The clinical significance of Oestrogen receptor expression in breast ductal carcinoma *in situ*

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ABSTRACT

Background: Oestrogen receptor (ER) in invasive breast cancer (BC) predicts response to endocrine therapy (ET) and provides prognostic value. In this study, we investigated the value of ER expression in ductal carcinoma *in situ* (DCIS) in terms of outcome and the impact on ET decision.

Methods: 643 pure DCIS, diagnosed at Nottingham University Hospitals, were assessed for ER. Clinicopathological data were correlated against ER status together with assessment of recurrence rate.

Results: ER-positivity was observed in 74% (475/643) of cases. ER positivity was associated with clinicopathological variables of good prognosis; however, outcome analysis revealed that ER status was not associated with local recurrence. In the intermediate and high-grade ER-positive DCIS, 58% (11/19) and 63% (15/24) of the recurrences were invasive, respectively, comprising 7% and 6% of all ER-positive DCIS, respectively. Invasive recurrence in low-grade DCIS was infrequent (2%) and none of these patients died of BC. The ER status of the recurrent invasive tumours matched the primary DCIS ER status (94% in ipsilateral and 90% of contralateral recurrence).

Conclusion: The strong correlation between DCIS and invasive recurrences ER status and the clinical impact of ET justify discussion of the use of ET in ER-positive DCIS treated by breast conserving surgery. The excellent outcome of low-grade DCIS, which was almost always ER-positive, does not, in the opinion of authors, justify the use of risk reducing ET. Therefore, the decision on ET for DCIS should be personalised and consider grade, ER status and other characteristics.

BACKGROUND

In countries with routine mammographic screening, one case of pure ductal carcinoma *in situ* (DCIS) is diagnosed for every four cases of breast cancer (BC) [1]. The management of DCIS continues to be a challenge. Although DCIS is predominantly associated with a low risk of mortality [2,3], it is well documented that it could progress into invasive BC (IBC), with an associated increased mortality risk [4,5]. Precise identification of the mortality risk has been difficult as most of the studies do not provide long term (i.e. >20 years) mortality data. For example, a woman in her 40s or 50s treated with breast conserving surgery (BCS) who subsequently died following DCIS would normally develop an initial local invasive recurrence, then subsequently metastatic disease before eventually dying of BC. Furthermore, in the last 20 years the average survival of patients with ER positive primary or metastatic BC have increased. In addition, the psychological impact of a recurrence, *in-situ* or invasive disease, must also not be underestimated. Currently, lack of a robust tool to identify low risk DCIS results in recommendation that all women with DCIS undergo treatment. Results of clinical trials that provide no active treatment to low risk DCIS are awaited [6,7] .

The optimal clinical management for women with newly diagnosed DCIS is controversial, with variable patterns of practice [8-10]. The standard management options for the treatment of DCIS in the UK currently are mastectomy or breast-conserving surgery (BCS) with or without postoperative whole breast radiotherapy (RT) [11].

Approximately 70% of women with DCIS will be treated with BCS followed by RT, because of its proven efficacy to reduce local recurrence (LR) risk [12-14]. RT may be omitted for women at low risk of recurrence, however clinical and pathological features have not reliably identified patients at low risk of LR following BCS alone, leading to variability in treatment and outcomes of women with DCIS [15].

Data on adjuvant endocrine therapy (ET) in DCIS continues to evolve debate. Its use has become more common in the United States, with approximately 60% of all DCIS cases having BCS receiving it in recent years [16]. Adjuvant ET for hormone receptor–positive DCIS tumours, with Tamoxifen or aromatase inhibitors (e.g. Anastrozole), may improve local control in hormone-responsive disease [12,17-20] and reduce the risk of BC recurrence but survival benefit is unproven [4,12]. Data on outcome by Oestrogen receptor (ER) status of the DCIS is absent in most randomised trials. However, a sub-study of 732/1799 (41%) of

patients in the NSABP B-24 trial reported that Tamoxifen significantly decreased BC recurrence in ER positive but not ER-negative DCIS [21].

The National Institute for Health and Care Excellence (NICE) has updated the recommendations for adjuvant ET for DCIS to offer it after BCS for women with ER-positive DCIS if RT is recommended but not received; and to consider ET after BCS for women with ER-positive DCIS if RT is not recommended [22]. The American Society of Clinical Oncology/College of American Pathologists also recommend testing of DCIS for ER to determine the potential benefit of ET to reduce risk of future BC [23]. Although the concept of ET for ER-positive DCIS patients who require RT, but cannot or choose not to receive it, is obvious as these patients are usually at high risk of disease progression and/or recurrence, this is currently a relatively small group. In reality, most women suitable to receive ET for DCIS also receive RT and/or are low risk [16]. Moreover, clinical application of these recommendations means that ER testing would be necessary for all DCIS in order to be considered for management decision. However, DCIS is not routinely stained with ER and the impact of ET on the outcome of DCIS, and whether this impact is limited to ER-positive DCIS, remains to be defined. Furthermore, routine measurement of ER would increase the burden on the pathology service. The effect of implementation of such recommendation on clinical practice and the effect on overall patient mortality and morbidity should be investigated.

In this study, we used a large retrospective cohort of DCIS treated in a single institution to address the outcome of ER-positive DCIS, especially invasive recurrence to consider the utility of routine ER testing in these patients. We have also reviewed the randomised studies reporting on adjuvant ET and clinical outcomes of DCIS.

