasive lobular carcinomas with an adjacent component of LCIS, it has been demontrated that the LCIS harbored the same E-cadherin mutation as the invasive component.[109] These results show that inactivation of the E-cadherin gene is an early event in the development of lobular carcinomas; and is specific to this subtype of breast cancer.[108] The classical form of LCIS can easily be distinguished histologically; there are also more pleomorphic variants, which can resemble DCIS. For these more pleomorphic variants of LCIS, E-cadherin may be valuable in characterizing cases of carcinoma in situ with indeterminate histological features.[107]

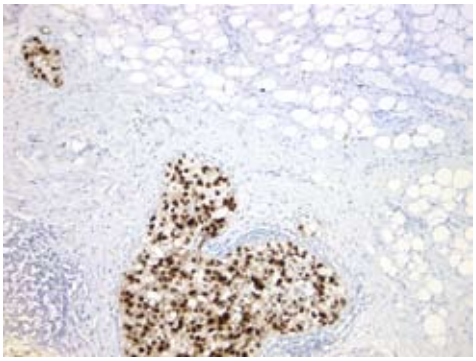

Figure 3. Poorly differentiated DCIS, stained immunohistochemically with antibodies directed against p53. P53 protein, residing in the nucleus, is overexpressed as a result of a mutation in the p53 gene, which inactivates normal p53 function. Mutated p53 protein has greater stability than wildtype p53 and can consequently be detected by immunohistochemistry

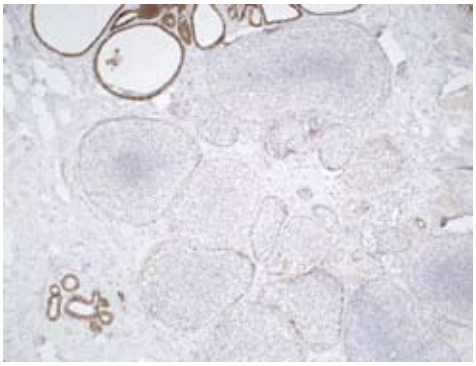

Figure 4. Lobular carcinoma in situ (LCIS), stained immunohistochemically with antibodies directed against E-cadherin. E-cadherin protein is absent in the LCIS cells, whereas a normal lobule shows normal membrane-located E-cadherin

Other tumor suppressor genes?

In DCIS there are several regions of frequent LOH, suggesting the presence of tumor suppressor genes in these regions. There are many reports on individual potential tumor suppressor genes in breast cancer and DCIS, including those on Rb[110], pTEN[111] and the IGF-II receptor gene.[112-114] Still, there is no convincing evidence that inactivation of any of these genes plays an important role in DCIS studies.

Genetic alterations detected by LOH and CGH

Since the modification of the CGH analysis for paraffin-embedded material, many studies on archival material of preinvasive disease have been performed.[115] Table 4 shows CGH studies that have been performed on DCIS. Gains have been found using CGH on DCIS on chromosomes 1q, 3p, 5p, 6q, 8q, 10q, 11q, 14q, 15q, 16, 17q, 19q, 20p, 20q, 21q, 22q, and Xq, and losses on chromosomes 2q, 4q, 5q, 6q, 8p, 9p, 11q, 13q, 14q, 16q, 17p, and 22q.[50,116-122] In DCIS, LOH was frequently identified at several loci on chromosomes 1, 3p, 11q, 8p, 13q, 16q, 17p, 17q, and 18q.[50,123-128] The various studies in which LOH was performed on DCIS are summarized in Table 5.

O'Connell et al. showed that 50% of the proliferative lesions and 80% of DCIS shared LOH with invasive carcinoma.[129] Stratton et al. were the first to show LOH in pure DCIS

Table 5. Loss of heterozygosity studies in DCIS

Author	No. of cases	Reference
Aldaz et al.	23	135
Munn et al.	19	124
Koreth et al.	83*	126
Radford et al.	61	128
Stratton et al.	132	123
Fujii et al.	41	127
O'Connell et al.	137	130
Amari et al.	23	137
Vos et al.	78	50
Moinfar et al.	16	134
Shen et al.	100*	131
Maitra et al.	13	125
Farabegoli et al.	53	132

*: including invasive ductal cancer

without adjacent invasive carcinoma,[123] providing further evidence for the fact that DCIS is likely to be a precursor of invasive carcinoma; several similar studies have confirmed these findings for DCIS[126,127,130-135] and for LCIS and synchronous invasive breast cancer.[135,136] The LOH identified at loci on 16q and 17p in invasive carcinoma and DCIS is also present in ADH[51,137,138] supporting the notion that ADH is not much distinct from (well-differentiated) DCIS. Moinfar et al. demonstrated LOH in 77% of cases of flat epithelial atypia, most commonly on chromosomes 11q, 16q, and 3p;[139] the losses at regions on 11q and 16q are similar in invasive tubular carcinoma and low grade DCIS.[140]

In addition to DCIS and ADH, genetic alterations have also been studied in UDH, where LOH is found in less than 20%.[130,138,141-143] These differences in the genetic alterations found in UDH corroborate the concept that UDH is not a direct precursor of DCIS and invasive ductal carcinoma.

A multi-step model for breast carcinogenesis

The multistep development of colon cancer has served as a model for the way in which accumulating genetic changes lead from a normal precursor cell through noninvasive neoplasms to an invasive, metastasizing malignancy.[144] Along similar lines, several different models of the evolution of DCIS to invasive breast cancer have been suggested.[32,50,116,119,145] Lakhani distinguishes a linear progression from normal epithelium to UDH to ADH to low-nuclear-grade DCIS to high-nuclear-grade DCIS to invasive carcinoma;[145] in this model, the relationship between DCIS and LCIS remains unclear. The simple model of Lakhani is,

more or less, supported by Krishnamurthy and Sneige, with evidence derived from animal experiments, epidemiology, and results from studies on genetic alterations.[146]

Buerger et al.[119] and Vos et al.[50] postulate that due to specific genetic aberrations, well-, intermediately, and poorly differentiated DCIS can arise as a result of distinctly separate pathways. They both found that LOH on chromosome 16q was present predominantly in well- and intermediately differentiated DCIS, whereas amplifications on chromosome 17 were predominant in, and restricted to poorly differentiated DCIS. The study by Buerger et al. described gain of chromosome 1q in combination with loss of 16q in intermediately differentiated DCIS and postulated that due to the gain of 1q DCIS can progress from well- to intermediately differentiated DCIS.[119] Poorly differentiated DCIS predominantly showed gains of 17q and 11q13. In the study of Vos et al., the most predominant alteration found in poorly differentiated DCIS in addition to amplification of 17q was LOH on chromosome 17.[50]

Boecker et al. postulated a morphological and genetic progression model of breast cancer in which benign proliferative breast disease is not an obligate direct precursor of DCIS.[116] Low-nuclear-grade DCIS and low-nuclear-grade invasive ductal carcinoma exhibit loss of 16q while high-nuclear-grade DCIS exhibits loss of 13q together with gains of 17q and 20q, which subsequently develop into high-grade invasive ductal carcinoma.

Allred et al. simplified the model by not distinguishing separate pathways for each histological grade, suggesting that some invasive ductal carcinomas arise directly from morphologically normal-appearing cells.[32] In addition, in this model, the progression of premalignant lesions to invasive carcinoma is not obligatory.

The biological nature of LCIS remains controversial. This has been supported by the lack of a strict definition for this lesion over time. Several studies investigated the risk of subsequent invasive disease, and LCIS was initially considered to be a high-risk marker for the development of invasive disease.[147,148] However, data demonstrated evidence for the concept that LCIS is

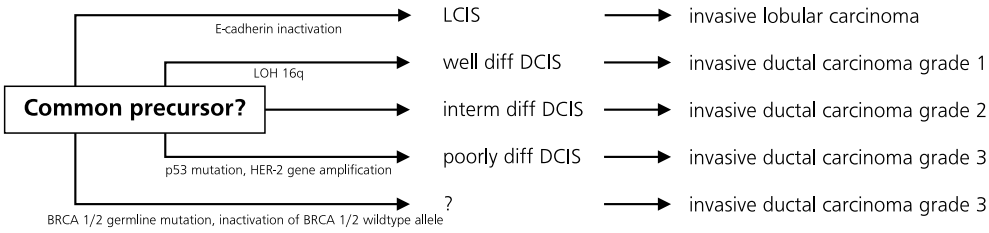

Figure 5. Multistep development of breast cancer. A common precursor cell precedes all lesions. There is a unique pathway for the development for breast carcinomas if patients with a BRCA1/2 germline mutation. Invasive lobular carcinoma has a distinct genetic development that is characterized by inactivation of the E-cadherin gene, which is already present in the LCIS stage (and never found in DCIS or invasive ductal cancer).[109] Loss of 16q is a characteristic feature in well-differentiated (*well diff*) DCIS and low nuclear grade invasive ductal carcinoma.[116] The progression of intermediately differentiated (*interm diff*) DCIS and grade 2 invasive ductal carcinoma represents a pathway that exhibits features of both well- and poorly differentiated (*poorly diff*) DCIS. The amplification of HER-2 and inactivation of p53 are involved in the development of poorly differentiated DCIS, which can progress to high-nuclear-grade invasive ductal carcinoma[35,47-51,99,100]

actually a direct precursor of invasive lobular carcinoma. LOH analysis showed the same mutations in LCIS as in the invasive component,[109] a concept that was further corroborated by findings from more recent studies.[108,149]

Future molecular genetic research will make it possible to test and refine the different models. Based on our current interpretation of the available data, we favor the model depicted in Figure 5.

Discussion and future prospects

The histological classification of DCIS has raised much interest over the past decade and there is general agreement that cytonuclear features (rather than architectural growth patterns) are eminent for classification. At the same time, it has become clear that the interobserver variation with each of the classification systems is too large to be used as a solid basis for the treatment of individual patients. Integration of genetic factors in the classification will hopefully lead to more objective criteria and reduction of this variability.

The genetic dissection of DCIS will continue and ultimately lead to a full understanding of all of the oncogene alterations and tumor suppressor gene inactivations that play a role in the development of DCIS. This will also shed more light onto the relationship between the various histological grades of DCIS, and to LCIS. New high-throughput techniques will speed up the discovery rate of genetic alterations in cancer, including DCIS. For example, gene expression profiling is already proving useful in prognostic classification of invasive breast cancer.[150]

The gene expression profiles of DCIS adjacent to invasive breast cancer have been studied by Ma et al., who used laser capture microdissection in combination with gene expression profiling.[151] Out of 36 patients, normal epithelium, ADH, DCIS or invasive ductal carcinoma was microdissected from the same patient followed by microarray analysis of these separate lesions. This study demonstrated that the gene expression profiles in the in situ component from the same tumor were very similar to that in the invasive component. However, a subset of genes that are expressed at higher levels in grade three DCIS relative to grade one DCIS are further elevated in invasive breast cancer, revealing an apparent link between tumor grade and stage progression.

Gene expression profiling of DCIS is hampered by the limited availability of frozen tissue for these lesions. Gene expression profiling using RNA isolated from paraffin-embedded tumors has been successful, and may be applied to DCIS in the future.[28]

All these developments will elucidate the genetic mechanisms leading to DCIS, but will they also help in guiding therapy? For this, large, well-annotated series of patients that have undergone various treatment protocols will be required. It will be a great challenge to acquire these patient series in the future, and the best setting to do these studies will be that of prospective randomized clinical trials of breast-conserving therapy of DCIS. As the recurrence rates are relatively low and the most relevant clinical endpoint, distant metastases, is indeed

very rare, large numbers of patients (hundreds to a few thousand) will need to be studied. This can and should be accomplished by incorporation of the collection of tissue into the clinical trial protocols of the future.

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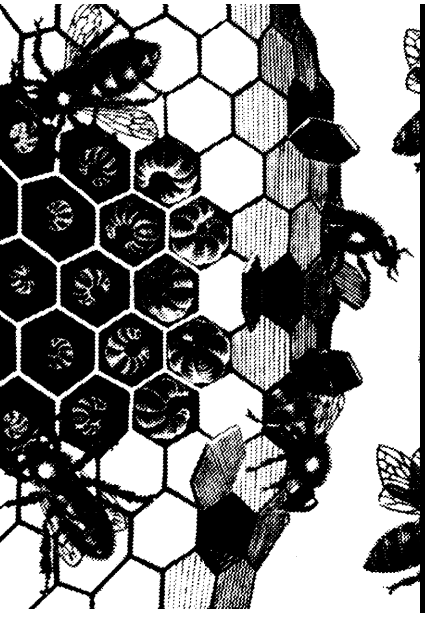

Chapter

3

Immunohistochemical categorization of ductal carcinoma in situ of the breast

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Abstract

Background: The aim of this study is to analyze whether immunohistochemistry (IHC) applying a broad set of markers could be used to categorize ductal carcinoma in situ (DCIS) of the breast in distinct subgroups corresponding the recently defined molecular categories of invasive carcinoma.

Methods: IHC of pure DCIS cases constructed in tissue arrays was performed with 16 markers (estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), Bcl-2, p53, Her2, insulin-like growth factor receptor (IGFR), E-cadherin, epithelial membrane antigen (EMA), CA125, keratins 5/6, 14, 19, epidermal growth factor receptor (EGFR), S100, and CD31). Results in 163 cases were analyzed by unsupervised hierarchical clustering. Histological classification was performed by review of whole tissue sections and identified 36 well-, 55 intermediately, and 72 poorly differentiated DCIS.

Results: Unsupervised hierarchical cluster analysis categorized DCIS in two major groups that could be further subdivided in subgroups based on the expression of six markers (ER, PR, AR, Bcl-2, p53, and Her2). In the major ER/Bcl-2-positive group three subgroups (AR-positive (n=33), AR-negative (n=40), and mixed (n=34)) could be identified and included 34 well-differentiated DCIS. Within the major predominantly ER/Bcl-2-negative group, a Her2-positive subgroup (n=34) was characterized by 31 poorly differentiated lesions. Eight triple negative lesions, including one positive for keratin 5/6 and two positive for p53, were encountered. Intermediately differentiated DCIS shared a comparable IHC staining pattern as well-differentiated DCIS distinct from poorly differentiated DCIS ($p<0.001$).

Conclusion: DCIS could be categorized by IHC into two major groups and five subgroups using six markers. Morphologically intermediately differentiated DCIS seems to have more biological similarities with well-differentiated lesions as compared to poorly differentiated lesions.

Introduction

Breast cancer encompasses a heterogeneous group of tumours, which vary in morphology, clinical presentation, and behaviour. Traditionally, breast cancers are morphologically typed according World Health Organisation (WHO) guidelines. The latest classification recognizes at least 30 different invasive tumour types.[1] There is no consensus about the classification of the non-invasive precursor of breast carcinoma, ductal carcinoma in situ (DCIS). As in other areas of pathology, a three-tier system is most often used, based on growth pattern and cytonuclear criteria, and dividing DCIS in well, intermediately, and poorly differentiated subtypes.[2] In prospective studies this classification has proven value in risk assessment of recurrence after breast-conserving treatment and progression into invasive carcinoma.[3] However, inter- and intraobserver variability is a problem inherent to morphologic tumour classification and grading and, moreover, heterogeneity within DCIS lesions is not uncommon resulting in variation in grade.[4,5] Perou et al. suggested a categorization of invasive breast cancers based on genetic profiles into ER-positive (luminal A and B) and ER-negative (non-luminal) subtypes with a further subdivision of the ER-negative types into Her2-positive and basal-like subtypes.[6] Luminal A tumours differ from luminal B tumours by a higher expression of ER-related genes and lower expression of proliferation associated genes. It was possible to make the same categorization by immunohistochemistry (IHC) using markers aimed at luminal, Her2, and basal-like features.[7] The objective of this study is to classify DCIS by marker expression in order to improve the current morphological classifications and gain insight in the biology underlying the heterogeneity in DCIS. Therefore, tissue micro arrays (TMA) were constructed from a series of pure DCIS, a large set of markers were used for IHC, and unsupervised hierarchical cluster analysis was performed to evaluate results; clustering was correlated with morphologic grade of DCIS as assessed on whole tumour slides.

Patients and methods

TMA sections were constructed taking three 0.6-mm tissue cores per case, from formalin-fixed, paraffin-embedded tumour blocks with pure DCIS of 238 patients using a tissue-arraying instrument (Beecher Instruments, Silver Spring, Maryland, USA). IHC was performed on an automated stainer after pretreatment in the autoclave in citratebuffer pH 6.0 according standardized protocols for the different antibodies at prescribed dilutions (see Table 1). Sixteen markers were used including estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), Her2, Bcl-2, p53, E-cadherin, epidermal growth factor receptor (EGFR), insulin-like growth factor receptor (IGFR), CD31, keratin 5/6, keratin 14, keratin 19, S100, epithelial membrane antigen (EMA), and CA125. The selection of antibodies was based on recent investigations in gene signature profiles in invasive breast cancer, suitability for DCIS, and availability. Staining results were semi-quantitatively scored according criteria in Table 1. The

Table 1. List of antibodies and tissue micro array scoring criteria

	Marker	Source	Dilution	Staining pattern	Cutoff point
1	ER	Neomarker	1:50	nuclear	any +
2	PR	ImmunoVision	1:500	nuclear	any +
3	AR	Neomarker	1:400	nuclear	strong >10%
4	Her2	Neomarker	1:80000	membranous	strong >10%
5	Bcl-2	DAKO	1:400	cytoplasmic	weak >10%
6	p53	DAKO	1:1000	nuclear	>25%
7	E-cadherin	Intermedico Zymed	1:2500	membranous	weak >10%
8	EGFR	Neomarker	1:200	membranous	strong >10%
9	IGFR	Neomarker	1:100	cytoplasmic and membranous	weak >10%
10	CD31	DAKO	1:50	cytoplasmic	any +
11	Keratin 5/6	DAKO	1:200	cytoplasmic	any +
12	Keratin 14	Neomarker	1:200	cytoplasmic	any +
13	Keratin 19	Neomarker	1:200	cytoplasmic	weak >10%
14	S100	DAKO	1:4000	cytoplasmic	any +
15	EMA	DAKO	1:1000	cytoplasmic and membranous	weak >10%
16	CA125	Biogenex	1:80	membranous	weak >10%

ER = estrogen receptor; PR = progesterone receptor; AR = androgen receptor; IGFR = insuline-like growth factor receptor; EMA = epithelial membrane antigen; EGFR = epidermal growth factor receptor. DAKO, Glostrup, Denmark. Neomarker, Fremont, CA, USA. Intermedico-Zymed, San Francisco, CA, USA. ImmunoVision, Springdale, AR, USA. Biogenex, San Ramon, CA, USA.

higher IHC score was considered as a final score in case of a difference between tissue cores. Cutoff points are shown in Table 1 and were directed to detect luminal and non-luminal (sub)groups. All cases were classified as well-, intermediately and poorly differentiated on the whole tumour slides according to the classification of Holland et al.[2]; in case of heterogeneity the highest grade was used for analysis. The distribution of markers and histological grade among (sub)groups was analyzed using the χ^2 test or Fisher's exact test. Tests were two-tailed and the significance level was taken 5 per cent. Discriminative markers underwent unsupervised hierarchical clustering analysis with average and complete linkage (Genesis 1.5.0, IGB-TUG, Graz, Austria) to organise TMA score data into meaningful structures, in accordance with the more complex method used for cDNA micro arrays.[8] The impact of the markers on hierarchical cluster group results was investigated to define a final set of markers for IHC classification. Correlation between markers was determined using the Spearman correlation coefficient. The agreement in classification of cases based on different hierarchical clustering methods (average linkage versus complete linkage) and different IHC classifications were assessed with the kappa statistic. A kappa value of 0.41 to 0.6 was considerate moderate agreement, 0.61 to 0.8 substantial agreement, and more than 0.8 near-perfect agreement. All analysis were performed in SPSS® 11.5 for Windows (SPSS, Chicago, Illinois, USA).

Results

Of the 238 DCIS samples, 27 (11%) did not contain tumour and 48 (20%) had incomplete IHC data due to loss of tissue. All analysis were performed on the remaining 163 cases. Median age of these patients was 50 years (range: 28-82). Seventy-three per cent of the lesions were screen-detected. Histological classification identified 36 (22%) well-, 55 (34%) intermediately and 72 (44%) poorly differentiated lesions.

Marker expression in DCIS

Table 2 presents the distribution of the different markers in DCIS. ER en PR were most frequently present in well- and intermediately differentiated DCIS ($p<0.001$), whereas Her2 expression was most frequently found in poorly differentiated DCIS ($p<0.001$). Also Bcl-2 and p53 expression was different among grades: well-differentiated DCIS were often Bcl-2 positive and p53 negative compared to poorly differentiated DCIS. Thirty-three out of 36 well-differentiated DCIS stained positive for Bcl-2 compared to 26 out of 72 poorly differentiated DCIS ($p<0.001$). Further, all well-differentiated DCIS were p53 negative while half of the poorly differentiated lesions were p53 positive ($p<0.001$). Moderately DCIS formed an intermediate group in expression of these markers with exception of AR. This marker was found positive in 28 intermediately differentiated DCIS compared to 13 well-differentiated and 19 poorly differentiated DCIS ($p=0.018$). The remaining markers showed no statistically significant association with grade of DCIS. The E-cadherin protein could be detected in all DCIS while markers for EGFR, CD31, keratin 14, S100, and CA125 showed negative staining results in all lesions. IGFR, keratin 19, and EMA were found positive in nearly all DCIS except seven. Five of these seven DCIS were poorly differentiated. Three poorly differentiated DCIS showed staining for keratin 5/6.

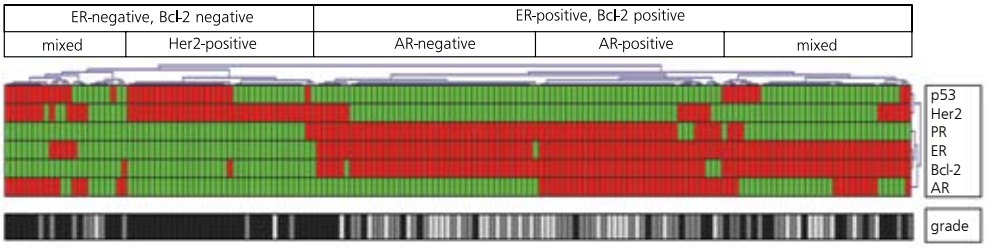

Figure 1. Two-dimensional unsupervised hierarchical cluster diagram (average linkage) of data consisting of 978 genes by 163 ductal carcinoma in situ samples. Clustergram of 6 markers and distribution of histological grade. Each column, a single case; each row, a single immunomarker. Green, negative immunostaining; red, positive immunostaining; white, well-differentiated lesion; grey, intermediately differentiated lesion; black, poorly differentiated lesion. The dendrogram shows the relatedness of the immunoprofiles of individual cases and suggests two major groups which are further subdivided in subgroups.

