# BREAST DUCTAL CARCINOMA IN SITU (DCIS): REPRODUCTIVE AND HORMONAL RISK FACTORS AND RELIABILITY OF HISTOLOGIC DIAGNOSES

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ABSTRACT

LYNETTE S. PHILLIPS: Breast Ductal Carcinoma *in Situ* (DCIS): Reproductive and Hormonal Risk Factors and Reliability of Histologic Diagnoses
(Under the direction of Robert C. Millikan)

Ductal carcinoma *in situ* (DCIS) accounts for nearly one-fifth of all newly diagnosed breast cancers in the United States. It comprises a heterogeneous collection of histopathologic characteristics, but reproducibility among pathologists is unknown due to lack of a uniform classification system. Recent data suggests that medium or low-grade (non-comedo type) DCIS differs from high-grade (comedo type) with regard to biological mechanisms, pathology, and risk factors and therefore may not require the same treatment intensity given to more aggressive forms of the disease.

Aims of this dissertation were to investigate: 1) agreement by pathologists on histopathologic diagnoses for DCIS subtypes, 2) which of the major diagnostic components contribute most often to each subtype diagnosis, and 3) whether known reproductive and hormonal risk factors for invasive breast cancer are risk factors for DCIS, and if associations differ between comedo and non-comedo DCIS. The dissertation utilized data from the Carolina Breast Cancer Study (CBCS), a population-based case-control study of *in situ* and invasive breast cancer.

When clinical pathologists rated DCIS histopathologic specimens, agreement was moderate for overall diagnosis and fair for the two most common cellular components characteristic of the disease, pattern of necrosis and maximum nuclear diameter. The

most advanced categories for each component were associated with comedo type DCIS, while no uniformity existed for non-comedo type.

Case-control analyses examined reproductive and hormonal risk factors for invasive breast cancer and both DCIS subtypes. In general, those decreasing estrogen exposure, such as parity, lactation, older age at menarche and younger age at menopause were inversely associated with disease, whereas factors causing increased estrogen exposure such as oral contraceptive use and postmenopausal hormone replacement therapy use showed increased associations with one or more breast cancer types. Results for comedo type DCIS were similar to invasive breast cancer and differed from non-comedo type for some risk factors. Although the lack of strong diagnostic reliability for DCIS subtypes suggests a potential for error in the risk factor analyses, these results support the division of DCIS cases by subtype for clinical and research purposes and reinforce the need for a universal DCIS classification system.

To my husband, Dan, without whose love and support

this dissertation might never have been completed.

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ABBREVIATIONS

| | |
|---|---|
| ADH | Atypical ductal hyperplasia |
| ALH | Atypical lobular hyperplasia |
| BCDDP | Breast Cancer Detection Demonstration Project |
| BMI | Body mass index |
| CBCS | Carolina Breast Cancer Study |
| CI | Confidence interval |
| CIS | Carcinoma *in situ* |
| DCIS | Ductal carcinoma *in situ* |
| DMV | Department of Motor Vehicles |
| ERT | Estrogen replacement therapy |
| H&E | Hematoxylin and eosin |
| HCFA | Health Care Finance Administration |
| HRT | Hormone replacement therapy |
| IBC | Invasive Breast Cancer |
| LCIS | Lobular carcinoma *in situ* |
| LN | Lobular neoplasia |
| LRT | Likelihood ratio test |
| OC | Oral contraceptive |
| OR | Odds ratio |
| SEER | Surveillance, Epidemiology and End Results |
| SMR | Standardized mortality ratio |
| VNPI | Van Nuys Prognostic Index |

# CHAPTER I: BACKGROUND AND LITERATURE REVIEW

## *Introduction*

Carcinoma *in situ* (CIS) of the breast is a broad classification for malignant cells confined to the epithelium of the breast lobules (lobular carcinoma *in situ*, or LCIS) or ducts (ductal carcinoma *in situ*, or DCIS). LCIS accounts for approximately 14% of all CIS cases in the United States [1], is often multicentric and/or bilateral [2], and is usually detected in conjunction with other benign or malignant diseases rather than on its own. LCIS has been considered a marker of invasive breast cancer risk rather than a direct precursor to malignancy [3, 4], although recent evidence suggests risk of invasive disease after LCIS is similar to risk of invasive cancer after a DCIS diagnosis [5].

Over 85% of CIS cases in the U.S. are DCIS. DCIS is usually detected by screening mammogram, is sometimes multicentric but infrequently bilateral, and is considered a direct precursor to invasive breast cancer in some cases [4]. DCIS is further defined by histopathologic characteristics, although no universal classification system for DCIS exists. Comedo-type or high-grade DCIS makes up only 20% of all U.S. DCIS cases but is likely to develop into invasive disease if left untreated [1]. The remaining 80% of DCIS cases consist of noncomedo-type or intermediate or low-grade DCIS. At the present, the severity of the latter lesions is unknown.

***Breast Carcinoma in Situ Pathology***

LCIS is the more severe component of lobular neoplasia (LN), with atypical lobular hyperplasia (ALH) representing similar but less well-developed lesions. Pathologic characteristics of DCIS are numerous and varied, due to the heterogeneity of the disease. Until recently, pathologists used architectural growth patterns to classify DCIS. Many pathologists now categorize DCIS into two subtypes based on patterns of necrosis, nuclear grade, and presence or absence of calcification. Those having "at least one duct in the breast…filled and expanded by large, markedly atypical cells and…abundant central luminal necrosis"[6] are considered comedo-type DCIS, while all other forms are classified as noncomedo. These include cribriform, micropapillary, clinging, and solid types [6]. Nuclear grade refers to the comparison of size and shape, chromatin distribution, and mitoses of tumor nuclei versus those of normal cells [7].

While nuclear grade, architectural growth pattern, and tumor size all vary in DCIS, it is not clear how much impact the various pathologic characteristics have on determining severity of the disease. In addition, very small foci of invasive breast cancer may be present with DCIS. Known as DCIS with microinvasion, this outcome has important clinical and possibly etiologic implications. Microinvasion is often associated with higher histologic grade [8]; specifically, 66-80% of the DCIS in cases with microinvasion are comedo type [9].

Classification of LCIS is relatively straightforward, but the more heterogeneous DCIS lesions do not easily lend themselves to a simple classification system. Pathologists in the United States and Europe have been working to develop one DCIS classification system that can be used universally with sufficient reliability and that

prioritizes the elements with the most clinical impact. The four most prevalent classification systems are listed in Table A.1. They differ based on how lesions are categorized and which components are most important for categorization. Nuclear grade and necrosis are features included in all systems, but their definitions and importance vary.

Studies in recent years have explored reproducibility, accuracy, and ability to predict disease progression and outcome among these systems and other combinations of DCIS pathology. The studies can be divided into three types: those examining reliability without regard to classification system, those investigating reliability for one specific CIS classification system, and those comparing reliability for multiple classification systems. Some studies included breast lesions other than CIS, such as benign breast lesions, various types of hyperplasia, and invasive disease. Table A.2 summarizes the pertinent details of previous reliability studies of benign breast disease and CIS pathology. They vary in number of raters from two to 466 and in cases from 12 to 180. Four studies used ratios (or percentages) to measure agreement, while the majority used the kappa statistic. Most reported only inter-rater statistics, but two also examined diagnostic accuracy [10, 11], and one measured intra-rater reliability [12].

Raters differentiated among DCIS subtypes in only two of these studies [13, 14]. Three divided hyperplasia into subtypes [15-17], two studied CIS characteristics such as nuclear pleomorphism and mitoses, and two delineated between high, intermediate, and low grade DCIS. Three studies provided training sets of slides to the participating pathologists before the study was conducted [16, 18, 19], and three others gave the raters information on particular rating systems before they participated in the study[10, 20, 21].

A uniform CIS classification system would be useful in selecting treatment that involves the least amount of invasiveness and trauma (both physical and emotional) while preventing recurrence. A number of studies have looked at predictors for DCIS recurrence or development of invasive disease after initial CIS diagnosis using current DCIS classification systems. Gupta et al (1997), Badve et al (1998), Denoux et al (2001), and Bijker et al (2001) all found significant correlation between the Van Nuys system and risk of recurrence of either DCIS or invasive breast cancer [22-26]. Gupta et al also showed an association between the Holland system and concurrent grade of invasive carcinoma, and Badve et al indicated good ability of that classification system to predict local DCIS recurrence when cell polarization was not included in the definition.

Additional studies that did not look at classification systems have shown that specific histologic features of DCIS are associated with poor prognostic grades of invasive carcinoma both concomitantly and in recurrences after initial DCIS diagnosis. Specifically, degree of epithelial proliferation in benign breast disease [27], poorly differentiated nuclei [26, 28], high nuclear grade [29-31], and comedo necrosis [29, 32-35] have all been found to correlate with local DCIS recurrence, risk of invasive cancer, and/or grade of concurrent invasive disease. In fact, in many of these studies, type of treatment had less impact on disease-free survival or mortality than subtype of DCIS. All of this information supports the theory that DCIS subtypes may differ enough from each other to be considered separate diseases clinically.

***Epidemiology of Breast Carcinoma in Situ***

Epidemiologic data on LCIS is fairly scarce. Autopsy series indicate prevalence of LCIS up to 4%, and Detroit SEER incidence rates for 1987-88 were 2.8 per 100,000 for LCIS [2]. Average age of diagnosis appears to be in the 40s, and diagnoses are primarily among premenopausal women [2]. A recent Danish study found rate of recurrence of invasive carcinomas, DCIS, or LCIS plus DCIS after LCIS diagnosis to be 17% [36], and Sasson et al discovered 5% of their invasive carcinoma population to have concurrent LCIS [3].

Treatment for LCIS often consists of the diagnostic biopsy only with follow-up observation and yearly mammograms [2, 37]. However, in high-risk patients local excision or mastectomy may be used. In addition, a regimen of Tamoxifen may be prescribed to decrease risk of subsequent breast cancers [37]. Because LCIS does not directly lead to death, mortality rates deal with subsequent invasive disease risk and death. Of 14 studies examining breast cancer deaths after LCIS diagnosis and treatment, mortality rates ranged from 0.8% to 16% for those treated with local excision and 0% to 3% for those who had mastectomies [2].

Incidence rates for DCIS have been increasing ever since the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program began recording such data in 1973, when the age-adjusted annual rates were 2.3 per 100,000 females [38]. By 1992, that figure had jumped to 15.8 per 100,000 [39]. The most dramatic increases have occurred since 1983, with a 17.5% annual increase in rates between 1983 and 1992 compared to increases of 3.9% annually from 1973 to 1983 [39]. Separate studies in Detroit [40], Connecticut [41], Vaud, Switzerland [42], and Florence, Italy [43] have

shown that most of this increase is due to the introduction of screening mammography in the early 1980s and subsequent increasing use in women over 40, even though screening programs targeted women 50 and over.

The SEER data has also been used to examine race and age differences in DCIS rates. Age-adjusted incidence rates for black women have been slightly lower than for whites nearly every year since 1973 but follow the same trends. Specifically, age-adjusted incidence rates in 1983 were 3.7 per 100,000 for whites and 3.8 for blacks, increasing to 15.8 and 14.4 per 100,000 respectively in 1992 [39]. Among white women, 12.1% of all newly diagnosed breast cancer cases in 1992 were DCIS, as were 12.5% among black women [44].

In comparison, age-adjusted incidence rates for invasive breast cancer in 1992 were 131.8 per 100,000 overall, 135.6 per 100,000 for white women and 123.2 per 100,000 for black women according to SEER data [45]. As of 2002, the most current year for which data is available, those rates are 133.8, 139.4, and 120.2 per 100,000, respectively [45].

As with LCIS, risk of subsequent invasive breast cancer and death from that disease is the most important concern with ductal carcinoma *in situ*, since DCIS does not directly cause death. Recent analyses from SEER data of breast cancer deaths among women diagnosed with DCIS between 1984 and 1989 showed five- and ten-year standardized mortality ratios (SMRs) of 1.6 and 1.9 respectively, compared with women in the general population [46]. In contrast, those same data for lesions diagnosed between 1978 and 1983 (prior to common use of screening mammography) were 3.1 and 3.4 [46].

Twenty-year survival rates for women diagnosed with *in situ* breast cancer between 1973 and 1980 in the Breast Cancer Detection Demonstration Project (BCDDP) were 78.5 observed (absolute) and 97.2 adjusted for deaths from other causes (relative) [47]. For invasive cases, these rates were 59.3 observed and 78.2 adjusted [47]. In this study, over 280,000 volunteer women in the U.S. were given annual breast cancer screening for five years, with a 96% follow-up rate after 20 years. While these data most likely are not representative of all U.S. women, they do provide the longest follow-up data to date and a method for comparing survival of those with DCIS and those who developed invasive breast cancer in the same study.

Recurrence of DCIS is also a concern. In one recent study, follow-up of over 1000 patients diagnosed with DCIS at Memorial Sloan-Kettering Cancer Center between 1978 and 1990 showed 157 subsequent recurrences for which follow-up data was available [29]. Univariate analyses of 6-year actuarial local recurrence rates showed that younger age (<40 yrs.), comedo histologic subtype, higher nuclear grade, and positive surgical margins were significantly associated with higher recurrence rates. However, positive margin status was the only statistically significant predictor in multivariate analyses. Other studies found associations of higher recurrence with comedo necrosis [34, 48-53], positive margin status [52, 54], and high nuclear grade [34, 48, 53].

While incidence data for invasive breast cancer come from SEER data, mortality rates for invasive disease are obtained from death certificates and computed at the National Center for Health Statistics. For 2003, those rates per 100,000 persons were 25.8 for all females, 25.3 for whites, and 34.3 for blacks [45]. The study of breast cancer mortality among those originally diagnosed with DCIS also used SEER data to examine

breast cancer deaths among women originally diagnosed with invasive disease [46]. Divided by severity of disease at diagnosis, five- and ten-year SMRs for 1978-83 were 17.8 and 16.8 for locally-invasive lesions, 64.3 and 55.5 for regionally invasive, and 290.4 and 254.2 for distant disease, respectively. For the period of 1984-89, these ratios decreased somewhat to 11.8 and 11.4 for local disease, 50.5 and 45.1 for regional invasion, and 258.3 and 232.8 for distant.

Current five-year relative survival rates for invasive breast cancer are 86% overall. Computed by race, those rates are 87% for white women and 72% for black women [55]. However, survival by stage of disease varies dramatically, with 96.4% of women with localized disease surviving to five years, 77.7% with regional metastasis, and 21.1% for those with distant metastasis [56]. Twenty-year adjusted survival rates in the BCDDP study by stage were reported as 86.8 for stage I, 75.4 for stage IIa, 71.7 for stage IIb, 70.1 for stage IIc, 59.6 for stage IId, and 40.3 for stage III [47].

***Endogenous Estrogen and Breast Cancer***

Endogenous estrogen stimulates breast cell growth and differentiation. Specifically, it binds to and activates estrogen receptors in the cell nucleus [57]. Experiments using human mammary cell lines and animal models provide biological and molecular evidence that lifetime events affecting estrogen levels, such as pregnancy, lactation, and length of time between menarche and menopause, cause sufficient cellular changes in the breast to affect breast cancer risk [58]. Epidemiologic studies support these findings for invasive breast cancer. Specifically, early age at first pregnancy, increasing number of pregnancies, long duration of lactation [59], late menarche, and

early menopause, all of which decrease lifetime estrogen exposure, decrease breast cancer risk. In addition, exogenous hormone use via hormone replacement therapy (HRT) or oral contraceptives (OC) have shown increased breast cancer risk in some studies [60-62]. Assuming that CIS is a precursor to invasive disease, these factors should affect risk for CIS as well.

***Carcinoma in Situ and Reproductive Events***

Table A.3 summarizes previously published studies of CIS reproductive risk factors. Results that were imprecise or showed weak or no association are not included in the table, unless they helped describe trends. Of the nine studies, six are population-based case-control designs, one is cross-sectional, and two are prospective cohorts. Nearly all of the populations studied consist of mostly White and/or Hispanic participants, and the Swedish study by Lambe, et al was the only one conducted on a non-U.S. population.

In general, any full-term births and births at young age (<20 years) were inversely associated with CIS whereas nulliparity and age at first full-term birth $\geq$30 years were positively associated with CIS in these studies. The two studies that included lactation suggest a positive association of CIS with lactation for more than 2 months [63, 64], but others found no association between CIS and lactation.