Patients and Methods

A pure DCIS cohort (n=1249) diagnosed at the Nottingham University Hospitals NHS Trust over a 30-year period (1990-2017) with at least 5 years follow-up time was identified. DCIS associated with invasive or micro-invasive carcinomas was excluded. All demographic, clinical, pathological, and outcome data were retrieved from patients' records. Nuclear grade was assessed using the previously published criteria [24-26]. In the current study glass slides from all cases were reviewed histologically by an observer and graded according to World Health Organisation (WHO) criteria of breast tumours classification. Grade was

compared with the originally reported grade and conflicted cases were reviewed by a consultant pathologist. Cases with more than one grade were reported and the higher grade was considered in the final analysis. Management details including the operation type (BCS or mastectomy), and RT data were collected. Over the period of the study, the management of DCIS showed significant changes with an increase in rate of BCS over mastectomy, and more frequent use of local RT as previously described [27]. ER status was not routinely assessed in DCIS unless indicated for diagnostic purposes and was not used to guide further management. Ten- and fifteen-year ipsilateral BC tumour recurrence (BCTR) was defined as any event of ipsilateral local tumour recurrence (either as DCIS (DCIS-BCTR) or invasive disease (I-BCTR)) occurring after 6 months from the first DCIS surgery and up to 120 and 180 months respectively. Contralateral BC (CBC) was assessed and defined as any contralateral breast event, either DCIS or IBC, identified after the primary diagnosis of DCIS. BC specific survival was defined as the time from the primary diagnosis of DCIS to death from BC. Patients were censored at the last time they were seen alive, died of other caused or the time they were lost to follow-up. ER status of the invasive recurrences either ipsilateral or contralateral was available as part of the routine management of patients.

In this study, ER status was assessed retrospectively on tissue microarray (TMA) sections of DCIS sample using immunohistochemistry for research purposes as previously described [28]. Briefly, 4µm sections were stained on the diagnostically valid Ventana Benchmark® ULTRA system (Tucson, Arizona, USA) using Ventana anti-ER (SP1) Rabbit Monoclonal Primary Antibody as per the recommended protocol. Sections were deparaffinised and antigen retrieval was performed with Cell Conditioner 1 (CC1) for 60 min. The primary antibody was applied for 16 minutes at 37°C followed by the OptiView HQ Linker for 8 minutes and the OptiView HRP Multimer for 8 minutes. Counter-staining was performed with Mayer's haematoxylin. Positive control cores were added to each TMA section. Only nuclear staining of DCIS cells was scored. ER positivity was considered when $\geq$1% of DCIS cells showed nuclear staining [23]. The final number of cases that were suitable for assessment for ER was 643/1249 (51%) cases, according to the availability of tumour tissue and informative cores in the TMA blocks. Data on Progesterone receptor (PR) and HER2 status based on retrospective staining of the TMA cores was available as previously described [27-29].

Statistical analysis

Statistical analyses were performed using SPSS v26 (Chicago, IL, USA) for Windows. Association between ER status and clinicopathological parameters was evaluated using Chi-squared test ER associated risk with ipsilateral and contralateral recurrence was evaluated individually as well as the overall risk combining both ipsilateral and contralateral events. Univariate survival rates were determined using the Kaplan–Meier method and compared by the log-rank test. A multivariate Cox regression hazards regression model was used to adjust confounding factors. All tests were 2-tailed and a *p* value of less than 0.05 was considered as statistically significant.

RESULTS

Study cohort

A total of 475/643 (74%) DCIS cases showed positive expression of ER defined as ≥1% of tumour cells showing nuclear positivity. In this study, only 4 cases (0.8%) showed ER positivity in 1-10% of tumour cells. There was a trend towards an increase in ER positivity over the period of the study (Figure 1). This was accompanied by increase rate of screen detected DCIS and lower rate of high-grade DCIS (Figure 1). High nuclear grade was observed in 60% of cases (388/643), while comedo necrosis was present in approximately two thirds of cases (64%). 300 (47%) patients were treated by BCS, while one third of them received RT (100/300). Over the period of the study, there was an increase in the rate of BCS as a primary surgical choice and rate of offering RT (Figure 1).

Association between ER and other clinicopathological factors

ER positivity was associated with features of good prognosis including smaller tumour size (<40mm), low nuclear grade, absence of comedo necrosis, positive PR status and lack of HER2 overexpression (all $p<0.0001$). ER-positive DCIS patients were more likely to be treated with BCS ($p<0.0001$), without adjuvant RT ($p=0.039$) compared to ER-negative cases ($p<0.0001$). Table 1 summarises the correlations between ER expression and other clinicopathological parameters.

Ipsilateral local recurrence and ER status

The number of cases who developed ipsilateral local recurrence (ILR) over a period of 10-year follow-up was 61 (9%), of which 35 patients (57% of recurred cases and 5% of the overall cohort) developed invasive ILR. 56 cases (92% of all recurrences) recurred after BCS (with or without RT) (56/300, 19%) and only five cases (8% of all recurrences) occurred after mastectomy (5/343, 1%). Within the BCS treated group, 55% of recurrences (31/56) were invasive recurrence. No statistically significant difference was observed between ER status and ILR in patients treated with BCS at 10-year (p=0.511) and at 15-year follow up (p=0.473); (Figure 2). Similar results were showed when the analysis was carried out the whole cohort regardless the surgical management (Supplementary Figure 1) and in multivariate analysis with other confounders factors including age at diagnosis, tumour size, grade and RT (Supplementary Table 1). In ER positive cohort, recurrence was mainly associated with nuclear grade and RT (Table 2). RT improved the outcome in the whole cohort and in ER-positive DCIS (p=0.039 and p=0.040, respectively).

80% of patients who developed invasive ILR within 10 years, were initially treated for ER-positive DCIS (28/35). Data on the ER status of the invasive ILR (n=30) showed that 94% of these tumours had the same ER status as the primary DCIS. 22 out of 23 ER-positive DCIS patients developed ER-positive invasive ILR (96%), whereas 6 out of 7 ER-negative DCIS patients developed ER-negative invasive carcinoma (86%). The discrepant ER-positive case was a patient who had intermediate and high-grade DCIS who subsequently developed ER-negative invasive disease, which was grade 3 ductal carcinoma of no special type (NST) associated with high grade DCIS (which was most likely a new primary). The discrepant ER-negative case was high grade DCIS with a triple negative phenotype whereas the subsequent tumour was ER-positive invasive lobular carcinoma, which could be representative of a new primary rather than being a true recurrence from the primary DCIS tumour.

Outcome of ER-positive DCIS based on nuclear grade

ER-positivity was more frequent in low and intermediate grade DCIS than high grade. 44 patients who had ER-positive DCIS and were treated with BCS developed ILR within 10 years. 59% (26/44) of these were invasive disease, (11 cases followed intermediate grade

DCIS and 13 cases followed high grade DCIS). Two low-grade ER-positive DCIS recurred as invasive disease (2% of low-grade DCIS), and both recurrences were low grade ER-positive invasive carcinoma associated with low grade DCIS. Both patients were alive at the end of follow up; the 10-year survival rate in this group was 100%. Table 3 summarises the percentage of ER-positive cases within the different grades of the DCIS cohort.