Table 2. Expression of markers in well-, intermediately, and poorly differentiated DCIS

Type and marker	Total (%)	Histological grade (%)			
		Well n=36 (22)	Intermediate n=55 (34)	Poor n=72 (44)	P
ER	111 (68)	34 (94)	47 (86)	30 (42)	<0.001
PR	75 (46)	26 (72)	33 (60)	16 (22)	<0.001
AR	60 (37)	13 (36)	28 (51)	19 (26)	0.018
Her2	64 (39)	1 (3)	11 (20)	52 (72)	<0.001
Bcl-2	105 (64)	33 (92)	46 (84)	26 (36)	<0.001
p53	42 (26)	0	7 (13)	35 (49)	<0.001
E-cadherin	163 (100)	36 (100)	55 (100)	72 (100)	-
EGFR	0	0	0	0	-
IGFR	157 (96)	35 (97)	54 (98)	68 (94)	0.513
Keratin 5/6	3 (2)	0	0	3 (4)	0.145
Keratin 14	0	0	0	0	-
S100	0	0	0	0	-
Keratin 19	162 (99)	35 (97)	55 (100)	72 (100)	0.170
EMA	162 (99)	36 (100)	55 (100)	71 (99)	0.529
CA125	0	0	0	0	-
CD31	0	0	0	0	-

Values in parentheses are percentages. ER = estrogen receptor; PR = progesterone receptor; AR = androgen receptor; EGFR = epidermal growth factor receptor; IGFR = insuline-like growth factor receptor; EMA = epithelial membrane antigen.

IHC categorization by unsupervised hierarchical analysis

Unsupervised hierarchical clustering analysis was applied to the IHC dataset. Based on the expression of ER and Bcl-2 the clustergram in figure 1 shows two major groups: an ER/Bcl-2-positive and an ER/Bcl-2-negative group. These two groups can be further subdivided using other marker results. The ER/Bcl-2-positive group demonstrated a completely AR-positive subgroup (n=33), a completely AR-negative subgroup (n=40), and a mixed subgroup of AR-positive and negative lesions (n=34) while the ER/Bcl-2-negative group included a completely Her2-positive cluster (n=34). The ER/Bcl-2-positive lesions included 34 (94%) well-differentiated, 46 (84%) intermediately differentiated, and 27 (38%) poorly differentiated DCIS. These poorly differentiated DCIS lesions showed markers positive for ER (all 27), Bcl-2 (n=25), PR (n=14), Her2 (n=12), AR (n=9), and p53 (n=4).

The ER/Bcl-2-negative subgroups had 45 poorly differentiated DCIS and 11 non-poorly differentiated lesions including 9 with intermediately and 2 with well-differentiated DCIS. These 11 lesions showed a marker pattern that was positive for ER (n=2), Bcl-2 (n=1), Her2 (n=7), AR (n=5), and p53 (n=2). In total, 8 ER-negative, PR-negative, and Her2-negative lesions, including one positive for keratin 5/6 and two positive for p53, were found. Table 3 shows the comparison of the distribution of histological grade among the identified subgroups.

Table 3. Distribution of markers and histological grade among cluster group after unsupervised hierarchical clustering analysis with six markers (estrogen receptor, progesterone receptor, androgen receptor, Bcl-2, Her2, and p53)

	Distribution of markers and grade by group and subgroup								
	ER positive, Bcl-2 positive group				ER negative, Bcl-2 negative group			P*	
	total	AR positive subgroup	AR negative subgroup	Mixed subgroup	total	Her2 positive subgroup	Mixed subgroup	two groups	sub-groups
No. of patients	107 (66)	33 (20)	40 (25)	34 (21)	56 (34)	34 (21)	22 (14)		
Markers									
ER	106 (99)	33 (100)	39 (98)	34 (100)	5 (9)	0	5 (23)	<0.001	<0.001
PR	73 (68)	30 (91)	40 (100)	3 (9)	2 (4)	2 (6)	0	<0.001	<0.001
AR	45 (42)	33 (100)	0	12 (35)	15 (27)	0	15 (68)	0.055	<0.001
Bcl-2	103 (96)	30 (91)	39 (98)	34 (100)	2 (4)	1 (3)	1 (5)	<0.001	<0.001
Her2	18 (17)	6 (18)	6 (15)	6 (18)	46 (82)	34 (100)	12 (55)	<0.001	<0.001
p53	9 (8)	0	0	9 (27)	33 (59)	20 (59)	13 (59)	<0.001	<0.001
Grade									
Well	34 (32)	11 (33)	15 (38)	8 (24)	2 (4)	1 (3)	1 (5)	0.190†	0.474†
Interm.	46 (43)	17 (52)	16 (40)	13 (38)	9 (16)	2 (6)	7 (32)		
Poor	27 (25)	5 (15)	9 (23)	13 (38)	45 (80)	31 (91)	14 (64)	<0.001‡	<0.001‡

Values in parentheses are percentages. ER = estrogen receptor; PR = progesterone receptor; AR = androgen receptor. *$\chi 2$ test. †Intermediately differentiated DCIS vs. well-differentiated DCIS. ‡Intermediately differentiated DCIS vs. poorly differentiated DCIS.

Intermediately differentiated DCIS significantly more often shared IHC features with well-differentiated DCIS than with poorly differentiated DCIS (p<0.001).

Reproducibility of cluster groups

For the assessment of variation in clustering results when using different hierarchical clustering methods, unsupervised hierarchical clustering by complete linkage was performed to the classification set of 6 markers. The concordance between designation of individual cases to one of the subgroups using average linkage versus complete linkage showed a near perfect agreement (kappa=0.876), with 16 mismatches of 163 paired cases.

Comparison with IHC categorization based on genetic profiles

A comparison of our results with the earlier findings from Perou et al. based on genetic profiles of invasive breast carcinoma is shown in Table 4. A classification of DCIS lesions into luminal A, luminal B, Her2, and basal-like subtypes was performed on the staining results of three markers (ER, PR, and Her2) and was compared with the findings of the present study. Both classifications showed a moderate agreement (kappa=0.411) mainly caused by

Table 4. Comparison of IHC classification of DCIS based on ER, PR, and Her2 expression (in analogy of Perou et al.) vs. IHC classification based on ER, Bcl-2, AR, and Her2 expression (present study) and relation with histological grade

	IHC classification based on ER, PR, and Her2 expression					
IHC classification based on ER, Bcl-2, AR, and Her2 expression	Luminal A (ER+,PR+, Her2-)	Luminal B (ER+, Her2+)	Her2 (ER-, Her2+)	Basal-like (ER-,PR-, Her2-)	Mixed	Total
ER+,Bcl2+,AR+	27	6	0	0	9	42
(well/interm./poor)	(10/15/2)	(0/4/2)			(2/3/4)	(12/22/8)
ER+,Bcl2+,AR-	34	10	0	0	16	60
(well/interm./poor)	(15/15/4)	(0/1/9)			(6/6/4)	(21/22/17)
ER-,Bcl2-,Her2+	0	0	42	0	0	42
(well/interm./poor)			(1/4/37)			(1/4/37)
ER-,Bcl2-,Her2-	0	0	0	7	0	7
(well/interm./poor)				(1/2/4)		(1/2/4)
Mixed	2	5	1	1	3	12
(well/interm./poor)	(1/1/0)	(0/2/3)	(0/0/1)	(0/1/0)	(0/1/2)	1/5/6)
Total	63	21	43	8	28	163
(well/interm./poor)	(26/31/6)	(0/7/14)	(1/4/38)	(1/3/4)	(8/10/10)	(36/55/72)

ER = estrogen receptor; PR = progesterone receptor; AR = androgen receptor.

the differentiation of the luminal types into A and B. If both the luminal types and ER/Bcl-2-positive lesions are considered as one group, the classifications demonstrated a substantial agreement (kappa=0.649). Nearly complete agreement is shown for the Her2-positive and basal-like type lesions. Mixed lesions from the Perou et al. classification frequently showed ER and Bcl-2 marker expression.

Discussion

The traditional histological classification of invasive breast cancer tumours has been debated by results from gene expression arrays leading to the molecular categorization of breast cancer into luminal and non-luminal tumours.[6] As invasive ductal breast cancers develop via the non-invasive precursor DCIS, these lesions may be categorized in the same way. High similarities were found among the different stages of breast tumour progression and it was suggested that gene expression alterations conferring the potential for invasive growth are already present in the pre-invasive stadium of breast cancer.[5] It has been shown that the molecular subgroups of invasive carcinoma can be distinguished using a set of IHC markers.[7] Using IHC, our analysis focused on the identification of subgroups in pure DCIS, to improve insight in the different pathways of tumour development, and to produce a classification

of DCIS based on marker expression. Sixteen markers were selected to distinguish luminal and non-luminal cell differentiation, or because their reported value to differentiate DCIS. Unsupervised hierarchical analysis, like in the evaluation of gene expression arrays, was performed to categorize DCIS. Ten markers were either positive or negative in nearly all DCIS and therefore not useful for classification.

ER, PR, AR, Her2, Bcl-2, and p53 were used for IHC classification of DCIS. ER, PR, and AR were positive in 68%, 46%, and 37% of the patients in our series, respectively. Others found ER, PR, and AR expression in 54-73%, 49-61%, and 33-44%, respectively.[9-12] We further found that well- and intermediately differentiated DCIS were predominantly ER-positive and PR-positive whilst poorly differentiated DCIS usually lacked steroid receptor expression and was correlated with Her2 overexpression. This finding became further evident by the unsupervised hierarchical clustering results that clearly divided DCIS into ER/Bcl-2-positive (luminal) lesions and ER/Bcl-2-negative (non-luminal) lesions. The luminal type DCIS was further divided into an AR-positive and AR-negative subtype.

Bcl-2, involved in apoptosis, was present in 64% of all DCIS while p53 was expressed in 26% of the cases in our series. These findings are in correspondence with results from others who reported Bcl-2 and p53 expression in 76% and 24% of DCIS cases, respectively.[13] The Bcl-2-positive/p53-negative phenotype, which is similar to normal epithelium and benign lesions, was observed in 95 cases originating from the ER/Bcl-2-positive (luminal) clusters. This phenotype might reflect a more favourable group of lesions.

AR expression was most frequently seen in intermediately and well-differentiated DCIS ($p=0.018$) in our series of patients. Not many studies investigated AR in DCIS. Moinfar et al.[14] reported a higher rate of AR expression in especially low grade DCIS as opposed to high grade DCIS although others did not find a correlation between AR expression and grade.[10] AR positive breast cancer patients have prolonged survival and a better response to hormonal treatment than AR negative patients.

Within the non-luminal type a Her2-positive/ER-negative subtype with 91% poorly differentiated DCIS could be identified. Her2 is known to be amplified and/or overexpressed in invasive breast cancer in 10-30% of cases and associated with poor outcome.[15,16] The absence of Her2 overexpression in normal ducts and atypical ductal hyperplasia, and the frequent Her2 amplification found in DCIS suggests that Her2 alterations are an early event in the pathway of development of Her2 positive invasive carcinomas. In our study 39% of the cases were positive for Her2. The higher frequency of Her2-positive lesions in DCIS compared to invasive breast cancer has been argued to occur due to loss of expression; however, it might indicate that in the breast cancer progression model there may be lesions that do not frequently evolve into invasive breast cancers including Her2-positive DCIS lesions. Moreover, the mammographic detection of poorly differentiated Her2-positive DCIS often occurs at an early stage due to the conspicuous microcalcifications.

Basal-like carcinomas have been identified in gene expression profiling studies as a subtype of invasive breast cancer. These lesions are ER-negative, PR-negative, and Her2-negative (triple negative). Bryan et al. studied 66 cases of high nuclear grade DCIS to determine the frequency

of the triple negative phenotype and showed that only four cases (6%) exhibited the triple negative phenotype.[17] We found eight (5%) triple negative lesions. Four of them were poorly differentiated. Given that invasive breast cancers typically share immunophenotypic features with the DCIS lesion from which they arise, these findings corroborate the possibility that the triple-negative DCIS lesions represent a precursor lesion to invasive basal-like carcinomas. In these (medullary-like and metaplastic) carcinomas, in situ components are usually minor or absent, suggesting a rapid progression from in situ to invasive stage. This is in keeping with the absence of basal-like in situ lesions in preventive mastectomy specimens of BRCA1 carriers, who are prone to develop basal-like tumours.[18]

Clustering analysis showed that the well-differentiated DCIS and intermediately differentiated DCIS share IHC features among the different clusters. It seems that intermediately differentiated DCIS shows more resemblance with well-differentiated DCIS as compared to poorly differentiated DCIS. A recent study from our institute investigating classification of DCIS by gene expression profiling confirms this finding and identified luminal, Her2-, and basal-like tumours in a series of 40 DCIS lesions.[19] A classification of DCIS by IHC might identify identical groups of luminal and non-luminal tumours which can be further subdivided reflecting the heterogeneous nature of DCIS. Therefore, IHC can assist in objectivation of variations in morphologic tumour classification of DCIS.

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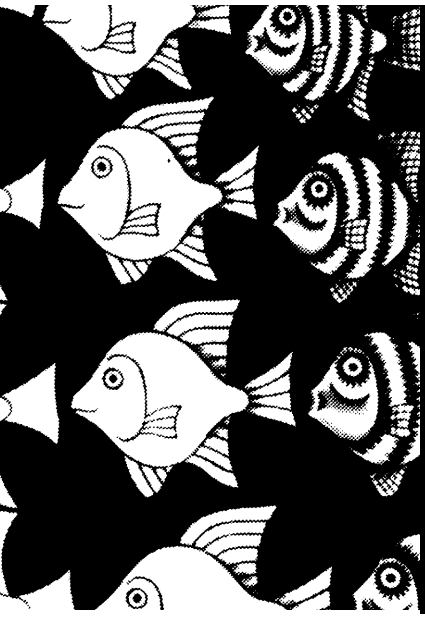

Chapter

4

Changing patterns in diagnosis and treatment of ductal carcinoma in situ of the breast

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Abstract

Background: The increased incidence of ductal carcinoma in situ (DCIS) of the breast and the emergence of new diagnostic and therapeutic tools like mammographic screening, stereotactic core biopsy and reconstructive surgery prompted us to investigate how these developments influenced diagnosis and treatment.

Methods: Clinical and pathological characteristics of 403 patients with DCIS consecutively treated at the Netherlands Cancer Institute between 1986 and 2002 were evaluated and the effect of introduction of mammographic screening, stereotactic core biopsy and reconstruction on diagnosis and treatment was studied.

Results: Following the nationwide introduction of mammographic screening the number of non-symptomatic DCIS increased from 47 to 77%. Introduction of stereotactic core biopsy resulted in a rise of one-step procedures from 26 to 52%. Mastectomy rate did not change over time: 59% overall. However, reconstruction rate increased from 17 to 39%.

Conclusion: This study shows a steep rise in diagnosis of non-symptomatic DCIS after introduction of screening. Further, the introduction of pre-operative diagnosis by stereotactic core biopsy resulted in a decrease of multiple surgical procedures. Mastectomy, with increasing application of breast reconstructions, remains an important treatment modality in the management of DCIS despite advancements in detection and diagnosis.

Introduction

Before mammographic screening, ductal carcinoma in situ (DCIS) usually presented as a symptomatic lesion, with a palpable mass, nipple discharge or Paget's disease. In the Netherlands nationwide mammographic screening has existed since 1990 for women between 50 and 69 years of age and was extended to an upper limit of 75 years of age in 1998. Of all screen-detected cancers between 1990 and 1997, 13% were DCIS.[1]

The histological diagnosis of DCIS requires tissue obtained by stereotactic core biopsy or surgical excision biopsy. Stereotactic core biopsy replaces increasingly surgical excision biopsy being an accurate, non-invasive diagnostic method,[2] although it may miss invasive breast cancer in 16% of patients initially diagnosed with DCIS.[3]

Opinions differ on the optimal treatment of DCIS. Patients can be cured from DCIS by complete excision. The extent of DCIS, however, is even in screen-detected lesions often wide and usually surpasses the area of radiological calcifications. Therefore, complete excision is technically challenging and may end up in ablative treatment after multiple local excisions. The introduction of screening and the application of new diagnostic tools like stereotactic core biopsy, as well as improvements in reconstructive surgery over the last decade, prompted us to investigate how these developments influenced diagnosis and management of DCIS.

Patients and methods

Files of a consecutive series of 426 patients, diagnosed and treated for pure DCIS at the Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital (NKI-AVL) between 1986 and 2002 were identified from the tumour-registration database. In the same period 4984 patients with invasive breast cancer were primarily treated at the NKI-AVL. Twenty-three patients with prior history of invasive breast cancer were excluded from this series. The following data were documented and analyzed: detection method (symptomatic, or by mammographic screening), methods used for diagnosis (fine needle aspiration cytology (FNAC), stereotactic core biopsy, biopsy of the nipple, or surgical excision biopsy), number of surgical steps (defined as separate operations) needed for optimal oncological result, definitive treatment (excision alone, excision plus radiotherapy, or mastectomy with or without immediate or delayed reconstructive surgery), and microscopic margin status (negative if ≥1mm or positive if <1mm, involved or doubtful). The population-based mammographic screening program was nationwide introduced in 1990. In 1998 stereotactic core biopsy was implemented in our hospital. Statistical analysis was done using the Statistical Package for Social Sciences 11.5 (SPSS Inc., Chicago, IL, USA). Data were analyzed by the chi-square test for categorical variables. Statistically significant differences were conferred by p values of < 0.05.

Results

Detection method

DCIS of 293/403 patients was screen-detected, non-symptomatic, and of 87 patients symptomatic. In 22 patients detection method was unknown and in one patient DCIS was an accidental finding after breast reduction. Figure 1 depicts the increase in the proportion of non-symptomatic lesions following the introduction of population-based screening: 27/57 patients were diagnosed without symptoms before 1990 (introduction of nationwide breast screening program in the Netherlands) and 266/346 patients were screen-detected after 1990.
The absolute number of patients with symptomatic lesions remained constant throughout the study period: 22 (1986-1989), 22 (1990-1993), 15 (1994-1997), and 28 (1998-2002). Figure 2 demonstrates the increased incidence of DCIS, as proportion of all breast cancers treated in our institute, up to more than 10% in 2001 and 2002.

Clinico-pathological characteristics

Table 1 shows the clinico-pathological characteristics of symptomatic and screen-detected DCIS patients (n=380). Median age at diagnosis was 51 years (range 24-81). Thirty-five of 87 symptomatic detected patients presented with palpable mass as first symptom, 38 patients were diagnosed with a surgical excision biopsy and 33 patients had a preoperative diagnosis by FNAC - part of triple diagnosis -, stereotactic core biopsy, or nipple biopsy in case of Paget's disease. Of the screen-detected patients 188/293 lesions were diagnosed by surgical excision biopsy, and in 104 patients lesions could be diagnosed as malignant pre-operatively by FNAC, stereotactic core biopsy, or nipple biopsy. After the introduction of stereotactic core biopsy in 1998 the number of pre-operative diagnoses increased from 22 to 57%. From 1998 to 2002, 44% of all diagnoses was done by stereotactic core biopsy (Figure 3).
Furthermore, the rise of pre-operative diagnoses resulted in a decline of the number of surgical procedures needed for definitive treatment. The number of one-step procedures increased from 26 to 52%.
Through the years, and among symptomatic and screen-detected DCIS histological grade was equally distributed: 26% well-, 24% intermediately, and 49% poorly differentiated lesions were

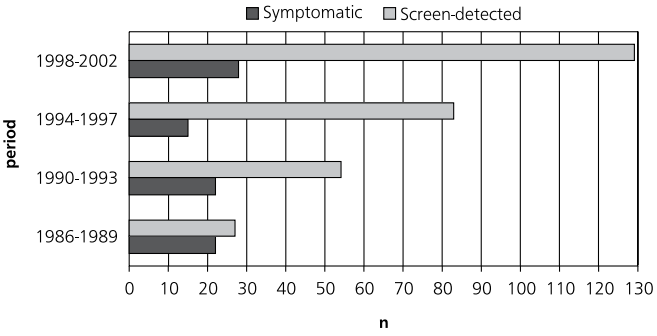


Figure 1. Symptomatic and screen-detected (non-symptomatic) ductal carcinoma in situ from 1986-2002 in the Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital

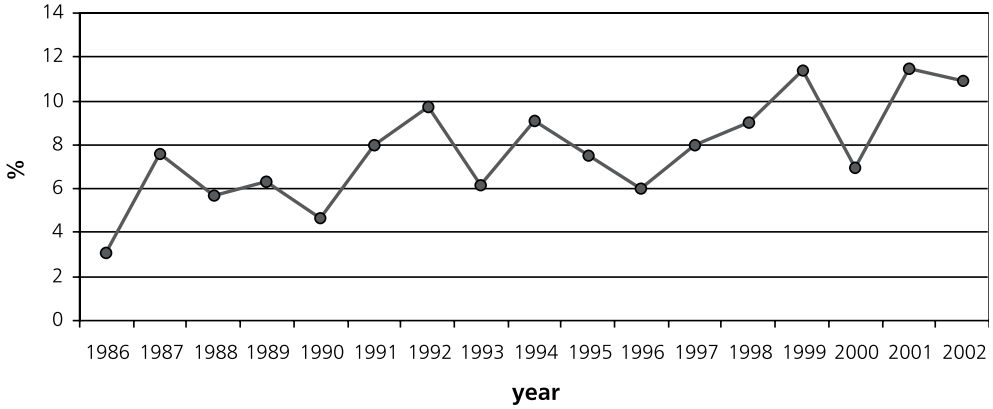
detected symptomatically and 22% well-, 31% intermediately, and 47% poorly differentiated lesions were screen-detected. Also completeness of definitive surgery, measured as margin status of the surgical specimen, did not exhibit a significant difference between detection method and time of diagnosis: 9/87 of the symptomatic patients and 22/293 of the screen-detected patients demonstrated positive margins at pathology examination (Table 1).