Wohlfahrt et al (2004) conducted the only other study to examine reproductive risk factors for comedo and non-comedo DCIS. Their analyses were limited to parity and age at first full-term birth and found an association between comedo DCIS and age at

first birth of 25 or higher. Neither parity nor age at first full-term birth were associated with noncomedo DCIS.

***Exogenous Hormones and Carcinoma in Situ***

Overall, the most consistent risk factors for CIS in these studies were having a first-degree relative with a history of breast cancer, previous benign breast disease or biopsy, and ever use of hormone replacement therapy. Two of the studies conducted separate analyses for pre- and postmenopausal subjects[65-67], and one studied only premenopausal women[66]. Two of the studies limited their analyses to those who were postmenopausal in order to study HRT as a risk factor[68, 69]. The remainder analyzed all ages together.

Kerlikowske et al [67] and Gapstur et al [70] focused only on DCIS cases, while the rest combined DCIS and LCIS in their analyses. Only the study by Claus et al [71] performed separate analyses for LCIS cases. Previous breast biopsy was the only variable found to be associated with LCIS, but that is most likely a result of high correlation between diagnostic method and diagnosis.

The prospective cohort study of CIS by Schairer et al [68], which looked at hormonal risk factors in postmenopausal women, studied 150 case subjects and found an association between any *in situ* breast cancer and ever-use of HRT (estrogen and progestin) as well as duration of use less than four years and current use. For estrogen-only replacement therapy (ERT), an association was found for current use of $\geq$10 years and a trend with increasing duration of use. For both total duration and past duration of estrogen-only use, only the 10-14 year category was statistically significant. While this

study included a number of variables in the regression model, including age, age at menopause and type, history of benign breast disease, family history of breast cancer, education, and parity, main effects were not presented. In addition, mean follow-up was only 6.4 years, and more time might be necessary to examine hormonal influences on CIS risk.

Longnecker et al [65] found age at menarche ≥14 years, previous benign breast disease, family history of mother or sister with breast cancer, and first full-term birth between the ages of 20 and 29 years to be risk factors for premenopausal women. The results for parity were not significant, but nulliparity could not be addressed because the authors did not include zero pregnancies as a category for this variable among premenopausal women. No explanation for this choice was given. For postmenopausal women in this population, previous benign breast disease, first degree relative with a breast cancer history, age at menopause of 55 years or more, and ever use of HRT were associated with DCIS and increasing number of full-term births beyond one showed an inverse association with DCIS. Age at menarche, BMI, age at first full-term birth, and ever use of ERT were all non-significant.

In the premenopausal population of Weiss et al, mother with history of breast cancer, previous breast biopsy, African-American race, and nulliparity were all associated with CIS, while some college education was inversely associated. Non-significant factors measured include age at menarche, number of full-term births, age at first full-term birth, interval since last birth, and alcohol use. Numbers of CIS studied by type were mentioned (156 DCIS, 46 LCIS), but separate analyses were not conducted.

Kerlikowske et al [67] found similar results with family history of breast cancer in at least one relative associated with DCIS in premenopausal participants. In addition, increasing age per 10-year increment was associated with DCIS in this study. Early menarche (<12 years old), previous breast surgery and palpable mass associated with diagnosis were all non-significant. Among postmenopausal women, only nulliparity or older age at first birth ($\geq$30 years) were associated with DCIS. Hysterectomy was not significant, and hormone replacement therapy was not included as a variable in this study.

The study by Henrich et al [69] was much smaller (32 *in situ* cases) and had a much lower participation rate (54%) than the other studies. Again, only postmenopausal hormonal risk factors were studied, and no association was found with either ERT or HRT use and CIS, even when categorized by recentness of use, type of estrogen, or duration of use. This study analyzed invasive and *in situ* breast cancer cases together, as well as invasive cases separately, but only reported ERT use results for *in situ* cases alone. ERT use and estrogen use alone were associated with IBC (OR 1.93, 95% CI 1.06-3.94; and OR 2.22, 95% CI 1.18-4.17 respectively) but not with *in situ* and invasive cancer cases combined. Most likely, this was due to the small sample size.

Gapstur et al [70] conducted the other prospective cohort that, like the Schairer study, focused on hormone replacement therapy among postmenopausal women with DCIS or invasive breast cancer. Although HRT was not found to be associated with DCIS, it was not separated by type as performed by Schairer et al. However, other potential risk factors were analyzed and 30 years or higher age at first birth and family history of breast cancer were found to be positively associated with DCIS.

The three largest and most recent studies, conducted in Sweden[72], Wisconsin[73], and Connecticut[71], all combined pre- and postmenopausal women in their analyses. Lambe et al [72] focused only on reproductive history risk factors, and there is no indication what potential confounders, if any, were controlled for in the analysis. They found parity to be protective, and increasing number of full-term births resulted in an increased protective effect (p for trend 0.005). Also, increasing age at first birth was associated with CIS (p for trend 0.05), but none of the risk estimates for the individual age categories were statistically significant. Although this study contained a very large number of subjects, its narrow focus and sparse analyses limit its usefulness for increasing knowledge about CIS risk factors.

A more robust CIS risk factor study was the one conducted by Trentham-Dietz et al [73]. Response rates for both cases and controls were very good (85% and 90% respectively). Data was obtained through telephone interview, with a small reliability study of the questionnaire conducted after 6-12 months showing high reproducibility. While they did not consider pre- and postmenopausal women separately in their analyses, they did adjust for a variety of confounders. In addition, this study conducted case-case analyses to compare exposure variables for *in situ* versus invasive cancer. Risk factors found in this study included family history of breast cancer, benign breast disease for which a biopsy had been conducted, and postmenopausal hormone use. Many other potential risk factors were examined, and parity, education, and BMI were all non-significant.

As mentioned above, Claus et al [71] included a large number of CIS cases and therefore were able to perform separate analyses for DCIS and LCIS. Specifically, there

were 875 DCIS cases and 123 LCIS subjects in the analyses. Results for LCIS were similar to those for DCIS, although the only statistically significant risk factor for LCIS was previous breast biopsy (adjusted OR 4.15 [95% CI 2.34-7.35]), and linear trend for age at menopause (1.07 [95% CI 1.01-1.12]). Telephone interviews were used in this study as well, but there was no mention of reliability of the questionnaire.

Even though numbers of DCIS research studies have increased in recent years, few explored risk factors or histopathologic characteristics of subtypes. Those distinguishing between comedo and non-comedo types included only a portion of the hormone-related exposures associated with invasive disease, and histopathologic reliability studies have not examined how DCIS diagnoses are made. The purpose of this dissertation is to:

1. Quantify inter-observer reliability on histopathologic diagnoses for DCIS cases among pathologists in clinical practice,
2. Examine agreement for histopathologic diagnostic components and how they contribute to the overall diagnosis,
3. Determine whether reproductive and hormonal risk factors for IBC are associated with DCIS in the CBCS population, and
4. Divide DCIS cases by histologic subtype and compare risk factors to invasive cancer.

# CHAPTER II: STUDY DESIGN AND METHODS

## *The Carolina Breast Cancer Study*

### *Overview of study*

All data for these analyses came from the Carolina Breast Cancer Study (CBCS), a population-based case-control study of women aged 20-74 residing in 24 contiguous counties of North Carolina [74]. These counties were chosen to maximize African-American and rural population representation and minimize identification and recruitment problems due to population mobility. Phase I of the study included cases diagnosed between May 1, 1993 and September 30, 1995, and was limited to invasive cases. Phase II encompasses those diagnosed between 1996 and 2001 and includes both invasive and *in situ* cases.

Among both cases and controls, randomized recruitment [75] was used to identify nearly equivalent numbers of African-American and white women as well as of women younger than age 50 and age 50 or older. Invasive cases were over-sampled for African-Americans and younger women (20-49 years). CIS cases were identified through the North Carolina Central Cancer Registry and were required to be first diagnoses of *in situ* breast cancer with less than two millimeters of microinvasion. All eligible CIS cases were included, with no over sampling on race or age. Controls were selected from a Department of Motor Vehicle (DMV) list for women under age 65 and from a Health

Care Finance Administration (HCFA) list for those aged 65-74. Controls were frequency-matched to the cases on race and five-year age group.

*Data Collection*

Permission to contact potential cases was obtained from their physician. A letter was sent to eligible cases and controls, followed by a telephone call if possible. If the woman did not want to participate in the full interview, she was asked to complete a mini questionnaire over the phone. A total of 705 CIS cases, 940 CIS controls, 2704 invasive cases and 3600 invasive controls were identified for contact. Reasons for non-participation after identification included inability to locate the person, ineligibility, patient or physician refusal, or death. Numbers of subjects in each of these categories and contact, cooperation, and response rates are shown in Table A.5. Contact, cooperation, and response rates were computed as follows: the contact rate is those able to be contacted divided by the total identified; the cooperation rate is the number who completed the full interview or mini questionnaire divided by those eligible to be contacted; and the response rate is the number who completed the interview or mini questionnaire divided by those eligible to be selected (total identified minus ineligible and deceased).

Upon agreement to participate in the full interview, trained nurse-interviewers scheduled in-person interviews, usually at the woman's home. At the interview, the nurse administered a detailed questionnaire and took height, weight, waist, and hip measurements. In addition, 30ccs of blood were drawn, and written permission to acquire medical records and paraffin-embedded tumor blocks was obtained from cases.

The tumor blocks were used to make 10 hematoxylin and eosin (H&E) stained slides to be used for histopathologic and molecular research.

Slides from CBCS cases diagnosed at the University of North Carolina (UNC) Hospitals were used to conduct a pathology study that examined inter-observer reliability of overall diagnosis and histopathologic components of DCIS. Pathologists from hospitals supplying tumor blocks and/or slides to the CBCS were recruited via a letter requesting volunteer participation in the reliability study and follow-up phone calls. Of 30 pathologists contacted, five agreed to participate in this study.

Only UNC cases were used for the reliability study due to ease of access to tumor blocks and pathology reports and so that IRB approval would only be required from one institution. Fifty-six of the CBCS DCIS cases were from UNC. Slides from 53 contained sufficient quality and size for inclusion in this reliability study. One slide per case was used, and the 53 slides were divided into four slide batches consisting of 13-14 slides each. Each pathologist received one batch at a time along with a one-page data entry form for each slide. When the current batch and completed forms were returned, the next batch was shipped.

For each slide, pathologists indicated the quality of the slide preparation and selected one of seven categories for overall diagnosis: comedo DCIS, non-comedo DCIS, lobular carcinoma *in situ* (LCIS), atypical ductal hyperplasia (ADH), other *in situ* proliferation, no proliferative lesion present, and unable to determine. If the pathologist assigned comedo or non-comedo DCIS as the overall diagnosis, the following additional information concerning components of the DCIS diagnosis was requested: pattern of necrosis (central/comedo-type, punctate/individual cell, and absent (no necrosis)),

maximum nuclear diameter (1.5 to 2 times, 2.1 to 2.5 times, or >2.5 times the diameter of a red blood cell), architectural growth pattern, nuclear chromatin pattern, and characteristics of nucleoli.

The pathologists were instructed to follow criteria and procedures used in their current practice and did not receive any special training prior to participating in the study, because we were interested in measuring clinical practice procedures. Pathologists also indicated whether they used an ocular micrometer to assess nuclear diameter for each slide. Only pattern of necrosis and maximum nuclear diameter data were analyzed for this study, because these components are included in the major classification systems and have been found to best predict clinical outcome [76].

*Description of Study Population*

When both phases of the invasive study were complete, data was available for 1808 invasive cases and 1564 controls. The CIS portion of the CBCS consisted of 508 cases, of which 446 were DCIS only, and 458 controls, identified and interviewed between 1996 and 2001. Table A.6 illustrates the main demographic characteristics for all invasive and DCIS participants. Age was computed from self-reported birth date. Because very few participants were American Indian/Eskimo, Asian or Pacific Islander, or Other (n=13 for DCIS, n=53 for invasive), they were combined with Whites, resulting in two race categories: African-American and non African-American.

### *Data Analysis*

*Ductal Carcinoma in Situ and Invasive Risk Factors*

The main outcome variables were ductal carcinoma *in situ*, which included all cases of pure DCIS, and invasive breast cancer. Initial diagnosis was assigned by the referring physician and verified by a pathologist employed by the CBCS using pathology reports and H&E stained slides. Fewer than two percent of the DCIS cases were reclassified as invasive based on the CBCS pathologist's evaluation. Main hormonal and reproductive risk variables included parity, age at first full-term pregnancy, lactation, oral contraceptive (OC) use and duration of use, hormone replacement therapy (HRT) use and duration of use, age at menopause, and age at menarche.

Univariate analyses were used to describe exposure and outcome variable distributions and identify missing values and possible outliers. Odds ratios and 95 percent confidence intervals were computed using unconditional logistic regression. In addition to analyses for all DCIS cases combined, univariate and multivariate analyses were conducted for DCIS cases stratified on histopathologic subtype (comedo vs. non-comedo). All regression models contained an offset term to adjust for age and race frequency matching. All evaluations of potential effect measure modification and confounding were conducted on the *in situ* data only, in order to obtain the model with the best fit for that data. The resulting model was used for the invasive data in order to make direct comparisons between *in situ* and invasive model estimates.

In order to determine the least biased estimates of the outcome-exposure relationship, a methodology using backward elimination was utilized to evaluate effect

measure modification and confounding. Potential effect measure modifiers were identified *a priori* and evaluated by comparing models including an interaction term and the main outcome and exposure effects with models containing the main effects only. These included age, race, education, income, and first-degree family history of breast cancer. Using the likelihood ratio test (LRT), a cut point of $\alpha$=0.10 determined whether or not the interaction term remained in the final model [77]. After this process had been performed for each of the main exposure covariates, no effect measure modification was found.

In addition to the main covariates, potential confounders included age, race, education, income, alcohol use, smoking, first-degree family history of breast cancer, and body mass index (BMI). If addition of the covariate to the model resulted in a 10 percent or larger change in stratum-specific regression coefficient ($\beta$), that variable was considered a confounder and remained in the final model. Only age and race created sufficient changes and were controlled for in the final model.

*Inter-observer Reliability*

The number of times a specific classification was chosen by each observer for overall diagnosis, pattern of necrosis, and maximum nuclear diameter was determined. In addition, number of cases for which the same classification category was selected by all five (100% agreement), four out of five (80% agreement), or three out of five (60% agreement) observers was determined, along with the number of cases for which there was "no majority agreement" (zero to two out of five) for a category.

Because no gold standard exists for CIS histopathologic diagnoses, a consensus rating was determined for each question based on the classification chosen by at least three pathologists for each case. If there was no majority classification, the consensus rating was missing for that case. Although this method is inherently biased toward agreement, since the individual ratings make up the consensus, it enables direct comparison among ratings for each slide. Cohen's kappa statistic ($\kappa$), a measure of agreement beyond chance, was used to assess inter-observer reliability for each of the categories for each rater compared to each of the other raters and to the consensus [78]. Kappa is zero if the observed agreement is consistent with a random assignment of classifications among observers, and 1.0 if there is perfect agreement.

The kappa statistic does not rely on knowledge of actual diagnosis, making it more informative than simple percent agreement [79]. However, a prevalence effect can occur with kappa when the proportion of agreement for one category differs from that of another [80]. A large difference in proportions (the prevalence index) results in a lower kappa, with greater attenuation occurring for larger kappa values [80]. In addition, the difference in proportions of disagreements (the bias index) results in a higher kappa than when there is little or no bias, and the bias effect has more impact when kappa is small [80]. Prevalence and bias indices were calculated for all kappa statistics in this study.

Simple pair-wise kappa statistics were computed for components with nominal categories, i.e. overall diagnosis and pattern of necrosis. Weighted kappas (which give more weight to cases in which disagreement varies by only one category versus two or more) were calculated for the ordinal component maximum nuclear diameter. For each component, an overall or "global" kappa was calculated that is a weighted average of all

ratings for each component as well as for every category of overall diagnosis, pattern of necrosis, and maximum nuclear diameter using a SAS® macro (http://ftp.sas.com/techsup/download/stat/magree.html). The resulting statistic estimates agreement among categories rather than individual observers and has been called a summary kappa in other reliability studies [12, 81, 82].