The invasive recurrence rate in the ER-positive intermediate and high-grade DCIS groups were 7% and 6% respectively. The ER status of the invasive recurrence was 100% identical for the intermediate grade group while 92% of invasive recurrences occurred after primary diagnosis of high-grade ER-positive DCIS showed similar ER positivity.

Interestingly, the figures of the overall recurrence rate up to the end of follow-up period for each nuclear grade were comparable with the 10-year recurrence rate in terms of ER positive recurrences (Table 3).

Contralateral breast cancer and ER status

Contralateral BC (CBC) was identified in 55/643 cases (9%); of which 37 cases were invasive disease (representing 6% of the overall cases and 67% of the contralateral events). In patients initially treated for ER-positive DCIS, 30 cases had invasive CBC. The primary DCIS in those cases were high grade in 14 patients (47%), intermediate grade in 11 (34%) and low grade in 5 cases (17%). 90% (27/30) of the contralateral invasive disease cases were ER-positive. The three discrepant cases that developed ER-negative contralateral invasive disease (high-grade NST type) initially presented with high-grade ER-positive DCIS.

The association between overall risk of developing a recurrent IBC event either ipsilateral or contralateral after primary diagnosis of DCIS and ER status

The 10-year risk of developing a recurrence episode either in the ipsilateral or contralateral breast was 17% (109/643), of which 69 events were invasive disease (11% of all cohort and 63% of all event). Within those 69 cases, the primary DCIS was ER-positive in 55 cases (80%). Most of the recurrent events after diagnosis of ER-positive DCIS showed positive ER expression, with few events recurring as ER-negative disease (Table 4).

Overall survival

The ten and twenty-year overall BC specific death rate was 0.9% (6/643) and 1.3% (9/643), respectively. Those patients had median age of 66 years, initially presented high grade DCIS, with comedo necrosis and half of them were treated with BCS. The ER status was positive in 6 cases (67%). The recurrent episode was invasive carcinoma for all cases prior to distant

metastasis and death. Thus, the overall death rate after primary diagnosis of ER-positive DCIS was less than 0.5% and 0.7% within 10 years and twenty years of the primary diagnosis of DCIS respectively. In addition, none of the patients initially presented with low grade DCIS died during the 20- year period of the study follow-up.

DISCUSSION

Optimal treatment of DCIS is still a controversial issue. Debate continues regarding potential overtreatment of DCIS; whether surgical excision is required for all cases, and the question as to whether adjuvant therapy, RT and/or ET can be avoided for low-risk subgroups [16]. Some multi-institutional randomised trials are underway comparing active monitoring with standard treatment for DCIS [7,30]. The LORIS (low-risk DCIS) trial, which started in 2014, is a randomised, non-inferiority trial of comparing surgery versus active surveillance in low-risk DCIS patients. Patients with low- or intermediate-grade DCIS are randomised to either surgery or active surveillance with no hormonal treatment. The Comparison of Operative versus Medical Endocrine Therapy for Low-Risk DCIS (COMET) trial is currently on-going and randomises low- and intermediate-grade ER-positive and HER2-negative DCIS to either standard management versus active surveillance. ET use is encouraged in the active monitoring arm. The European Organisation for Research and Treatment of Cancer (EORTC)-sponsored LOw Risk DCIS (LORD) trial, is due to open, and this will randomise patients with low-grade DCIS to conventional treatment versus an active monitoring strategy. The principle objective of these trials is to avoid over treatment of low risk DCIS and to provide evidence that active surveillance is a management option for these patients.

In this study, there was a steady increase in the proportion of ER positive DCIS over the time, which was correlated with lower rate of HER2 positivity and higher rate of breast conservation as the primary option of DCIS management as illustrated in Figure 1. This constellation of observed features could be a reflection of the presence of a well-established screen programme throughout the period of the study that led to increasing detection of DCIS of small size and low grade and decease rate of high-risk DCIS (based on tumour grade, size and patient age at diagnosis) [31,32]. Another possibility for higher rate of ER positivity throughout the time of the study that cannot be entirely excluded is that the tissue specimens were fresher, and the integrity of the tissue was better. However, the

Nottingham cohorts of breast cancer including DCIS follow a standardised protocol of specimen fixation and processing in addition to tissue block storage. Based on our experience with hundreds of biomarkers tested using IHC in the invasive and the in situ diseases, no significant trend in the rate of positivity was observed between different time points and the rate of positivity of different markers is mainly related to tumour and tissue characteristics rather than the age of the specimen or the time period of storage. Using Benchmark IHC auto-stainer and ER antibody, which is used in routine clinical workflow, to stain ER in this study reduce the possible technical errors and false staining results.

These findings were addressed in our previous work using on the same cohort which where we showed lower rates of high risk DCIS over the time which was reflected by higher rate of BCS as a primary surgical management, lower rate of second operation [27] and decrease the proportion of HER2 positive DCIS over time [29]. Moreover, the protocol for management of DCIS in routine practice changed over the time. Overall, in our series, 41% of BCS treated patients were offered post-operative RT. However, prior to 2008, it was a common practice in our centre not to offer RT to DCIS patients with clear pathological margins 10 mm or more. Following evidence and that showed that closer margins are acceptable, the margin width was reduced, and this was followed by increasing use of RT in BCS treated DCIS patients. Selective RT regimen was also introduced. RT was then recommended after BCS to those with high grade DCIS, women younger than 50 years old and lesions more than 30mm, regardless of tumour grade, following a multidisciplinary team discussion even if the margin is clear [27]. In this study, there were 6 cases of low-grade DCIS which showed ER expression negativity. Although this is an unusual observation, assessment of ER on TMA sections which might underestimate the heterogeneity of ER expression in terms of morphological type and grade within the whole tumour. Interestingly, the PR status of these cases was negative. From our clinical experience, we came across few cases of low nuclear grade DCIS that are ER negative in routine practice and the diagnosis is usually based on the cytonuclear and architecture features which were sufficient for the diagnosis of DCIS and did not fit any other entity included in the differential diagnosis. In addition, we diagnosed occasional cases of ER negative low nuclear grade apocrine type DCIS that show typical architecture pattern of DCIS. Despite the low nuclear grade features, the cytoplasm was abundant and eosinophilic mimicking apocrine type cells. In our cohort, some of the low-grade DCIS that showed ER negativity had such apocrine morphology (Supplementary Figure

3). This indicates that ER negative low nuclear grade DCIS exists but are extremely rare however, the false negative ER expression resulting from the use of TMA in this study may have exaggerated this phenomenon.