Table 1. Clinicopathological characteristics of symptomatic and screen-detected ductal carcinoma in situ

	Symptomatic (n=87)	Screen-detected (n=293)	Total (n=380)
Age (years)			
Median	50	51	51
Mean	52.5	51.6	51.8
Range	24-81	27-79	24-81
Method of detection			
Palpable mass	35		
Nipple discharge	29		
Paget's disease	23		
Method of diagnosis			
Cytopathological (FNAC)	24	36	60
Stereotactic core biopsy	5	66	71
Nipple biopsy	4	2	6
Surgical excision biopsy	38	188	216
Unknown	16	1	17
Treatment			
Excision alone	17	76	93
Excision and radiotherapy	15	49	64
Mastectomy	55	168	223
Surgical procedures			
One-step	27	110	137
Two-step	52	158	210
Three-step	7	24	31
Four-step	1	1	2
Margins			
Negative	78	271	349
Positive	9	22	31
Histological grade			
Well	23	65	88
Intermediately	21	90	111
Poorly	43	138	181

FNAC, fine needle aspiration cytology.

Figure 2. Incidence of ductal carcinoma in situ* as proportion of total breast cancer incidence from 1986-2002 in the Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital



*: includes 23 lesions preceded by an invasive carcinoma.

Breast-conserving therapy vs mastectomy

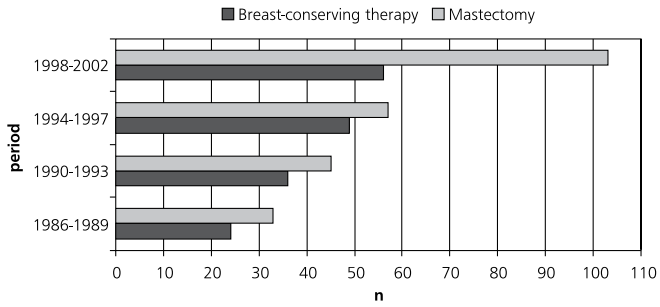
Thirty-seven percent of the patients with symptomatic DCIS underwent breast-conserving surgery versus 43% of the patients with screen-detected lesions. Breast-conserving therapy and mastectomy rates are depicted in Figure 4. The observed mastectomy rate showed a slight decrease up to 1997 followed by an increase to 65%. Overall mastectomy rate was 59%. In 36% of the patients a one-step procedure, in 55% a two-step, and in 9% more than two steps were needed.

Reconstruction

Reconstructive surgery was performed in 75 patients (32% of all patients treated with mastectomy). Compared with the 17% of all mastectomies before 1994, reconstruction rate increased to 39% after 1994. Breast reconstruction consisted of the initial implantation of a temporary tissue expander (n=51), the immediate implantation of a definitive silicon-filled endoprosthesis (n=8), the transposition of a latissimus dorsi flap in combination with an endoprosthesis (n=4), or the transposition of a transverse rectus abdominus myocutaneous flap (TRAM) (n=11). One patient underwent a delayed reconstruction using a deep inferior epigastric perforator flap (DIEP). Sixty of the 75 (80%) patients had an immediate reconstruction and 15 (20%) of them underwent a delayed reconstruction. Complications with loss of reconstruction occurred in 3 patients; in 2 of these patients a delayed reconstruction was successful. The third patient declined further reconstruction.

Figure 3. Surgical excision biopsy vs. stereotactic core biopsy from 1986-2002

Figure 4. Treatment of ductal carcinoma in situ from 1986-2002



Discussion

Screening

In this series of 403 consecutive cases of DCIS, 73% of all lesions were mammographically detected, due to screening. Detection of DCIS within a breast cancer screening program is worthwhile although it contributes in a limited way to the mortality reduction gained by screening.[4] Others assert that DCIS is a disease wherein a certain percentage of cases will never progress to invasive breast cancer and, therefore, will not ultimately result in death, so that its detection mainly constitutes overdiagnosis.[5] As this is based on the idea that symptomatic detected tumours have a greater potential to become aggressive than screen-detected tumours, one would expect more, favourable, slowly growing, well-differentiated or low grade lesions to be detected by screening in a non-symptomatic stage.[6] However, we observed no differences in relative distribution of grade between screen-detected and symptomatic detected lesions. This has also been described by others.[7,8]

Diagnosis

Although FNAC is well established for the diagnosis of invasive breast cancer, it cannot reliably differentiate between invasive and in situ cancer.[9] We used the so-called triple diagnosis:

palpable suspicious tumour, suspicious mammography and tumour positive cytology for palpable cancer of which some turned out to be DCIS. Yearly, a few patients with non-palpable lesions were diagnosed with duplo measures: suspicious imaging and positive cytology. Meanwhile, stereotactic core biopsy has become the standard technique to establish diagnosis pre-operatively.[2,10,11] In our series, 77% of the patients were diagnosed with this non-invasive method in 2002. When compared with a surgical excision biopsy, pre-operative diagnosis of breast cancer by stereotactic core biopsy facilitated fewer surgical procedures to accomplish definitive treatment. Other series reported similar results.[12-14] The number of one-step surgical procedures increased steeply from 26 to 52% after the introduction of this new diagnostic tool. The beneficial influence of stereotactic core biopsy on the number of procedures is corroborated by results from a study where the use of stereotactic core biopsy for non-palpable lesions was compared to needle localized open (surgical excision) biopsy: 1.31 and 1.91 procedures were needed for definitive surgery after stereotactic core biopsy and surgical excision biopsy, respectively.[15] In addition a therapeutic excision after a pre-operative diagnosis by stereotactic core biopsy leads more frequently to clear margins as compared to surgical excision biopsy as diagnostic procedure.[16]

Treatment

Breast cancer screening changed the number and presentation of DCIS over nearly two decades. The number of DCIS patients showed a steep rise of the non-symptomatic cases. Despite this last feature mastectomy, which involves a risk of overtreatment, remained an important treatment modality for DCIS reflecting the malignant potential and extensiveness of DCIS. Screening does not appear to have had a positive effect on the mastectomy rate, which is undoubtly due to the extensive nature of DCIS. In addition, the comprehensive histological work up of excisional specimen in our institute may have led to a better assessment of margins resulting more frequently to positive margins and thus to a higher mastectomy rate. Furthermore, 58% of non-palpable lesions reported in a series of 279 patients from our institute[17] proved to be malignant suggesting a higher threshold in selecting non-palpable lesions for stereotactic core biopsy and subsequent surgery as compared to reports by others.[18,19] However, all large institutes participating in the EORTC DCIS 10853 trial had a mastectomy rate varying from 26 to 54% (average 40% in 910 consecutive patients with DCIS).[20] A mastectomy rate of 58% was reported in a study of 304 women treated between 1989 and 1994 in the UK,[21] and in the southeast of the Netherlands a proportion of around 50% underwent ablative treatment during the period 1984-1997[22] while a recent study in the USA established a decrease in mastectomy rate from 53% in 1988 to 32% in 1999.[23] These numbers reflect the absence of an uniform approach in the management of DCIS.

Reconstructive surgery was increasingly applied: 17% of all mastectomies before 1994 and 39% after that were followed by a reconstruction. Immediate or delayed breast reconstruction has no ontoward oncological consequences,[24] and it does not interfere with the detection of recurrence of cancer.[25,26] The main advantage of breast reconstruction is its positive psychological impact on the patient. This is reflected by a decreased post-mastectomy stress

and a more positive body image.[24,27,28] Moreover, for women diagnosed with DCIS the possibility of breast reconstruction may ease their difficult choice between a mastectomy or breast conserving therapy.

Conclusion

Nowadays most DCIS is diagnosed by mammographic screening. Comprehensive management of DCIS should consist of preoperative histological diagnosis by stereotactic core biopsy resulting in less operative procedures and more clear margins. As mastectomy remains an important modality to reach optimal local control, breast reconstruction should be available, offered, and performed with limited complications to meet the needs of women in achieving the best possible aesthetic result.

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Chapter

5

The risk of invasion and axillary lymph node metastases in ductal carcinoma in situ diagnosed by core-needle biopsy

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Abstract

Background: The aim of the study was to assess the risk of invasion and axillary lymph node metastases in patients with ductal carcinoma in situ (DCIS) diagnosed by preoperative core-needle biopsy to select criteria for patients in whom sentinel node (SN) biopsy might be indicated.

Methods: One hundred and seventy-one women with 172 DCIS lesions diagnosed by core-needle biopsy were analysed. Axillary staging was performed by SN biopsy, axillary node sampling, or level 1-2 axillary lymph node dissection.

Results: Invasive breast cancer was found in the surgical specimens of 45 tumours (26.2%). Risk factors for invasion were a palpable lesion (odds ratio (OR) 2.95 (95% confidence interval 1.20-7.26), p=0.019), presence of a mass on mammography (OR 3.06 (1.43-6.56), p=0.004), and intermediate (OR 5.81 (1.18-28.57), p=0.030) or poorly differentiated (OR 5.46 (1.17-25.64), p=0.031) tumour grade. Lymph node metastases were found in ten women with DCIS and invasion on final pathology. Factors associated with metastases were age 55 years or less (p=0.030), invasion of 1.0 cm or more (p<0.001) and the presence of vascular invasion (p=0.001).

Conclusion: SN biopsy should be considered in women with an initial diagnosis of DCIS on core-needle biopsy who are at risk for invasion; this includes women with a palpable lump, a mass on mammography, and intermediate or poor tumour grade.

Introduction

In ductal carcinoma in situ (DCIS) of the breast the tumour is confined within the ducts without invading the periductal stroma. Tumour spread has not yet occurred, as reflected by the favourable long-term breast cancer specific mortality rate of 1-2%.[1,2] DCIS can be diagnosed reliably only after careful histological investigation requiring extensive tumour sampling to exclude invasion. Core-needle biopsy is increasingly used for the assessment of non-palpable, mammographically detected breast lesions. DCIS is often diagnosed radiologically, and stereotactically guided core biopsies can be used to obtain tissue for histological diagnosis.[3] A drawback of this technique is the limited sampling of a lesion. In series of patients with DCIS diagnosed by core-needle biopsy, the risk of invasion varied between 13 and 47%.[4-11] These patients are at risk of having (axillary) metastases.

Lymphatic mapping by sentinel node (SN) biopsy is an accurate technique with a low morbidity for the investigation of nodal involvement in invasive breast cancer. Thus, SN biopsy may also be considered in the initial management of patients with DCIS diagnosed by core-needle biopsy.[12] However, most patients will not have metastatic disease, and at present there are no criteria to determine which patients may benefit from SN biopsy.

The aims of the study were to analyse patients with core-needle biopsy-diagnosed DCIS to determine the risk of invasion and axillary lymph node metastasis, and to identify criteria that might aid in the selection of patients for whom SN biopsy is warranted.

Patients and methods

The medical records of all patients with DCIS diagnosed by core-needle biopsy between 1999 and 2005 were used. Patients were excluded if they had not received further treatment at the authors' institution. Clinical, radiological and pathological characteristics were analysed.

Core-needle biopsy technique

Most core-needle biopsies were performed stereotactically on a dedicated prone stereotactic unit (StereoGuide, Lorad®; Trex Medical Corporation, Danbury, Iowa, USA) with an automated biopsy gun (Bard Magnum®; C.R. Bard, Covington, Georgia, USA) and a 14-G core tissue biopsy needle. Usually, six core biopsy specimens were obtained; specimens were checked radiologically and further core biopsies were taken, if necessary.

Axillary lymph node staging

Axillary lymph node staging was done by SN biopsy, axillary node sampling, or a delayed level 1-2 axillary lymph node dissection (ALND). SN biopsies were performed selectively according to clinical judgement, for both mastectomy and breast-conserving surgery. The technique used for SN biopsy has been reported previously.[13] Axillary node sampling was achieved at

mastectomy by harvesting one or more lymph nodes from level 1 of the axilla. A selection of these patients underwent delayed ALND after finding invasion on final pathology.

Pathological assessment

After breast-conserving surgery, a specimen radiograph was performed to confirm complete excision of microcalcifications. Surgical specimens were sampled at 0.5-cm intervals to exclude invasive disease, and DCIS was classified according to Holland et al..[14] Sentinel nodes were assessed using the same protocol: the nodes were bisected or sectioned in 2-mm slices and embedded completely. All paraffin blocks were cut at three levels at 150-μm intervals. Haematoxylin and eosin staining was performed for all samples; immunohistochemical keratin staining was carried out for tumour-negative SNs. Lymph nodes obtained from axillary node sampling or ALND were evaluated at one level and stained with haematoxylin and eosin; immunohistochemical staining was not used routinely.

Statistical analysis

Descriptive statistics were used to evaluate frequency distributions. Differences in the distribution of categorical variables were analysed using the χ^2 test, and differences between continuous variables with the Mann-Whitney U test. A logistic regression model was used for univariable and multivariable analysis. Variables with complete data that were statistically significant in univariable analysis were used for multivariable analysis. $P \leq 0.050$ was considered statistically significant. SPSS® 11.5 for Windows (SPSS, Chicago, Illinois, USA) was used for statistical analysis.

Results

During the study interval, 180 patients had a preoperative diagnosis of DCIS on core-needle biopsy. After exclusion of nine patients who did not undergo surgery at this hospital, the study group comprised 172 tumours in 171 women (one bilateral). Clinical and radiological characteristics are summarized in Table 1. Eighty-four patients underwent a wide local excision as initial treatment. Nine needed re-excision and 20 had a subsequent mastectomy after incomplete excision. An invasive component was found in the surgical specimens of 45 (26.2% (95% confidence interval (CI) 19.6-32.7%) of the patients (Table 2). The presence of invasion did not differ between type of treatment. Axillary staging was performed for 91 lesions (52.9%) patients by SN biopsy, axillary node sampling or delayed ALND. Nodal involvement was detected in 11 patients.

Predictors of invasion

On univariable analysis, women with a palpable lesion were more likely to have invasion than those with a non-palpable lesion (p=0.010). Thirteen of 28 palpable lesions were upstaged

Table 1. Presentation and treatment of ductal carcinoma in situ diagnosed by core-needle biopsy

	Wide local excision (n = 64)	Mastectomy (n = 108)	P
Palpable lesion	5 (8)	23 (21)	0.021†
Calcifications and mammographic mass	15 (23)	32 (30)	0.378†
Median (range) size of calcification (mm)*	20 (6-45)	40 (4-100)	<0.001‡

Values in parentheses are percentages unless indicated otherwise. *Analysis based on 137 lesions (79.7%) for which the extent of microcalcification had been determined (wide local excision, 51; mastectomy, 86). †χ^2 test; ‡Mann-Whitney U test.

to invasive breast cancer. Further, the finding of a visible mass on mammography was more frequently associated with invasion (p=0.001). In 21 of 47 patients with calcification and a mammographic mass, the surgical specimen showed invasion. Invasion was more frequent when the mammographic area of microcalcification was larger than 2.5 cm in diameter. Invasion was encountered in 30 DCIS lesions larger than 2.5 cm on mammography (odds ratio (OR) 2.88 (95% CI 1.20-6.91), p=0.018). The median extent of microcalcifications was 2.1 cm in the DCIS group, compared with 3.0 cm in the group with invasion (p=0.986). Size at microscopy was also not associated with invasion (p=0.563).

Histological classification demonstrated that intermediate or poorly differentiated DCIS was more likely to be underestimated. Of 30 patients with well differentiated DCIS, two (7%) patients had an invasive component, whereas 17 (31%) of 54 patients with intermediate and 26 (30%) of 88 with poorly differentiated DCIS had invasion on final pathology.

Multivariable analysis showed that a palpable lesion (OR 2.95 (1.20-7.26), p=0.019), presence of a mass on mammography (OR 3.06 (1.43-6.56), p=0.004), and intermediate (OR 5.81 (1.18-28.57), p=0.030) or poorly differentiated (OR 5.46 (1.17-25.64), p=0.031) tumour grade were independent predictors of invasive disease.

Axillary staging

Axillary staging was performed predominantly in lesions that were incompatible with breast-conserving treatment: 82 (75.9%) of tumours treated by mastectomy had axillary staging, compared with nine (14%) of those that had a wide local excision (Table 2). Three of the nine patients initially treated conservatively had an ALND after invasion was found in the surgical excision specimen, and the other six women underwent SN biopsy for a palpable lesion (three patients), a lesion with a mammographic mass (two) or a lesion larger than 3.0 cm in diameter (one). Initial axillary staging by SN biopsy was performed in a total of 30 patients: the SN(s) could be identified in 29. A median of 2 (range 1-5) SNs was retrieved. Fourteen of these patients showed invasion on final pathology including five women with a tumour positive SN detected by haematoxylin and eosin staining. One of these five patients had a SN located in the internal mammary chain without axillary SN involvement. This patient was spared an ALND. In the other four patients subsequent ALND was performed resulting in the identification of four positive lymph nodes in three patients. In two patients additional

Table 2. Staging and treatment of ductal carcinoma in situ diagnosed by core-needle biopsy

	Wide local excision (n = 64)	Mastectomy (n = 108)	P
Type of axillary staging			
Sentinel node biopsy	6 (9)	24 (22.2)	0.032†
Axillary node sampling	0 (0)	52 (48.1)	<0.001‡
Delayed axillary node dissection	3 (5)	6 (5.6)	0.805‡
Final pathology			
Invasion	18 (28)	27 (25.0)	0.652†
> 1 mm	14 (22)	21 (19.4)	
≤ 1 mm	4 (6)	6 (5.6)	
Median (range) size (mm)*	17.5 (5-75)	30.0 (4-200)	<0.001§
Detection of nodal metastasis	2 (3)	9 (8.3)	0.215‡
Haematoxylin and eosin	1 (2)	3 (2.8)	
Immunohistochemistry	1 (2)	6 (5.6)	

Values in parentheses are percentages unless indicated otherwise. *Analysis based on 149 lesions (86.6%) for which the pathological size had been determined (wide local excision, 52; mastectomy, 97). †χ^2 test unless indicated otherwise; ‡Fisher's exact test. §Mann-Whitney U test.

macrometastases were detected by haematoxylin and eosin staining, and in one a cluster of positive tumour cells was detected by immunohistochemistry alone.

Axillary node sampling was done at initial mastectomy in 52 patients. A median of 4 (range 1-14) lymph nodes was removed. Nine patients had invasive breast cancer; two were found to have micrometastatic disease detected by immunohistochemistry alone, including one patient with a confirmed DCIS lesion. No further treatment of the axilla was performed in these two patients.

Nine patients had ALND as delayed axillary staging procedure after invasion was found on definitive pathology. A median of 16 (range 10-22) lymph nodes was retrieved. Haematoxylin and eosin staining demonstrated nodal involvement in two of these and immunohistochemistry resulted in the detection of micrometastases in a further two patients. Thirteen patients with DCIS and an invasive component did not undergo axillary staging, including two with microinvasion, one patient with invasive tubular carcinoma, two patients with lobular carcinoma in situ and invasive lobular carcinoma, and eight patients with invasive ductal carcinoma. The median size of the largest invasive component was 3 (range 1-6) mm in these 13 patients; three underwent mastectomy as final treatment, and the other ten patients had breast-conserving surgery followed by radiotherapy.

Nodal involvement was found in ten of 32 patients with invasive tumour on final pathology who underwent axillary staging; this was associated with age 55 years or less (p=0.030), invasion of 1.0 cm or more in diameter (p<0.001) and the presence of vascular invasion (p=0.001). The chance of finding invasive breast cancer including metastases by initial axillary staging was 9% (seven of 82 patients). These unfavourable lesions were found primarily in patients with microcalcifications greater than 2.5 cm in diameter (p=0.037).

Discussion

The introduction of mammographic screening and core-needle biopsy for detecting and diagnosing non-palpable breast lesions has led to an increase in the preoperative diagnosis of DCIS.[15] In the present study, a preoperative diagnosis of DCIS on core-needle biopsy was associated with a 26.2% risk of finding invasion in the surgical specimen. Other similar recent studies have reported underestimation rates of 13-38%.[7-10] Yen et al.[10] reported a comparable invasion rate of 25% for DCIS diagnosed by core-needle biopsy, but found that patients who had diagnosis by this method were more likely to have invasion than those who had an excision biopsy. Indeed, many DCIS lesions carry a risk of invasion and nodal involvement, leaving the difficult choice of when to perform axillary staging. Axillary staging by SN biopsy is preferable and has already become part of initial treatment in selected women with a preoperative diagnosis of DCIS.

Patients in whom axillary staging is indicated can be selected by a variety of clinicopathological characteristics. Tumour size is, according to many, a risk factor for invasion,[10,16-18] which explains the high rate of axillary staging procedures in combination with mastectomy in the present series. In addition, SN biopsy is simply not possible after mastectomy and considered less accurate following breast-conserving surgery.[19] In the present series, lesions with microcalcification greater than 2.5 cm in diameter were more prone to invasion (and metastasis), although median mammographic (and pathological) extent did not differ significantly between the groups. The transition from DCIS to invasive breast cancer is not limited to large lesions, but might be considered as a genetic change.[20] This is corroborated by the fact that most features of breast cancer are already present at the time DCIS has evolved.[21,22] Nonetheless, many clinicians consider SN biopsy to be warranted for large DCIS tumours.

Furthermore, lesions that are palpable, have a mammographically visible mass or are high grade are considered to be at a relative high risk for invasion.[7,9,10,16,18] Goyal et al.[7] showed that the presence of a palpable or mammographic mass increased the risk of invasion by fivefold and sevenfold respectively. In the present study, both features were associated with a threefold increase. A similar finding was reported by Wilkie and colleagues,[9] who showed that patients with a mammographically visible mass were twice as likely to be upstaged to invasive breast cancer. Ultrasonographically guided core biopsy directed at the mass, rather than stereotactic core biopsies aimed at microcalcifications, may assess the risk of invasion more accurately and should be used for breast abnormalities visible on ultrasonography.[23] The benefit of MRI seems to be limited to ruling out tumour invasion.[24]

Tumour invasion was associated with poorly differentiated lesions; this accords with the finding that patients with poorly differentiated DCIS had a higher risk of distant metastasis following an invasive local recurrence.[25]

In the present study younger patients were more likely to have invasion followed by metastasis. Younger age was also described by Yen et al.[10] as a predictor of invasion. These authors found that patients under 55 years of age had a higher risk of tumour invasion. Younger age

is associated with a more aggressive biological tumour behaviour and an increased risk of local recurrence after breast-conserving treatment.[2]

Some 3-13% of patients with presumed DCIS who are scheduled for SN biopsy have invasion and a positive SN.[7-10] The majority of these positive lymph nodes have metastases that can be detected by haematoxylin and eosin staining. In the present series, 82 (47.7%) of 172 tumours had initial axillary staging. Seven (9%) of these showed nodal involvement; five detected by haematoxylin and eosin. The overall nodal involvement rate increased to 12% after delayed ALND was performed in nine patients with invasion. In a comparable series of 587 patients diagnosed by core-needle biopsy, nodal involvement was demonstrated in 35 (13.0%) of 269 patients who had initial axillary staging by ALND; all 35 were shown to have invasion on final pathology.[7]

The presence of invasion in 26.2% of DCIS lesions diagnosed by core-needle biopsy poses the problem of identifying which patients with DCIS warrant axillary staging by SN biopsy. Some suggest performing SN biopsy in all patients with a core-needle biopsy diagnosis of DCIS, or at least in all patients who undergo mastectomy.[8,26] Patients could be selected more carefully to prevent axillary staging in those who have little to gain. SN biopsy should be considered in patients with an initial diagnosis of DCIS on core-needle biopsy who have a palpable lesion, a mass on mammography, or an intermediate or poorly differentiated tumour grade.