# CHAPTER III: INTER-OBSERVER RELIABILITY OF DUCTAL CARCINOMA *IN SITU* HISTOPATHOLOGY IN CLINICAL PRACTICE


## *Abstract*

Ductal carcinoma *in situ* (DCIS) of the breast is a heterogeneous disease with no single histopathologic classification system. It is unclear which features pathologists use to categorize DCIS in clinical practice, and the consistency of categorization among pathologists is unknown. Five pathologists from separate North Carolina hospitals rated 53 slides of DCIS on overall classification (comedo or non-comedo type DCIS), pattern of necrosis (central/comedo-type, punctate/individual cell, or absent) and maximum nuclear diameter to determine inter-observer reliability using the kappa ($\kappa$) statistic. Agreement on overall diagnosis was moderate ($\kappa$=0.48, 95% CI=0.41-0.55), while reliability was fair for necrosis pattern ($\kappa$=0.35, 95% CI=0.30-0.41) and maximum nuclear diameter ($\kappa$=0.22, 95% CI=0.16-0.28). These results indicate the inconsistency in DCIS classification and add to the growing evidence that a universal classification system is needed to ensure accurate, reliable DCIS diagnoses.


## *Introduction*

As U.S. incidence rates of ductal carcinoma *in situ* (DCIS) of the breast have increased with the increase in mammographic screening in the early 1980s, pathologists have struggled to define and categorize the disease. DCIS are malignant breast tumors that are confined to the lactiferous ducts, without stromal invasion across the basement

membrane. Pathologic characteristics of DCIS are heterogeneous, with variation by nuclear grade, amount and type of necrosis, and growth pattern. When combined, these characteristics determine the grade of DCIS and its likelihood to develop into invasive disease. However, pathologic classification of DCIS is not uniform, and final diagnoses may differ depending upon the classification system used [51, 83-86]. Variation in the methods, reliability and validity of pathologic diagnostic schemes used in actual practice settings may influence the reliability of DCIS subtype classifications, which in turn may affect treatment decisions and the design and conduct of DCIS research studies.

Previous studies of inter-observer reliability of breast histopathology included few DCIS cases [11, 13, 15, 16, 21, 81, 87] or compared reliability using one specific classification system rather than reproducing actual clinical practice procedures [10, 12, 18-20, 88]. However, the heterogeneous nature of DCIS requires that a sufficient number of cases be included in a reliability study to capture the variety of pathologic characteristics that comprise a DCIS diagnosis.

Knowing which components lead a pathologist to a specific diagnosis and how they compare with other pathologists' ratings of the same components would clarify the diagnostic process, independent of the specific classification scheme used. For instance, comparing agreement on specific components with agreement on overall diagnosis would clarify which components are most important for consistency in determining DCIS subtype diagnoses across pathologists. This information could simplify diagnostic criteria and lead to greater consensus and ideally to the development of a universal classification system. In addition, it could identify areas where additional training is needed.

The current study was conducted to (1) examine how well pathologists from university and community hospital settings agree on overall histopathologic diagnoses for DCIS cases, particularly for the two most common subgroups, comedo (high-grade) DCIS and non-comedo (low-grade) DCIS; (2) determine which diagnostic components have the highest and lowest agreement among pathologists; and (3) explore which of the major diagnostic components contribute most often to the diagnosis of comedo versus non-comedo DCIS subtypes.

## *Methods*

### *Specimen Selection*

Carcinoma in situ specimens were selected from those already obtained for participants in the Carolina Breast Cancer Study (CBCS), a population-based case-control study of in situ and invasive breast cancer among women aged 20 to 74 residing in 24 North Carolina counties [74]. New carcinoma in situ (CIS) cases diagnosed between 1996 and 2001 were identified by rapid ascertainment in conjunction with the North Carolina Central Cancer Registry [89]. Only cases diagnosed at the University of North Carolina (UNC) were included in this study. A formalin-fixed, paraffin-embedded tumor block was provided by UNC Hospitals for each CIS case, from which ten sections were cut and hematoxylin and eosin (H&E) stained. For uniformity, the tenth H&E stained slide of each tumor block was used for this reliability study; the other nine were used to confirm the diagnosis or were designated for other research studies. Of 56 total UNC cases included in the CBCS, 53 were of sufficient quality and size for inclusion in

this reliability study. Potential raters for this study were 30 pathologists who had supplied tumor blocks and/or slides to the CBCS.

*Pathologist Recruitment*

Pathologists were recruited via a letter requesting volunteer participation in the reliability study. Five North Carolina pathologists from five different hospitals agreed to participate in this study, including three from large urban teaching hospitals with cancer centers and two from smaller suburban community-based hospitals. The pathologists were instructed to follow criteria and procedures used in their current practice and did not receive any special training prior to participating in the study.

*Study Procedures*

The 53 case slides were divided into four batches consisting of 13-14 slides each. Each pathologist received one batch at a time along with a one-page data entry form for each slide. For each slide, pathologists indicated the quality of the slide preparation and selected one of seven categories for overall diagnosis: comedo DCIS, non-comedo DCIS, lobular carcinoma *in situ* (LCIS), atypical ductal hyperplasia (ADH), other *in situ* proliferation, no proliferative lesion present, and unable to determine. If more than one lesion type was present, pathologists chose one at their discretion. Additional information concerning components of the DCIS diagnosis was requested for cases assigned comedo or non-comedo DCIS as the overall diagnosis, including pattern of necrosis (central/comedo-type, punctate/individual cell, and absent (no necrosis)), maximum nuclear diameter (1.5 to 2 times, 2.1 to 2.5 times, or >2.5 times the diameter of a red blood cell), architectural growth pattern, nuclear chromatin pattern, and

characteristics of nucleoli. Pathologists also indicated whether they used an ocular micrometer to assess nuclear diameter for each slide. Only pattern of necrosis and maximum nuclear diameter were analyzed for this study, because these components are included in the major classification systems and have been found to best predict clinical outcome [76].

*Statistical Methods*

Data were double entered into a database, error checked, and imported into the SAS statistical program (version 8.0, SAS Institute, Cary, NC) for statistical analyses. The number of times a specific classification was chosen by each observer for overall diagnosis, pattern of necrosis, and maximum nuclear diameter was determined. In addition, the number of cases for which the same classification category was selected by all five (100% agreement), four out of five (80% agreement), or three out of five (60% agreement) observers, or where there was "no majority agreement" was determined.

Because no gold standard exists for CIS histopathologic diagnoses, a consensus rating was determined based on the classification chosen by at least three pathologists for each case. If there was no majority classification, the consensus rating was missing for that case. Although this method is inherently biased toward agreement, since the individual ratings make up the consensus, it enables direct comparison among ratings for each slide. It is possible to determine whether some raters agree with each other more consistently than others and if raters are more likely to choose one category versus another compared with the other raters. A consensus value has been used in other reliability studies when no gold standard was available [90, 91].

Cohen's kappa statistic (κ), a measure of agreement beyond chance, was used to assess inter-observer reliability for overall diagnosis, pattern of necrosis, and maximum nuclear diameter [78]. Kappa is zero if the observed agreement is consistent with a random assignment of classifications among observers, and 1.0 if there is perfect agreement. The following table specifies qualitative ranges that can be used for subjective interpretation of agreement measured by kappa [92]:

| κ Statistic | Level of Agreement |
|---|---|
| ≤0.20 | Poor |
| 0.21-0.40 | Fair |
| 0.41-0.60 | Moderate |
| 0.61-0.80 | Good/Substantial |
| 0.81-1.00 | Very Good/Almost Perfect |

The kappa statistic does not rely on knowledge of actual diagnosis, making it more informative than simple percent agreement [79]. However, a prevalence effect can occur with kappa when the proportion of agreement for one category differs from that of another [80]. Using the example 2x2 table shown below, the prevalence index is computed by dividing the absolute value of the difference between frequencies in the agreement cells ('a' and 'd') by the total number of ratings (n=a+b+c+d):

| | | Observer 1 | |
|---|---|---|---|
| | | Diagnosis 1 | Diagnosis 2 |
| Observer 2 | Diagnosis 1 | a | b |
| | Diagnosis 2 | c | d |
| Total | | a+c | b+d |

$$\text{Prevalence index} = \left| \frac{a-d}{n} \right|$$

A large prevalence index results in a lower kappa, with greater attenuation occurring for larger kappa values [80]. In addition, a bias effect occurs when there is a difference in

proportions of disagreements. The bias index is the absolute value of the difference between frequencies in the disagreement cells ('b' and 'c') divided by the total number of ratings (n):

$$\text{Bias index} = \left| \frac{b-c}{n} \right|$$

Kappa tends to increase with increasing bias, and the bias effect has more impact when kappa is small [80]. Prevalence and bias indices were calculated for all kappa statistics in this study.

Simple pair-wise kappa statistics were computed for components with nominal categories, i.e. overall diagnosis and pattern of necrosis, and weighted kappas (which give more weight to cases in which disagreement varies by only one category versus two or more) were calculated for maximum nuclear diameter, which is an ordinal component. For each component, an overall or "global" kappa was calculated that is a weighted average of all ratings for each component as well as for every category of overall diagnosis, pattern of necrosis, and maximum nuclear diameter using a SAS® macro (http://ftp.sas.com/techsup/download/stat/magree.html). The resulting statistic estimates agreement among categories rather than individual observers. Other reliability studies have used this statistic to describe agreement among all pathologists within the study, sometimes calling it a summary kappa [12, 81, 82].

## ***Results***

### *Frequencies and Percent Agreement*

All five pathologists rated the same H&E stained section from each of the 53 individual cases, resulting in 265 total ratings. Each pathologist classified at least 85% of

the cases as either comedo or non-comedo DCIS, for a total of 236 ratings (Table 3.1); however, the classification of individual cases as comedo or non-comedo DCIS differed among the observers. Pathologists B and C classified more cases as comedo type than any other, D and E chose non-comedo most frequently, and A selected the two categories with equal frequency. The most common necrosis pattern assigned to comedo and non-comedo cases was the central/comedo-type necrosis pattern (Table 3.2). Maximum nuclear diameter classifications varied the most among observers, especially for the small (1.5 to 2) and large (>2.5) categories (Table 3.3). Three observers (A, C, and E) used the ocular micrometer to measure maximum nuclear diameter for at least 95% of the cases, one (D) used it for just over half the cases, and one observer (B) only used it for three cases (data not shown). Micrometer use did not affect results for maximum nuclear diameter.

Consensus agreement, represented by three or more pathologists concurring, was obtained for 52 of the 53 cases for overall diagnosis (Table 3.4). Complete agreement by all five observers on overall diagnosis occurred for 20 of the 53 cases. Of these, nine were diagnosed as comedo, nine as non-comedo, and two as other. For pattern of necrosis, complete agreement occurred for 22 cases, of which all but one was comedo-type. All observers agreed on only six cases for maximum nuclear diameter, with five of those in the large category.

Of the 236 ratings with an overall diagnosis of DCIS, six were missing data for pattern of necrosis, maximum nuclear diameter, or both. For the 230 observations with complete data, necrosis pattern and maximum nuclear diameter categories were compared between observations classified as comedo and non-comedo DCIS subtypes

(Figure 3.1). Over 95 percent of the slides rated as comedo-type DCIS were classified as central/comedo-type necrosis, and almost 75 percent were also classified as large maximum nuclear diameter. In contrast, necrosis and nuclear size characteristics of slides classified as non-comedo DCIS were more heterogeneous, with 42%, 20%, and 38% classified as central/comedo, punctate or no necrosis (respectively), and 29%, 45% and 27% classified as small, medium and large maximum nuclear diameter. The most common joint classification was central/comedo-type necrosis and medium nuclear diameter (19%), followed by no necrosis and medium nuclear diameter (18%).

*Inter-observer Reliability*

Prevalence and bias indices were low for all comparisons and therefore did not greatly influence the magnitude of kappa statistics. Inter-observer reliability analyses for each diagnostic component generated four pair-wise kappa statistics that were averaged for each observer (Table 3.5). Mean inter-observer kappa statistics were highest for overall diagnosis, with values for three pathologists indicating moderate reliability and two showing fair reliability when compared with the other four observers; however, individual pair-wise kappas for overall diagnosis ranged from 0.21 to 0.66. Inter-observer reliability was lowest for maximum nuclear diameter, with average kappa statistics indicating only fair agreement for all observers.

Results for each observer also were compared with consensus values based on simple or weighted kappa statistics as appropriate (Table 3.5). In general, kappa statistics for agreement with the consensus value for each characteristic were higher than pair-wise (inter-observer) kappas. With inter-observer comparisons, agreement was highest for

overall diagnosis and lowest for maximum nuclear diameter. For the consensus kappas, agreement was highest for overall diagnosis but nearly equal for the other components.

Global kappa estimates for all observers combined indicated that agreement was highest for overall diagnosis ($\kappa$=0.48) and lowest for maximum nuclear diameter ($\kappa$=0.22) (Table 3.6). Reliability results were similar for the three categories of DCIS overall diagnosis but varied among necrosis categories ($\kappa$=0.57 for central/comedo type and $\kappa$=0.18 for punctate/individual call) and nuclear diameter categories ($\kappa$=0.33, 0.16 and 0.15 for large, medium and small maximum diameter, respectively).

***Discussion***

The incidence of ductal carcinoma *in situ* (DCIS) of the breast has increased dramatically over the past 15 years due to increased use of routine mammography screening. Lesions defined as DCIS vary with regard to cytonuclear grade, necrosis, architecture, growth pattern, and progression to invasive carcinoma. Proposed DCIS classification systems emphasize different histopathologic characteristics, with Bellamy et al focusing on architecture [93], Holland et al on cytological grade [83], Poller et al on necrosis [51], and the Van Nuys Prognostic Index (VNPI) on a combination of nuclear grade and necrosis [86].

In 1997, an international conference convened to establish consensus guidelines for evaluation of DCIS pathology, including the classification of DCIS and identification of features affecting prognosis in DCIS patients [94]. The committee recommended that specific items be included in a DCIS pathology report, but no single classification system

was endorsed due to insufficient evidence that one system could be replicated easily and reliably.

Since then, reliability studies have examined which of the many DCIS pathology rating systems are most repeatable among pathologists, both with training [18, 19, 82] and without [12, 88]. Each study included different numbers of observers and cases, and agreement was higher if pathologists were trained before the study. For the VNPI, arguably the most popular system, results from four different studies ranged from a high of $\kappa$=0.66 (for pathologists who received a tutorial on the diagnostic criteria plus supporting written information and accompanying photographic aids) to a low of $\kappa$=0.26 (for pathologists given written information on the system only) [12, 18]. Similar results were reported for the Holland system ($\kappa$=0.57 for observers given a tutorial and written material, $\kappa$=0.37 for those receiving only a written description) [18, 88], and for the system developed by Lagios ($\kappa$=0.46 with a training set of slides and written criteria, $\kappa$=0.26 with only written information) [12, 19]. These results suggest that specific instruction and written aids are necessary for pathologists to achieve more than moderate agreement on DCIS histopathology.

The results of the current study indicate that without specific rating guidelines, pathologists in a variety of clinical practices differed substantially in their assessments of DCIS histopathology. Among five observers using the same data entry form but their own system of rating for pathology slides from 53 separate DCIS cases, agreement on overall diagnosis was only moderate ($\kappa$=0.48). Previous studies that also included two categories of DCIS (comedo vs. noncomedo or high grade vs. low grade) found similar results. With only written descriptions of the classification systems, 23 observers rating

slides from 33 cases resulted in an overall kappa of 0.34 [88], and 23 observers of 107 cases showed slightly higher agreement with an overall kappa of 0.43 [95]. For six observers rating 125 cases with training that included practice slides and written criteria, overall kappa was 0.46 [19].

Reliability in the current study was fair for the two component categories used in most classification systems, pattern of necrosis ($\kappa$=0.35) and maximum nuclear diameter ($\kappa$=0.22). Within each component, agreement was highest for those category choices considered most severe, specifically central/comedo-type necrosis and largest nuclear diameter. Because the most severe categories of each component represent the greatest cellular change, they are more easily distinguished from normal or benign tissue, and therefore higher agreement is expected for these categories. In fact, studies comparing reliability across classification schemes have shown substantially lower kappa scores for intermediate and low-grade lesions than for those rated as high grade or comedo-type [19, 95].