Although prognosis following a diagnosis of DCIS is excellent, the goal of ET is to reduce invasive recurrence, which occurs in up to half of the recurrent cases [16]. Few clinical trials reported in the literature have evaluated the response of DCIS patients to ET following BCS and RT with or without comment on ER status, either comparing Tamoxifen versus placebo [20,33] or comparing the difference of clinical benefit between Tamoxifen and Anastrozole [18,19]. For each study, updated results were published afterwards and were included in Supplementary Table 2.

In the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-24 trial, all women with DCIS (n=1804) received RT before being randomly assigned to ET or placebo. After a median follow-up of 6 years, a significant 37% reduction in BC recurrence was observed with ET compared with placebo. BC events were also lower in the Tamoxifen treated group (6.0%) compared to the placebo arm (9.3%) in the ipsilateral breast (p=0·0009). The cumulative incidence of all IBC events in the ET group was 2.1% in the ipsilateral breast at 5 years compared to 4.2% in the placebo arm [20].

In a retrospective evaluation of ER and PR in 732/1804 patients from the B-24 trial, 449 tumours had ER and PR measured at a central lab while the remaining 283 tumours had results from the enrolling institutions. 76% of DCIS was ER-positive, 24% were ER-negative. Benefit of ET by receptor status at 10 years was evaluated with overall median follow-up of 14.5 years. Patients with ER-positive DCIS treated with ET (versus placebo) showed significant 51% reduction in subsequent any BC (ipsilateral and contralateral, invasive and non-invasive) at 10 years (HR=0.5; p=0.001). No significant benefit was observed in ER-negative DCIS [21]. They concluded that the use of adjuvant ET (Tamoxifen) offered an additional therapeutic option for patients with ER-positive DCIS [21].

In the UK/ANZ DCIS trial, 1578 women with DCIS were randomly assigned to receive Tamoxifen with or without RT. After a median of 13 years of follow-up, Tamoxifen significantly reduced all new BC events by 29%, with a significant impact on ipsilateral DCIS recurrence and contralateral tumours, but no effect on ipsilateral invasive recurrence [12,33]. ER was not an entry criterion and there was no analysis of the invasive recurrence by ER status of the initial DCIS.

Overall, the evidence from the former studies is that ET significantly decreases BC recurrence. Based on the sub-study on B-24 this would appear to be limited to ER-positive DCIS and not ER-negative DCIS. These findings would be supported by the BC prevention studies of Tamoxifen [34] and aromatase inhibitors [35,36] versus placebo, which have reported a significant reduction in ER-positive BC (both invasive and DCIS) but no significant reduction in ER-negative BC.

In an observational study of a prospective artificially randomised cohort [37], low dose Tamoxifen showed 30% reduction of any type of recurrence in women with high risk ER-positive DCIS (p=0.005). However, when ipsilateral invasive recurrence only was considered the difference was not significant (p=0.21).

However, it is noted that these studies were not large enough nor were they designed to assess survival benefit. Importantly, the NSABP B-24 and UK/ANZ studies were on patients unselected by ER status. Although the subset of cases with known ER status in the NSABP-24 and showed that adjuvant Tamoxifen significantly reduced subsequent ipsilateral BC only in patients with ER-positive DCIS after standard treatment with lumpectomy and RT and not ER negative, there was no association, in subgroup analysis, with ipsilateral invasive recurrence (p=0.1) or contralateral invasive recurrence (p=0.06), which are more important than the overall recurrence rate. Similar results were shown in NSABP B-35 where no obvious reduction of invasive recurrence was shown. It is noteworthy that neither of the randomised controlled trials considered DCIS grade as an inclusion criterion for patients. However, the current results showed that DCIS grade is more important than ER status to consider in prediction of recurrence risk after BCS for DCIS patients (Supplementary Figure 2) independent of other confounding factors (Supplementary Table 1) .

Therefore there remains confusion regarding treatment of DCIS with one trend toward avoiding surgical treatment (and subsequently no RT) and another trend to offer more ET therapy to DCIS patients and to make ER status assessment mandatory in all DCIS to allow clinicians to offer ET to ER-positive patients [22,23].

Importantly, the studies which evaluated the benefit of ET therapy in DCIS considered both ipsilateral (likely a true recurrence but could be a new event) and contralateral events (a new event). Therefore, it was difficult to differentiate whether the benefits obtained by ET following the diagnosis of DCIS is related to treating the index DCIS itself to prevent its recurrence as invasive disease or if the use of ET in these cases was more prophylactic to

reduce the overall risk of invasive disease in patients with ER-positive disease. The former aims treat DCIS as an index lesion akin to adjuvant ET of invasive disease whereas the latter aims at treating DCIS as a marker of subsequent risk [38]. The results of the current study support the fact that ER-status of the primary DCIS correlates strongly with the ER-status of recurrent invasive disease in the same or contralateral breast and therefore justify the use of ET in these patients. On the other hand, ER-negative DCIS is associated with development of ER-negative invasive disease, so ET is unlikely to influence the risk of invasive disease development. We also showed that RT in general and RT regardless of the ER status, offered to ER-positive DCIS cases have improved outcome after BCS. Therefore, the current results support NICE guidelines to provide ET for patients with high risk ER-positive DCIS when RT cannot be given for any reason [22]. In ER negative DCIS, RT reduce the risk of recurrence from 28% (9/32) in patients who did not receive RT, to 19% (5/26) in patients who receive post-operative RT, however this difference was not statistically significance likely due to small number of ER negative cases included in the analysis.