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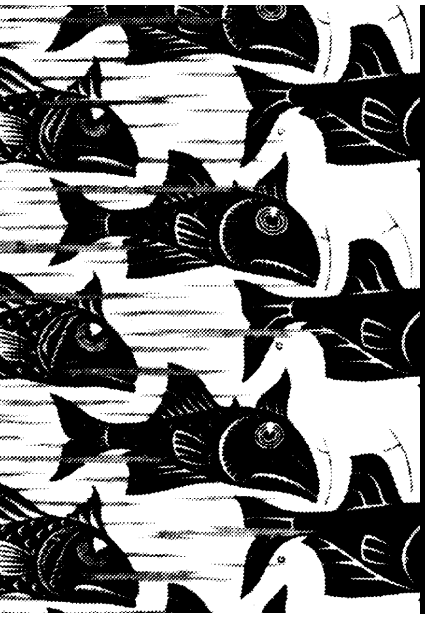

Chapter

6

Clinical outcome after selective treatment for patients diagnosed with ductal carcinoma in situ of the breast

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Abstract

Background: The effect of treatment of patients diagnosed with ductal carcinoma in situ (DCIS) of the breast was evaluated and factors associated with local recurrence were assessed.

Methods: The study involved 504 patients treated by wide local excision alone (WLE) (n=91), wide local excision and radiotherapy (WLE+RT) (n=119), or mastectomy (n=294) at the Netherlands Cancer Institute from 1986 to 2005. Clinical, pathological and follow-up data were evaluated.

Results: The median time to follow-up was 6.7 years. The 8-year overall local recurrence rate was 12% after breast-conserving treatment (BCT) (15.6% after WLE and 8.8% after WLE+RT (p=0.161)), and 0.9% after mastectomy (p<0.0001). In total, 18 (66.7%) invasive local recurrences and 9 (33.3%) DCIS local recurrences occurred. The 8-year distant metastasis rate was 4% after BCT (4.3% after WLE and 4.2% after WLE+RT (p=0.983)) and 0.9% after mastectomy (p=0.048). Median tumor extent was 10, 15, and 35 mm for patients treated with WLE, WLE+RT, and mastectomy, respectively. Margins were involved in 6.4% of all patients. Factors associated with local recurrence were age younger than 40 years (HR 8.66), surgical margins involvement (HR 5.75), WLE (HR 26.77), and WLE+RT (HR 7.42).

Conclusion: BCT of DCIS bears the risk of residual disease progressing into invasive local recurrence and distant metastasis. A re-excision or mastectomy is therefore wanted in all patients with unclear margins. Mastectomy treatment is associated with optimal local control and might be considered for patients younger than 40 years who are at high risk of local recurrence.

Introduction

Ductal carcinoma in situ (DCIS) is a proliferation of morphologically malignant cells within the ducts and lobules of the breast without evidence, by light microscopy, of invasion through the basement membrane into the surrounding stroma. The optimal treatment of DCIS is controversial. In the pre-screening era DCIS was rare and accounted for 2-3% of all breast cancers. At that time, mastectomy was the treatment of choice. Nowadays the proportion of DCIS among screen-detected breast cancers has increased to 20%.[1] It is not known whether all these DCIS will progress into symptomatic and/or invasive lesions.[2] Since breast-conserving treatment (BCT) became an appropriate alternative for mastectomy in women diagnosed with invasive breast cancer,[3] it is self evident that this treatment modality was also tested in DCIS. In several randomized clinical trials wide local excision alone (WLE) was compared with wide local excision and radiotherapy (WLE+RT) and it was shown that by adding RT to WLE an approximately 50% reduction of local failure could be achieved compared to WLE alone with a local recurrence rate of 15% at 10-12 years.[4,5] Despite this obvious beneficial local effect of RT, the breast cancer-specific death rate is the same for all treatment options.[4-6] Identification of patients with a high or low risk of local recurrence has become important to tailor individual treatment strategies. The aim of this study was to evaluate the effect of different treatment modalities on the clinical outcome of patients with DCIS and to identify factors for local recurrence.

Patients and methods

The files of 504 consecutive patients with no prior history of breast cancer who underwent (final) surgical treatment for DCIS at the Netherlands Cancer Institute between 1986 and 2005 were reviewed. Clinical, pathological, and follow-up data were collected. Patients with a diagnosis of invasive carcinoma or micro-invasion were excluded from the analysis. Final treatment was by WLE (n=91), WLE+RT (n=119), or mastectomy (n=294). Treatment selection was based on clinical and pathological characteristics, and was subject to patient and/or physician preferences. In case of WLE+RT (n=119), the entire breast was irradiated to a dose of 50 Gy, divided in fractions of 2 Gy in 5 weeks; more recently a boost (16 Gy in fractions of 2 Gy) was added to the tumor bed in 36 patients. One hundred and nine patients of the total study group were referred after previous excision(s) elsewhere. Thirty patients treated by BCT were entered in the EORTC 10853 DCIS trial (WLE with or without RT).[4] The histological slides of all patients with an initial biopsy elsewhere were reviewed. Postoperative specimen X-rays were performed to confirm complete excision of microcalcifications. Margins were microscopically evaluated and scored as free when exceeding a tumor free width of 1 mm, and as involved when less. DCIS was graded in three categories (well-, intermediately, and poorly differentiated DCIS) according to the classification of Holland et al.[7] Well-differentiated DCIS

was further subdivided in clinging or micropapillary, and cribriform or solid type lesions. The relation between architectural growth patterns and local recurrence were further analyzed within the group of well-differentiated DCIS comparable to the earlier EORTC trial.[4] When possible the maximum tumor diameter was estimated by radiologic-microscopic correlation. After completion of treatment, patients were followed up every 3 or 6 months for the first 2 years (BCT and mastectomy), every 6 (BCT) or 12 months (mastectomy) for the next 3 years, and at 1-year intervals thereafter (only BCT). Mammograms were performed annually, unless warranted by an intercurrent finding. After ten event-free years, patients who underwent BCT were offered the possibility of continuing follow-up care in the national screening program.
The differences in clinical and pathological characteristics and in outcome between patients treated by WLE, WLE+RT, and mastectomy were evaluated. The χ^2 test and Mann-Whitney U test were used to investigate differences in possible prognostic characteristics, e.g. age at diagnosis, method of detection and biopsy, number of operations, size of tumor, grade of tumor, and margin status. Survival analysis was performed in terms of time to local recurrence (including chest wall recurrences), distant metastases, and contralateral breast cancer. Time to local recurrence, distant metastases, and contralateral breast cancer was calculated from date of diagnosis to the date of first event or date of last follow-up or death. Breast cancer-specific survival and overall survival were calculated from date of diagnosis until date of death of breast cancer or last follow-up and death of any cause or last follow-up, respectively. A log rank test was used to investigate the association between treatment modality and survival. Event free rates were estimated by the Kaplan-Meier method. A multivariable Cox proportional hazard model was fitted to investigate the association between clinical and pathological factors (age, method of detection, treatment, margins, grade of DCIS) and time to local recurrence. For all analyses statistically significant differences were conferred by p values of <0.05. All analyses were performed in Statistical Package for the Social Sciences 11.5 (SPSS Inc., Chicago, IL, USA).

Results

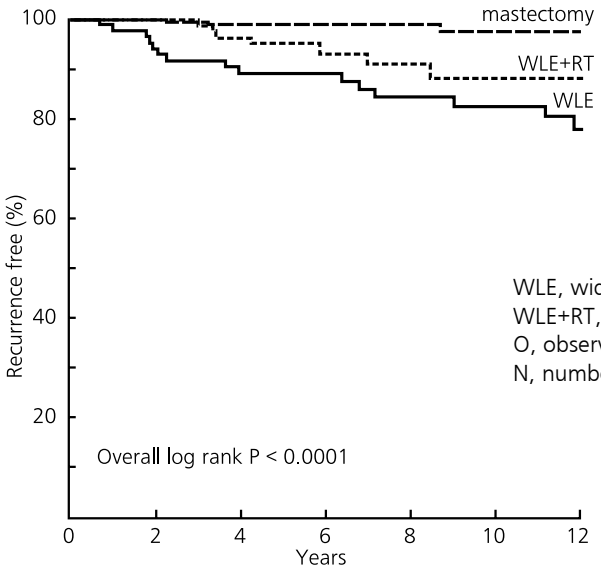
Patient and tumor characteristics

Table 1 summarizes the distribution of patient and tumor characteristics among the three treatment modalities. Median length of follow-up was 6.7 years (range 0.5-20.1) for all patients. Patients treated with WLE had a significant longer follow-up than those treated with WLE+RT: 10.6 vs. 5.8 years, respectively ($p<0.001$). Time of follow-up did not differ between the patients treated by BCT and those who underwent mastectomy. Seventy-nine per cent of the patients were screen-detected. Synchronous bilateral DCIS occurred in 6 patients. DCIS was in the majority of cases (60%) diagnosed by excision biopsy at a median age of 51 years (range 22-81). Younger women were primarily treated by mastectomy. Out of 64 patients younger than 40 years, 9 (14%) patients had BCT (WLE+RT in 7 patients) and

55 (86%) underwent mastectomy. Tumor extent could be estimated in 271 cases, and varied among the different treatment categories. The median size was 21 mm (range 2-200) with a significant difference between patients treated with BCT and mastectomy: 15 mm and 35 mm, respectively (p<0.001). Nearly 70% of DCIS treated by mastectomy measured more than 20 mm including 21% larger than 50 mm. WLE was choice of treatment for smaller lesions with a median size of 10 mm. The median extent of DCIS in younger patients was 1.5 times larger than in older patients (30 mm vs. 20 mm, p=0.029). DCIS treated by WLE was well-differentiated in 57% of the cases compared to 9% and 17% of the lesions treated by WLE+RT and mastectomy, respectively. Ninety-six per cent of poorly differentiated DCIS was treated by WLE+RT or mastectomy. Poorly differentiated DCIS was evenly distributed among younger and older patients: 48% and 47%, respectively.

In 59% of all patients at least two surgical procedures were performed to pursue complete excision of DCIS. Especially diagnostic excision biopsy for DCIS was frequently (81%) incomplete, warranting mastectomy in 53% of the 304 cases. In the 135 patients with a preceding core biopsy the percentage of incomplete first excisions was 30 (p<0.001). Ninety-seven (46%) of the 210 patients who underwent BCT received a re-excision to optimalize clearance of margins which resulted in negative margins in 92% of these patients. Finally, surgical margins were involved in 32 (6.4%) patients: 11 after WLE, 16 after WLE+RT, and 5 after mastectomy. From the 11 patients with margins reported not free after WLE, 8 women were diagnosed with well-differentiated DCIS and 2 with intermediately differentiated DCIS. One patient with poorly differentiated DCIS and unclear margins refused further treatment.

Figure 1. Time to local recurrence by treatment



O	N	No. of patients at risk:					
16	91	79	64	58	52	44	32
8	119	100	74	53	35	23	13
3	294	247	202	150	110	78	48

Table 1. Patient, tumor, and treatment characteristics

	total	breast-conserving treatment	
		WLE	WLE+RT
Number of patients	504	91 (18)	119 (24)
Median follow-up in years (range)	6.7 (0.5-20.1)	10.6 (0.6-19.5)	5.8 (0.5-18.2)
Median age in years (range)	51 (22-81)	51 (29-79)	56 (34-76)
Screen-detected	399 (79)	75 (82)	95 (80)
Method of biopsy			
cytology	65 (13)	6 (7)	13 (11)
core biopsy	135 (27)	11 (12)	36 (30)
excision biopsy	304 (60)	74 (81)	70 (59)
Number of operations			
one	205 (41)	43 (47)	70 (59)
two	258 (51)	47 (52)	46 (39)
three	41 (8)	1 (1)	3 (2)
Median pathologic size in mm (range)*	21 (2-200)	10 (5-55)	15 (2-70)
≤ 10 mm	55 (20)	22 (58)	21 (31)
11-20 mm	79 (29)	11 (29)	29 (43)
21-50 mm	99 (37)	4 (11)	15 (22)
> 50 mm	38 (14)	1 (3)	2 (3)
Histologic tumor grade			
well	114 (23)	52 (57)	11 (9)
intermediate	154 (31)	30 (33)	34 (29)
poor	236 (47)	9 (10)	74 (62)
Margins not free	32 (6)	11 (12)	16 (13)

BCT, breast-conserving treatment; WLE, wide local excision; WLE+RT, wide local excision with radiotherapy. Values in parentheses are percentages unless otherwise indicated. *Pathologic size was reported in 271 (54%) patients: 38 (42%) after WLE, 67 (55%) after WLE+RT, and 166 (56%) after

Local recurrences and other events

Table 2 shows the 8-year event free rates for local recurrence (both invasive and non-invasive (DCIS)), distant metastasis, and contralateral breast cancer. Also overall and breast cancer-specific mortality is listed in this table. With a median follow-up of 6.7 years (range, 0.5-20.1 years), 24 (11.4%) local recurrences (16 invasive and 8 DCIS) occurred after BCT: 16 (17.6%) patients developed a local recurrence after WLE and 8 (6.7%) patients after WLE+RT. The median time to local failure was 4.1 years (range, 0.7-13.2). Chest wall recurrences were observed in three (1%) patients (2 invasive and 1 DCIS) after mastectomy at intervals of 2.3, 3.3, and 8.7 years. The overall local recurrence free rates of all three treatment groups (WLE, WLE+RT, and mastectomy) are plotted in Figure 1 ($p<0.0001$). Exclusion of 91 patients treated by WLE alone, still demonstrated mastectomy to be superior as opposed

	all treatments		
p†	BCT	mastectomy	p†
	210 (42)	294 (58)	
<0.001	7.5 (0.5-19.5)	6.2 (0.5-20.1)	0.067
0.058	53 (29-79)	50 (22-81)	<0.001
0.636	170 (81)	229 (78)	0.404
	19 (13)	46 (16)	
0.002	47 (22)	88 (30)	0.004
	144 (69)	160 (54)	
	113 (54)	92 (31)	
0.150	93 (44)	165 (56)	<0.001
	4 (2)	37 (13)	
0.030	15 (2-70)	35 (6-200)	<0.001
	43 (41)	12 (7)	
0.061	40 (38)	39 (24)	<0.001
	19 (18)	80 (48)	
	3 (3)	35 (21)	
	63 (30)	51 (17)	
<0.001	64 (31)	90 (31)	0.002
	83 (40)	153 (52)	
0.771	27 (13)	5 (2)	<0.001

mastectomy. †The differences in the distribution of categorical variables were analysed using the $\chi 2$ test, and the differences between continuous variables were tested using the Mann-Whitney U test.

to WLE+RT (p=0.0004). The beneficial effect of RT after WLE showed a reduction of DCIS local recurrences (p=0.030) but not of invasive local recurrences (p=0.861) in our selection of patients (Table 2). Of 43 patients with lesions ≤ 10 mm, 22 women treated with WLE and 21 women treated with WLE+RT, four and one patient(s) developed a local recurrence, respectively (p=0.177). A salvage mastectomy was performed in 17 of the 24 patients with local recurrences after BCT. During time, when the number of patients diagnosed with DCIS became larger, BCT was increasingly performed by WLE+RT while mastectomy remained the treatment of choice in the majority of cases. The 4-year local recurrence free rates varied from 94.7% in the period from 1986-1990 (median follow-up: 15.9 years) to 100% in the recent period from 2001-2005 (median follow-up: 2.5 years) (Table 3).

Distant metastases occurred in nine (1.8%) patients. Six of them following an invasive local recurrence after BCT. Four of these six patients had surgical margins involvement. Another

Table 2. Outcome data after breast-conserving treatment and mastectomy

	breast-conserving treatment		
	WLE	**WLE+RT**	**log rank p***
Number of patients	91	119	
Local recurrences			
total	16	8	
invasive	9	7	
non-invasive (DCIS)	7	1	
8-year overall local recurrence free rate	84.4	91.2	0.161
8-year invasive local recurrence free rate	91.6	92.5	0.861
8-year DCIS local recurrence free rate	92.1	98.6	0.030
Distant metastases			
total	4	3	
after invasive local recurrence	4	2	
8-year distant metastasis free rate	95.7	95.8	0.983
Contralateral breast cancer			
total	5	1	
8-year contralateral breast cancer free rate	95.5	100	0.324
Death			
total	4	4	
breast cancer-specific	3	3	
8-year overall survival	95.7	96.9	0.584
8-year breast cancer-specific survival	96.8	98.0	0.487

patient developed distant metastasis after a second primary breast cancer, that was localized in a different quadrant than the DCIS and demonstrated to be Her-2/Neu negative in contrast with the Her-2/Neu positive DCIS lesion. Two patients developed distant metastasis after mastectomy, one after an invasive chest wall recurrence, the other without evidence of local failure. Re-examination of the slides of the mastectomy specimen of the last patient revealed no invasive growth. Both patients were diagnosed with DCIS at young age, respectively 32 and 39 years. Seven of the nine patients with distant metastases died of disease. The 8-year distant metastasis free rate was 96% after BCT and 99.1% after mastectomy (p=0.048). Five patients died from non breast cancer-specific causes like cardiac disease (n=2), lung cancer (n=2), or colon cancer (n=1).

Factors associated with local recurrence

Factors associated with local recurrence are shown in Table 4. Multivariate analysis demonstrated that age younger than 40, involved margins, WLE, and WLE+RT were associated with an increased risk of local recurrence. Four (44.4%) out of nine younger patients developed a local recurrence after BCT (1 after WLE and 3 after WLE+RT) and two

all treatments		
BCT	**mastectomy**	**log rank p***
210	294	
24	3	
16	2	
8	1	
88	99.1	<0.0001
92.2	99.6	0.0001
95.5	99.5	0.005
7	2	
6	1	
96	99.1	0.048
6	15	
97.4	93.5	0.120
8	5	
6	2	
96.1	99.4	0.266
97.3	99.4	0.110

DCIS, ductal carcinoma in situ; WLE, wide local excision; WLE+RT, wide local excision with radiotherapy; BCT, breast-conserving treatment.
*The event free rates for local recurrence, contralateral breast cancer, distant metastasis, and (breast cancer-specific) survival were calculated by the Kaplan-Meier method.

(3.6%) out of 55 younger patients after mastectomy (p=0.0009). Clinical and pathological characteristics of younger patients showed no marked differences as compared to older patients after BCT for DCIS. At 8 years, 36.5% of younger patients developed a local recurrence after BCT compared to 10.8% of older patients (p=0.010) (Table 5).

Ten (31.3%) of the 32 patients with local failure had involved margins. Margins involvement was associated with a five to six times higher risk of local recurrences (HR 5.75 (2.44-13.56)), both after BCT and mastectomy. The number of local recurrences was not affected by the number of excisions. Twelve local recurrences occurred both after one and two procedures.

Although histological grade was not correlated with risk of recurrence, we identified - within the group of well-differentiated DCIS - a subgroup of patients with a very low risk of local recurrence. Table 6 shows 38 well-differentiated lesions with a clinging or micropapillary architecture and 76 well-differentiated lesions with a cribriform or solid architecture. Both groups demonstrated similarities in patient, tumor, and treatment characteristics but showed different 8-year local recurrence free rates (p=0.031). All 11 patients with local failures had a cribriform or solid architecture, whereas none of the 38 patients with clinging or micropapillary architecture had recurrent disease after a median follow-up of 6.6 years (range, 0.8-18.4).

Table 3. Treatment and outcome patterns of 504 patients diagnosed with ductal carcinoma in situ in the period 1986-2005

	Period			
	1986-1990	1991-1995	1996-2000	2001-2005
Number of patients	76	107	144	177
Treatment				
WLE	22 (29)	37 (35)	16 (11)	16 (9)
WLE+RT	14 (18)	15 (14)	43 (30)	47 (27)
mastectomy	40 (53)	55 (51)	85 (59)	114 (64)
Median follow-up in years (range)	15.9 (5.7-20.1)	11.3 (1.9-15.3)	6.9 (0.5-10.2)	2.5 (0.5-5.5)
Local recurrences	5	14	8	0
4-year local recurrence free rate*	94.7	94.3	96.3	100

WLE, wide local excision; WLE+RT, wide local excision with radiotherapy. Values in parentheses are percentages unless otherwise indicated. *The local recurrence free rate was calculated by the Kaplan-Meier method

Table 4. Multivariate analysis of risk factors related to local recurrence

Variable	Events/patients	HR (95% CI)	p*
Age			<0.001
≥ 40	21/440	1	
≤ 39	6/64	8.66 (2.63-28.56)	
Method of detection			0.137
screen	23/399	1	
symptomatic	4/105	0.42 (0.13-1.32)	
Treatment			<0.001
mastectomy	3/294	1	
WLE+RT	8/119	7.42 (1.76-31.20)	
WLE	16/91	26.77 (5.50-130.31)	
Margins			<0.001
free	17/472	1	
not free	10/32	5.75 (2.44-13.56)	
Histologic tumor grade			0.851
well	11/114	1	
intermediate	8/154	0.96 (0.35-2.66)	
poor	8/236	1.30 (0.39-4.27)	

HR, hazard ratio; WLE, wide local excision; WLE+RT, wide local excision with radiotherapy. *A Cox proportional hazard model was fitted for the multivariate analysis of local recurrence.

Table 5. Clinical, pathological, and outcome characteristics of patients ≤ 39 years vs. patients ≥ 40 years who underwent breast-conserving treatment for ductal carcinoma in situ

	≤ 39 years	≥ 40 years	p†
Number of patients	9	201	
Screen-detected	6 (67)	164 (82)	0.377
WLE+RT	7 (78)	112 (56)	0.305
Median age in years (range)	37 (29-39)	54 (40-79)	<0.0001
Median pathologic size in mm (range)*	16 (8-20)	15 (2-70)	0.644
Poorly differentiated grade	3 (33)	80 (40)	0.643
Margins not free	2 (22)	25 (12)	0.326
Local recurrences	4 (44)	20 (10)	0.011
8-year local recurrence free rate	63.5	89.2	0.010‡

WLE+RT, wide local excision with radiotherapy. Values in parentheses are percentages unless otherwise indicated. *Pathological size was reported in 105 (50%) patients: 6 (67%) patients with age ≤ 39 years and 99 (49%) patients with age ≥ 40 years. †The differences in the distribution of categorical variables were analyzed using the $\chi 2$ test, and the differences between continuous variables were tested using the Mann-Whitney U test unless otherwise indicated. ‡The local recurrence free rate was calculated by the Kaplan-Meier method.