While a number of histopathologic reliability studies of breast lesions have been conducted in the past 15 years, this study is the first among published studies to focus on DCIS cases and specifically on comedo and non-comedo DCIS subtypes. In addition, we examined the components of diagnosis used by pathologists to delineate between comedo and non-comedo DCIS subtypes. A previous study that estimated kappa values for architectural growth patterns and nuclear grade for a small number of DCIS cases reported a lower inter-observer kappa value for comedo-type growth pattern ($\kappa$=0.38) and higher values for nuclear grade (high nuclear grade, $\kappa$=0.41, and low nuclear grade, $\kappa$=0.51) [96]. Pathologists were given specific rating guidelines for the cases, which

could account for the higher agreement on nuclear grade but does not explain the difficulty distinguishing comedo-type lesions.

Only one other study examined pathologist reliability for DCIS subtypes and found slightly lower reliability for comedo versus non-comedo diagnoses compared to our study ($\kappa$=0.44) [13]. However, the authors examined reliability across all major types of breast disease, so while more than 180 observers participated, only 17 DCIS cases were included. The same research group determined reliability among different classification systems but included only two systems that attempted to distinguish DCIS subtypes [88].

Other reliability studies compared *in situ* diagnoses with those of invasive and/or other proliferative breast diseases and found much higher agreement on overall diagnosis for DCIS, with kappas ranging from 0.59 to 0.87 [11, 13, 81, 87]. However, distinctions between carcinoma *in situ* and benign diseases or invasive carcinomas are much clearer than those within each disease category. Therefore, better inter-observer reliability is expected in studies that examine histopathologic agreement differentiating CIS from proliferative lesions.

Observers in the current study were not required to use a specific rating system. This allowed for simulation of actual clinical practice and permitted a reliability analysis of necrosis and nuclear diameter histopathologic components included in the different classification schemes. In this way, we were able to explore similarities and differences in methods of classifying comedo versus non-comedo DCIS subtypes. While observers usually diagnosed comedo-type as having high-grade nuclei and comedo-type necrosis, less agreement occurred for non-comedo type lesions. DCIS cases that did not have

severe characteristics of necrosis and nuclear grade seemed to be the hardest to classify. The implications for clinical decision are that diagnosis and treatment decisions for CIS may be based upon less than reliable diagnostic criteria.

Pathologists often utilize an ocular micrometer to obtain precise measurements for maximum nuclear diameter. Observers in this study recorded whether or not they used a micrometer in their evaluations, but inter-observer agreement for this component was low, suggesting that differences in the choice of representative nuclei for diameter measurements may be a source of variation between pathologists. One possibility for the anomaly is length and amount of experience reading breast cancer slides. All five pathologists had been in practice for at least 15 years, and three worked at hospitals with large cancer centers. However, no more detailed information on the pathologists' training or experience was obtained. Future studies of this type should consider doing so in order to possibly control for this variable.

Slides from seven of the cases were rated as poor quality by at least three observers. To assess whether slide quality was associated with agreement, ratings from those cases were analyzed separately, but agreement among all observers was similar to those for slides rated as adequate or good quality for any of the categories.

Before a universal classification system for DCIS pathology is developed, a comprehensive study of actual pathology practices must be conducted to determine where diagnostic discrepancies occur among pathologists. Future studies could use the same pathologists and slides to examine reliability without training first, then conduct training for a particular existing classification system and reassess reliability. The current study

suggests that one universal system, accompanied by training, is necessary to ensure the highest possible agreement across pathologists in clinical practice.

Table 3.1. Frequencies of overall diagnosis by observer, including consensus

| Observer | DCIS, comedo-type N** (%) | DCIS, non-comedo type N (%) | Other* N (%) | Unable to Determine N (%) | Total N |
|---|---|---|---|---|---|
| A | 23 (43) | 23 (43) | 6 (11) | 1 (2) | 53 |
| B | 26 (49) | 22 (42) | 5 (9) | 0 (0) | 53 |
| C | 27 (51) | 19 (36) | 7 (13) | 0 (0) | 53 |
| D | 20 (38) | 25 (47) | 5 (9) | 3 (6) | 53 |
| E | 17 (32) | 34 (64) | 2 (4) | 0 (0) | 53 |
| Average*** | 22.6 (42.6) | 24.6 (46.4) | 5 (9.4) | 0.8 (1.5) | 53 |
| Consensus | 24 (45.3) | 25 (47.2) | 3 (5.7) | 1 (1.9) | 53 |

*Includes LCIS, ADH, other *in situ* proliferation, and no proliferative lesion present
**N=number of slides; percentages may not equal 100 due to rounding
***Average=Total for column ÷ 5

Table 3.2. Frequencies of pattern of necrosis by observer, including consensus

| Observer | Central/ comedo-type N* (%) | Punctate/ individual cell N (%) | Absent N (%) | Unable to Determine N (%) | Total N |
|---|---|---|---|---|---|
| A | 30 (65) | 10 (22) | 5 (11) | 1 (2) | 46 |
| B | 33 (69) | 3 (6) | 11 (23) | 1 (2) | 48 |
| C | 32 (70) | 9 (19) | 5 (11) | 0 (0) | 46 |
| D | 26 (59) | 2 (4) | 15 (33) | 2 (4) | 45 |
| E | 36 (71) | 4 (8) | 11 (21) | 0 (0) | 51 |
| Average** | 31.4 (66.5) | 5.6 (11.9) | 9.4 (19.9) | 0.8 (1.7) | 47.2 |
| Consensus | 32 (65.3) | 5 (10.2) | 7 (14.3) | 5 (10.2) | 49 |

*N=number of slides; total number varies by observer and is less than 53 because cases not diagnosed as DCIS do not have answers for pattern of necrosis.
**Average=Total in column ÷ 5

Table 3.3. Frequencies of maximum nuclear diameter by observer, including consensus

| Observer | 1.5 to 2 (Small) N** (%) | 2.1 to 2.5 (Medium) N (%) | >2.5 (Large) N (%) | Unable to determine N (%) | Total N |
|---|---|---|---|---|---|
| A | 2 (4) | 17 (37) | 27 (59) | 0 | 46 |
| B | 5 (11) | 16 (33) | 27 (56) | 0 | 48 |
| C | 4 (9) | 9 (19) | 33 (72) | 0 | 46 |
| D | 20 (45) | 15 (33) | 10 (22) | 0 | 45 |
| E | 10 (20) | 18 (35) | 21 (41) | 2 (4) | 51 |
| Average*** | 8.2 (17.4) | 15 (31.8) | 23.6 (50.0) | 0.4 (0.9) | 47.2 |
| Consensus | 6 (12.2) | 11 (22.5) | 27 (55.1) | 5 (10.2) | 49 |

NOTE: Maximum nuclear diameter=nuclear diameter of largest red blood cells
**N=number of slides
***Average=Total in column ÷ 5

Table 3.4. Frequencies of agreement by number of slides for each DCIS component

| Frequencies | Overall Diagnosis N* (%) | Pattern of Necrosis N (%) | Maximum Nuclear Diameter N (%) |
|---|---|---|---|
| 100% agreement (5 out of 5 observers) | 20 (38) | 22 (44) | 6 (12) |
| 80% agreement (4 out of 5 observers) | 20 (38) | 9 (18) | 16 (32) |
| 60% agreement (3 out of 5 observers) | 12 (23) | 10 (20) | 21 (42) |
| No majority agreement | 1 (2) | 9 (18) | 7 (14) |
| Total | 53 | 50 | 50 |

*N=number of cases

Table 3.5. Mean kappa for each observer versus all other observers and kappa versus consensus

| Component<br>**OBSERVER** | **Observer vs. All Other Observers<br>Mean Kappa* (Range)** | **Observer vs. Consensus<br>Kappa** (95%CI)** |
|---|---|---|
| Overall Diagnosis | | |
| A | .55 (.45-.66) | .78 (.63, .92) |
| B | .50 (.34-.65) | .74 (.57, .90) |
| C | .55 (.40-.66) | .78 (.63, .92) |
| D | .39 (.21-.49) | .53 (.33, .72) |
| E | .36 (.21-.48) | .55 (.35, .76) |
| Pattern of Necrosis | | |
| A | .41 (.27-.50) | .65 (.49, .81) |
| B | .41 (.37-.50) | .58 (.41, .75) |
| C | .37 (.24-.50) | .56 (.40, .72) |
| D | .28 (.23-.37) | .30 (.14, .45) |
| E | .34 (.23-.37) | .51 (.31, .71) |
| Maximum Nuclear Diameter | | |
| A | .33 (.19-.46) | .65 (.49, .81) |
| B | .30 (.25-.38) | .53 (.26, .79) |
| C | .30 (.21-.46) | .53 (.28, .78) |
| D | .23 (.19-.28) | .30 (.12, .48) |
| E | .28 (.26-.31) | .54 (.30, .77) |

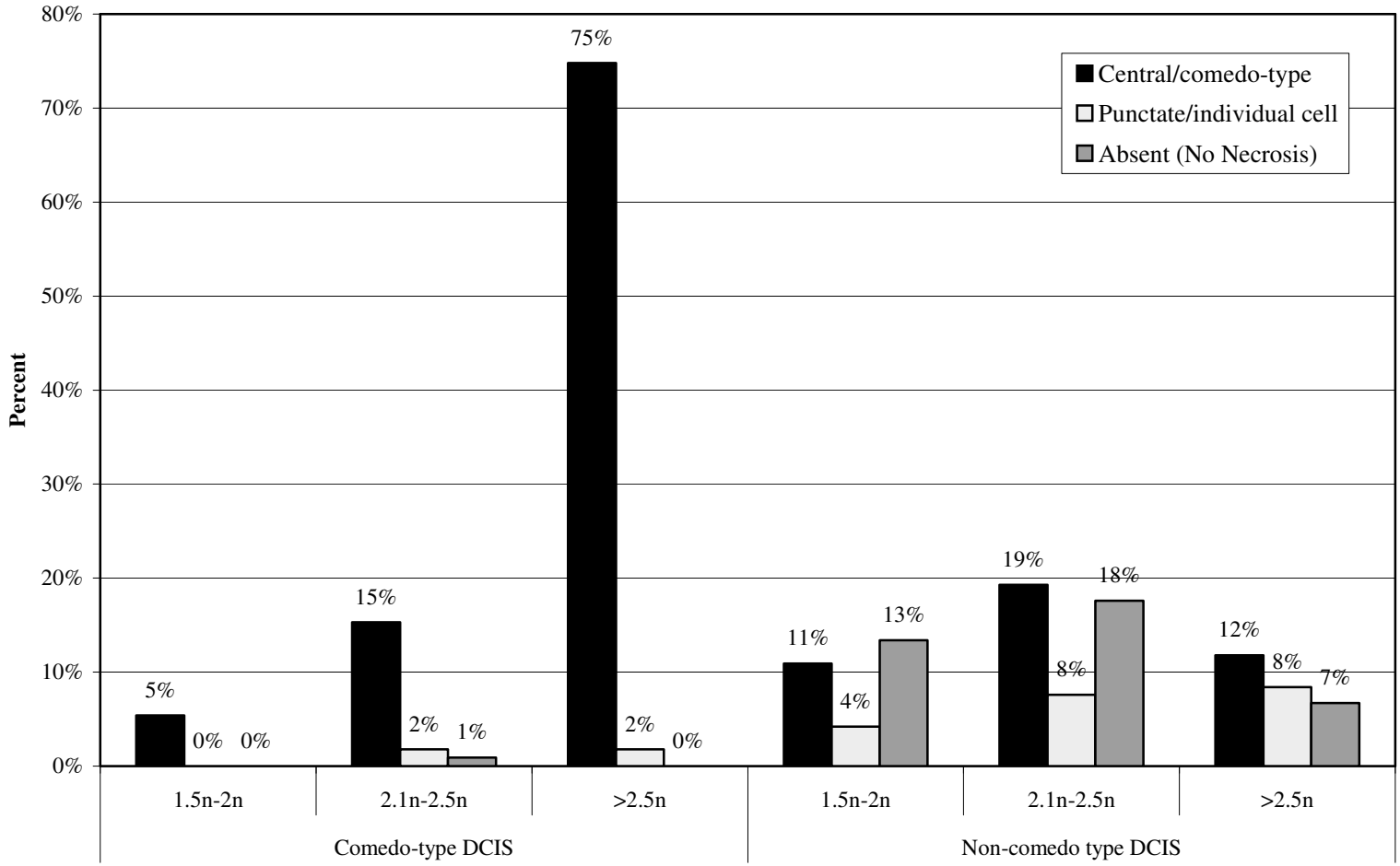
*Mean kappa = average of all comparisons between that observer and the other four observers

**Kappa=simple kappa for overall diagnosis and pattern of necrosis, weighted kappa for maximum nuclear diameter

Table 3.6. Overall agreement measured by global kappa, all observers combined for each component and category

| **DCIS components and categories** | **Global Kappa (95% CI)** |
|---|---|
| Overall Diagnosis | .48 (.41-.55) |
| Comedo-type DCIS | .52 (.44-.61) |
| Non-comedo type DCIS | .43 (.35-.52) |
| Other | .51 (.43-.60) |
| Pattern of Necrosis | .35 (.30-.41) |
| Central/comedo-type | .57 (.48-.66) |
| Punctate/individual cell | .18 (.09-.26) |
| Absent (no necrosis) | .28 (.19-.37) |
| Maximum Nuclear Diameter | .22 (.16-.28) |
| 1.5 to 2.0 | .15 (.07-.24) |
| 2.1 to 2.5 | .16 (.07-.25) |
| >2.5 | .33 (.24-.41) |

Figure 3.1. Pattern of necrosis and maximum nuclear diameter combinations for all slides and observers by DCIS subtype



# CHAPTER IV: REPRODUCTIVE AND HORMONAL RISK FACTORS FOR DUCTAL CARCINOMA *IN SITU* OF THE BREAST: A COMPARISON WITH INVASIVE BREAST CANCER


***Abstract***

Carcinoma *in situ* (CIS) of the breast now accounts for one-fifth of all newly diagnosed breast cancer cases. Cases of the most prevalent CIS type, ductal carcinoma *in* situ (DCIS), are treated using the same aggressive methods as those for invasive disease, but recent research indicates some subtypes of DCIS (high grade, or comedo) share histopathologic and epidemiologic characteristics with invasive disease, while others (medium or low grade, or non-comedo) show different patterns. Estrogen exposure, measured by reproductive and hormonal factors, has been associated with invasive breast cancer (IBC) risk, but its connection with DCIS is less clear.

To investigate whether reproductive and hormonal risk factors differ among comedo and non-comedo types of DCIS and invasive breast cancer, we used a population-based case-control study of 1808 invasive and 446 DCIS breast cancer cases aged 20-74 diagnosed in North Carolina between 1993 and 2001. Controls (N=1564 for invasive, 458 for DCIS) were frequency-matched to cases by age and race. Odds ratios (ORs) and 95% confidence intervals (CI) were used to evaluate risk factors for each subtype of DCIS as well as invasive disease. Two or more full-term pregnancies and breastfeeding were inversely associated with comedo-type DCIS and invasive breast cancer (IBC) but showed no effect for non-comedo DCIS. Postmenopausal hormone

replacement therapy (HRT) use was not associated with either DCIS subtype or IBC. Increasing duration of OC use was associated with IBC but not associated with DCIS. Our results support the theory that DCIS is a heterogeneous disease and the etiology of comedo-type DCIS is more similar to invasive breast cancer than non-comedo DCIS.

### *Introduction*

Carcinoma *in situ* (CIS) of the breast is a classification for malignant cells that have not moved beyond the epithelium to invade the basal membrane and is further categorized as either lobular (LCIS) or ductal (DCIS), depending on its location [97]. DCIS can be classified into comedo (high grade) and non-comedo (medium or low grade) subtypes based on histopathologic characteristics such as pattern of necrosis and maximum nuclear diameter. Both biologic and epidemiologic evidence show that some DCIS develop into invasive disease, but the distinction between those that will and those representing neoplasms distinct from invasive breast cancer (IBC) is unclear [22, 35, 98-101].