In the current study, our results showed that there is no significant association between ER status and the development of an ipsilateral breast event, either as DCIS or invasive recurrence. This was similar when we carried out the analysis on BCS treated patients only or when we included the whole cohort regardless the surgical management. We focused mainly on BCS group as they are the group of interest in NICE guidelines. the rate of recurrence after mastectomy was very low (1.2% recurrence rate which was ~6% of all recurrences in the study cohort). Patients' treated with mastectomy usually receive no further therapy (neither RT nor ET) thus they are not included under the recommendation of NICE guidelines and. The current finding is similar to that of the sub-study of NSABP B-24 [21] and also of other studies [37,39], where the recurrence rates in the placebo group were similar for both ER-positive and ER-negative DCIS. We also showed that ER was not a contributing factor affecting the development of CBC in DCIS patients.

It is important to rationalise the use of prophylactic or preventive therapy considering the side effects [40,41], cost and the magnitude of risk when planning such therapies; patients who are at high risk of disease development or those likely to develop high risk disease will derive the most benefit from such therapy. The results in the current study showed that low grade DCIS treated with BCS, had excellent prognosis with 100% survival rate even in the occasional patients who developed invasive disease, which was low grade ER-positive

invasive carcinoma. In addition, all ER-positive DCIS that recurred as invasive disease and showed subsequent BC related mortality incident were of high-grade, and they were treated with BCS only. Therefore, we consider adjuvant ET for low risk DCIS is questionable as in view of the excellent outcome event in the recurrent cases. In RTOG 9804, there are so few events at the time of analysis that the data could not support or refute the role of ET in the treatment of low risk DCIS [42]. Another important finding in this study is the difference between low-nuclear grade DCIS and the intermediate and high-nuclear grade DCIS group of patients regarding the development of invasive carcinoma risk and that the binary distinction of DCIS for risk stratification should be between low and intermediate / high-nuclear grade group rather than between low/intermediate and high-nuclear grade groups of DCIS.

The current study has some potential limitations. None of the patients included in this cohort were offered adjuvant ET. Management of DCIS was following local institutional protocols, and this was in line with the local and national UK guidelines present at the time of the study. The National Comprehensive Cancer Network (NCCN) and NICE guidelines for offering ET in DCIS were published in 2017 and 2019, respectively [22,43] which were not available during the period of the study. Second, the study was conducted on DCIS cases spanning a long time period with potential bias regarding the availability of tumour tissue for ER assessment. Thirdly, ER was evaluated on TMA sections which might underestimate the heterogeneity of ER expression within the whole tumour. The interobserver variability in DCIS grading is another potential limitation. Although DCIS grading similar to other biological features of differentiation is subjective and distinction between intermediate and high-grade DCIS is often challenging, diagnosis of low-grade DCIS is considered to be the easiest and more objective.

CONCLUSIONS: There is a tendency for overtreatment of DCIS which can be by surgery and/or RT and/or ET. Although the impact of ET on the overall survival of DCIS patients is not demonstrated, ET reduces the risk of development of an invasive disease by up to 40%. Recommending ET to DCIS patients who do not receive RT can be justifiable however this should be based on ER status (positive) and grade (intermediate and high) of DCIS. ET appears to reduce the risk of invasive disease in general and not just limited to reducing the incidence of recurrence of the index DCIS and as such its use may contrast with the purpose of its use in IBC patients. The routine management of all DCIS including the low risk group

with ET does not appear to be justified by current data. There is an urgent need for molecular biomarkers and evidence-based guidelines to further refine the recurrence risk assessment and treatment decision-making in DCIS patients.

ADDITIONAL INFORMATION

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AUTHORS' CONTRIBUTIONS

Conception and design: IMM, MT, ER
Cohort Identification, Collection and assembly of data: IMM, MT
Staining and scoring analysis: IMM, MT
Data analysis and interpretation: IMM, MT, AG, JR, ER
Manuscript writing: IMM, MT, SS, GO, BS, HK, QT, AG, RDM, JR, ER
All authors contributed to revise and approve the final version of the manuscript

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This work obtained ethics approval by the North West – Greater Manchester Central Research Ethics Committee under the title; Nottingham Health Science Biobank (NHSB), reference number 15/NW/0685. All patients included were consented to participate in the study and to use their materials in research. All samples from Nottingham used in this study were pseudo-anonymised and stored in compliance with the UK Human Tissue Act. The study was performed in accordance with the Declaration of Helsinki.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm the data that has been used is available on reasonable request.

COMPETING INTEREST

The authors declare no conflict of interest.

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Figures' legends

Figure 1: The annual rates of the various clinicopathological parameters of the study cohort, over the period between 1987 and 2017. The graph shows a slightly increase in ER positivity rate over the time accompanied by a quite similar change in breast conserving surgery (BCS) rates. There was a steady increase of screen detected DCIS and radiotherapy rates over the time. There was a slightly lower rates of high-grade DCIS from the start of the study till end which was reflected on HER2 positivity rate. Low risk DCIS rate increased over time as well.

*Radiotherapy rate for BCS treated patients only,
** DCIS risk estimated based on tumour size, grade, and age at diagnosis)

Figure 2: Kaplan-Meier curve shows the association between ER expression and ipsilateral local recurrence rate in patients treated with breast conservative surgery (Horizontal axis: local recurrence free interval in months, vertical axis: probability of recurrence). Number of cases at risk after 15 years becomes smaller for meaningful statistical analysis.