Table 6. Patient, tumor, treatment, and outcome characteristics of different growth patterns in well-differentiated DCIS

	clinging/micropapillary	cribriform/solid	p†
Number of patients	38	76	
Screen-detected	33 (87)	54 (71)	0.062
Breast-conserving treatment	20 (31)	43 (32)	0.966
Median age in years (range)	50.5 (37-70)	51.0 (24-81)	0.398
Median pathologic size in mm (range)*	20 (5-80)	20 (5-100)	0.957
Margins not free	2 (5)	6 (8)	0.717
Local recurrences	0	11 (14)	0.016
8-year local recurrence free rate	100	89.2	0.031‡

Values in parentheses are percentages unless otherwise indicated. *Pathological size was reported in 58 (51%) patients: 16 (42%) patients with well-differentiated DCIS of clinging or micropapillary type and 42 (55%) patients with well-differentiated DCIS of cribriform or solid type. †The differences in the distribution of categorical variables were analyzed using the $\chi 2$ test, and the differences between continuous variables were tested using the Mann-Whitney U test unless otherwise indicated. ‡The local recurrence free rate was calculated by the Kaplan-Meier method.

Discussion

This study presents the clinical outcome of 504 patients who underwent WLE, WLE+RT, or mastectomy for DCIS at the Netherlands Cancer Institute from 1986 to 2005. The majority of lesions were mammographically detected due to the introduction of a nationwide screening program around 1990. Choice of treatment remains a dilemma in DCIS. Mastectomy may

be overtreatment whereas BCT bears the risk of residual tumor progressing into invasive recurrence and distant metastasis. Moreover, BCT of DCIS is technically difficult, as most lesions are non-palpable. Optimal treatment therefore is challenging as it requires a balance between obtaining local control by excision with sufficient tumor-free margins and good cosmetic outcome by minimalisation of excised tissue volume.

In the randomized EORTC DCIS and NSABP B-17 studies treatment with WLE alone was associated with local recurrence rates of 17-26% at 4-10 years and 27-32% at 8-12 years.[4,5] The 16% local recurrence rate at 8-years for WLE in the current series reflects the influence of patient selection when compared with the results from randomized studies.[8] For example, in our series, the majority of women who underwent WLE only had well-differentiated lesions and/or lesions smaller than 10 mm. On the other hand, patients with poorly differentiated DCIS were more frequently considered for WLE+RT (or mastectomy) and were overrepresented compared to the WLE+RT arm of the EORTC trial (62% in current series vs. 40% in EORTC trial). Due to this selection, the specific role of adjuvant RT in the prevention of local recurrences cannot be determined based on non-randomized series like the current one.

None of the patients in this series received hormonal treatment. The conflicting findings of the two randomized clinical trials investigating the role of tamoxifen in patients with DCIS resulted in our reserve to prescribe tamoxifen.[5,9] Only the NSABP B-24 study showed an absolute local recurrence reduction of 3% ($p=0.02$).[5] In addition, the benefit attributable to tamoxifen is confined to those tumors that are estrogen receptor (ER) positive while about half of the (poorly differentiated) DCIS seem to lack ER expression.[10,11]

For invasive ductal carcinoma, an additional boost has proven to be of benefit in especially patients younger than 50 years.[12] An additional boost to the tumor bed is also being applied more frequently in patients with DCIS.[13,14] Recently, a retrospective study assessed the effect of boost RT in patients with DCIS younger than 46 years and observed a decreased risk of local recurrence for those treated with a boost (10-years local recurrence rate with boost: 14%, with no boost: 28%).[15] This study seems to confirm that particularly young patients benefit most from a more extensive treatment. In accordance with other studies, we also found young age to be an independent risk factor for local recurrence.[13,14,16] Nonetheless, caution should be taken when interpreting the local recurrence rate after BCT for younger patients in our series as the absolute numbers are very small. Although we did not find a higher proportion of poorly differentiated DCIS in younger patients, their tumors may be biologically different by Her-2/Neu overexpression which partially contributes to an increased risk of recurrence.[17] These patients should be informed about their individual risks associated with BCT. For them a skin-sparing mastectomy with immediate reconstruction might be a reasonable alternative, particularly in light of their long-term life expectancy.

The most important factor associated with local failure is margin involvement.[4,5,16] Many patients (including a large number of patients referred from elsewhere) with involved margins after prior excision were not amenable to re-excision and underwent mastectomy. This explains the relative high mastectomy rate in our series throughout the whole period. Patients who underwent radical re-excision showed similar local recurrence rates as those

treated radical with one excision, an observation which is confirmed by others.[18] These results underline the necessity to perform a complete excision in all patients with DCIS. The number of second (or more) operations to allow for BCT in our series was higher compared to the EORTC study: 46% vs. 35%, respectively.[19] This further indicates that the surgery in our series aimed at complete excision of the DCIS lesion and, if not possible, a conversion to mastectomy. In 13% of the patients of the current study treated by BCT free margins were not obtained as opposed to 22% in the EORTC study.[4]

Although there is no evidence in literature that local treatment of DCIS is a significant factor for distant metastasis or survival,[4-6] in our series the 8-year distant metastasis free rate between BCT and mastectomy was of borderline significance (p=0.048). Six (2.9%) patients treated by BCT developed distant metastasis after invasive local failure, and after mastectomy this number was one (0.3%). The majority of these patients featured risk factors for local failure like age at diagnosis younger than 40 and margins not free. After exclusion of 34 (16.2%) high risk patients (age younger than 40 or margins not free), the 8-year local recurrence and distant metastasis free rate after BCT increased to 92.6% and 98.1%, respectively. This figure seems acceptable and improvement of BCT might therefore only be possible if these and other risk factors for local recurrence are recognized and taken into consideration for treatment choice.

On the other side of the spectrum, identification of patients with a low risk of developing a local recurrence is also warranted. WLE alone should only be reserved for low risk patients with prognostic favorable lesions. In our series, none of the 38 patients with well-differentiated clinging or micropapillary DCIS developed recurrent disease whereas 11 of the 65 patients with well-differentiated cribriform or solid DCIS did. The well-differentiated clinging type represents one of the earliest, morphologically recognizable, neoplastic alterations of the breast and is also defined as flat epithelial atypia by others.[20-22] The micropapillary type can be considered an intermediate type in the spectrum of development to the cribriform type. These findings are supported by the EORTC study in which was concluded that the benefit of radiotherapy on reduction of local recurrence will be very small for these lesions.[4] So far, no other low risk factors have been identified that warrant treatment by WLE alone.[23]

Finally, also after selection of patients, BCT of DCIS is associated with the risk of residual disease progressing into invasive local recurrence and distant metastasis. A re-excision or mastectomy is therefore wanted in all patients with unclear margins. Age younger than 40 years has shown to be an important risk factor for local recurrence. Younger patients should be informed about their individual risks and treatment options including the possibility of a skin-sparing mastectomy with immediate reconstruction.

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Chapter

7

Breast-conserving treatment with or without radiotherapy in ductal carcinoma in situ: ten-year results of EORTC randomized phase III trial 10853

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Abstract

Background: The European Organization for Research and Treatment of Cancer conducted a randomized trial investigating the role of radiotherapy (RT) after local excision (LE) of ductal carcinoma in situ (DCIS) of the breast. We analyzed the efficacy of RT with 10 years follow-up on both the overall risk of local recurrence (LR) and related to clinical, histologic, and treatment factors.

Methods: After complete LE, women with DCIS were randomly assigned to no further treatment or RT (50 Gy). One thousand and ten women with mostly (71%) mammographically detected DCIS were included. The median follow-up was 10.5 years.

Results: The 10-year LR-free rate was 74% in the group treated with LE alone compared with 85% in the women treated by LE plus RT (logrank $p<0.0001$, hazard ratio (HR)=0.53). The risk of DCIS and invasive LR was reduced by 48% ($p=0.0011$) and 42% ($p=0.0065$) respectively. Both groups had similar low risks of metastases and death. At multivariate analysis, factors significantly associated with an increased LR risk were young age (≤ 40 years, HR=1.89), symptomatic detection (HR=1.55), intermediately or poorly differentiated DCIS (as opposed to well-differentiated DCIS, HR=1.85 and HR=1.61 respectively), cribriform or solid growth pattern (as opposed to clinging/micropapillary subtypes, HR=2.39 and HR=2.25 respectively), doubtful margins (HR=1.84), and treatment by LE alone (HR=1.82). The effect of RT was homogeneous across all assessed risk factors.

Conclusion: With long-term follow-up, RT after LE for DCIS continued to reduce the risk of LR, with a 47% reduction at 10 years. All patient subgroups benefited from RT.

Introduction

Since the introduction of mammographic screening in the Western world, ductal carcinoma in situ (DCIS) has changed from being a rare disease to a lesion detected in up to 20% to 30% of breast cancers in screening programs.[1] Before the advent of screening, this preinvasive form of breast cancer was normally treated by mastectomy. After the proven success of radiotherapy (RT) in breast-conserving treatment (BCT) for invasive breast cancer,[2,3] in the mid and late 1980s, several randomized clinical trials in Europe and North America were initiated to evaluate optimal BCT for patients with DCIS. Three studies investigated the role of breast RT after local excision (LE) of the lesion.[4-6] In the European Organization for Research and Treatment of Cancer (EORTC) 10853 study, more than 1,000 women were randomly allocated to RT to the whole breast or no further treatment after complete LE of DCIS. The early results, published in 2000,[6] indicated an overall reduction of the risk of local recurrence (LR) as a result of RT. With this report, we analyzed the efficacy of RT with 10 years follow-up on both the overall risk of LR and related to various clinical, histologic and treatment factors.

Patients and methods

Women with DCIS of the breast were randomly assigned between RT and no further treatment following complete LE of the lesion. Extent of free margins was not specified other than that DCIS, should not be present at microscopic examination of the margins. Patients with lesions up to 5 cm in diameter, without evidence of (micro)invasion or Paget's disease, were eligible for the study. The prescribed irradiation dose was 50 Gy in 25 fractions to the whole breast. No boost was advised (5% of the patients randomized to RT received a boost). The use of tamoxifen was not recommended. The primary end points were both invasive and DCIS LR in the treated breast. Secondary end points included metastasis, death, and contralateral breast cancer (CLBC). Further information about study design, eligibility criteria, surgery and RT protocols, quality assurance, and follow-up procedures has been given previously.[6]

The data obtained from a general review, during which mammographic, surgical, histologic, and follow-up data were checked in the patients' medical files, served as a basis for the previous and current analyses. In the 16% for which no detailed review data were available, the original data reported to the EORTC Data Center were used for the analyses.

All patients were required to have bilateral mammograms preoperatively and annually during follow-up. Although the protocol did not require postoperative mammograms, a specimen x-ray was made in 90% of the patients with non-palpable DCIS.[7]

The trial included a central pathology review, available on 863 patients.[8] For the current analysis, we have used the data of the pathology review for analyses related to the risk of recurrence. At pathology review, invasive growth was found in 27 cases, and in 13 there was

suspicion of invasion. In 48 cases, benign proliferative lesions or lobular carcinoma in situ were diagnosed at review. These cases have been included in all analyses of the effect of RT on the primary and secondary end points. Analysis restricted to confirmed DCIS cases yielded the same results (data not shown).

Because the extent of the lesion and the width of the tumor-free margin could not reliably be assessed by review of the histologic slides, the pathology reports were reviewed. The size of the DCIS was mentioned in the pathology report in only 193 cases (25%). The margin status was considered free if it was reported free (> 1 mm), or if a re-excision was performed and no residual DCIS was found. When margins were reported to be close (≤ 1 mm) or involved, and when the margin status was not specified, the margin status was classified under not free. A previous analysis had shown that at a median follow-up of 5.4 years, the first groups, as well as the last three groups, had similar recurrence rates.[8]

All analyses are based on the intent-to-treat principle with recurrence-free intervals defined as the time between the date of random assignment and the date of recurrence. The time-to-recurrence curves were calculated using the Kaplan-Meier technique[9] and compared using a two-sided log-rank test with 5% type I error.[10] An estimate of the size of the treatment effect was calculated based on the hazard ratio (HR) and its 95% two-sided CI. The HRs are presented with the level of the variable considered best as the baseline. A Cox proportional hazards regression model[11] was fitted for the multivariate analysis of LR, using variables with significant p values (< 0.05) in the univariate analysis.

Results

Between March 1986 and July 1996, 1,010 women were randomly assigned to no further treatment (503 patients) or to RT (507 patients) after LE. The previous report demonstrated that patient, tumor, and treatment characteristics according to treatment group were well balanced between the arms.[6] The median age of the women was 53 years; 71% of them were mammographically detected. The present analysis was done in August 2005, at a median duration of follow-up of 10.5 years.

LR-free interval

One hundred thirty-two patients developed LRs in the LE group and 75 in the LE+RT group. The risk of LR was reduced with 47% in the LE+RT group compared with that in the LE group (log-rank p<0.0001), the 10-year LR-free rates were 85% and 74%, respectively (Table 1, Figure 1). One hundred three patients had LRs of DCIS, and 106 patients developed invasive LRs. Two patients with a DCIS LR subsequently developed an invasive LR. There was a similar reduction in the risk of DCIS and invasive LR. The 10-year DCIS LR-free rate was 93% in the LE+RT group versus 86% in the LE group (p=0.0011); the 10-year invasive LR-free rates were 92% and 87% respectively (p=0.0065) (Table 1, Figure 1).

Table 1. Event-free estimates at 10 years and hazard ratios according to treatment

Event	Number of patients*	10-year event free estimate† (%)	Hazard ratio‡	95% CI	Log-rank p
Local recurrence			0.53	0.40 to 0.70	< 0.0001
LE	132	74			
LE+RT	75	85			
DCIS recurrence			0.52	0.34 to 0.77	0.0011
LE	67	86			
LE+RT	36	93			
Invasive recurrence			0.58	0.39 to 0.86	0.0065
LE	66	87			
LE+RT	40	92			
Regional recurrence			0.46	0.20 to 1.07	0.064
LE	17	97			
LE+RT	8	99			
Distant metastasis			1.14	0.63 to 2.08	0.66
LE	20	96			
LE+RT	23	96			
Death			1.18	0.70 to 1.96	0.53
LE	27	95			
LE+RT	32	95			
Contralateral breast cancer			1.41	0.87 to 2.30	0.16
LE	28	96			
LE+RT	39	92			
Contralateral DCIS			1.10	0.47 to 2.59	0.82
LE	10	98			
LE+RT	11	98			
Contralateral invasive			1.48	0.83 to 2.65	0.18
LE	19	97			
LE+RT	28	94			
Event free survival			0.72	0.57 to 0.91	0.0066
LE	160	70			
LE+RT	123	76			

Abbreviations: LE, local excision alone; RT, radiotherapy; DCIS, ductal carcinoma in situ. *Overall totals 503 on LE and 507 on LE+RT. †Kaplan-Meier estimate at 10 years. ‡Values < 1 indicate a better outcome for LE+RT.

A salvage mastectomy was performed in 144 (70%) of the 207 LRs, whereas 56 patients underwent BCT. Thirty patients, originating from the LE group, received adjuvant RT. In seven patients, treatment of LRs was not reported.

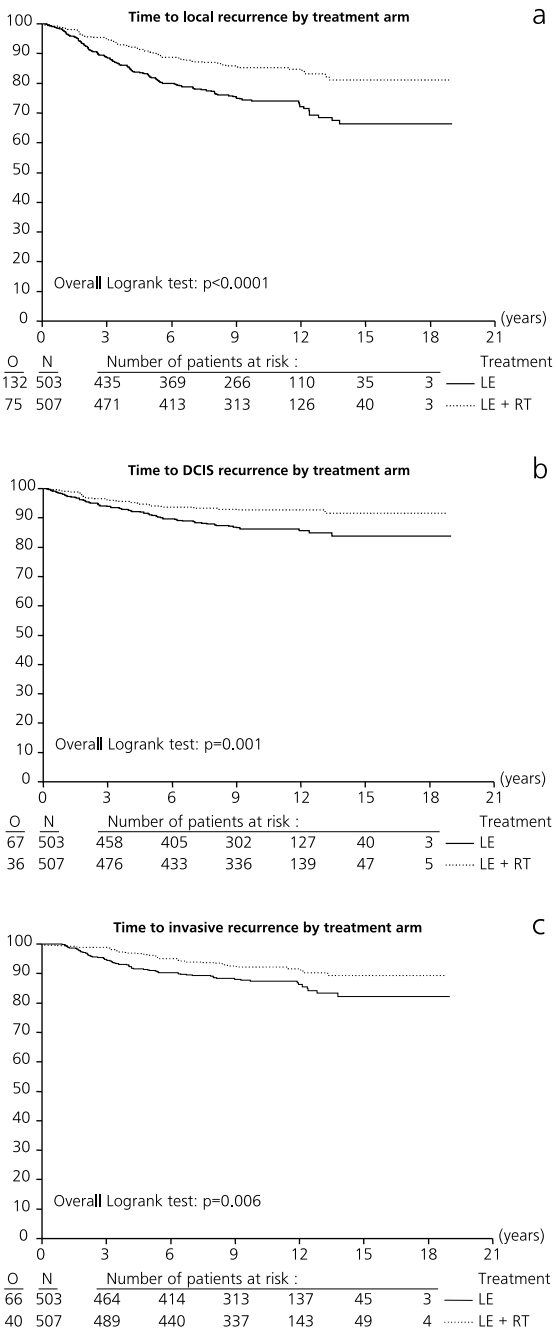


Figure 1. (a) Time to local recurrence by treatment arm; (b) time to ductal carcinoma in situ recurrence by treatment arm; and (c) time to invasive recurrence by treatment arm. O, observed; N, number of patients; LE, local excision; RT, radiotherapy.

Other events

Table 1 demonstrates no significant difference in the 10-year CLBC-free interval. The 10-year metastasis-free rate was the same in the two treatment arms (96%). In 25 patients, metastases originated from an invasive LR (15 in the LE group and 10 in the LE+RT group). Two patients (in the LE+RT group) developed metastases after a DCIS LR. In five patients, distant metastasis developed without a prior LR or CLBC, and in nine patients, metastatic disease was preceded by a CLBC. Two patients developed metastases simultaneously with a regional recurrence (without an LR).

Of the 59 deaths, 32 were breast cancer-related (15 in the LE group and 17 in the LE+RT group): 23 patients died as a result of metastatic disease after an LR, four patients from metastases as first event without prior LR, and another five patients after an invasive CLBC. Another malignancy was the cause of death in 13 patients, seven died of cardiovascular disease, five because of various other causes, and for two patients the cause of death was unknown. The 10-year overall survival rate was 95% in both arms.

Risk factors associated with recurrence

The analyses on risk factors were performed on 775 cases in which the diagnosis of DCIS was confirmed. Table 2 shows results of the univariate analysis. Women 40 years of age or

Table 2. Univariate analyses of clinical and histologic characteristics related to local recurrence

Characteristic	Number of patients	Number of events	10-year event-free %	Hazard ratio	95% CI	Log-rank p
Age, years						
> 40	945	184	81	1		0.0021
≤ 40	65	23	66	1.95	1.26-3.01	
Method of detection						
X-ray finding only	723	134	82	1		0.0095
Clinical symptoms	275	72	74	1.46	1.09-1.94	
Size*						
<10 mm	134	25	82	1		0.12
10-20 mm	42	11	79	1.37	0.67-2.80	
>20 mm	17	7	59	2.37	1.02-5.47	
Histologic type*						
Well	284	39	86	1		0.0001
Intermediate	199	57	73	2.26	1.50-3.39	
Poor	292	77	74	2.08	1.41-3.05	
Architecture*						
Clinging/micropapillary	204	20	91	1		< 0.0001
Cribriform	269	69	74	2.83	1.72-4.65	
Solid/comedo	299	83	73	3.13	1.92-5.10	
Margins*						
Free	578	110	81	1		0.0001
Not free	163	55	68	1.89	1.37-2.63	

*: in ductal carcinoma in situ-confirmed patients.

younger were at high risk for developing an LR (34% at 10 years). In the LE group, 16 of 38 young women developed an LR (43% at 10 years). In the LE+RT group, seven of 27 women had an LR (23% at 10 years). Young women had a higher rate of symptomatically detected lesions (66%, compared with 25% of women older than 40), mostly because they were not in the screening age range. Twenty-seven percent (11 of 41) of the young patients had margins that were not free, compared with 22% (152 of 700) of the women older than 40 years of age. Of the younger patients, 37% had poorly differentiated lesions, compared with the 38% of patients older than 40 years of age.

Also at a high risk of LR were patients with not-free margins (32% LRs at 10 years). The LR rate after LE was 39%, and after LE+RT 24% at 10 years. Low LR rates were observed in lesions with a clinging/micropapillary growth pattern; overall, 9% LRs at 10 years were found, with 13% in the LE and 5% in the LE+RT group. A further analysis of the well-differentiated lesions according to architectural pattern demonstrated that four of 58 and eight of 99 patients with, respectively, clinging and micropapillary growth patterns developed an LR. If the well-

Table 3. Multivariate analysis of risk factors related to local recurrence

Variable	Hazard ratio	95% CI	P
Age, years			
> 40	1		
≤ 40	1.89	1.12 to 3.19	0.026
Method of detection			
X-ray finding only	1		
Clinical symptoms	1.55	1.11 to 2.16	0.012
Histological type			
Well	1		
Intermediate	1.85	1.18 to 2.90	0.024
Poor	1.61	0.93 to 2.79	
Architecture			
Clinging/micropapillary	1		
Cribriform	2.39	1.41 to 4.03	0.002
Solid/comedo	2.25	1.21 to 4.18	
Margins			
Free	1		
Not free	1.84	1.32 to 2.56	0.0005
Treatment			
LE+RT	1	1.33 to 2.49	
LE	1.82		0.0002

Abbreviations: LE, local excision; RT, radiotherapy.