When the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program began recording incidence rates for DCIS in 1973, the age-adjusted rate was 2.3 per 100,000 females [38]. By 1992, that figure had jumped to 15.8 per 100,000 [39]. The most dramatic increases have occurred since 1983, with a 17.5% annual increase in incidence rates between 1983 and 1992 compared to increases of 3.9% annually from 1973 to 1983 [39]. Separate studies in Detroit [40], Connecticut [41], Vaud, Switzerland [42], and Florence, Italy [43] have shown that most of this increase was due to the introduction of screening mammography in the early 1980s and its

subsequent increasing use in women age 40 and over. However, since 1992 the proportional change in incidence rates for DCIS has slowed, especially for comedo DCIS [1]. In addition, 80% of all DCIS diagnosed in the US since 1980 were non-comedo type.

Whether or not DCIS lesions found through increased detection will progress to invasive disease is unknown. It is generally believed that comedo-type DCIS is more similar to invasive disease than is the non-comedo-type. Studies of women with concomitant DCIS and IBC found higher grade DCIS correlated with higher grade IBC [102-104]. The same correlation has been observed in long-term follow-up studies of women with DCIS who developed invasive disease [28, 29, 33]. Autopsy series studies of women who died from causes other than breast cancer have found prevalence rates of DCIS ranging from 0.2% to 14.7%, compared with 0-1.8% for invasive breast cancer [105]. Therefore, some *in situ* lesions may take much longer to develop invasive traits or may never become invasive during a woman's normal lifespan. Because of the uncertainties regarding DCIS progression, most lesions are treated aggressively. Knowing which DCIS types are less likely to progress to invasive cancer could lower morbidity by limiting unnecessary surgical and adjuvant treatment.

Many of the accepted risk factors for invasive breast cancer involve hormonal exposure, in particular estrogen, whether directly through exogenous use (oral contraceptives, hormone replacement therapy) or through reproductive events such as timing of menarche and menopause, pregnancy, and lactation. Previous studies have found nulliparity, late age at first pregnancy, early menarche, late menopause, no

lactation, and exogenous hormone use associated with invasive breast cancer [106]. The connection between estrogen and *in situ* breast cancer is less clear.

We examined known hormonal and reproductive risk factors for invasive breast cancer to determine whether they are risk factors for DCIS, and to determine whether risk factors differ for comedo and non-comedo DCIS subtypes. Odds ratios for DCIS as well as for DCIS subtypes (comedo, non-comedo) were compared directly with those of invasive breast cancer in the same North Carolina study population.

## *Methods*

### *Study Design*

The Carolina Breast Cancer Study (CBCS) is a population-based case-control study of *in situ* and invasive breast cancer in African-American (AA) and Caucasian women [74]. Eligible study participants were aged 20 to 74 at time of diagnosis (cases) or selection (controls) and residing in 24 contiguous counties of eastern and central North Carolina. Women with first breast cancer diagnoses (*in situ* or invasive) were identified through a rapid-ascertainment system in conjunction with the North Carolina Central Cancer Registry [89], and controls were located via computerized lists from the Department of Motor Vehicles (under age 65) and the Health Care Finance Administration (age 65 and over). Controls were frequency-matched to cases on race and 5-year age intervals.

Invasive cases were enrolled in two phases, between 1993 and 1996 (Phase 1) and from 1996 through 2001 (Phase 2), and were over-sampled for African-Americans and younger age (20-49 years). Specifically, a process of randomized recruitment using

predetermined probabilities [75] was used to balance four groups based on age and race: younger African Americans, older African-Americans, younger non-African Americans, and older non-African Americans. In order to accomplish this, 100% of African American cases younger than 50 years, 75% of African American cases at least 50 years old, 67% of non–African American cases younger than age 50 years, and 20% of non–African American cases at least 50 years old were sampled [74].

*In situ* case enrollment occurred between 1996 and 2001 and included pure ductal carcinoma *in situ* (DCIS), DCIS with microinvasion to a depth of 2mm, and lobular carcinoma *in situ* (LCIS). All *in situ* cases matching the age and geographic constraints mentioned above were eligible for the study, with no oversampling on race or age.

*Study Population*

A total of 705 carcinoma *in situ* (CIS) cases were identified during the enrollment period. Seven hundred (99.3%) could be contacted, of which 50 were ineligible or deceased (7.1%), physicians refused participation for 51 (7.3%), and 58 declined to participate (8.3%), resulting in a cooperation rate of 83.2% and an overall response rate of 82.6%. Thirty-eight of the participants completed only a mini questionnaire, which did not include hormonal or reproductive questions. For the current study, 28 cases of pure LCIS and 29 cases of DCIS with microinvasion were excluded leaving 446 pure DCIS cases. Of the 940 controls sampled, 852 were located (90.6%), 122 were ineligible or deceased (13.0%), and 197 refused participation (21.0%). Cooperation and overall response rates for controls were 73.0% and 65.2%, respectively. After excluding 75

women who completed the mini questionnaire only, 458 DCIS study controls remained for this study.

Risk factor distributions were similar for invasive cancer cases enrolled in both phases of data collection, so data for all IBC cases from Phase 1 and Phase 2 were combined for a total of 2704 identified cases. Of those, 2640 (97.6%) were locatable (contact rate). Two hundred and one were ineligible or deceased (7.4%), physicians refused participation for 175 (6.5%), and 361 declined to participate (13.4%), resulting in a cooperation rate of 78.0% and an overall response rate of 76.0%. Ninety-five completed only the mini questionnaire, leaving 1808 IBC cases for analysis. A total of 3600 controls for IBC cases were identified, of which 2911 were located (80.9%), 427 were ineligible or deceased (11.9%), and 739 declined participation (20.5%). The cooperation rate for controls was 70.3%, and the overall response rate was 55.0%. Removal of the 175 who did not complete the full questionnaire left 1564 controls for the IBC study analyses.

*Data Collection*

Trained female nurses conducted in-person interviews using a structured questionnaire. Topics covered by the questionnaire include sociodemographic factors, menstrual and pregnancy history, medical history, hormone use, family history of cancer, physical activity and occupational history. The nurse measured height and weight at the time of the interview; all other questions were answered via self-report. Participants were given visual aids to assist with recall, such as pictures of common prescription and non-prescription drugs and calendars to pinpoint dates.

*Statistical Analyses*

All statistical analyses were conducted using SAS version 8.2 (SAS Institute Inc., Cary, NC, USA). The main outcome variable was ductal carcinoma *in situ*, which included all cases of pure DCIS. Initial diagnosis was assigned by the referring physician and verified by a pathologist employed by the CBCS based on a review of pathology reports and H&E stained slides. Less than two percent of the cases were reclassified based on the CBCS pathologist's evaluation. Main hormonal and reproductive risk variables included parity (no full-term pregnancies, one, two, three or more), age at first full-term pregnancy (<26, 26+), lactation (never, ever), oral contraceptive (OC) use (never, ever) and duration of OC use (<5 years, 5 to 10 years, >10 years), hormone replacement therapy (HRT) use (never, ever) and duration of HRT use (<5 years, 5-10 years, >10 years), age at menopause (<40, 40-49, 50+), and age at menarche ($\leq$11, 12, 13, 14+).

Univariate analyses were used to evaluate exposure and outcome variable distributions and identify missing values and possible outliers. Participants who classified their race as American Indian/Eskimo, Asian or Pacific Islander, or Other (n=13 for DCIS, n=53 for invasive) were combined with Whites, resulting in two race categories: African-American and non African-American. Age at the time of interview was computed from self-reported birth date and included in all analyses as a continuous variable. Women under age 50 were considered postmenopausal if they had undergone natural menopause (menstruation cessation), bilateral oophorectomy, or irradiation to the ovaries. In women aged 50 or older, menopausal status was assigned based on

menstruation cessation. We combined natural and surgical menopause for analysis, since we were interested in duration of estrogen exposure.

Odds ratios and 95 percent confidence intervals were computed using unconditional logistic regression. Case-control analyses were conducted for all data in order to estimate main effects of the risk factors. In addition, case-case analyses were used to identify factors with different relationships between comedo and non-comedo DCIS [107]. All regression models contained an offset term to adjust for age and race frequency matching. Potential effect measure modifiers were identified *a priori* and evaluated by comparing models including an interaction term and the main outcome and exposure effects with models containing the main effects only. These included age (continuous), race (African American, non-African American), education (less than college, college or greater), income (<$30,000/year, $30,000+/year), and first-degree family history of breast cancer (yes, no). Backward elimination with a cut point of $\alpha$=0.10 determined whether or not the interaction term remained in the final model. In addition to the main covariates, potential confounders included age, race, education, income, alcohol use (never, ever), smoking (never, previous, current), first-degree family history of breast cancer, and body mass index (BMI) (<25 kg/m$^2$, 25-29.9 kg/m$^2$, 30+ kg/m$^2$). If removal of the covariate from the model resulted in a 10 percent or larger change in stratum-specific regression coefficients, that variable was considered a confounder and remained in the final model.

All evaluations of potential effect measure modification and confounding were conducted on the *in situ* data only, in order to obtain the model with the best fit for that

data. The resulting model was used for the invasive data in order to make direct comparisons between *in situ* and invasive model estimates.

In addition to analyses for all DCIS cases combined, univariate and multivariate analyses were conducted for DCIS cases stratified on histopathologic subtype (comedo vs. non-comedo). The study pathologist classified DCIS subtype based on a detailed microscopic examination of an H&E stained slide for each case. Comedo DCIS was defined as having comedo-type pattern of necrosis as well as two of the following three characteristics: large or very large nuclear diameter (>2 times the diameter of a red blood cell), vesicular nuclear pleomorphism, and prominent nucleoli. All others were categorized as non-comedo. Fifty-six DCIS cases did not have this examination performed and were excluded from the subtype analyses, leaving a total of 393 DCIS cases (163 comedo and 230 non-comedo).

***Results***

*Distributions*

Characteristics of both the DCIS and IBC cases and controls are shown in Table 4.1. A total of 904 women participated in the DCIS study, of which 18.1 percent were African-American (N=164). The mean age of CIS cases (55.16 $\pm$ 11.07 SD) was slightly higher than that of controls (54.46 $\pm$ 10.26 SD), and a higher percentage of CIS cases than controls were African-American (21.1 vs. 15.3%). A larger proportion of IBC participants than DCIS subjects, both cases and controls, were African-American because of over-sampling by race in the IBC study but not in the DCIS portion. IBC cases and controls were slightly younger than their DCIS counterparts, as reflected in both mean

and median ages. Participants in the DCIS portion were more likely to be postmenopausal than those in the IBC group.

Table 4.2 displays reproductive and other characteristics of DCIS study controls and cases stratified by histopathologic subtype (comedo versus non-comedo). More non-comedo cases than controls were African-American, and fewer non-comedo cases reached menopause before age 40 than controls. More comedo cases than non-comedo cases used oral contraceptives for more than ten years; otherwise, risk factors were distributed evenly among comedo and non-comedo cases.

Age and race, the only confounders of the associations between cancer outcomes and hormonal and reproductive risk variables, were included in all multivariate models along with the offset terms to account for probability sampling by race and age. There was no significant effect measure modification by any of the evaluated covariates. Final modeling results for all DCIS and IBC are shown in Table 4.3, and results for comedo and non-comedo analyses are shown in Table 4.4.

*Ductal Carcinoma in Situ Cases vs. Controls and Invasive Cases vs. Controls*

Parity was inversely associated with both DCIS and IBC, although the inverse association increased with number of full-term pregnancies in the DCIS group (p for trend=0.02) but remained relatively constant for IBC regardless of number of pregnancies. Ever having breastfed was not associated with DCIS. However, any lactation was inversely associated with any number of full-term pregnancies in the invasive cancer group.

Odds ratios for OC use decreased with increasing duration of use among DCIS subjects (p for trend=0.05). The opposite was true for invasive cancers: increasing duration of OC use showed a suggested increasing association with IBC (p for trend=0.09). HRT use was not associated with DCIS in this study, whereas any HRT use was inversely associated with invasive disease, especially among long-term users.

Younger age at menopause (<40) and older age at menarche (14+) showed an inverse association with IBC, and each increase of age at menarche was associated with a decrease in odds ratio (p for trend=0.001). Older age at menopause ($\geq$50) was associated with invasive disease. There was no association with age at menarche and DCIS but an inverse association between younger age at menopause (<40) and DCIS.

*Ductal Carcinoma in Situ Comedo vs. Non-comedo*

Table 4.4 displays odds ratios and 95% confidence intervals for comedo DCIS cases vs. controls, non-comedo DCIS cases vs. controls, and comedo cases vs. non-comedo cases (case-case odds ratios). Risk estimates were less precise than for all DCIS due to fewer numbers of cases when stratified by histology. Any number of full-term pregnancies was inversely associated with comedo DCIS, with a trend of increasing inverse association as number of full-term births rose (p for trend=0.02). Non-comedo DCIS showed an inverse association trend with parity, but ORs were closer to the null. Ever breastfeeding was inversely associated with comedo DCIS but was not associated with non-comedo DCIS. Ever use of hormone replacement therapy was inversely associated with comedo DCIS but not associated with non-comedo DCIS.

When comedo and non-comedo DCIS cases were compared in a case-case analysis, duration of oral contraceptive use had a different association with the two DCIS subtypes. Ten or more years of OC use showed an increased association with comedo DCIS but an inverse association with non-comedo.

***Discussion***

Using a large, population-based study of carcinoma *in situ* of the breast and invasive breast cancer, we compared known hormonal and reproductive risk factors for invasive breast cancer in both groups to determine whether they are risk factors for DCIS as well and to make direct comparisons of odds ratios in the two groups. Parity and younger age at first full-term pregnancy, and younger age at menopause (<40) were inversely associated with both DCIS and IBC, while older age at menopause was positively associated with each. Older age at menarche was inversely associated with IBC only. Increasing duration of oral contraceptive use showed a trend of increasing association with IBC. Menopausal status showed no association with either DCIS or invasive disease.

When DCIS cases in our study were separated into the two main histologic subtypes, comedo and non-comedo, comedo-type DCIS associations paralleled invasive results more frequently than non-comedo DCIS. Parity, lactation, and HRT use were inversely associated with comedo DCIS and IBC but showed no association with non-comedo DCIS. These results support the theory that DCIS is not a uniform disease and are in agreement with data showing that comedo DCIS is more likely than non-comedo to become invasive [108]. Wohlfahrt et al (2004) conducted the only other published study

to examine reproductive risk factors for comedo and non-comedo DCIS [109]. Their analyses were limited to parity and age at first full-term birth and found an association between comedo DCIS and age at first birth of 25 or higher. In contrast, parity showed a stronger inverse association with comedo DCIS than with non-comedo or all DCIS combined in our study, especially for two or more full-term pregnancies.

Many studies have examined reproductive risk factors for invasive breast cancer and DCIS. However, differences in study designs, methods, and populations make comparisons of results difficult. Including both DCIS and invasive cases from the same population circumvents many of those issues, allowing for direct comparison between odds ratios. Eight previous studies of DCIS reproductive or hormonal risk factors have included invasive cases as well [64-67, 71, 73, 109, 110]. As with our current study, these eight found few differences in risk factors between DCIS and invasive disease. Parity [64-67, 71, 73, 109], young age at first full-term pregnancy [65, 67, 71, 73, 109, 110], older age at menarche [65, 67], and higher body mass index [67, 73] were inversely associated with both outcomes, while older age at menopause and postmenopausal hormone replacement therapy [65, 73] have been positively associated with both forms of cancer.

Evidence suggests that a woman's breasts reach full maturity after a full-term pregnancy, making the cells less vulnerable to neoplastic changes [58]. In the current study, ever having a full-term pregnancy was inversely associated with invasive breast cancer, as was any lactation. For DCIS, the protective association was limited to those with age at first full-term pregnancy under age 26. Nine previous DCIS risk factor

studies included parity and age at first full-term pregnancy, all of which found results similar to ours [64-66, 70-73, 109].

Only three other DCIS studies assessed associations with lactation [64, 66, 73]. Weiss et al and Tretham-Dietz et al found no association between breastfeeding and either DCIS or IBC, but in the Meeske et al study, lactating for 24 months or more was associated with DCIS. In the current study, lactation was inversely associated with IBC but showed no overall association with DCIS. These varied findings may be due to differences in lactation practices in the underlying populations. For instance, breastfeeding is more prevalent and done for longer periods of time in China, where a significant inverse association with lactation for more than 24 months was found for invasive breast cancer [111]. The predominant practice in Western populations of breastfeeding for a maximum of one year and often supplementing with formula might dilute any protective effect in a population such as ours.