Table 1: Correlation between ER expression and the clinicopathological variables of DCIS cases

Parameter	ER Expression			
	Total (n=643) n (%)	**Negative (n=168) n (%)**	**Positive (n=475) n (%)**	**χ^2 (*p* value)**
Age (years)*				
<40	23 (4)	6 (26)	17 (74)	0.077
40 to 60	354 (55)	94 (27)	260 (73)	(0.962)
>60	266 (41)	68 (26)	198 (74)	
Presentation				
Screening	336 (52)	96 (29)	240 (71)	2.178
Symptomatic	307 (48)	72 (23)	235 (77)	(0.140)
Size*				
<16mm	210 (33)	42 (20)	168 (80)	17.272
16 to 40mm	248 (39)	57 (23)	191 (77)	(**<0.0001**)
>40mm	182 (28)	68 (37)	114 (63)	
Grade				
Low	88 (14)	6 (7)	82 (93)	25.194
Intermediate	165 (26)	10 (6)	155 (94)	**(<0.0001)**
High	390 (60)	152 (39)	238 (61)	
Comedo necrosis				
Yes	412 (64)	145 (35)	267 (65)	48.844
No	231 (36)	23 (10)	208 (90)	(**<0.0001**)
Management+				
Mastectomy	342 (53)	110 (32)	232 (68)	13.617
BCS	300 (47)	58 (19)	242 (81)	(**<0.0001**)
Radiotherapy**				
Yes	100 (33)	26 (26)	74 (74)	4.275
No	200 (67)	32 (16)	168 (84)	**(0.039)**
PR Status				
Positive	342 (58)	4 (1)	338 (99)	274.791
Negative	246 (42)	154 (63)	92 (37)	**(<0.0001)**
HER2 Status***				
Negative	447 (81)	73 (16)	374 (84)	84.379
Positive	107 (19)	63 (59)	44 (41)	**(<0.0001)**

DCIS: Ductal carcinoma *in situ*, N: Number, X^2: Chi square, ***p* value in bold: significant**.
ER: Oestrogen receptor, BCS: Breast conserving surgery, PR: Progesterone Receptor.
*Age and size: categorised according to the Van Nuys Prognostic Index (VNPI),
+Management is according to the final operation
**Radiotherapy status is for cases treated with BCS,
***HER2 final status is achieved using combination of IHC and chromogenic *in situ* hybridisation (CISH).

Table 2: Correlation between various clinicopathological factors and recurrence in ER positive DCIS treated with breast conserving surgery.

Parameter	ER Positive DCIS in BCS treated patients (n=242)		
	No recurrence (n=190)	**Recurrence (n=52)**	χ^2 **(p value)**
Age (years)*			
<40	3 (2)	3 (6)	2.979
40 to 60	101 (53)	26 (50)	(0.226)
>60	86 (45)	23 (44)	
Presentation			
Screening	121 (64)	26 (50)	3.206
Symptomatic	69 (36)	26 (50)	(0.073)
Size*			
<16mm	101 (53)	28 (54)	0.127
16 to 40mm	76 (40)	20 (39)	(0.966)
>40mm	13 (7)	4 (7)	
Grade			
Low	46 (24)	4 (8)	7.028
Intermediate	61 (33)	19 (36)	**(0.030)**
High	82 (43)	29 (56)	
Comedo necrosis			
Yes	104 (55)	24 (46)	1.207
No	86 (45)	28 (54)	(0.272)
Radiotherapy			
Yes	68 (36)	6 (12)	11.311
No	122 (64)	46 (88)	**(0.001)**
Margin status (mm)			
< 2	9 (5)	2 (4)	0.058
≥ 2	169 (95)	44 (96)	(0.971)
PR Status			
Positive	144 (83)	38 (84)	0.038
Negative	29 (17)	7 (16)	(0.846)
HER2 Status**			
Negative	125 (85)	40 (84)	0.011
Positive	23 (15)	7 (16)	(0.915)

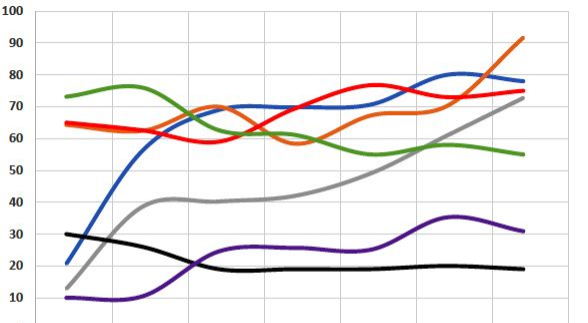
DCIS: Ductal carcinoma *in situ*, N: Number, X^2: Chi square, ***p* value in bold: significant**.
PR: Progesterone Receptor.
*Age and size: categorised according to the Van Nuys Prognostic Index (VNPI),
**HER2 final status is achieved using combination of IHC and chromogenic *in situ* hybridisation (CISH)

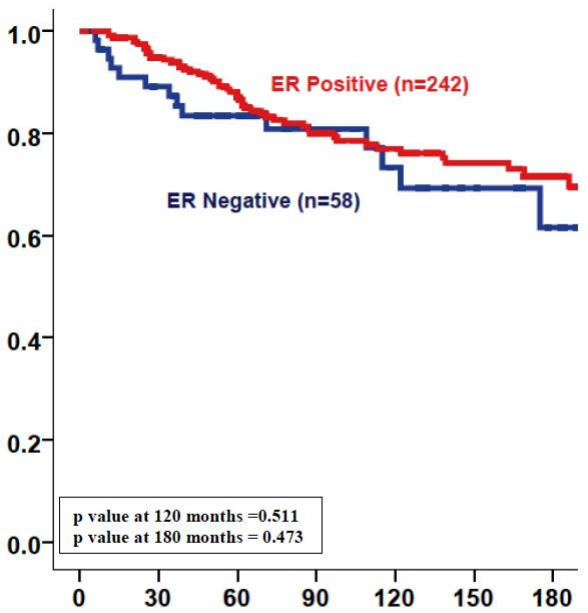
Table 3: Oestrogen receptor status among the different DCIS grades and the corresponding recurrence rate

DCIS Grade	**Number of ER-positive cases (n=475; 74%)**	**10-year overall (DCIS and invasive) recurrence rate in the ER-positive group (n=47; 10%)**	**10-year invasive recurrence rate (n=28; 6%)**	**ER-positive invasive recurrence (n=22; 5%)**	**Overall recurrence (DCIS and invasive) recurrence rate in the ER-positive group (n=55; 12%)**	**overall invasive recurrence rate (n=34; 7%)**	**ER-positive invasive recurrence (n=25; 5%)**
Low (n=88, 14%)	82 (94% of low grade)	4 cases (5% of all low-grade ER-positive cases)	2 (2%)	2 (100%)	5 cases (6% of all low-grade ER-positive cases)	3 (3%)	2 cases with available ER data all ER-positive (100%)
Intermediate (n=165, 26%)	155 (94% of intermediate grade)	19 (12% of all intermediate grade ER-positive cases)	11 (7%)	9 cases with available ER data all ER-positive (100%)	20 (13% of all intermediate grade ER positive cases)	11 (7%)	9 cases with available ER data all ER-positive (100%)
High (n=390, 60%)	238 (61% of high grade)	24 (10% of all high-grade ER-positive cases)	15 (6%)	11/12 cases with available ER data were ER-positive (92%)	30 (13% of all high-grade ER positive cases)	20 (8%)	14/15 cases with available ER data were ER-positive (93%)