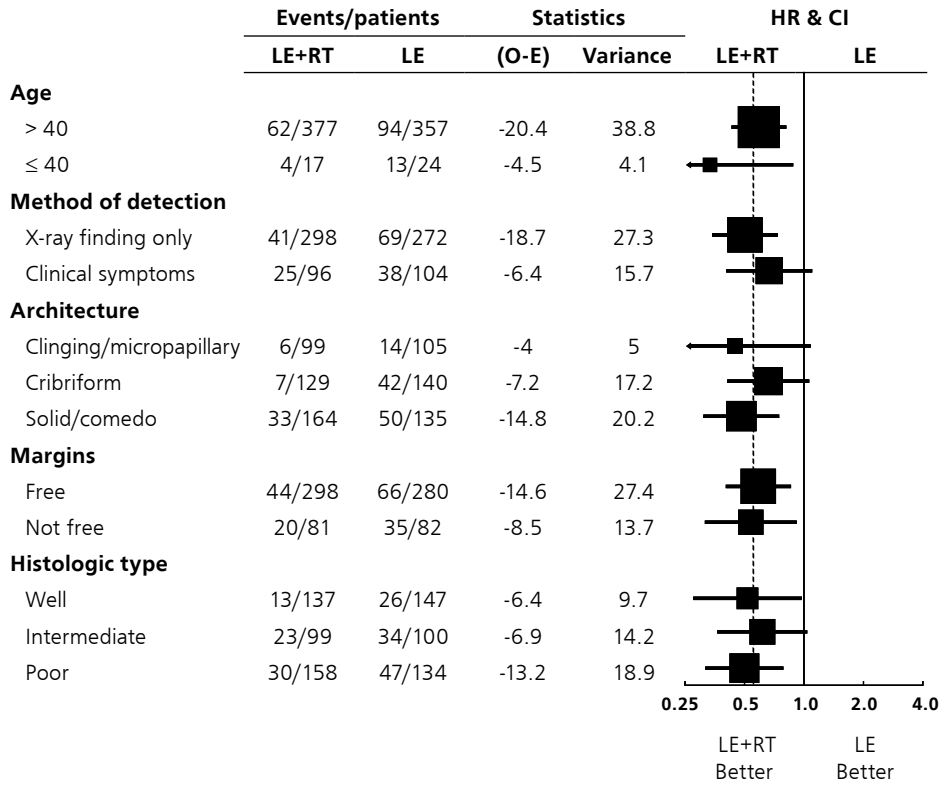
differentiated DCIS had cribriform or a solid/comedo architecture, 24 of 115 and three of 10 women developed an LR, respectively.

Figure 2 shows in a Forrest plot that RT reduced the risk of LR in all subgroups, with the effect of RT being homogeneous across all risk factors.

At multivariate analysis, factors significantly associated with an increased risk of LR were young age, symptomatic detection of the lesion, intermediately or poorly differentiated DCIS (as opposed to well-differentiated DCIS), solid or cribriform growth pattern (as opposed to clinging/micropapillary subtypes), margins that were not free, and treatment by LE alone (Table 3).

The histologic type was related to the risk of DCIS and invasive LR, metastases, and death (Table 4). Well-differentiated DCIS had a lower risk of DCIS LR but not of invasive LR. Overall, the histologic type was not significantly related to the risk of distant metastases or death. Of note is that all causes of metastases and death are included in this analysis (eg, also resulting from CLBC). Twenty-three of 106 patients with an invasive recurrence developed metastasis; the corresponding Kaplan-Meier estimate of the metastasis-free rate at 10 years after an invasive recurrence is 74% (counting from the invasive recurrence).

Figure 2. Effect of radiotherapy on local control by subgroup



Abbreviations: LE, local excision; RT, radiotherapy; HR, hazard ratio; CI, 95% CI.

CHAPTER 7

Discussion

With a median follow-up of 10.5 years, the results of this randomized trial continue to show that RT after LE of DCIS of the breast reduces the risk of LR as compared to LE alone. The magnitude of the reduction has become slightly larger compared with the analysis performed at 4.25 years (HR now = 0.53, HR then = 0.62). The EORTC 10853 trial is the second to publish its long-term results. The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-17 study, including 818 women, published long-term results at 10.8 years follow-up.[4] The UK Co-ordinating Committee on Cancer Research (UKCCCR) DCIS trial, randomly assigning 1,701 patients to RT, tamoxifen, or both, presented its first results in 2003 at 4.4 years.[5] All three trials demonstrate a reduction in the risk of LR as result of RT. The EORTC study demonstrates similar reductions by RT for DCIS and invasive LR: a 10-year DCIS LR rate reduction from 14% to 7% and from 13% to 8% of invasive LR. All three trials, as well as many nonrandomized studies,[12-14] show that in both treatment groups about half of the LRs are DCIS and half of them are invasive.

Table 4. Histological type related to DCIS/invasive recurrence, metastasis and death

Event	Number of patients	Events		Hazard ratio	(95% CI)	P
		Number	(%)			
DCIS recurrence						0.0006
Histological type						
Well	284	15	5	1		
Intermediate	199	31	16	3.13	1.69 to 5.80	
Poor	292	41	14	2.82	1.56 to 5.09	
Invasive recurrence						0.35
Histological type						
Well	284	26	9	1		
Intermediate	199	26	13	1.45	0.84 to 2.51	
Poor	292	36	12	1.35	0.81 to 2.23	
Distant metastasis*						0.24
Histological type						
Well	284	8	3	1		
Intermediate	199	8	4	1.47	0.55 to 3.91	
Poor	292	17	6	2.04	0.88 to 4.73	
Death†						0.17
Histological type						
Well	284	11	4	1		
Intermediate	199	10	5	1.35	0.57 to 3.18	
Poor	292	23	8	1.96	0.95 to 4.02	

Abbreviation: DCIS, ductal carcinoma in situ. *As a result of ipsilateral breast cancer: four well, five intermediate, 13 poorly-differentiated. †As a result of ipsilateral breast cancer: three well, three intermediate, 12 poorly-differentiated.

Whereas in the EORTC study, at the first analysis a surprisingly higher rate of CLBCs was found in the RT arm,[6] in the current update a significant difference could no longer be observed. As was assumed in the first analysis, this update seems to confirm that the original finding was a false positive.

Our analysis of risk factors for LR showed that young women (≤40 years of age) are at a particularly high risk for LR. A similar phenomenon is seen for invasive breast cancer.[15-18] Other studies have also found that young age is an adverse prognostic factor for LR after BCT for DCIS.[12,14,19,20] The cases of DCIS in the young age group are a mixture of lesions detected in high-risk women who underwent individual screening, and of symptomatic lesions, which have been shown to grow more extensively.[21] Possible biological differences of DCIS in young women are subject of research.[22] In our study, the young women did not have a higher frequency of poorly differentiated DCIS compared with the older women.

The completeness of excision of the DCIS remains one of the most important predictors for LR. Many studies have shown that margin status is an independent factor for LR after BCT for DCIS.[12-14,20,23] The current trial required excision margins to be free of tumor for trial

entry. Thus, by review of the medical files, strictly, patients with involved margins would have become ineligible. However, only seven patients were entered while the pathologist stated the margins to be involved with tumor. When margins are really involved with tumor, one can expect even higher LR rates. Therefore, the performance of a complete excision with tumor-free margins is one of the mainstays of BCT for DCIS. In 1999, a retrospective series suggested that with a margin width of at least 10 mm, the risk of LR was very low, with possibly a limited absolute additional benefit of RT in BCT of DCIS. Recently, a prospective study of 158 patients with small (≤ 2.5 cm) grade 1 or 2 DCIS, excised with margins of 10 mm or larger, still found a high LR rate after LE only of 12% at 5 years.[24] In our study, those patients who underwent a re-excision in which no residual DCIS was found (also considered ≥ 10 mm) did not have a lower LR rate compared with those who had free margins without further specification of the margin width. Currently, the Radiation Therapy Oncology Group (RTOG) 9804 study randomly assigns women with 'good risk' DCIS between RT plus tamoxifen and tamoxifen only.

The risk factor analysis of the NSABP study at 8 years follow-up yielded the presence of comedo necrosis as the most important risk factor related to LR.[25] Although margin status was of borderline significance in this analysis, the authors still stressed the importance of a microscopic complete excision.

Current practice, to ensure complete removal of all microcalcifications, includes a postoperative mammogram that was not part of the protocol because, at the time this study was designed, there was limited experience with BCT for nonpalpable lesions.

Our analysis shows that well-differentiated DCIS had a lower risk of LR than intermediately and poorly differentiated subtypes. Nevertheless, also in the well-differentiated group, RT reduced the risk of LR (Figure 2). As can be seen in Table 4, well-differentiated DCIS had a lower risk of DCIS LR but not of invasive LR. From our data, high-grade DCIS does not seem to progress into invasive carcinoma more rapidly than low-grade DCIS. Table 4 shows that a higher number of women (n=12) with poorly differentiated DCIS died as a result of invasive LR, compared with three women with a well and three with an intermediately differentiated DCIS.

The groups with an exceptionally low risk of recurrence were the well-differentiated DCIS with clinging or micropapillary growth pattern, with, in both groups, overall less than 10% LRs at 10 years. In these groups, although the relative benefit of RT is similar to the other groups, the absolute benefit of RT on the LR risk will become very small.

The reduced risk of LR caused by RT has, at 10 years follow-up, not resulted in a survival difference between the two arms. The death rate attributable to metastasized breast cancer after an invasive LR is with 2% the same in both arms and is, with this time of follow-up, similar to death rates reported after mastectomy.[26] However, this study was not powered for finding a difference in metastasis or survival. Perhaps only long-term follow-up from combined clinical trials can give answer to these questions. For women with DCIS who are at a high risk of invasive LR, such as those 40 years of age or younger or those with lesions that cannot be excised with tumor-free margins, the subsequent risk of eventually dying from metastasized disease after an invasive LR could become unacceptably high.

Both the NSABP B-17 and the EORTC 10853 trial show relatively high 10-year LR rates of about 15% after RT. In the RT arms of these trials, the whole breast was irradiated to a dose of 50 Gy, without a boost dose administered to the original tumor bed. Recently, a large randomized trial has demonstrated that, in invasive breast cancer, an additional dose of 16 Gy directed at the tumor bed further reduced the risk of LR, with a HR of 0.59.[27] This additional dose to the tumor bed might also further reduce the risk of LR in DCIS.

The joint randomized trial NSABP B-39/RTOG 0413 compares whole-breast RT with partial breast RT in patients with early stage breast cancer, including DCIS. Due to the sometimes discontinuous spread of DCIS within the branching ducts, residual disease may not be in the immediate vicinity of the biopsy cavity. Therefore, women with DCIS might not be good candidates for partial breast RT.

Two randomized trials have investigated tamoxifen in the treatment of DCIS.[5,28] The UKCCCR reported only a slight effect of tamoxifen on the reduction of DCIS LR, and concluded that there is little evidence for treatment with tamoxifen in women with DCIS. The NSABP B-24 study showed a reduction of mainly invasive LRs and CLBCs caused by tamoxifen. In neither the UKCCCR nor the NSABP B-24 trial was information available on the estrogen receptor (ER) of the DCIS. Our data demonstrate that patients with a higher risk of metastases are those with a poorly differentiated DCIS. These lesions lack ER overexpression in 52-61%.[29-31] Tamoxifen is known to be ineffective for preventing recurrence in ER-negative breast tumors. The NSABP B-35 study, comparing tamoxifen with anastrozole in postmenopausal women with DCIS, is ongoing.

In summary, the updated results of our trial confirm that, at long-term follow-up, the effectiveness of RT in BCT for DCIS persists. In addition, we have observed that RT reduced the risk of LR in all clinical and pathological subgroups of patients, with a homogeneous treatment effect of RT across the levels of all factors considered. Hence, RT should be considered in all women treated with BCT for DCIS. However, some subgroups are at very low risk for LR; patients with clinging/micropapillary well-differentiated DCIS might be offered excision without additional irradiation in view of their excellent prognosis with surgery alone. In contrast, some women are, even after RT, at high risk of LR, such as young women, and/or those with involved excision margins; in these patients, conservation of the breast should be weighted against a relatively high risk of developing distant metastases caused by an invasive LR from a curable disease.

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Appendix

The following institutions, listed with their principal investigators, participated to this study: Istituto Nazionale Dei Tumori, Milano, Italy (M Gennaro), Institut Bergonié, Bordeaux, France (A Avril), Center Henri Becquerel, Rouen, France (J-P Julien), CRCL Val D'Aurelle, Montpellier, France (P Rouanet), Institut Jules Bordet, Brussels, Belgium (M Coibion), University Hospital Gasthuisberg, Leuven, Belgium (J Wildiers), Radiotherapeutisch Instituut, Arnhem, the Netherlands (AH Westenberg), The Netherlands Cancer Institute - Antoni van Leeuwenhoek Ziekenhuis, Amsterdam, the Netherlands (EJT Rutgers), Rotterdams Radiotherapeutisch Instituut, the Netherlands (PCM Koper/C Griep), Dr Bernard Verbeeten Instituut, Tilburg, the Netherlands (HP Hamers), Ospedale Civile, Genova, Italy (G Canavese), Integraal Kankercentrum West, Leiden, the Netherlands (MB Lagaaij), Policlinico di Careggi, Florence, Italy (L Cataliotti), University Hospital Leiden, the Netherlands (JWH Leer), Maastro Clinic, Heerlen, the Netherlands (JJ Jager), Guy's Hospital, London, England (IS Fentiman), Universitair Medisch Centrum St Radboud, Nijmegen, the Netherlands (T Wobbes), Intergraal Kankercentrum Amsterdam, the Netherlands (HW Siebbeles), UMCU Utrecht, the Netherlands (H Struikmans), Center Eugène Marquis, Rennes, France (C Chenal), St Savas Hospital, Athens, Greece (I Karydas), IPO, Porto, Portugal (J Cardoso da Silva), Western Gerenal Hospital, Edinburgh, Scotland (U Chetty/HJ Stewart), Istituto Europeo di Oncologia, Milano, Italy (V Sacchini), Center Georges-François Leclerc, Dijon, France (JC Horiot), Hôpital Jean Minjoz, Besançon, France (S Schraub/JF Bosset), Academisch Medisch Centrum, Amsterdam, the Netherlands (CW Taat), Istituto de Ginecologia e Ostetrica, Torino, Italy (D Katsaros), Universitätsklinik für Chirurgie, Graz, Austria (MG Smola), Marika Eliadi Hospital, Athens, Greece (S Vassilaros), I Universitäts Klinik für Chirurgie, Innsbruck, Austria (M Duenser), Radiotherapeutisch Instituut Friesland, Leeuwarden, the Netherlands (G Botke), Policlinico di Borgo Roma, Verona, Italy (S Modena), Ospedale de Noale, Italy (M Bari), Instituto Oncologia San Sebastian, Spain (JA Alberro), Onze Lieve Vrouwe Gasthuis, Amsterdam, the Netherlands (KJ Roozendaal), Diakonessenhuis, Utrecht, the Netherlands (JWR Nortier), Ninewells Hospital, Dundee, Scotland (PE Preece), Erasmus MC, Rotterdam, the Netherlands (TAW Splinter), Sint Lucas Andreas Ziekenhuis, Amsterdam, the Netherlands (R Weller), Streekziekenhuizen Gooi-Noord, Blaricum, the Netherlands (MAN Gerrits), Universitätsklinik, Göttingen, Germany (H Rauschecker), Hôpital Cantonal de Genève, Switzerland (L Perey), Tygerberg Hospital, South Africa (J Van Zijl), Rambam Medical Center, Haïfa, Israël (A Kuten), CHU Vaudois, Lausanne, Switzerland (R Mirimanoff)

Comment and authors' reply

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Radiation Therapy for Ductal Carcinoma In Situ: Is It Really Worth It?

To the Editor: Ductal carcinoma in situ (DCIS) is almost a non–life-threatening disease with 10-year cancer-specific survival of more than 97% as reported in European Organisation for Research and Treatment of Cancer trial 10853.[1] The potential benefit of preventing local recurrences must be weighed in the context of potential morbidity of such preventive treatment (local radiation therapy [RT]) because these women are most likely to fulfill their normal life expectancy. Although this trial showed significant reduction in local recurrences, it reported slightly more (nonsignificant) cancer-specific deaths and distant-metastasis events in the RT arm. Because there is no difference in breast-cancer–specific deaths in two arms, let us examine the number needed to treat for preventing metastatic disease. Approximately 25 invasive cancer recurrences were prevented with RT and approximately one quarter of patients with invasive cancer further developed metastatic disease. This means six to seven of such events were prevented when more than 500 patients received RT—a number needed to treat of almost 100. A recent Early Breast Cancer Trialists' Collaborative Group pooled analysis[2] shows that RT increases risk of death due to heart disease by 27% (after 15 years). Median age of patients in this trial was 53, which means after 15 years, the majority of them will be in their late 70s. Data from patients with invasive breast cancer[3] shows that at this age their likelihood of dying due to breast cancer is less compared with death due to other causes, like cardiovascular diseases. Please note that we are not even counting other causes of death like lung and esophageal cancers caused by RT and all of these hazards of RT have not disappeared with the newer radiotherapy machines.[4] Expecting RT to save lives of DCIS patients will not be appropriate. On the contrary, it may increase all-cause mortality. Using local RT very cautiously and judiciously only in the high-risk group seems to be the appropriate message to be drawn from this trial.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST
The authors indicated no potential conflicts of interest.

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In Reply: On behalf of the European Organisation for Research and Treatment of Cancer (EORTC) ductal carcinoma in situ (DCIS) study team, we thank Thorat et al for their valuable comments on the recently updated results of the EORTC 10853 DCIS trial. We completely agree that the benefit of a treatment should always be weighed against a possible harm. However, this study showed that radiotherapy reduced the number of invasive local recurrences (48%; $P = .0011$) without observing an increased risk of cardiovascular death due to radiotherapy at 10.5 years of follow-up. Seven patients died of cardiovascular disease: four in the local excision alone group (0.8%) and three in the local excision plus radiotherapy group (0.6%).[1] We found an invasive local recurrence rate of 13% for patients treated with excision alone at 10 years. The National Surgical Adjuvant Breast and Bowel Project B-17 study reported a rate of 17% for these patients at 12 years.[2] It is therefore likely that this rate will be at least 16% in the EORTC study at 15 years. If one quarter of these patients will develop additional distant metastases, radiotherapy will prevent 2% of such events. In addition, recurrent disease is known to have a negative effect on the quality of life of women treated for DCIS.[3] Despite that the findings from the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) analysis could not be confirmed in our study, we acknowledge the possible long-term risks associated with radiotherapy.[4] The EBCTCG analysis reported that some of the radiotherapy regimens used in the older trials of postmastectomy radiotherapy appreciably increased nonbreast cancer mortality more than 5 years later, with most of this excess mortality involving heart disease and lung cancer. The radiotherapy regimens of the early 1970s involved greater hazards than many recent regimens. This is reflected by a decrease in the cardiac mortality ratio 5 to 9 years after breast cancer diagnosis for left-sided versus right-sided breast cancer from 1.21 for patients diagnosed between 1973 and 1982 to 0.99 for patients diagnosed between 1993 and 2001.[5] In addition, it should be noted that the EBCTCG study reporting on excess 15-year nonbreast cancer mortality effect of radiotherapy does not specify this risk by type of radiation fields used. Regional lymph node irradiation, with notable radiotherapy of the internal mammary nodes, which can lead to relatively high cardiac doses, is irrelevant to DCIS. Finally, the favorable prognostic outcome of DCIS compels identification of patients who benefit most from radiotherapy by selecting those patients with a high risk of local recurrence. However, none of the large randomized clinical trials have identified a large subgroup that clearly does not benefit from radiotherapy. This is corroborated by results from the early stopped prospective study of wide excision alone.[6]

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST
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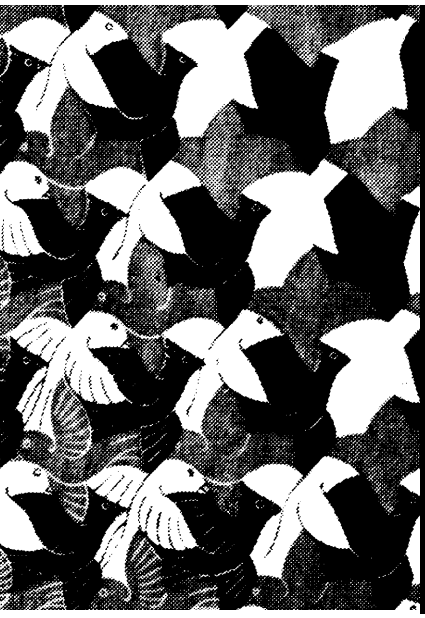

Chapter

8

Ductal carcinoma in situ of the breast. An overview of the randomized clinical trials

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Abstract

Since 1985, 6478 patients were randomized in five large randomized clinical trials assessing the role of adjuvant radiotherapy and/or tamoxifen in breast conserving surgery for patients with ductal carcinoma in situ (DCIS).

First the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-17 trial proved relevance of postoperative radiotherapy in breast conserving surgery in DCIS patients: an approximately 50% reduction of ipsilateral tumour recurrences. This is confirmed by longer time of follow-up and validated by data from the European Organization for Research and Treatment of Cancer (EORTC) 10853, UK Coordinating Committee on Cancer Research (UKCCCR) DCIS, and Swedish Breast Cancer Cancer Group DCIS (SweDCIS) trials. A subset of patients who do not benefit of adjuvant radiotherapy can not be identified. Completeness of excision proved to be the most important treatment variable to reduce local recurrence.

Both the NSABP B-24 and UKCCCR DCIS trials investigated the effect of tamoxifen resulting in contradictory findings. In addition, a possible benefit seems to be restricted to estrogen receptor-positive DCIS lesions nourishing the existent reserve in prescribing tamoxifen for all patients with DCIS.

The failure to materialize a difference in survival by the effect of either adjuvant radiotherapy or tamoxifen endorses the favourable outcome of DCIS. Currently, four other randomized clinical trials investigate the role of radiotherapy, tamoxifen, anastrazole, or no further treatment for a selected group of patients.