Inverse associations between breast cancer and both later age at menarche and younger age at menopause may be related to length of estrogen exposure. Increase in estrogen leads to menarche, and decreasing levels precipitate menopause. Estrogen augmented by progesterone has been shown to promote cell division, which increases the chance of mutant cell growth [112]. Our results support this theory for both IBC and DCIS. Increasing age at menarche was inversely associated with IBC in our study, with the oldest category (age 14+ at menarche) showing the strongest association compared with the referent category of age 11 or younger at first menses. Compared with women who experienced menopause during the most common time period, ages 40-49, younger

age at menopause (<40) was inversely associated with both IBC and DCIS, and older age at menopause (50 and older) was positively associated with IBC in our study.

Other studies that examined age at menarche and menopause found mixed results. The studies by Claus et al (2001) and Weiss et al, as well as one involving only postmenopausal women [70], found no association between age at menarche and DCIS or invasive disease. Age at menarche of 14+ was protective for premenopausal women with DCIS and all women with invasive breast cancer in the study by Longnecker et al. Two studies used the oldest age at menarche group as a referent, and both found an increased association with invasive disease for the youngest age at menarche group [67, 73]. Menopause at age 55+ was associated with DCIS and IBC in the Longnecker et al study, and for DCIS only in the study by Claus et al (2001). Age 45+ at menopause showed an increased association with IBC only in the study by Trentham-Dietz et al. One other study reported no association between age at menopause and either DCIS or invasive disease [70].

The link between oral contraceptive use and breast cancer risk is less clear than with other hormonal risk factors, especially among earlier-stage cancer. OC use showed no association with DCIS in our study, although there was a trend of increasing association with increased duration of use for invasive disease. As with IBC, use of oral contraceptives for more than ten years was associated with comedo DCIS. However, long-term OC use showed an inverse association with non-comedo DCIS. All other studies that included both invasive and DCIS cases found OC use was positively associated with IBC but not associated with DCIS [60, 73, 110, 113, 114].

Although most other studies found that postmenopausal hormone replacement therapy was associated with either DCIS or IBC [65, 68-71, 73], HRT was inversely associated with IBC in our study, especially among those using HRTs for longer than 10 years. While this difference is puzzling, one explanation may be that we did not differentiate between estrogen-only and estrogen-plus-progestin regimens. Two studies which did examine HRT (estrogen and progesterone) and ERT (estrogen only) separately found HRT associated with CIS but not with IBC, while ERT was not associated with either outcome [65, 68]. A third study found ERT associated with IBC but not with CIS, and that HRT was not associated with either outcome [69].

Strengths of this study include analyses of both DCIS and invasive data from the same study, the inclusion of many African-American women, analyses by DCIS histopathologic subtype, and inclusion of a wide spectrum of reproductive and hormonal risk factors. Selection bias was a potential issue for this study, since case participants could have had better and more frequent access to healthcare and therefore mammography screening. However, we analyzed the data stratified on frequency of doctor's visits and having had a mammogram in the two years previous to participation in the study, and neither affected the odds ratios (data not shown).

For all DCIS combined, our results indicated many similarities between reproductive and hormonal risk factors for DCIS and invasive breast cancer, in agreement with previous studies. Separating DCIS into categories according to histopathologic type showed comedo-type risk factors more closely resembled those of invasive cancer than risk factors for the non-comedo type did, supporting the theory that higher-grade comedo DCIS is the most likely type to progress to IBC. In particular, evidence of protective

effects of some reproductive and hormonal risk factors seen for both DCIS and invasive disease did not emerge for non-comedo DCIS.

It has already been established that women with non-comedo type DCIS should be evaluated and treated using criteria different from those of the more aggressive types of DCIS. Our results support this conclusion, suggesting that the two may be distinct subtypes with potentially different underlying etiologies. However, future studies will need to include larger numbers of both DCIS subtypes in order to clarify associations between each subtype and potential risk factors. With more women being diagnosed at earlier stages of breast cancer, more precise estimates of these relationships are possible.

Table 4.1. Characteristics of ductal carcinoma *in situ* (DCIS) and invasive cases and controls

| | DCIS Cases (N=446) | | DCIS Controls (N=458) | | Invasive Cases (N=1808) | | Invasive Controls (N=1564) | |
|---|---|---|---|---|---|---|---|---|
| **Covariate** | **No.** | **%** | **No.** | **%** | **No.** | **%** | **No.** | **%** |
| Age at selection/diagnosis (years) | | | | | | | | |
| Mean ± SD | 55.16±11.07 | | 54.46±10.26 | | 51.01±11.67 | | 51.99±11.47 | |
| Median | 55 | | 53 | | 49 | | 49 | |
| Range | 27-74 | | 27-74 | | 23-74 | | 21-74 | |
| Race | | | | | | | | |
| Non African-American | 352 | 78.9 | 388 | 84.7 | 1020 | 56.4 | 846 | 54.1 |
| African-American | 94 | 21.1 | 70 | 15.3 | 788 | 43.6 | 718 | 45.9 |
| Parity (Number of full-term pregnancies) | | | | | | | | |
| None (nulliparous) | 69 | 15.5 | 56 | 12.2 | 275 | 15.2 | 174 | 11.1 |
| One | 74 | 16.6 | 62 | 13.5 | 316 | 17.5 | 279 | 17.8 |
| Two | 159 | 35.7 | 175 | 38.2 | 557 | 30.8 | 496 | 31.7 |
| Three or more | 144 | 32.3 | 165 | 36.0 | 660 | 36.5 | 615 | 39.3 |
| Age at first full-term pregnancy | | | | | | | | |
| Nulliparous | 69 | 15.5 | 56 | 12.2 | 275 | 15.2 | 174 | 11.1 |
| <26 years | 250 | 56.1 | 105 | 22.9 | 521 | 28.8 | 490 | 31.3 |
| 26+ | 127 | 28.5 | 297 | 64.8 | 1005 | 55.6 | 897 | 57.4 |
| Missing | 0 | 0.0 | 0 | 0.0 | 7 | 0.4 | 3 | 0.2 |
| Lactation | | | | | | | | |
| Never | 261 | 58.5 | 273 | 59.6 | 1174 | 64.9 | 950 | 60.7 |
| Ever | 185 | 41.5 | 185 | 40.4 | 634 | 35.1 | 614 | 39.3 |
| Oral contraceptive (OC) use | | | | | | | | |
| Never | 161 | 36.1 | 156 | 34.1 | 625 | 34.6 | 572 | 36.6 |
| Ever | 382 | 63.2 | 300 | 65.5 | 1177 | 65.1 | 981 | 62.7 |
| Missing | 3 | 0.7 | 2 | 0.4 | 6 | 0.3 | 11 | 0.7 |
| Age at first OC use | | | | | | | | |
| Never | 161 | 36.1 | 156 | 34.1 | 625 | 34.6 | 572 | 36.6 |
| <20 | 78 | 17.5 | 101 | 22.1 | 444 | 24.6 | 347 | 22.1 |
| 20+ | 202 | 45.3 | 198 | 43.2 | 730 | 40.4 | 632 | 40.4 |
| Missing | 5 | 1.1 | 3 | 0.7 | 9 | 0.5 | 13 | 0.8 |
| Duration of OC use | | | | | | | | |
| Never | 161 | 35.1 | 156 | 34.1 | 625 | 34.6 | 572 | 36.6 |
| <5 years | 140 | 31.4 | 136 | 29.7 | 538 | 29.8 | 489 | 31.3 |
| 5-10 years | 94 | 21.1 | 107 | 23.4 | 411 | 22.7 | 323 | 20.7 |
| >10 years | 48 | 10.8 | 57 | 12.4 | 228 | 12.6 | 169 | 10.8 |
| Missing | 3 | 0.7 | 2 | 0.4 | 6 | 0.3 | 11 | 0.7 |
| Age at menarche | | | | | | | | |
| ≤11 | 98 | 22.0 | 87 | 19.0 | 405 | 22.4 | 306 | 19.7 |
| 12 | 131 | 29.4 | 136 | 29.7 | 516 | 28.6 | 413 | 26.5 |
| 13 | 105 | 23.5 | 140 | 30.6 | 484 | 26.8 | 422 | 27.1 |
| 14+ | 111 | 24.9 | 95 | 20.7 | 401 | 22.2 | 415 | 26.7 |
| Missing | 1 | 0.2 | 0 | 0.0 | 2 | 0.1 | 8 | 0.5 |
| Menopausal Status* | | | | | | | | |
| Premenopausal | 142 | 31.8 | 153 | 33.4 | 873 | 48.3 | 718 | 45.9 |
| Postmenopausal | 304 | 61.2 | 305 | 66.6 | 935 | 51.7 | 846 | 54.1 |

Table 4.1, cont. Characteristics of ductal carcinoma *in situ* (DCIS) and invasive cases and controls

| | DCIS Cases (N=446) | | DCIS Controls (N=458) | | Invasive Cases (N=1808) | | Invasive Controls (N=1564) | |
|---|---|---|---|---|---|---|---|---|
| **Covariate** | **No.** | **%** | **No.** | **%** | **No.** | **%** | **No.** | **%** |
| Age at menopause** | | | | | | | | |
| <40 | 47 | 15.5 | 67 | 22.0 | 185 | 19.8 | 213 | 25.2 |
| 40-49 | 138 | 45.4 | 123 | 40.3 | 440 | 47.1 | 388 | 45.9 |
| >=50 | 111 | 36.5 | 105 | 34.4 | 290 | 31.0 | 212 | 25.1 |
| Missing | 8 | 2.6 | 10 | 3.3 | 20 | 2.1 | 33 | 3.9 |
| Postmenopausal hormone replacement therapy (HRT) use** | | | | | | | | |
| Never | 122 | 40.1 | 110 | 36.1 | 518 | 55.4 | 420 | 49.6 |
| Ever | 182 | 59.9 | 195 | 63.9 | 417 | 44.6 | 426 | 50.4 |
| Duration of HRT use** | | | | | | | | |
| Never | 122 | 40.1 | 110 | 36.1 | 518 | 55.4 | 420 | 49.6 |
| <5 years | 64 | 21.1 | 88 | 28.9 | 202 | 21.6 | 204 | 24.1 |
| 5-10 years | 60 | 19.7 | 50 | 16.4 | 115 | 12.3 | 98 | 11.6 |
| >10 years | 55 | 18.1 | 55 | 18.0 | 94 | 10.1 | 121 | 14.3 |
| Missing | 3 | 1.0 | 2 | 0.7 | 6 | 0.6 | 3 | 0.4 |

*Postmenopausal women: <50 years who had undergone natural menopause, bilateral oophorectomy, or irradiation to the ovaries; age 50+ for whom menstruation had ceased.
**Among postmenopausal women only.

Table 4.2. Characteristics of reproductive risk factors among comedo and non-comedo DCIS cases and all DCIS controls

| Covariate | Comedo DCIS N=163 No. | % | Non-comedo DCIS N=230 No. | % | Controls N=458 No. | % |
|---|---|---|---|---|---|---|
| Age at selection/diagnosis (years) | | | | | | |
| Mean ± SD | 55.36 ± 11.46 | | 55.56 ± 11.11 | | 54.46 ± 10.26 | |
| Median | 55 | | 55 | | 53 | |
| Range | 27-74 | | 29-74 | | 27-74 | |
| Race | | | | | | |
| Non African-American | 128 | 78.5 | 179 | 77.8 | 388 | 84.7 |
| African-American | 35 | 21.5 | 51 | 22.2 | 70 | 15.3 |
| Parity (Number of full-term pregnancies) | | | | | | |
| None (nulliparous) | 29 | 17.8 | 31 | 13.5 | 56 | 12.2 |
| One | 27 | 16.6 | 43 | 18.7 | 62 | 13.5 |
| Two | 53 | 32.5 | 84 | 36.5 | 175 | 38.2 |
| Three or more | 54 | 33.1 | 72 | 31.3 | 165 | 36.0 |
| Age at first full-term pregnancy | | | | | | |
| Nulliparous | 29 | 17.8 | 31 | 13.5 | 56 | 12.2 |
| <26 years | 94 | 57.7 | 129 | 56.1 | 110 | 24.0 |
| 26+ years | 140 | 24.5 | 170 | 30.4 | 292 | 63.8 |
| Lactation | | | | | | |
| Never | 102 | 62.6 | 135 | 58.7 | 273 | 59.6 |
| Ever | 61 | 37.4 | 95 | 41.3 | 185 | 40.4 |
| Oral contraceptive (OC) use | | | | | | |
| Never | 61 | 37.4 | 86 | 37.4 | 156 | 34.1 |
| Ever | 101 | 62.0 | 142 | 61.7 | 300 | 65.5 |
| Missing | 1 | 0.6 | 2 | 0.9 | 2 | 0.4 |
| Age at first OC use | | | | | | |
| Never | 61 | 37.4 | 86 | 37.4 | 156 | 34.1 |
| <20 | 27 | 16.6 | 40 | 17.4 | 101 | 22.1 |
| 20+ | 73 | 44.8 | 101 | 43.9 | 198 | 43.2 |
| Missing | 2 | 1.2 | 3 | 1.3 | 3 | 0.7 |
| Duration of OC use | | | | | | |
| Never | 61 | 37.4 | 86 | 37.4 | 156 | 34.1 |
| <5 years | 52 | 31.9 | 76 | 33.0 | 136 | 29.7 |
| 5 to 10 years | 26 | 16.0 | 52 | 22.6 | 107 | 23.4 |
| >10 years | 23 | 14.1 | 14 | 6.1 | 57 | 12.4 |
| Missing | 1 | 0.6 | 2 | 0.9 | 2 | 0.4 |
| Age at menarche | | | | | | |
| ≤11 | 37 | 22.7 | 49 | 21.3 | 87 | 19.0 |
| 12 | 46 | 28.2 | 67 | 29.1 | 136 | 29.7 |
| 13 | 37 | 22.7 | 54 | 23.5 | 140 | 30.6 |
| 14+ | 43 | 26.4 | 59 | 25.7 | 95 | 20.7 |
| Missing | 0 | 0.0 | 1 | 0.4 | 0 | 0.0 |
| Menopausal status* | | | | | | |
| Premenopausal | 51 | 31.3 | 71 | 30.9 | 153 | 33.4 |
| Postmenopausal | 112 | 68.7 | 159 | 69.1 | 305 | 66.6 |
| Age at menopause* | | | | | | |
| <40 | 21 | 18.8 | 21 | 13.2 | 67 | 22.0 |
| 40 to 49 | 48 | 42.9 | 79 | 49.7 | 123 | 40.3 |
| 50+ | 42 | 37.5 | 54 | 34.0 | 105 | 34.4 |
| Missing | 1 | 0.9 | 5 | 3.1 | 10 | 3.3 |

Table 4.2, cont. Characteristics of reproductive risk factors among comedo and non-comedo DCIS cases and all DCIS controls

| Covariate | Comedo DCIS N=163 No. | % | Non-comedo DCIS N=230 No. | % | Controls N=458 No. | % |
|---|---|---|---|---|---|---|
| Postmenopausal HRT use** | | | | | | |
| Never | 50 | 44.6 | 62 | 39.0 | 110 | 36.1 |
| Ever | 62 | 55.4 | 97 | 61.0 | 195 | 63.9 |
| Duration of postmenopausal HRT use** | | | | | | |
| Never | 50 | 44.6 | 62 | 39.0 | 110 | 36.1 |
| <5 years | 23 | 20.5 | 35 | 22.0 | 88 | 28.9 |
| 5 to 10 years | 20 | 17.9 | 34 | 21.4 | 50 | 16.4 |
| >10 years | 19 | 17.0 | 25 | 15.7 | 55 | 18.0 |
| Missing | 0 | 0.0 | 3 | 1.9 | 2 | 0.7 |

NOTE: This table excludes 56 DCIS cases not classified into subtype by the study pathologist.
*Postmenopausal women: <50 years who had undergone natural menopause, bilateral oophorectomy, or irradiation to the ovaries; age 50+ for whom menstruation had ceased.
**Among postmenopausal women only.