Table 4: Oestrogen receptor status of the primary DCIS and the subsequent invasive episodes either ipsilateral or contralateral

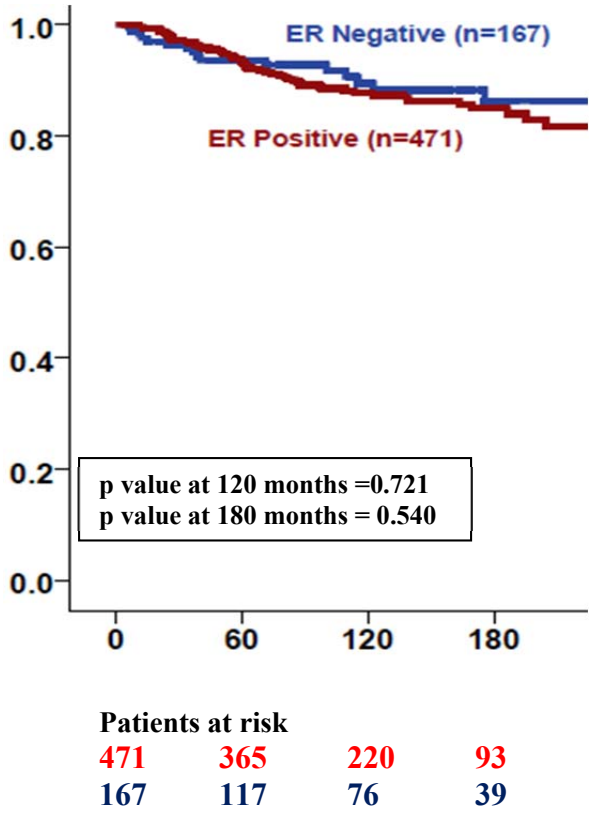
ER status within the primary DCIS that had subsequent invasive episode (n=69; 10%)	**ER status within the invasive disease (ipsilateral and/or contralateral)**		
	Positive	**Negative**	**Unknown**
Positive (n=55; 12%)	46 (92% of valid cases)	4 (8% of valid cases)	5
Negative (n=14; 8%)	6 (43%)	8 (57%)	0


100
90
80
70
60
50
40
30
20
10
0
1985
1990
1995
2000
2005
2010
2015
2020
Screen detected DCIS
Breast Conserving Surgery
Radiotherapy*
ER Positivity
HER2 positivity
High Grade DCIS
Low Risk DCIS**



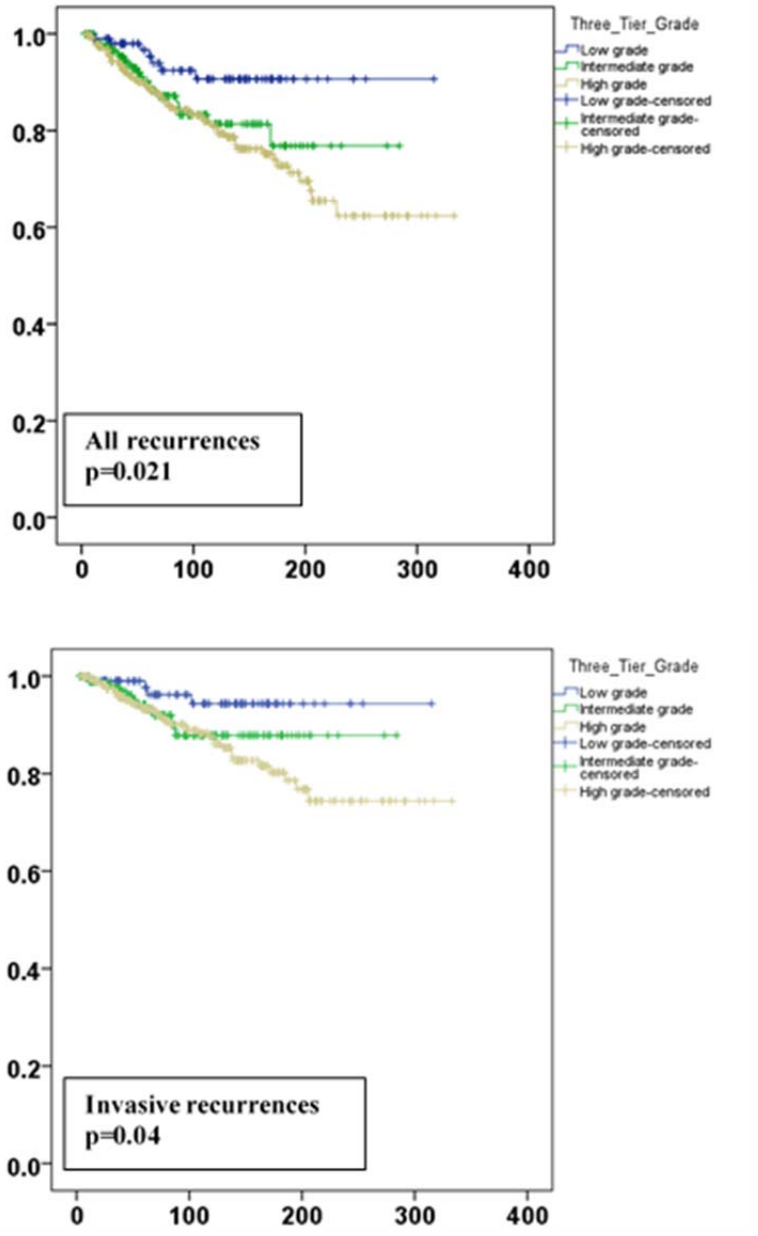
Patients at risk						
242	215	172	118	93	70	39
58	48	36	28	18	12	7