Introduction

Although many people believe the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-17 trial (1985-1990) was the first randomized clinical trial to study the effect of postoperative radiotherapy in patients with breast conserving surgery in ductal carcinoma in situ (DCIS), the NSABP B-06 trial (1976-1984) comparing total mastectomy and segmental mastectomy (lumpectomy) with or without radiotherapy inadvertently included 78 DCIS lesions.[1] Seventy-six of them were followed for 83 months following treatment by lumpectomy only (n=21), lumpectomy and radiotherapy (n=27), or mastectomy (n=28). Nine of 21 (43%) exhibited recurrences after lumpectomy, two of 27 (7%) after lumpectomy and radiotherapy and none after mastectomy.[2] The B-17 trial was designed with the same purpose as the B-06 in a period that DCIS had been treated in many different ways, ranging from local excision with or without adjuvant radiotherapy to unilateral or bilateral mastectomy; both studies suggest a beneficial effect of radiation in local control.[3,4] In contrast to (and as a result of) the trials comparing outcomes after mastectomy with those after breast conserving surgery in invasive breast cancer, it is unlikely that a similar trial for DCIS will ever be designed. Local recurrence rates of 22.5%, 8.9%, and 1.4% after conservative surgery alone, conservative surgery with adjuvant radiotherapy and mastectomy, respectively were reported in a meta-analysis of 4174 patients.[5] Lack of difference in survival between these treatment modalities led to the intricacy of patient tailored management in DCIS. Considering invasive recurrence as the most potent violator of local failure and keeping in mind the possibility of a salvage mastectomy, breast conserving surgery should - in general - be the standard treatment for all DCIS lesions. Unfortunately, medicine is not so straight forward since DCIS lesions are very heterogeneous in their presence and behaviour. Multicentric and large lesions are not uncommon and bear a higher risk of harbouring a (micro)invasive component. So far, five large randomized clinical trials have been designed and carried out to evaluate the role of adjuvant radiotherapy and/or hormonal treatment in breast conserving therapy of DCIS patients: the NSABP B-17 and B-24 trials, the European Organization for Research and Treatment of Cancer (EORTC) 10853 trial, the UK Coordinating Committee on Cancer Research (UKCCCR) DCIS trial, and the recent Swedish Breast Cancer Cancer Group DCIS (SweDCIS) trial. (Table 1).

NSABP B-17 trial

The NSABP B-17 trial was the first large trial to investigate the effect of postoperative radiotherapy in patients diagnosed with this noninvasive lesion. Primary endpoints were ipsilateral, contralateral, regional, or distant recurrences. From 1985-1990, a group of 818 women with DCIS were randomly assigned to undergo lumpectomy or lumpectomy followed by radiotherapy (50 Gy). Eighty-one per cent of all lesions was detected by mammography. Hence, the term lumpectomy is obsolete for most of the procedures where no palpable lump

Table 1. Five randomized clinical trials in DCIS

Protocol and publication dates	No. of patients	Median follow-up (yrs.)	No. of patients	Treatment	Number of local recurrences		Local recurrence free survival	
					invasive	DCIS	(%)	years
NSABP B-17								
1993[4]	818	3.6	399	LX	8	20	93	5
			391	L	32	32	84	
1998[6]		7.5 (mean)		LX	17	30	88	8
				L	53	51	73	
2001[7]		12		LX	29	32	84	12
				L	66	57	68	
EORTC 10853								
2000[8]	1010	4.3	502	LX	24	29	91	4
			500	L	40	44	84	
2006[9]		10.5		LX	40	36	85	10
				L	66	67	74	
UKCCCR								
2003[10]	1701	4.4	522	LX	15	14	94	4
			508	L	30	38	86	
		4.4	794	LT	45	57	87	4
			782	LP	35	77	85	
NSABP B-24								
1999[11]	1804	5	899	LT	23	40	94	5
			899	LP	40	47	91	
2001[7]		7		LT	27	45	92	7
				LP	49	51	98	
SweDCIS								
2006[12]	1067	5.2	526	LX	21	23	93	5
			520	L	48	69	78	

NSABP, National Surgical Adjuvant Breast and Bowel Project; EORTC, European Organization for Research and Treatment of Cancer; UKCCCR, UK Coordinating Committee on Cancer Research; SweDCIS, Swedish Breast Cancer Group DCIS trial; LX, lumpectomy and radiotherapy; L, lumpectomy alone; LT, lumpectomy and tamoxifen; LP, lumpectomy and placebo

could be detected. Margins of the resected specimen were histologically tumour-free, defined as tumour-filled ducts not touching an inked surface. Axillary dissection was obligatory at the onset of the study, but subsequently became optional on the basis of evidence, indicating that it was not necessary in the treatment of DCIS.[13] Three-hundred (38%) patients underwent an axillary dissection ultimately. Results after 3.6 years of median follow-up were reported in 1993 leading to the recommendation that radiotherapy after lumpectomy is more appropriate than lumpectomy alone for women with localized DCIS: 7% vs. 16.4% risk of ipsilateral

tumour recurrence, respectively (p<0.001).[4] These findings were affirmed by longer time of follow-up: 12.1% vs. 26.8% at 8 years[6] and 15.7% vs. 31.7% at 12 years, respectively.[7] Invasive ipsilateral recurrence rate was reduced by 47% (p=0.01), and noninvasive by 15% (p=0.48). In total, 413 patients were assigned to radiotherapy resulting in 29 invasive, and 32 noninvasive ipsilateral local recurrences compared to 405 patients treated with excision alone resulting in 66 invasive, and 57 noninvasive ipsilateral local recurrences. The 12-year local recurrence free survival rate was 84.3% and 68.3% for the radiotherapy and excision alone group, respectively. Overall survival was 86% for the patients who underwent radiotherapy and 87% for the patients treated with excision alone.

First analysis of possible pathologic discriminants predictive of ipsilateral recurrence resulted in the association of positive margins and comedonecrosis with higher risk for ipsilateral recurrence.[14] Nevertheless, these features failed in selecting a subgroup of patients who benefit most of adjuvant radiotherapy. Following this first large DCIS trial margin width has become subject of many studies[15-17] but its role in selecting patients has not yet been confirmed by a prospective study. Wong et al. reported the findings of an early stopped prospective study investigating the role of local excision alone with margins of ≥ 1cm for small, grade 1 or 2 DCIS. The accrual goal of 200 patients could not be achieved as the study was closed to further accrual at 158 patients because of the high number of ipsilateral local recurrences (5 year rate: 12%) suggesting benefit of radiotherapy for all patients treated with conservation.[18] The B-17 trial was criticized on the histological data reflecting the apparent assumption that all DCIS are histologically the same.[19] However, the heterogeneity of this lesion became more evident.[20,21] Secondly, partial tissue sampling in this trial may have harboured a risk of missing invasion. Further, dichotomous stratification of grade and size hampered comparison with other studies, and made it likely that many cases were larger and invasive. In addition, the definition of histologically free margins was doubtful leading to residual disease in many cases which made it more difficult to assess the benefit of radiotherapy.[19] An update of the pathologic findings reported in 1995 was published in 1999 and represented the results of 623 patients.[22] Unlike the first analysis of the B-17 study, uncertain/involved margins became a borderline risk factor for ipsilateral recurrence at 8 years, although, its early importance resembles the need for clear margins. The most favourable subgroup, comprised of patients with absent or slight comedonecrosis and negative margins, still demonstrated a 7% reduction in recurrence at 8 years leading to the conclusion that the use of adjuvant radiotherapy is appropriate for all patients with DCIS.

EORTC 10853 trial

Identical in study design and results is the EORTC 10853 trial which randomized 1010 patients by 46 institutes from 13 different countries between 1986 and 1996. Seventy-one per cent were detected by mammography only. At a median follow-up of 4.25 years, 11%

of the patients treated with radiotherapy developed an ipsilateral recurrence compared to 17% of the patients without further treatment.[8] Five-hundred and seven patients received radiotherapy resulting in the development of 24 invasive, and 29 noninvasive ipsilateral recurrences. Five-hundred and three patients were assigned to no further treatment of which 40 patients developed an invasive ipsilateral recurrence, and 44 patients a noninvasive ipsilateral recurrence. The 4-year local recurrence free survival rate was 91% vs. 84% in the radiotherapy and excision alone group, respectively. These findings confirm the favourable effect of radiotherapy in the conservative treatment of DCIS as reported by the NSABP B-17.[4,6,7] In view of the comparable follow-up of the B-17 data published in 1993, the effect on the reduction of invasive ipsilateral recurrence was larger in the American B-17 trial (2.9% vs. 4%) than in the EORTC study. The findings in non-randomized studies that about half of all local recurrences after conservative treatment with radiotherapy are invasive corresponds with the EORTC findings.[16,23-25] The observation of a significant higher rate of contralateral breast cancers diagnosed in the radiotherapy group of the 10853 trial could not be clearly explained. Epidemiological data indicate that the median induction period after radiotherapy for Hodgkin's disease is around 15 years (range 4-20)[26] suggesting another cause or, more likely, a result of chance. The 8 years update of the B-17 trial reported also a higher, but not significant, rate of contralateral breast cancer occurrence reported as first event while the overall number of contralateral breast cancers was equally distributed. The risk of eventually dying from breast cancer when DCIS is treated with conservative surgery is still minimal: four patients died of ipsilateral recurrence in the radiotherapy group, and seven patients in the no further treatment group. The 4-year overall survival rate was 99%. Longer follow-up is needed to investigate whether the beneficial effect of radiotherapy on local control will improve survival rates.

In search of risk factors predictive for invasive recurrence a central review of 863 (85%) patients was undertaken: in 10% DCIS could not be confirmed. Five per cent of the patients were classified as benign proliferative lesions, 1.5% as suspicious of invasion, and 3% as invasive.[27] In the B-17 trial 2% invasive lesions were found.[14] In the EORTC study lesions classified as atypical ductal hyperplasia did not come across compared to 7% in the B-17 trial. This likely reflects the existing problems with the classification of DCIS.[28] Further, as result of the finding that no recurrences were observed in 59 patients with well-differentiated DCIS with a clinging architecture, the authors seemed it justified to classify this group of lesions as 'columnar alteration with prominent apical snouts and secretion', or 'atypical ductal cells with apocrine snouts', and recommended close follow-up and excision only for these favourable lesions. Poorly defined margin status in the study design did not require the assessment of margin width. For this reason the opportunity to select patients eligible for excision alone was missed. Factors significantly associated with recurrence were age (≤ 40 years), cribriform or solid/comedo architecture, symptomatic detection, and close/involved or unknown margins. In addition, poorly differentiated DCIS was correlated with noninvasive local recurrence. Further, relapses of poorly differentiated DCIS was more frequently associated with distant metastasis and death after invasive local recurrence.

At a median follow-up of 10.5 years, the EORTC study has been updated and continues to show that radiotherapy after local excision of DCIS of the breast reduces the risk of local recurrence as compared to local excision alone.[9] The magnitude of the reduction has become slightly larger compared with the analysis performed at 4.25 years (HR now = 0.53, HR then = 0.62). The 10-year local recurrence free rate was 74% in the group treated with local excision alone compared with 85% in the women treated by local excision plus radiotherapy (logrank p<0.0001, hazard ratio (HR)=0.53). Whereas at the first analysis a surprisingly higher rate of contralateral breast cancers was found in the radiotherapy arm, in the current update a significant difference could no longer be observed. For further details of the long-term results of this study we refer to chapter seven of this thesis.

NSABP B-24 trial

The NSABP B-24 protocol was written after the observation that many women were ineligible for the B-17 trial because they had been treated by mastectomy in case of extensive calcifications, or because margins remained positive after multiple excisions. In addition, the favourable effect of hormonal treatment by tamoxifen for patients with invasive breast cancer was already reported but not yet investigated for DCIS.[29,30] Between 1991 and 1994, 1804 patients, including those whose margins were involved with tumour were randomized for lumpectomy, radiotherapy (50 Gy), and placebo, or tamoxifen (20 mg daily for 5 years) instead of placebo. Sixteen per cent of the patients had positive margins and in 10% margins were uncertain. Thirty-one percent (n=564) of the patients showed a lack of compliance because of side effects (n=146 in tamoxifen group, n=98 in placebo group), personal reasons (n=124 in tamoxifen group, n=146 in placebo group), or unspecified reasons (n=25 in both groups). Seventy per cent of all breast cancer events were ipsilateral. The likelihood of ipsilateral tumour recurrence at 5 years of follow-up was 6% after administration of tamoxifen and 9% after placebo (p=0.04).[11] The latter was almost identical with the 5 years result of the B-17 trial indicative for the relationship between these two trials.[14] Seven years of follow-up demonstrated an 8% (3% invasive, 5% noninvasive) ipsilateral recurrence rate after tamoxifen compared to 11% (5% invasive. 6% noninvasive) with placebo (p=0.02).[7] Noninvasive local recurrences seem unaffected by the administration of tamoxifen (p=0.48). In addition, the benefit seems to be restricted to estrogen receptor (ER)-positive DCIS lesions with a relative risk of 0.41 (p<0.001) in the ER-positive group compared to 0.8 (p=0.51) in the ER-negative group. ER status was determined for 628 patients of which 482 (77%) were positive.[31] Contralateral breast cancer as first event was reduced with 53% (p=0.01). In total, 39% fewer breast cancer events were observed in the tamoxifen group. Young age (≤ 49 years) was associated with local recurrence, as were positive margins, symptomatic detected lesions, and comedonecrosis. Systemic treatment with tamoxifen showed no survival benefit so far (95% in both groups after 7 years).

The NSABP P-1 trial demonstrated that tamoxifen reduced the incidence of invasive, and noninvasive cancer in high risk populations.[32] Patients with a history of atypical ductal hyperplasia or lobular carcinoma in situ had about a 50% reduction in the risk of invasive breast cancer overall. Acceptance of the profit of this intervention for these high risk patients can not be taken without the consideration of administration of tamoxifen for DCIS patients as well; even if they had been treated with radiotherapy. Since patients with DCIS have twice a higher risk of invasive breast cancer than those with lobular carcinoma in situ, and an even three times higher risk than patients with atypical ductal hyperplasia lesions.[11]
The occurrence of adverse events included nine cases of deep vein thrombosis and two cases of nonfatal pulmonary embolism in the tamoxifen group vs. two cases and one case, respectively, in the placebo group. These numbers were expanded with a report of six strokes (five tamoxifen, one placebo).[33] The P-1 study showed a nonsignificant increase in the number of strokes for patients receiving tamoxifen. Although endometrial cancer was increased in the tamoxifen arm compared to the placebo, total number of events were minimal (1.53 vs. 0.45 per 1000 patients per year, respectively) in the B-24 study. The toxicity profile of tamoxifen indicates selection of patients who have the least risk of serious side effects.

UKCCCR DCIS trial

A second randomized trial to assess the role of tamoxifen in combination with adjuvant radiotherapy or not, for the treatment of patients with DCIS was set up by the UKCCCR (now the National Cancer Research Institute [NCRI]) DCIS working party in collaboration with the Australian-New Zealand Breast Cancer Trials Group, and consisted of a 2x2 factorial design. Again, the primary endpoint was invasive ipsilateral recurrence. Between 1990 and 1998, 1701 patients were randomized to both treatments in combination or singly, or to none, or to either one with an elective decision to give or to withhold the other. Complete surgical excision of the lesion was confirmed by specimen radiography and histology. There was no evidence of an interaction for any of the endpoints. After a median follow-up of 4.4 years, ipsilateral tumour recurrence was reduced from 15% to 13% ($p=0.42$) by the prescription of tamoxifen at a dose of 20 mg daily for 5 years. Eleven percent of the patients in the tamoxifen arm did not take the treatment for the complete 5 years. The comparison of radiotherapy and no radiotherapy revealed an ipsilateral tumour recurrence of 6% and 14% ($p<0.0001$) in favour of adjuvant radiotherapy.[10] In contrast to the B-24, the UKCCCR DCIS trial did not demonstrate a favourable effect of tamoxifen contributing the reserve in prescribing this hormonal agent for DCIS patients. This could be explained by a difference in distribution of age between the two trials demonstrating at least a beneficial effect of hormonal administration in patients less than 50 years of age in both studies. Authors' reply on complaints about the lack of histological information[34,35] added a possible explanation for the failure in validating the B-24 results based on a difference in the histological characteristics

of lesions from patients entered in to these two trials. The higher frequency of high-grade lesions included in the UKCCCR trial is likely a result from differences in clinical assessment and management of screen-detected lesions. High-grade DCIS has a lower frequency of ER-positivity than do low- or intermediate-grade DCIS, which would explain the differences in proportions of hormone-receptor status in these two trials and consequently the differences in tamoxifen effect. In view of the possible negative effects of tamoxifen: two endometrial, four ovarian, and two unspecified gynaecological tumours were reported; only one did not receive tamoxifen as adjuvant treatment. This difference was not significant. No other adverse events were reported. A central histopathological review is undertaken and the results are eagerly awaited.

SweDCIS trial

Recently, the Swedish Breast Cancer Group reported the findings of a randomized trial including 1046 patients that received postoperative radiotherapy after breast conserving surgery or not between 1987 and 1999. Seventy-nine per cent of the patients were screen-detected. After a median follow-up of 5.2 years there were 44 recurrences in the radiotherapy group vs. 117 in the control group. The 5-year local recurrence free rate was 93% and 78%, respectively giving an overall hazard ratio of 0.33 (95%CI 0.24-0.47, $p<0.0001$).[12] An additional risk factor analysis confirmed the findings from earlier trials and could not identify a subgroup of patients that did not benefit from radiotherapy.[36]

Recently closed and ongoing trials

ECOG E-5194

The American Eastern Cooperative Oncology Group (ECOG) E-5194 trial investigates local excision alone for patients with DCIS lesions that were 2.5 cm or less and low- or intermediate grade, or 1 cm or less and high-grade. Margins width of at least 3 mm was necessary for all patients. Approximately 1000 patients were included to close this trial. Five- and ten-year local recurrence rates will hopefully provide more information on the efficacy of lumpectomy alone for low risk DCIS leading to criteria for selecting patients who do not need adjuvant radiotherapy.[37]

RTOG 9804

Still ongoing is the RTOG (Radiation Therapy Oncology Group) 9804 study. Patients are assigned either to adjuvant radiation therapy or not with the option of tamoxifen. Lesions must be 2.5 cm or less in diameter, low- or intermediate grade, and have a minimum margin width of 3 mm. In total 1790 patients are required.[37]

NSABP B-35 trial

The development of newer hormonal drugs has led to the start of the NSABP-B35 trial in January 2003. ER-positive postmenopausal patients are assigned to adjuvant anastrozole (aromatase inhibitor) or tamoxifen after local therapy. Unlike tamoxifen, anastrazole actually prevents the production of estrogen in postmenopausal women with fewer side effects. Anastrazole has already proven its efficacy in postmenopausal patients with hormone sensitive invasive breast cancer.[38] In total, 3500 postmenopausal patients with ER-positive DCIS lesions will be randomized to adjuvant anastrazole or tamoxifen. Primary endpoints are local recurrence and the development of contralateral breast cancer.

IBIS-II trial

A similar background as the previous trial has the IBIS-II (International Breast Cancer Intervention Study) which started in September 2003. The IBIS-II trial aims to identify the role of the relatively new drug anastrazole in 10000 women at a higher risk of developing breast cancer including 4000 patients with DCIS. Women at high risk are randomized to anastrazole vs. nil, while patients with ER-positive DCIS are randomized between anastrazole and tamoxifen after complete excision and radiotherapy.

Conclusion

Important take home messages are as follows:

- Adjuvant radiotherapy reduces significantly the incidence of ipsilateral recurrences after breast conserving surgery in patients with completely excised DCIS.
- A subset of low risk patients can not be selected to whom radiotherapy is of no benefit.
- A complete excision is the most important treatment variable to reduce recurrences.
- There is not a difference in survival between the adjuvant treatment modalities indicative for the relative favourable prognosis of most DCIS lesions.

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Chapter

9

Future prospects and concluding remarks

Future prospects

Although DCIS is associated with a favourable prognosis, still some patients treated by breast-conserving treatment will develop invasive ductal carcinoma including the risk of distant metastasis and death over time.[1] The main challenge for the future is the identification of patients at low and high risk of local recurrence in order to adjust individual treatment strategies. Based on individual risk assessment, treatment should be tailored by performing a wide local excision alone in low risk patients while high risk patients should be given more extensive treatment including the use of radiotherapy or mastectomy. The clinical presentation of DCIS has changed from palpable to screen-detected lesions during the last decades. Therefore, the results from the early randomized clinical trials for DCIS can not easily be translated into current clinical practice.

The effect of a treatment in a certain group of patients compared to controls without treatment is often expressed in relative or absolute risk reduction. For clinical decision-making, absolute measures are more meaningful. The number needed to treat (NNT), the reciprocal of the absolute risk reduction, is a powerful estimate of the effect of treatment.[2] Defining a threshold for the NNT in patients with DCIS is difficult. First of all because the endpoint of randomized clinical trials for DCIS is the local recurrence rate. So far, no treatment modality has shown a difference in distant metastasis or death rate for patients with DCIS. Nonetheless, a recent update of the Early Breast Cancer Trialists' Collaborative Group showed that differences in local treatment for invasive breast cancer that substantially affect local recurrence rates, would prevent about one breast cancer death for every four invasive local recurrences avoided.[3] If we extrapolate these numbers to the local treatment for DCIS, the overall absolute reduction of 11% local recurrences (6% DCIS and 5% invasive) caused by radiotherapy in the EORTC DCIS study would - hypothetically - lead to 1.25% less mortality over time.[1] This means that for every 80 patients who undergo radiotherapy as part of breast-conserving treatment for DCIS, 9 local recurrences (5 DCIS and 4 invasive, or 1 death) are prevented. These patients are spared a salvage mastectomy. The prevention of a local recurrence is thus of substantial benefit to these patients.
A second point of interest is the variability in thresholds for NNT. Patients often will accept remarkable low degrees of benefit in change of more intensive treatments. For example, patients accepted treatment with adjuvant chemotherapy for invasive breast cancer when a median reduction in risk of recurrence of 0.5 to 1.0% was achieved.[4] The impact of a local recurrence on the quality of life should therefore not be ignored.[5] In particularly, in younger patients with a long life expectancy and increased life time risk of developing a local recurrence. These patients need to be informed adequately about their higher risk of local recurrence and should be involved in a shared clinical decision making process.[6]

Some patients are, even after radiotherapy, at high risk of local recurrence, such as younger women, and/or those with involved surgical margins. Involved surgical margins is an avoidable

risk factor and need to be handled by re-excision or mastectomy while the latter should also be considered a valuable treatment option in younger women diagnosed with resectable DCIS. An additional boost of 16 Gy has shown benefit in patients with invasive breast cancer by reducing the local recurrence rate by a factor 2 (HR 0.55) and might also be beneficial in especially younger patients with DCIS.[7,8] However, there is no evidence from randomized studies that can confirm this hypothesis. Nonetheless, in analogy with invasive breast cancer an additional boost is often part of breast-conserving treatment for DCIS in some centres.