Table 4.3. Multivariate adjusted odds ratios of reproductive risk factors for ductal carcinoma *in situ* and invasive breast cancer

| Variable | Ductal carcinoma in situ (DCIS) | | | Invasive | | |
|---|---|---|---|---|---|---|
| | Cases N=446 | Controls N=458 | OR (95% CI) | Cases N=1808 | Controls N=1564 | OR (95% CI) |
| Parity (No. of full-term pregnancies) | | | | | | |
| None | 69 | 56 | 1.00 | 275 | 174 | 1.00 |
| One | 74 | 62 | 0.98 (0.60, 1.61) | 316 | 279 | 0.76 (0.59, 0.98) |
| Two | 159 | 175 | 0.73 (0.48, 1.12) | 557 | 496 | 0.78 (0.62, 0.98) |
| Three or more | 144 | 165 | 0.62 (0.40, 0.97) | 660 | 615 | 0.79 (0.63, 0.99) |
| Age at First Full-Term Pregnancy | | | | | | |
| Nulliparous | 69 | 56 | 1.00 | 275 | 174 | 1.00 |
| <26 years | 250 | 297 | 0.63 (0.42, 0.95) | 1124 | 1057 | 0.77 (0.62, 0.95) |
| 26+ years | 127 | 105 | 0.99 (0.64, 1.55) | 403 | 330 | 0.80 (0.63, 1.03) |
| Missing | 0 | 0 | | 7 | 3 | |
| Lactation | | | | | | |
| Never | 261 | 273 | 1.00 | 1174 | 950 | 1.00 |
| Ever | 185 | 185 | 1.02 (0.78, 1.34) | 634 | 614 | 0.77 (0.67, 0.89) |
| Oral Contraceptive (OC) Use | | | | | | |
| Never | 161 | 156 | 1.00 | 625 | 572 | 1.00 |
| Ever | 282 | 300 | 1.11 (0.80, 1.53) | 1177 | 981 | 1.11 (0.94, 1.32) |
| Missing | 3 | 2 | | 6 | 11 | |
| Age at first OC use | | | | | | |
| Never | 161 | 156 | 1.00 | 625 | 572 | 1.00 |
| <20 | 78 | 101 | 0.74 (0.46, 1.18) | 444 | 347 | 1.04 (0.83, 1.31) |
| 20+ | 202 | 198 | 1.18 (0.85, 1.63) | 730 | 632 | 1.13 (0.95, 1.34) |
| Missing | 5 | 3 | | 9 | 13 | |
| Duration of OC Use | | | | | | |
| Never | 161 | 156 | 1.00 | 625 | 572 | 1.00 |
| <5 years | 140 | 136 | 1.21 (0.85, 1.74) | 538 | 489 | 1.06 (0.88, 1.28) |
| 5 to 10 years | 94 | 107 | 1.03 (0.69, 1.53) | 411 | 323 | 1.15 (0.93, 1.42) |
| >10 years | 48 | 57 | 0.95 (0.59, 1.55) | 228 | 169 | 1.21 (0.94, 1.56) |
| Missing | 3 | 2 | | 6 | 11 | |
| Age at menarche | | | | | | |
| ≤11 | 98 | 87 | 1.00 | 405 | 306 | 1.00 |
| 12 | 131 | 136 | 0.85 (0.58, 1.25) | 516 | 413 | 0.95 (0.78, 1.16) |
| 13 | 105 | 140 | 0.66 (0.45, 0.98) | 484 | 422 | 0.86 (0.70, 1.05) |
| 14+ | 111 | 95 | 0.98 (0.65, 1.47) | 401 | 415 | 0.72 (0.59, 0.89) |
| Missing | 1 | 0 | | 2 | 8 | |
| Age at menopause* | | | | | | |
| <40 | 47 | 67 | 0.61 (0.39, 0.95) | 185 | 213 | 0.68 (0.54, 0.87) |
| 40-49 | 138 | 123 | 1.00 | 440 | 388 | 1.00 |
| ≥50 | 111 | 105 | 0.89 (0.61, 1.28) | 290 | 212 | 1.25 (1.00, 1.57) |
| Missing | 8 | 10 | | | | |
| Postmenopausal HRT use** | | | | | | |
| Never | 122 | 110 | 1.00 | 518 | 420 | 1.00 |
| Ever | 182 | 195 | 0.94 (0.66, 1.32) | 417 | 426 | 0.81 (0.66, 0.99) |

Table 4.3, cont. Multivariate adjusted odds ratios of reproductive risk factors for ductal carcinoma *in situ* and invasive breast cancer

| Variable | Ductal carcinoma in situ (DCIS) | | | Invasive | | |
|---|---|---|---|---|---|---|
| | Cases N=446 | Controls N=458 | OR (95% CI) | Cases N=1808 | Controls N=1564 | OR (95% CI) |
| Duration of HRT use* | | | | | | |
| Never | 122 | 110 | 1.00 | 518 | 420 | 1.00 |
| <5 years | 64 | 88 | 0.75 (0.49, 1.15) | 202 | 204 | 0.80 (0.63, 1.02) |
| 5-10 years | 60 | 50 | 1.27 (0.79, 2.04) | 115 | 98 | 0.99 (0.73, 1.35) |
| >10 years | 55 | 55 | 0.94 (0.59, 1.49) | 94 | 121 | 0.67 (0.49, 0.91) |
| Missing | 3 | 2 | | | | |

NOTE: All odds ratios adjusted for age, race, and frequency-matching offset terms.
*Among postmenopausal women only.

Table 4.4. Multivariate-adjusted odds ratios for DCIS reproductive risk factors, stratified by histology

| Variable | Comedo DCIS N | Non-comedo DCIS N | Controls N | Comedo vs. Controls OR (95% CI) | Non-comedo vs. Controls OR (95% CI) | Comedo vs. Non-comedo OR (95% CI) |
|---|---|---|---|---|---|---|
| Parity (# of full-term pregnancies) | | | | | | |
| None (nulliparous) | 29 | 31 | 56 | 1.00 | 1.00 | 1.00 |
| One | 27 | 43 | 62 | 0.81 (0.42, 1.55) | 1.36 (0.74, 2.47) | 0.67 (0.33, 1.35) |
| Two | 53 | 84 | 175 | 0.57 (0.33, 1.00) | 0.91 (0.54, 1.54) | 0.68 (0.37, 1.25) |
| Three or more | 54 | 72 | 165 | 0.53 (0.30, 0.95) | 0.73 (0.42, 1.27) | 0.82 (0.43, 1.54) |
| Age at first full-term pregnancy | | | | | | |
| Nulliparous | 29 | 31 | 56 | 1.00 | 1.00 | 1.00 |
| <26 years | 94 | 129 | 297 | 0.55 (0.33, 0.94) | 0.77 (0.47, 1.29) | 0.79 (0.44, 1.42) |
| 26+ years | 40 | 70 | 105 | 0.71 (0.39, 1.28) | 1.29 (0.74, 2.23) | 0.61 (0.32, 1.16) |
| Lactation | | | | | | |
| Never | 102 | 135 | 273 | 1.00 | 1.00 | 1.00 |
| Ever | 61 | 95 | 185 | 0.82 (0.57, 1.20) | 1.02 (0.72, 1.42) | 0.85 (0.56, 1.29) |
| Oral Contraceptive (OC) Use | | | | | | |
| Never | 61 | 86 | 156 | 1.00 | 1.00 | 1.00 |
| Ever | 101 | 142 | 300 | 1.08 (0.69, 1.69) | 1.10 (0.75, 1.64) | 1.00 (0.62, 1.59) |
| Missing | 1 | 2 | 2 | | | |
| Age at first OC use | | | | | | |
| Never | 61 | 86 | 156 | 1.00 | 1.00 | 1.00 |
| <20 | 27 | 40 | 101 | 0.67 (0.34, 1.32) | 0.78 (0.44, 1.40) | 0.93 (0.45, 1.92) |
| 20+ | 73 | 101 | 198 | 1.14 (0.78, 1.79) | 1.16 (0.78, 1.73) | 1.00 (0.62, 1.62) |
| Missing | 2 | 3 | 3 | | | |
| Duration of OC use | | | | | | |
| Never | 61 | 86 | 156 | 1.00 | 1.00 | 1.00 |
| <5 years | 52 | 76 | 136 | 1.21 (0.74, 1.98) | 1.31 (0.85, 2.03) | 0.96 (0.57, 1.62) |
| 5 to 10 years | 26 | 52 | 107 | 0.78 (0.43, 1.39) | 1.09 (0.67, 1.76) | 0.70 (0.38, 1.31) |
| >10 years | 23 | 14 | 57 | 1.31 (0.70, 2.47) | 0.51 (0.25, 1.04) | 2.33 (1.06, 5.09) |
| Missing | 1 | 2 | 2 | | | |

Table 4.4, cont. Multivariate-adjusted odds ratios for DCIS reproductive risk factors, stratified by histology

| Variable | Comedo DCIS N | Non-comedo DCIS N | Controls N | Comedo vs. Controls OR (95% CI) | Non-comedo vs. Controls OR (95% CI) | Comedo vs. Non-comedo OR (95% CI) |
|---|---|---|---|---|---|---|
| Age at menarche | | | | | | |
| <11 | 37 | 49 | 87 | 1.00 | 1.00 | 1.00 |
| 12 | 46 | 67 | 136 | 0.76 (0.45, 1.28) | 0.88 (0.56, 1.40) | 0.91 (0.51, 1.60) |
| 13 | 37 | 54 | 140 | 0.61 (0.36, 1.04) | 0.66 (0.41, 1.06) | 0.91 (0.50, 1.65) |
| 14+ | 43 | 59 | 95 | 1.00 (0.58, 1.71) | 1.01 (0.62, 1.65) | 0.97 (0.54, 1.73) |
| Missing | 0 | 1 | 0 | | | |
| Age at menopause** | | | | | | |
| <40 | 21 | 21 | 67 | 0.83 (0.46, 1.52) | 0.48 (0.27, 0,84) | 1.67 (0.83, 3.40) |
| 40 to 49 | 48 | 79 | 123 | 1.00 | 1.00 | 1.00 |
| 50+ | 42 | 54 | 105 | 0.97 (0.59, 1.59) | 0.75 (0.48, 1.17) | 1.27 (0.27, 2.22) |
| Missing | 1 | 5 | 10 | | | |
| Postmenopausal hormone replacement therapy (HRT) use* | | | | | | |
| Never | 50 | 62 | 110 | 1.00 | 1.00 | 1.00 |
| Ever | 62 | 97 | 195 | 0.78 (0.49, 1.23) | 1.00 (0.66, 1.52) | 0.77 (0.46, 1.30) |
| Duration of postmenopausal HRT use* | | | | | | |
| Never | 50 | 62 | 110 | 1.00 | 1.00 | 1.00 |
| <5 years | 23 | 35 | 88 | 0.66 (0.37, 1.18) | 0.82 (0.49, 1.38) | 0.81 (0.41, 1.58) |
| 5 to 10 years | 20 | 34 | 50 | 1.03 (0.54, 1.95) | 1.48 (0.84, 2.61) | 0.71 (0.35, 1.43) |
| >10 years | 19 | 25 | 55 | 0.78 (0.42, 1.47) | 0.86 (0.48, 1.54) | 0.90 (0.44, 1.87) |
| Missing | 0 | 3 | 2 | | | |

NOTE: All odds ratios adjusted for age, race, and frequency-matching offset terms.
*Among postmenopausal women only.

# CHAPTER V: CONCLUSIONS

## *Summary*

The studies detailed above contribute additional and necessary information to the literature regarding histopathology reliability and reproductive and hormonal risk factors for ductal carcinoma *in situ* of the breast. Until recently, DCIS was treated as a single entity. However, strong biological and epidemiological evidence suggests it consists of at least two subgroups that differ with regard to pathologic characteristics, progression to invasive disease, and prognosis.

Using the Carolina Breast Cancer Study, DCIS histopathology among practicing pathologists was examined, with the result that when pathologists ascertain overall diagnosis by subtype for DCIS, agreement is only moderate in current practice conditions. Reliability for two common histologic components, comedo and non-comedo, was fair. In addition, exploring what specific categories of the components pathologists use to distinguish between comedo and non-comedo subtypes led to the discovery that the most severe category of each component usually accompanies a comedo type diagnosis. However, no similar uniformity exists for non-comedo type tumors.

In the second study, CBCS data was used to divide DCIS cases into comedo and non-comedo subtypes for risk factor analyses. Specifically, these were reproductive and hormonal risk factors that contribute to endogenous and exogenous estrogen exposure

over a woman's lifetime, which are known risk factors for IBC. Results for comedo DCIS cases were similar to those for IBC, whereas non-comedo cases showed numerous differences from either comedo DCIS or IBC. In particular, at least one full-term pregnancy and any amount of breastfeeding were inversely associated with comedo DCIS and IBC but showed no association with non-comedo. Long-term OC use showed a tendency toward increased association with both comedo DCIS and IBC and an inverse association with non-comedo DCIS, but the estimates were imprecise. These results support both the cumulative estrogen exposure hypothesis for increased breast cancer risk and the theory that comedo and non-comedo DCIS may be distinct subtypes.

The most intriguing outcome of this dissertation is that the results of the reliability study call into question the validity of the case-control study results. If the reliability rates of comedo and non-comedo DCIS diagnoses found in our reliability study represent those of clinical practice, the odds ratios in the risk factor study may be wrong. Specifically, this would result in misclassification of at least a portion of the DCIS subtypes. Misclassification by subtype was not examined for this dissertation but could be in the future using the data from the reliability analyses.

***Biologic Plausibility***

Breast cell growth and development occurs when estrogen binds to and activates receptors in the cell nucleus [57]. Changes that occur throughout a female's reproductive and menopausal stages alter the amount of endogenous estrogen and how it functions. A current theory suggests that cumulative estrogen exposure increases the chances of mutant breast cell proliferation, leading to cancer [115].

Pregnancy and lactation reduce the number of cumulative ovulatory cycles, which decreases the amount of estrogen exposure. Although estrogen levels are high during early pregnancy, they decrease as the pregnancy continues and are lowest by 38 weeks (full-term). An additional theory suggests breast tissue may not reach full maturity until after a full-term pregnancy, making the cells more resistant to neoplastic changes [58].

The inverse associations between breast cancer and both later age at menarche and younger age at menopause may be related to length of estrogen exposure. Increase in estrogen leads to menarche, and decreasing levels precipitate menopause. Both oral contraceptives and hormone replacement therapy are methods of introducing exogenous estrogens at different times in a women's reproductive history. Because each drug contains distinct regimens of estrogen and progestagens, associations are less clear. A majority of women on HRT use combined estrogen/progestagen regimens due to risk of endometrial cancer in women with intact ovaries. Estrogen augmented by progesterone has been shown to promote cell division, which increases the chance of mutant cell growth [112]. However, not all progestagens act on estrogen-metabolizing enzymes in breast cancer cells in the same way, so it is possible that those with less or no metabolic activity may neutralize estrogenic effects [116].

### *Public Health Implications*

Improvements in ductal carcinoma *in situ* diagnosis and accurate risk factor identification for the disease will only help physicians and patients make informed decisions regarding both prevention and treatment. The results of these analyses support

previous studies and give additional information that indicates two important areas of improvement: diagnostic accuracy and further investigation of DCIS subtypes.

***Strengths***

The Carolina Breast Cancer Study included a large catchment area that was arranged to maximize data completeness. The population-based design allowed for generalizability and enabled identification and recruitment of a large number of DCIS cases and African-American participants, both of which have been lacking in previous studies. Even without sufficient numbers of African-American women for stratified analyses, our results can be generalized to at least African-American women from the study area and arguably to all in North Carolina. Most invasive breast cancer risk factor studies and nearly all DCIS risk factor studies to date have not achieved such inclusion because their base populations were predominantly White and/or Hispanic.

The CBCS questionnaire was thorough and included a wide range of potential exposures and confounders. Because data was collected for invasive and DCIS subjects in the same manner, we were able to compare DCIS results directly with those for invasive disease. Visual prompts were used for accuracy during recall of past and present prescription and non-prescription drug use, and a timeline was used to place past events. In addition, we were able to combine analyses for parity and lactation as well as age at first use of oral contraceptives and duration of OC use to determine whether particular combinations were more likely to affect DCIS or IBC risk.

***Limitations***

Statistical analysis for the reliability study was complicated. While the kappa statistic improves upon percent agreement, using it to compare results among studies must be done with caution. Knowing prevalence and bias percentages for other studies makes comparisons more accurate, and these can usually be computed using data provided. Kappa is not an ideal reliability measure for studies that include multiple raters and categories such as this; however, no other statistic has been shown to improve upon it.