Supplementary Figure 1



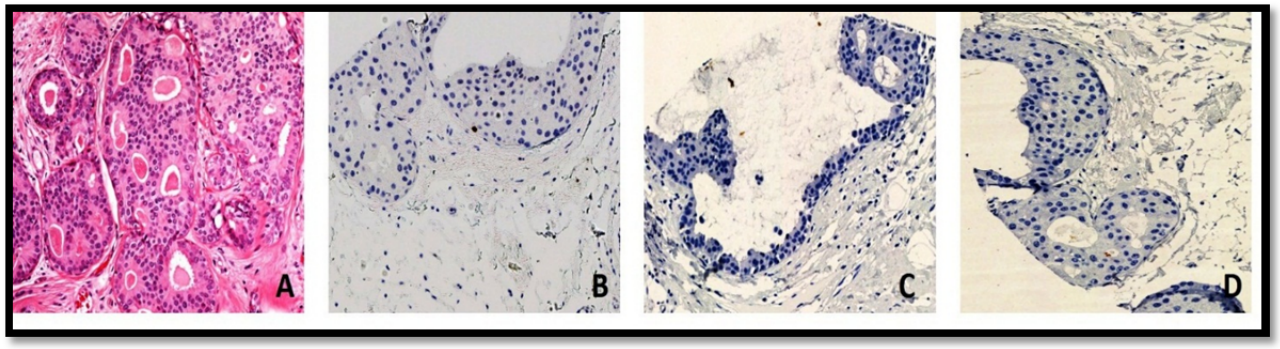
Supplementary Figure 1: Kaplan-Meier curve shows the association between ER expression and ipsilateral local recurrence rate in the whole cohort regardless the surgical treatment (Horizontal axis: local recurrence free interval in months, vertical axis: probability of recurrence).

Supplementary Figure 2



Supplementary Figure 2: Kaplan-Meier curve shows the association between DCIS grade and ipsilateral local recurrence (all recurrence in the upper curve and invasive recurrence in the lower curve). (Horizontal axis: local recurrence free interval in months, vertical axis: probability of recurrence).

Supplementary Figure 3



Supplementary Figure 3: Photomicrographic examples of low-grade DCIS that show negative expression of oestrogen receptor (ER); A) H&E stained section, B-D) immunohistochemistry stained sections showing negative ER expression. Note the apocrine changes (abundant esinophilic cytoplasm) and low-grade morphology.

Supplementary Table 1: Multivariate analysis of various variables associated with DCIS recurrence in patients treated with breast conserving surgery

	Hazard Ratio	95.0% CI for Exp(B)		P value
		Lower	Upper	
Patient age at diagnosis	1.5	1.3	1.8	**0.008**
DCIS size	1.5	0.8	2.5	0.170
DCIS Grade	1.5	1.1	2.2	**0.027**
Radiotherapy	0.4	0.2	0.9	**0.018**
Oestrogen receptor status	0.9	0.5	1.9	0.985

Significant p values are in **bold**

Supplementary Table 2: Clinical trials for the role of hormonal therapy in DCIS after breast conserving treatment.

Study	ER status as inclusion criterion	Primary End points	Outcome of the study
Radiotherapy (RT) and Tamoxifen in women with completely excised ductal carcinoma *in situ* of the breast in the UK, Australia, and New Zealand: randomised controlled trial [30].	No prior knowledge of the hormone receptor status of the patients.	To compare the efficacy of complete local excision alone with excision followed by RT to the residual ipsilateral breast, or excision followed by Tamoxifen for 5 years, or both, in reducing the incidence of subsequent ipsilateral invasive breast carcinoma in patients with DCIS.	Ipsilateral invasive disease was not reduced by Tamoxifen, but recurrence of overall DCIS was decreased. RT reduced the incidence of ipsilateral invasive disease and ipsilateral DCIS, but there was no effect on the occurrence of contralateral disease. There was no evidence of interaction between RT and Tamoxifen.
Tamoxifen in treatment of intraductal BC: National Surgical Adjuvant Breast and Bowel Project B-24 randomised controlled trial [20].	No prior knowledge of the hormone receptor status of the patients.	Occurrence of invasive or non-invasive tumours in the ipsilateral or contralateral breast following Tamoxifen therapy.	The effectiveness of Tamoxifen in treating DCIS without RT is speculative. The value of Tamoxifen used in combination with RT to lower the occurrence of invasive cancer could justify the suggestion that combined therapy replaces mastectomy for the treatment of DCIS patients in whom radiological findings are unlikely to be related to an invasive tumour
Adjuvant Tamoxifen reduces subsequent BC in women with oestrogen receptor-positive ductal carcinoma in situ: a study based on NSABP protocol B-24 [21].	ER and PR evaluation was carried out later for a subset of cases.	Time to the occurrence of any BC as a first event ipsilateral or contralateral subsequent to the original diagnosis of DCIS.	Adjuvant Tamoxifen significantly reduced subsequent ipsilateral BC only in patients with ER-positive DCIS after standard treatment with lumpectomy and RT. Tamoxifen reduced contralateral BC in patients with ER-positive and -negative DCIS. No ipsilateral benefit was observed in ER-negative disease.
Primary results, NSABP B-35/NRG Oncology: A clinical trial of Anastrozole vs Tamoxifen in postmenopausal patients with DCIS undergoing lumpectomy plus RT A randomized clinical trial [18].	ER or PR positive	BC-free interval, the time from randomisation to any BC event including local, regional, or distant recurrence or contralateral disease, invasive or DCIS.	There was no significant decrease in ipsilateral cancer, either invasive or non-invasive.
Anastrozole versus Tamoxifen for the prevention of locoregional and contralateral BC in postmenopausal women with locally excised ductal carcinoma in situ (IBIS-II DCIS): a double-blind, randomised controlled trial [19].	ER and PR positivity was determined as greater than or equal to 5% positive cells (equivalent of Quick-score of three or above and H-score of ten or above).	The development of histologically confirmed BC, both invasive and new or recurrent DCIS.	No clear efficacy differences were seen between the two treatments.

BC=Breast cancer, RT=Radiotherapy