On the other side of the spectrum we need to identify patients with such a low risk of local recurrence that the absolute benefit of radiotherapy will become very small. These patients could be treated safely by wide local excision only. A small subgroup of patients in the EORTC study with well-differentiated DCIS of the clinging or micropapillary type was associated with an absolute risk reduction of invasive local recurrences as low as 5% to 2.5% at 10 years by radiotherapy. This - hypothetically - results in a NNT to prevent one death of 160, and therefore for these tumours radiotherapy is not applied in most institutes.
Another possible low risk group for local recurrence after breast-conserving treatment consists of older patients.[9,10] A recent study of more than 3000 women aged 66 years or older treated by wide local excision and radiotherapy reported a NNT to prevent one local recurrence (DCIS or invasive) of 11 in older patients with high risk features (age 66-69 years, tumor larger than 2.5 cm, comedo histology, and/or high grade DCIS) and of 15-16 in older patients with low risk features.[11] These data could result in a NNT by radiotherapy to prevent one death of 88 and 120, respectively. Further, in contrast with results (12% local recurrence rate at 5 years) from a prematurely stopped prospective study investigating treatment with wide local excision alone[12], Hughes et al. reported a 6.8% local recurrence rate for non-high grade, widely excised (5-10 mm), small (<10 mm) DCIS lesions.[13] The NNT by radiotherapy to prevent one death in this group would become 118. It seems that if pathologically clear margins can be obtained, wide local excision without adjuvant radiotherapy is warranted in only a very selected group of patients but a clear definition of such a group is still part of debate.

Integration of genetic factors into the classification of DCIS will hopefully lead to more objective criteria and might help in selecting patients who can be treated by wide local excision alone. The use of gene expression profiles can distinguish between well- and poorly differentiated DCIS. These profiles seem to indicate that intermediately differentiated DCIS is genetically more associated with well-differentiated DCIS than with poorly differentiated DCIS.[14] Nonetheless, the EORTC DCIS study observed similar risks of local recurrence for intermediately (HR, 1.85) and poorly (HR 1.61) differentiated DCIS.[1] It seems therefore that other features are involved that characterize and possible predict long term biological tumour behaviour of DCIS. This is reflected by gene expression profiles that identified basal-like type, Her-2 type, and luminal type tumors in DCIS similar to invasive ductal carcinoma.[14] Another study investigating DCIS adjacent to invasive breast cancer demonstrated that the gene expression profiles in the in situ component were very similar to that of those of

the invasive component.[15] These findings hopefully will elucidate step by step the genetic mechanisms leading to DCIS, but could also help in guiding therapy. It will therefore, be a great challenge to incorporate gene expression profiles in future prospective studies. Some of these developments might also be of use in detecting invasive components in patients with apparently pure DCIS on initial histological diagnosis who might require axillary staging by a sentinel node procedure.

Although MRI might not be superior to mammography for detecting invasion[16], this imaging tool can be very helpful for the accurate assessment of extent of the lesion and to reflect the heterogeneous presentation of DCIS.[16,17]

Finally, the role of adjuvant hormonal treatment with tamoxifen seems limited as most estrogen receptor positive DCIS are low grade lesions in which treatment benefit is very small.[18] The role of aromatase inhibitors in DCIS is still under investigation.[19]

Concluding remarks

Ductal carcinoma in situ is a heterogeneous disease and warrants a patient tailored and multidisciplinary approach, both regarding diagnosis and treatment. The introduction of screening, stereotactic core biopsy, and breast-conserving treatment for DCIS is associated with an improvement in clinical management but also has downstream consequences which should be monitored carefully. This dissertation focuses on these consequences but also demonstrates the favourable prognosis of this - still not completely - unraveled disease.

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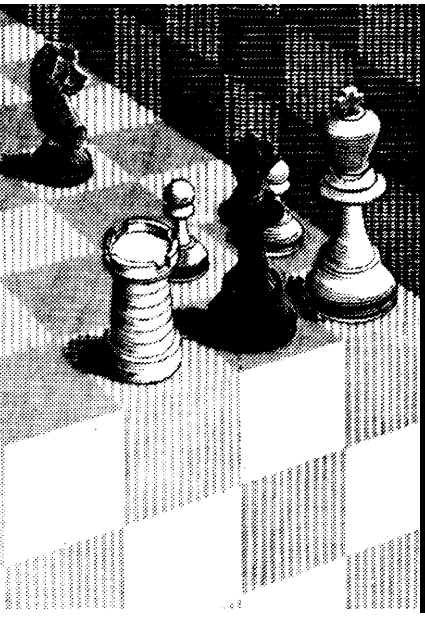

Chapter

10

Summary / Samenvatting

Summary

Ductal carcinoma in situ (DCIS) of the breast refers to a proliferation of abnormal epithelial cells within the basement membrane of the mammary ductal system, without the presence of stromal invasion. It is a non-obligate precursor of invasive carcinoma and does not fully express the malignant phenotype of unlimited growth, invasiveness, angiogenesis, and metastatic potential. It is therefore associated with a favourable prognosis although a proportion of patients still develop invasive local recurrences after breast-conserving treatment for this disease including the risk of distant metastasis. In the recent decades new developments like mammographic screening, stereotactic core-needle biopsy, breast-conserving treatment, and sentinel node biopsy have been introduced for breast cancer including DCIS. This dissertation focuses on the various aspects of these changed patterns in the diagnosis and treatment of DCIS.

DCIS is a heterogeneous spectrum of lesions, varying in morphology, extent and clinical presentation. Genetic alterations in DCIS have become an important research area in recent years. Chapter 2 gives an overview on the histological classification systems and genetic alterations in DCIS. In this chapter we report a marked inter-observer variability for the assessment of the histological type in DCIS, especially for lesions in the intermediately differentiated group. Histological classification alone is therefore probably insufficient to guide therapy in individual patients.

In Chapter three the use of immunohistochemistry for classification of DCIS in comparison with the current histological classification system is addressed. We analyzed whether immunohistochemistry applying a broad set of markers could be used to categorize DCIS in distinct subgroups. Immunohistochemical staining of 163 pure DCIS cases constructed in tissue arrays was performed with 16 markers and analyzed by unsupervised hierarchical clustering. Histological classification was performed by review of whole tissue sections and identified 36 well-, 55 intermediately and 72 poorly differentiated DCIS. Morphologically intermediately differentiated DCIS seems to have more biological similarities with well-differentiated lesions as compared to poorly differentiated lesions.

The increased incidence and/or detection of DCIS of the breast and the emergence of new diagnostic and therapeutic tools like mammographic screening, stereotactic core biopsy and reconstructive surgery influenced diagnosis and treatment. In Chapter four the clinical and pathological characteristics of 403 patients with DCIS, consecutively treated at the Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital between 1986 and 2002, were evaluated. Following the nationwide introduction of mammographic screening the percentage of non-symptomatic DCIS increased from 47% to 77% and the introduction of stereotactic core biopsy resulted in a rise of one-step procedures from 26% to 52%. The mastectomy rate did not change over time: 59% overall, while the breast reconstruction rate increased from 17% to 39%.

Chapter five deals with the risk of invasion and axillary lymph node metastases in 172 patients with DCIS diagnosed on core biopsy to select criteria to determine for which patients sentinel node biopsy might be warranted. Axillary staging was performed by sentinel node biopsy, axillary node sampling, or level 1-2 axillary lymph node dissection. Invasive breast cancer was found in the surgical specimens of 45 (26%) patients. Risk factors for invasion were a palpable lesion (OR: 2.95 (1.20-7.26), p=0.019), presence of a mass on mammography (OR: 3.06 (1.43-6.56), p=0.004), intermediately (OR: 5.81 (1.18-28.57), p=0.030), or poorly differentiated tumour grade (OR: 5.46 (1.17-25.64), p=0.031). Lymph node metastases were found in 10 patients with DCIS and invasion on final pathology. Factors associated with metastases were age up to 55 years (p=0.030), invasive area larger than 1.0 cm (p<0.001), and presence of vascular invasion (p=0.001). Sentinel node biopsy should be considered in patients with an initial diagnosis of DCIS on core-needle biopsy who have a palpable lesion, a mass on mammography, or intermediately or poorly differentiated tumour grade.

Chapter six describes the clinical outcome and factors associated with local recurrence of 504 patients who underwent final surgery at The Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital and outlines the current practice of clinical management for DCIS. After a median follow-up of 6.7 years, 24 (11.4%) patients developed a local recurrence after breast-conserving treatment. This occurred in 16 out of 91 patients (17.6%) after wide local excision alone and in 8 out of 119 patients (6.7%) after wide local excision and radiotherapy. Three out of 294 patients (1%) had a chest wall recurrence after mastectomy. The 8-year invasive local recurrence free rate was 92.2% after BCT and 99.6% after mastectomy (p=0.0001). Seven patients experienced distant metastases following invasive local failure: six (2.9%) after breast-conserving treatment, and one (0.3%) after mastectomy. Median tumor extent was 10, 15, and 35 mm for patients treated with wide local excision alone, wide local excision and radiotherapy, and mastectomy, respectively. Fifty-two out of 63 (83%) patients treated for well-differentiated DCIS by breast-conserving treatment underwent wide local excision alone, while 74 out of 83 (89%) patients with poorly differentiated DCIS underwent breast-conserving treatment by wide local excision and radiotherapy. Margins were involved in 6.4% of all patients. All 38 patients treated for clinging or micropapillary well-differentiated DCIS remained local recurrence free. Factors associated with local recurrence were age younger than 40 years, surgical margins involvement, and breast-conserving treatment. Breast-conserving treatment of DCIS bears the risk of residual disease progressing into invasive local recurrence and distant metastasis. A re-excision or mastectomy is therefore wanted in all patients with unclear margins. Mastectomy treatment is associated with optimal local control and might be the best treatment for patients younger than 40 years who are at high risk of local recurrence.

The 10 year results of the EORTC 10853 randomized trial investigating the role of radiotherapy after local excision of DCIS are presented in Chapter seven followed by a comment and authors' reply with a discussion on the long-term effects of radiotherapy. We analyzed the efficacy of radiotherapy after 10 years follow-up on the overall risk of local recurrence and

related to clinical, histological and treatment factors. After complete local excision, women with DCIS were randomly assigned to no further treatment or radiotherapy (50 Gy). One thousand and ten women with mostly mammographically (71%) detected DCIS, were included. The median follow-up was 10.5 years. The 10-year local recurrence-free rate was 74% in the group treated with local excision alone compared with 85% in the women treated by local excision plus radiotherapy (logrank $p<0.0001$, HR=0.53). Radiotherapy reduced the risk of DCIS local recurrence and invasive local recurrence by 48% ($p=0.0011$) and 42% ($p=0.0065$), respectively. Both groups had similar low risks of metastases and death. At multivariate analysis, factors significantly associated with an increased local recurrence risk were young age (≤ 40 years, HR=1.89), symptomatic detection (HR=1.55), intermediately or poorly differentiated DCIS (as opposed to well-differentiated DCIS, HR=1.85 and HR=1.61 respectively), cribriform or solid growth pattern (as opposed to clinging/micropapillary subtypes, HR=2.39 and HR=2.25 respectively), doubtful margins (HR=1.84), and treatment by local excision alone (HR=1.82). With long-term follow-up, radiotherapy after local excision for DCIS continued to reduce the relative risk of local recurrence, with 47% at 10 years. All patient subgroups benefited from radiotherapy.

This thesis ends with an overview of the randomized clinical trials for DCIS in Chapter eight. Since 1985, 6478 patients have been randomized in five large randomized clinical trials assessing the role of adjuvant radiotherapy in breast-conserving treatment for patients with DCIS. In short, these studies demonstrated approximately 50% reduction of local recurrences (half invasive and half DCIS) when adjuvant radiotherapy was given. A subset of patients who do not benefit from adjuvant radiotherapy could not be identified.

Samenvatting

Het ductaal carcinoom in situ (DCIS) van de borst bestaat uit een proliferatie van abnormale epitheliale cellen in de afvoergangen van de melkklier. De laesie wordt begrensd door de ductale basaal membraan en wordt gekarakteriseerd door afwezigheid van stromale invasie. DCIS kan worden beschouwd als een - mogelijk - voorstadium van invasieve borstkanker zonder de volledige kenmerken van het maligne fenotype, zoals onbeperkte groei, invasiviteit, angiogenese, en metastasering. Het kenmerkt zich dan ook door een gunstige prognose ondanks het feit dat een aanzienlijk aantal patiënten na borstsparende behandeling een lokaal recidief ontwikkelt en risico loopt op metastasering. De afgelopen decennia hebben op het gebied van borstkanker verschillende ontwikkelingen plaats gevonden zoals de introductie van het bevolkingsonderzoek, de dikkenaaldbiopsie, de borstsparende behandeling en de schildwachtklierbiopsie. In dit proefschrift wordt beschreven welke effecten deze ontwikkelingen hebben op de diagnostiek en behandeling van het DCIS.

DCIS is een heterogene ziekte die wordt gekenmerkt door variatie in morfologie, omvang en klinisch beeld. Het opsporen van genetische veranderingen, die ten grondslag liggen aan het DCIS, is in de laatste jaren een belangrijk onderzoeksterrein geworden. Hoofdstuk twee beschrijft de histologische classificaties zoals die bij DCIS worden gehanteerd en geeft een overzicht van de tot nu toe aangetoonde genetische veranderingen. In dit hoofdstuk wordt tevens een inter-observer variabiliteit voor de histologische beoordeling van het DCIS beschreven, met name voor het matig gedifferentieerde type. Gezien deze subjectieve beoordeling van DCIS, is de gangbare histologische classificatie waarschijnlijk ontoereikend voor betrouwbare individuele behandelvoorschriften.
In hoofdstuk drie wordt het gebruik van immunohistochemie voor de classificatie van DCIS vergeleken met de huidige histologische classificatie. We hebben onderzocht of d.m.v. immunohistochemisch onderzoek DCIS in verschillende groepen kan worden onderverdeeld. Hierbij werd d.m.v. 16 markers immunohistochemische kleuring verricht van tissue arrays van 163 DCIS tumoren. De analyse vond plaats door middel van ongesuperviseerde hiërarchische clustering. De histologische classificatie werd verricht op volledige weefsel coupes en 36 goed, 55 matig en 72 slecht gedifferentieerde DCIS werden geïdentificeerd. Uit dit onderzoek blijkt dat het matig gedifferentieerde DCIS meer biologische overeenkomsten lijkt te hebben met het goed gedifferentieerde DCIS dan met het slecht gedifferentieerde DCIS.
De toename in incidentie en/of detectie van het DCIS en de opkomst van de genoemde nieuwe diagnostische en therapeutische middelen zijn van invloed geweest op de diagnostiek en behandeling van DCIS. In hoofdstuk vier worden de klinische en pathologische karakteristieken van 403 patiënten, behandeld voor DCIS in het Nederlands Kanker Instituut – Antoni van Leeuwenhoek Ziekenhuis in de periode van 1986 tot en met 2002, geëvalueerd. Als gevolg van de introductie van het bevolkingsonderzoek naar

borstkanker nam het aantal asymptomatisch gedetecteerde DCIS tumoren toe van 47% naar 77%; de introductie van het dikkenaaldbiopt leidde tot een toename van het aantal een-staps procedures van 26% naar 52%. Het aantal mastectomie procedures bleef gelijk (59%), terwijl het aantal borstreconstructies steeg van 17% naar 39%. Het is aannemelijk dat deze trend zich na 2002 heeft voortgezet.

Hoofdstuk vijf beschrijft het risico op invasie en axillaire lymfekliermetastasen en mogelijke indicaties voor een schildwachtklierbiopsie bij 172 patiënten bij wie met een dikkenaaldbiopsie de diagnose DCIS was vastgesteld. Axillaire stadiëring vond plaats door middel van schildwachtklierbiopsie, het verwijderen van de basale okselklieren of een niveau 1-2 axillaire klierdissectie. Invasieve borstkanker werd gevonden in de chirurgische preparaten van 45 (26%) patiënten. Risico factoren voor invasie waren: een palpabele laesie (OR: 2,95 (1,20-7,26), p=0,019), een massa op het mammogram (OR: 3,06 (1,43-6,56), p=0,004), matig gedifferentieerd DCIS (OR: 5,81 (1,18-28,57), p=0,030) of slecht gedifferentieerd DCIS (OR: 5,46 (1,17-25,64), p=0,031). Lymfekliermetastasen werden gevonden bij 10 patiënten met DCIS en een invasieve component. Deze patiënten werden gekenmerkt door: leeftijd jonger dan 55 jaar (p=0,030), een invasieve component groter dan 1,0 cm (p<0,001) of de aanwezigheid van vasculaire invasie (p=0,001). Geconcludeerd wordt dat schildwachtklierbiopsie overwogen dient te worden bij DCIS patiënten in geval van een palpabele tumor, een massa op het mammogram, of een matige of slechte differentiatiegraad.

Hoofdstuk 6 bespreekt de klinische resultaten van 504 patiënten met DCIS die behandeld werden in het Nederlands Kanker Instituut – Antoni van Leeuwenhoek Ziekenhuis. Ook werd gekeken naar factoren die mogelijk van invloed zijn op het ontwikkelen van een lokaal recidief. Na een mediane follow up van 6,7 jaar kregen 24 (11,7%) patiënten een lokaal recidief na borstsparende behandeling (16 (17,6%) na lumpectomie alleen en 8 (6,7%) na lumpectomie en radiotherapie) en drie (1%) patiënten kregen een thoraxwand recidief na mastectomie. Het 8-jaar invasief lokaal recidief-vrije percentage was 92,2% na borstsparende behandeling en 99,6% na mastectomie (p=0,0001). Zeven patiënten ontwikkelden afstandsmetastasen na een eerder invasief lokaal recidief: zes (2,9%) na borstsparende behandeling en één (0,3%) na mastectomie. De mediane tumor omvang was respectievelijk 10, 15 en 35 mm voor patiënten behandeld met lumpectomie alleen, lumpectomie en radiotherapie en mastectomie. Tweeënvijftig van de 63 (83%) patiënten, die behandeld waren voor een goed gedifferentieerd DCIS door middel van een borstsparende behandeling, ondergingen alleen een lumpectomie, terwijl 74 van de 83 (89%) patiënten met een slecht gedifferentieerd DCIS borstsparende behandeling kregen door middel van lumpectomie en radiotherapie. Bij 6,4% van alle patiënten waren de snijvlakken positief. Alle 38 patiënten die behandeld waren voor een clinging of micropapillair goed gedifferentieerd DCIS bleven vrij van lokaal recidief. Factoren die gepaard gingen met een lokaal recidief waren leeftijd jonger dan 40 jaar, positieve snijvlakken en borstsparende behandeling. De borstsparende behandeling van het DCIS herbergt het risico dat niet verwijderde tumor zich kan ontwikkelen tot een invasief lokaal recidief en kan metastaseren. Een re-excisie

of mastectomie is derhalve gerechtvaardigd bij alle patiënten met positieve snijvlakken. Door middel van mastectomie kan een optimale lokale controle worden verkregen en deze ingreep dient overwogen te worden bij patiënten jonger dan 40 jaar die een hoog risico lopen op het ontwikkelen van een lokaal recidief.

De 10-jaar resultaten van de gerandomiseerde EORTC 10853 studie, waarin het effect van radiotherapie na lumpectomie bij 1010 patiënten met DCIS werd onderzocht, worden gepresenteerd in hoofdstuk zeven, gevolgd door een commentaar met antwoord van de auteurs over de langetermijngevolgen van radiotherapie. Het effect van radiotherapie na 10 jaar follow-up op het totale risico voor het krijgen van een lokaal recidief, mede in relatie tot klinische presentatie, histologie en behandeling, werd geanalyseerd. Na een complete lumpectomie werden de patiënten gerandomiseerd tussen geen verdere behandeling of radiotherapie (50 Gy). Duizendentien vrouwen met voornamelijk (71%) mammografisch gedetecteerd DCIS werden geïncludeerd. De mediane follow-up was 10,5 jaar. Het 10-jaar lokaal recidief-vrije percentage was 74% in de groep behandeld met lumpectomie alleen, vergeleken met 85% in de groep vrouwen behandeld met lumpectomie en radiotherapie (logrank $p<0,0001$, HR=0,53). Het risico op een DCIS lokaal recidief en invasief lokaal recidief werd gereduceerd met respectievelijk 48% ($p=0,0011$) en 42% ($p=0,0065$). Beide groepen hadden een laag risico voor het ontwikkelen van metastasen of voor overlijden t.g.v. de ziekte. Factoren die na multivariate analyse significant geassocieerd waren met een verhoogd risico voor het krijgen van een lokaal recidief waren: jonge leeftijd ($\leq$ 40 jaar, HR=1,89), symptomatische detectie (HR=1,55), matig of slecht gedifferentieerd DCIS (ten opzichte van goed gedifferentieerd DCIS, respectievelijk HR=1,85 and HR=1,61), cribriforme of solide type (ten opzichte van het clinging of micropapillaire type, respectievelijk HR=2,39 and HR=2,25), positieve snijvlakken (HR=1,84) en behandeling door middel van lumpectomie alleen (HR=1,82). Na lange follow-up blijkt radiotherapie het risico op een lokaal recidief te verminderen met 47%. Dit voordeel wordt gezien bij alle subgroepen.

Dit proefschrift eindigt met een overzicht van de tot nu toe gerandomiseerde klinische studies die verricht zijn voor DCIS. Sinds 1985 zijn 6478 patiënten gerandomiseerd in vijf grote studies waarin de rol van radiotherapie bij de borstsparende behandeling van patiënten met DCIS is onderzocht. Samengevat, toonden deze studies ongeveer een 50% reductie van het aantal lokale recidieven (de helft invasief en de helft DCIS). Een subgroep van patiënten die geen baat had van radiotherapie kon niet worden aangewezen.

METAMORPHOSE

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Curriculum Vitae

Philip Meijnen is geboren op 28 augustus 1978 te Heerenveen. Na het behalen van zijn VWO diploma aan de Rijksscholen Gemeenschap te Heerenveen startte hij in 1996 met de studie Geneeskunde aan de Rijksuniversiteit Groningen. De co-schappen heeft hij grotendeels in het Sint Elisabeth Hospitaal op Curaçao doorlopen. In 2003 behaalde hij het artsexamen en begon hij als research fellow voor de European Organisation for Research and Treatment of Cancer (EORTC) Breast Cancer Group waarbij hij werkzaam was als studie monitor van de EORTC AMAROS studie (studie coördinator, prof.dr. E.J.Th. Rutgers). Tegelijkertijd startte hij zijn promotieonderzoek naar het ductaal carcinoom in situ op de afdeling heelkunde van het Nederlands Kanker Instituut - Antoni van Leeuwenhoek Ziekenhuis te Amsterdam (afdelingshoofd, prof. dr. B.B.R. Kroon). In december 2006 is hij begonnen aan de opleiding tot radiotherapeut-oncoloog in het Nederlands Kanker Instituut - Antoni van Leeuwenhoek Ziekenhuis te Amsterdam met voormalig opleider prof.dr. G.M.M. Bartelink en huidig opleider prof.dr. M. Verheij.