Although response rates for IBC and DCIS cases were comparable to those in previous similar studies, response rates for IBC and DCIS controls were quite a bit lower. While we do not have information on most of those who did not participate, 10% of the IBC controls and 16% of the DCIS controls who declined to complete the full interview answered a mini questionnaire over the phone. The women with and without complete data were similar on race, parity, education, and age at menarche and menopause. However, women who completed the full interview were less likely to have used oral contraceptives or hormone replacement therapy. If these results represent the characteristics of the other non-participating controls, selection bias would be a factor and we would have underestimates of the true associations between both breast cancer types and OC and HRT use.

Recall bias is a potential issue in the case-control study because cases may put more effort into remembering events they think are related to their diagnosis. The exposures focused on in our analyses have been mentioned in the media as potentially associated with breast cancer, so these are the ones most likely to be recalled differently

between cases and controls. Cases also have the potential to inflate their exposures for the same reason. If this type of bias exists, the odds ratios would be inflated with respect to the true risk estimate. As well, older women may have remembered past events with less accuracy than younger ones. The further a woman is from her reproductive years at the time of interview, the more chance for error when recalling pregnancy and breastfeeding events as well as oral contraceptive use and age at menarche. However, this is equally as likely to occur for cases as controls, so the error would be nondifferential. In any case, the methods used to trigger accurate memories that were incorporated into the interview process most likely minimized these errors as much as can be expected in this type of study.

The possibility always exists that variables not included in the study were confounders or effect measure modifiers. The bias could be in either direction, causing an overestimation or underestimation of the true associations. Even though we used an assortment of variables to represent estrogen exposure, we were unable to measure estrogen levels metabolically. The variables probably do not represent all estrogen sources, so conclusions based on estrogenic effect are limited. We did not differentiate by OC and HRT regimens, so characteristics particular to individual drugs could have divergent effects that we did not control for. However, each risk factor is important on its own merit and, when combined, they give an overall picture of how factors related to estrogen expression link to DCIS risk.

***Future Directions***

The studies described in this dissertation provide further evidence that DCIS is a heterogeneous disease that requires a comprehensive agreed-upon classification system

and should be considered two at least potentially distinct subgroups (comedo and non-comedo). Our results regarding the heterogeneity of DCIS, when added to those of previous studies, can encourage pathologists to develop a classification system that can be validated and replicated and to implement it worldwide as soon as possible. Then, more complete epidemiologic studies can be carried out to determine differences in risk factors to address prevention, and clinical trials can elucidate treatment programs tailored to the subtype.

APPENDIX: ADDITIONAL TABLES

Table A.1. DCIS Classification Systems

| Name and Categories | Description |
|---|---|
| *Van Nuys Classification [97]* | |
| Group 3 (High grade) | Nuclei with a diameter greater than two red blood cells with vesicular chromatin and one or more nucleoli; necrosis may be present or absent; any architectural pattern may be present |
| Group 2 (Non-high grade with necrosis) | Any architectural pattern in which central lumina contain substantial amounts of necrotic neoplastic cells of duct origin; occasional desquamated or individually apoptotic cells are ignored; nuclear grade must be intermediate or low |
| Group 1 (Non-high grade without necrosis | Intermediate or low nuclear grade cases without evidence of intraductal necrotic material |
| *European Pathologists Working Group (EPWG) (aka Holland et al) [97]* | |
| Poorly differentiated | Nuclei: very pleomorphic w/variation in size, irregular; Chromatin: coarse and clumped<br>Nucleoli: prominent, mitoses often present; Polarization of cells: absent or minimal<br>Central necrosis: usually present, often prominent, individual cell necrosis usually present<br>Growth pattern: solid, clinging, pseudomicropapillary or cribriform; Calcifications: amorphous |
| Intermediately differentiated | Nuclei: Mildly pleomorphic cells; some size, outline and spacing variation; Chromatin: Fine to coarse<br>Nucleoli: Visible, mitoses occasionally present; Polarization of cells: Present<br>Central necrosis: Variable, individual cell necrosis may be focally present<br>Growth pattern: Variable; Calcifications: Amorphous or laminated |
| Well-differentiated | Nuclei: Monomorphic cells, uniform size, regular nuclear outline & spacing; Chromatin: Uniform, fine<br>Nucleoli: Insignificant, rare mitoses; Polarization of cells: Marked<br>Central necrosis: Absent or minimal, no individual cell necrosis<br>Growth pattern: Clinging, micropapillary, cribriform or rarely solid<br>Calcifications: Psammoma-like or rarely amorphous |

Table A.1. cont, DCIS Classification Systems

| **Name and Categories** | Description |
|---|---|
| *Nottingham Classification [97]* | |
| Pure comedo | Central lumina containing necrotic debris surrounded by large pleomorphic cells in solid masses |
| DCIS with necrosis (nonpure comedo) | Central lumina containing necrotic debris, cribriform, micropapillary, or variable architectural pattern |
| DCIS without necrosis | No evidence of intraluminal necrosis, occasional apoptotic desquamated cells or mucus ignored |
| | |
| *Lagios Nuclear Grading System [19]* | |
| High Grade (Grade 3) | Nuclear diameter: >2.5-3 times diameter of a red blood cell (RBC)<br>Pleomorphism: Prominent<br>Chromatin pattern: Vesicular<br>Nucleoli: Often prominent<br>Mitoses: Frequently demonstrated |
| Intermediate Grade (Grade 2) | Nuclear diameter: 2-2.5 times diameter of RBC<br>Pleomorphism: More uniform<br>Chromatin pattern: Coarse<br>Nucleoli: Small<br>Mitoses: Infrequent |
| Low Grade (Grade 1) | Nuclear diameter: <2 times diameter of RBC<br>Pleomorphism: Absent<br>Chromatin pattern: Diffuse<br>Nucleoli: Absent<br>Mitoses: Rare |

Table A.2. Summary of previously published reliability studies for CIS pathology

| First Author, year published | No. of Raters | No. of Cases | Categories or Classification System | Results* |
|---|---|---|---|---|
| Rosai J, 1991 | 5 | 17 | Normal, hyperplasia, atypical hyperplasia, atypical lobular hyperplasia, CIS | No cases with 100% agreement, 18% with 80% agreement, 33% with 0% agreement |
| Schnitt SJ, 1992 | 6 | 24 | Usual hyperplasia, atypical hyperplasia, CIS | 58% with 100% agreement, 71% with $\geq$ 83% agreement, 92% with $\geq$ 67% agreement |
| Bodian CA, 1993 | 2 | 63 | Page system for benign breast disease | 100% agreement for LCIS cases |
| Sloane JP, 1994 | 186-251 | 72 (17 DCIS) | All CIS<br>All DCIS<br>Comedo DCIS | $\kappa$=0.62<br>$\kappa$=0.23<br>$\kappa$=0.44 |
| Frierson HF, 1995 | 6 | 75 | Nottingham modification | $\kappa$=0.55 |
| Douglas-Jones AG, 1996 | 2 | 180 | Van Nuys<br>Holland et al<br>Nottingham | 78.9% agreement; 63.8% agreement**<br>69.5% agreement; 59.2% agreement**<br>77.8% agreement; 65.6% agreement** |
| Palli D, 1996 | 16 | 81 | CIS | $\kappa$=0.69 |
| Bethwaite P, 1998 | 11 | 25 | Van Nuys<br>Holland et al | $\kappa$=0.66<br>$\kappa$=0.57 |
| Giardina C, 1998 | 12 | 88 | CIS | $\kappa$=0.64** |
| Sidawy MK, 1998 | 6 | 12 | Low nuclear grade DCIS | $\kappa$=0.35 |

Table A.2., cont. Summary of previously published reliability studies for CIS pathology

| First Author, year published | No. of Raters | No. of Cases | Categories or Classification System | Results* |
|---|---|---|---|---|
| Sloane JP, 1998 | 23 | 33 | Van Nuys | κ=0.42 |
| | | | Holland et al | κ=0.37 |
| | | | Comedo vs. noncomedo necrosis | κ=0.34 |
| | | | High vs. low nuclear grade | κ=0.46 |
| Wells WA, 1998 | 26 | 30 | Noninvasive malignant | κ=0.59 |
| Sloane JP, 1999 | 23 | 107 | All DCIS | κ=0.87 |
| | | | High grade DCIS | κ=0.43 |
| | | | Intermediate grade DCIS | κ=0.17 |
| | | | Low grade DCIS | κ=0.49 |
| Sneige N, 1999 | 6 | 125 | Lagios | κ=0.46 |
| Wells WA, 2000 | 7 | 40 | Van Nuys | κ***=0.26, 0.29, 0.29 |
| | | | Modified Lagios | κ***=0.26, 0.57, 0.29 |
| | | | Holland et al | κ***=0.46, 0.49, 0.53 |
| Ellis IO, 2006 | 220-466 | 62 | CIS | κ=0.36 |
| | | | Comedo DCIS | κ=0.45 |
| | | | High grade DCIS | κ=0.51 |
| | | | Intermediate grade DCIS | κ=0.23 |
| | | | Low grade DCIS | κ=0.31 |

*Unless otherwise specified, all results are for inter-rater comparisons
**Diagnostic accuracy
***Inter-rater kappa, intra-rater kappa, kappa for diagnostic accuracy

Table A.3. Summary of CIS Reproductive Risk Factor Studies

| First author, year published | Study design | Number of participants | Major findings |
|---|---|---|---|
| Brinton, 1983 | Cross-sectional | 199 CIS | Age at first full-term birth 20+ increased risk |
| Dubin, 1984 | Case-control | 112 CIS*<br>2143 controls | Age at first live birth 10-19 yrs. and 2+ children nursed 2+ months increased risk of CIS |
| Longnecker, 1996 | Case-control | 233 CIS<br>2203 controls | Age at menarche 13+ protective for premenopausal; age at first full-term pregnancy ≥20 yrs. increased risk for premenopausal CIS; any full-term pregnancy protective for postmenopausal |
| Kerlikowske, 1997 | Cross-sectional | 102 DCIS<br>39,177 controls | Nulliparous or ≥30 yrs. at first birth increased risk for postmenopausal |
| Lambe, 1998 | Nested case-control | 1,368 CIS<br>6837 controls | Any full-term pregnancies protective for CIS; age at first full-term birth ≥25 yrs. increased risk for CIS |
| Gapstur, 1999 | Prospective cohort | 175 DCIS<br>371,477 person-years of follow-up | Age at first full-term birth ≥30 yrs. increased risk for DCIS |
| Trentham-Dietz, 2000 | Case-control | 238 DCIS, 63 LCIS<br>3999 controls | 2+ full-term pregnancies protective for all CIS; age at first full-term birth ≥30 yrs. increased risk for DCIS |
| Claus, 2001 | Case-control | 875 DCIS, 123 LCIS<br>999 controls | Any full-term pregnancy protective for DCIS; age at first live birth ≥20 and age at menopause ≥55 increased risk for DCIS |
| Meeske, 2004 | Case-control | 567 CIS<br>614 controls | 3+ full-term pregnancies protective for CIS; lifetime breastfeeding ≥ 24 mos. increased risk for CIS |
| Wohlfahrt, 2004 | Prospective cohort | 694 DCIS, 242 LCIS<br>22.5 million person-years of follow-up | 4+ full-term births protective for DCIS; age at first birth 25+ increased risk for DCIS and LCIS |

*Included cases with ≤1 cm microinvasion.

Table A.4. Summary of CIS Hormonal Risk Factor Studies

| First author, year published | Study design | Number of participants | Major findings |
|---|---|---|---|
| Stanford JL, 1989 | Case-control | 279 CIS<br>2183 controls | >5 years OC use decreased risk |
| Schairer C, 1994 | Prospective cohort | 150 CIS<br>313,902 person-years of follow-up | Any HRT increased risk; current use of ERT ≥10 yrs. increased risk |
| Brinton LA, 1995 | Case-control | 227 CIS<br>1505 controls | No association with OC use |
| Longnecker MP, 1996 | Case-control | 233 CIS<br>2203 controls | Age at menopause 55+ and ever use of ERT or HRT increased risk for postmenopausal |
| Henrich JB, 1998 | Matched case-control | 32 CIS<br>160 controls | No association with ERT use |
| Gapstur SM, 1999 | Prospective cohort | 175 DCIS<br>371,477 person-years of follow-up | No association with HRT use and DCIS |
| Trentham-Dietz A, 2000 | Case-control | 238 DCIS<br>63 LCIS<br>3999 controls | Any HRT use increased risk for DCIS; no association with OC use and DCIS or LCIS |
| Claus EB, 2003 | Case-control | 875 DCIS<br>999 controls | No association with OC use or HRT use |
| Gill JK, 2006 | Case-control | 547 CIS<br>614 controls | No association with OC use |

Table A.5. Reasons for non-participation in CBCS and contact, cooperation, and response rates for CIS and invasive breast cancer (IBC) cases and controls

| | **CIS Cases** | **CIS Controls** | **IBC Cases** | **IBC Controls** |
|---|---|---|---|---|
| Total identified for contact | 705 | 940 | 2704 | 3600 |
| Not able to locate/non-responsive | 5 | 88 | 64 | 689 |
| Ineligible | 47 | 100 | 165 | 348 |
| Deceased | 3 | 22 | 36 | 79 |
| Physician refused | 51 | 0 | 175 | 0 |
| Patient/subject refused | 58 | 197 | 361 | 738 |
| Completed mini questionnaire only | 38 | 75 | 95 | 182 |
| Completed interview | 503 | 458 | 1808 | 1564 |
| Contact rate | 99.3% | 90.6% | 97.5% | 80.9% |
| Cooperation rate | 83.2% | 73.0% | 78.0% | 70.3% |
| Overall response rate | 82.6% | 65.2% | 76.0% | 55.0% |

NOTE: Invasive cases and controls include phase 1 and phase 2 combined.

Table A.6. Demographic characteristics of the CBCS participants

| | **DCIS Cases N=446 N (%)** | **DCIS Controls N=458 N (%)** | **Invasive Cases N=1808 N (%)** | **Invasive Controls N=1564 N (%)** |
|---|---|---|---|---|
| Race | | | | |
| Non African-American | 352 (78.9) | 388 (84.7) | 1020 (56.4) | 846 (54.1) |
| African-American | 94 (21.1) | 70 (15.3) | 788 (43.6) | 718 (45.9) |
| Age | | | | |
| 20-24 | 0 (0.0) | 0 (0.0) | 6 (0.3) | 1 (0.1) |
| 25-29 | 2 (0.5) | 1 (0.2) | 23 (1.3) | 10 (0.6) |
| 30-34 | 8 (1.8) | 4 (0.9) | 90 (5.0) | 63 (4.0) |
| 35-39 | 24 (5.4) | 29 (6.3) | 176 (9.7) | 123 (7.9) |
| 40-44 | 53 (11.9) | 42 (9.2) | 279 (15.4) | 250 (16.0) |
| 45-49 | 65 (14.6) | 82 (17.9) | 402 (22.2) | 340 (21.7) |
| 50-54 | 58 (13.0) | 94 (20.5) | 171 (9.5) | 174 (11.1) |
| 55-59 | 61 (13.7) | 57 (12.5) | 181 (10.0) | 162 (10.4) |
| 60-64 | 63 (14.1) | 56 (12.2) | 159 (8.8) | 133 (8.5) |
| 65-69 | 62 (13.9) | 47 (10.3) | 178 (9.8) | 157 (10.0) |
| 70-74 | 50 (11.2) | 46 (10.0) | 143 (7.9) | 151 (9.7) |
| Education | | | | |
| Less than College | 310 (69.5) | 319 (69.7) | 1300 (71.9) | 1158 (74.1) |
| College + | 135 (30.3) | 139 (30.3) | 508 (28.1) | 405 (25.9) |
| Missing | 1 (0.2) | 0 (0.0) | 0 (0.0) | 1 (0.1) |
| Income | | | | |
| <$15,000 | 72 (16.1) | 46 (10.0) | 404 (22.3) | 321 (20.5) |
| $15,000-$30,000 | 81 (18.2) | 114 (24.9) | 392 (21.7) | 322 (20.6) |
| $30,00-$50,000 | 99 (22.2) | 101 (22.1) | 399 (22.1) | 350 (22.4) |
| >$50,000 | 162 (36.3) | 159 (34.7) | 484 (26.8) | 439 (28.1) |
| Missing | 32 (7.2) | 38 (8.3) | 129 (7.1) | 132 (8.4) |

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