

ORIGINAL ARTICLE

Clinical characteristics and management of a Greek female patient cohort with breast ductal carcinoma *in situ*

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Summary

Purpose: The purpose of this retrospective single-centre study was to examine the histopathological characteristics of breast ductal carcinoma *in situ* (DCIS) lesions in a cohort of Greek female patients and describe our experience regarding the clinical management of the disease.

Methods: The medical records from 1995 up to mid-2014 were scanned in order to trace DCIS cases. One hundred and seventy two patients (6.8% of all breast cancer cases) were diagnosed with pure DCIS and no invasive components; 32.0% underwent a second surgery, mainly due to first surgery positive margins.

Results: Age at first surgery ranged from 27 to 79 years (mean±SD 50±11) and median tumor size was 10mm (interquartile range/IQR=12mm). Comedo necrosis (CN) was identi-

fied in 28.5% of the cases. The detection of CN was significantly associated with older age at diagnosis, larger tumor size and lower probability of highly differentiated tumors. Radiotherapy (RT) and hormonotherapy (HT) were applied to 44.8% and 63.4% of the patients, respectively.

Conclusions: We implemented international practices (surgery, radiotherapy and prophylactic hormonal therapy) to patients diagnosed with DCIS and have observed only two relapses. It is our belief that DCIS requires a multidisciplinary approach and patient-tailored therapy which can potentially contribute to minimization of the local recurrence risk.

Key words: breast cancer, DCIS management, ductal carcinoma *in situ*

Introduction

By definition, DCIS is a specific pattern of cellular growth consisting of a premalignant proliferation of neoplastic epithelial cells evolving within the lumen of mammary ducts, lined by a layer of myoepithelial cells and confined by an intact basement membrane; however, there is no evidence of invasion into the surrounding stroma [1]. The notable rise in the detection rate of new DCIS cases from 2-5% in the past to 20-25% nowadays is mainly attributed to the introduction and wide use of screening mammography programs. With the increasing use of digital mammography and MRI the majority of DCIS lesions are impalpable, asymptomatic and detected upon mammographic microcalcifications [2]. Of note,

in Greece there are no such screening programs supported by the public health system and the incidence rate mentioned later in the current study was largely based on data extracted by the records kept in our private medical institution which also is a referral breast clinic.

It is generally accepted that DCIS is not a uniform entity but rather consists of a group of lesions which are clinically, radiologically, morphologically and genetically heterogeneous. The natural history of DCIS remains poorly understood.

The initial theory that DCIS are non-obligatory malignant precursors of invasive breast cancer (IBC), proposed by Wellings and Jensen [3], was

based on the evidence of a marked histological continuity observed in the sequence of progression from normal to abnormal breast tissues. Succeeding works on molecular level revealed genetic similarities and possible common origins between DCIS and IBC [4-7].

Furthermore, various observational clinical studies have confirmed the hypothesis that DCIS is a precursor of IBC. Perhaps the most convincing evidence suggesting that DCIS and IBC are progressive stages of the same evolutionary process is that they affect the same anatomical site. Retrospective analyses of several studies focusing on patients with DCIS misdiagnosed as benign conditions, and therefore managed with biopsy only, demonstrated that 33% of this population developed later on IBC in the same quadrant of the ipsilateral breast where the original DCIS was traced [8-15].

DCIS can be classified into similar molecular subtypes as IBC, based primarily on the expression of estrogen receptors (ER), progesterone receptors (PR), HER2 and cytokeratin 5/6 [16-19]. More often than not, associated *in situ* and invasive components exhibit a similar immunophenotype [20,21]. In addition, nuclear grade is generally concordant between *in situ* and invasive components of invasive carcinomas, which have comparable nuclear morphology. Consequently, the cytonuclear grade is defined as low, intermediate or high [22,23].

Although there have been numerous attempts to develop clinical or molecular tests able to predict which patients are most likely to develop invasive disease following a diagnosis of DCIS [24-28], there is currently no test with demonstrated clinical utility to identify this sub-population. This results in the vast majority of patients still being subjected to surgical treatment followed by RT and/or prophylactic systemic therapies (e.g. tamoxifen).

Despite extensive efforts to unveil the biological underpinnings of the phenomenon of progression from *in situ* to invasive disease and to develop biomarkers predictive of the likelihood of progression to IBC, several biological aspects of the former transition have yet to be elucidated.

The aim of this study was, on one hand, to examine the characteristics of DCIS lesions in terms of histopathological features and, on the other hand, to describe our experience regarding the clinical management of the aforementioned disease among a cohort of Greek female patients from a single institution.

Methods

This was a retrospective, single-centre study regarding a Greek female population. All patients of this referral breast clinic of Athens diagnosed with pure DCIS were included in the study, whereas DCIS patients with an invasive component were excluded. The date of the DCIS diagnosis in the samples ranged from July 1996 to June 2014 so as to allow a minimum follow up period of one year. The study protocol was approved by the participating institution's ethics committee.

The following data were analysed for each patient according to their records: age at diagnosis, tumor size, tumor grade, as well as ER, PR, C-erbB-2 status and cytokeratin 6/7. The last three parameters were measured in a scale 0 up to 3+ [29]. Furthermore, we examined paraffin blocks from each specimen for the expression of Ki-67. Presence or absence of CN was recorded in each case as well as multifocality and microcalcifications. Emphasis was put on tracing the data relative to the surgical margins at the time of first surgery. Finally, information on the assigned treatment type (mastectomy, RT, HT with tamoxifen) was also registered.

It should be noted that there was a number of missing values in ER, PR and C-erbB-2 mainly in the oldest patient records (i.e. before 2000) because it was not common practice to perform immunohistochemistry measurements for these parameters those days.

For the immunohistochemical study we used the Ventana complete rabbit monoclonal breast panel which includes estrogen receptor antibody (ER-SP1), progesterone receptor antibody (PR-1E2), HER2/neu-(4B5)PMA and Ki-67(930-9). Sections 5 mm thick of paraffin embedded breast tissue were placed in positively charged slides and were put in a Ventana automated slide strainer, using the Ventana detection kits and ancillary reagents. Interpretation was done using light microscopy. For the hormonal receptors (ER,PR) interpretation, a positive internal control was used and we evaluated the presence of nuclear staining, its intensity (weak, moderate, intense) and the percentage of positive cells. The *HER2/neu* immunostaining was interpreted as follows:

0 (Negative):no staining was observed;

1+ (Negative) : a fair, partly membrane staining was observed in >10% of the tumor cells;

2+ (Equivocal) : a weak to moderate membrane staining was observed in >10% of the tumor cells;

3+ (Positive) : a strong, complete membrane staining was observed in >10% of the tumor cells.

The Ki-67 index was estimated as the percentage of the nuclear positively stained cells. The evaluation was made in the so called "Hot spots" of the tumor.

Statistics

Statistical analyses were performed using R Statistical Software, version 3.1.2 (The R Foundation for Statistical Computing, Vienna, Austria) and all figures were produced using the *ggplot2* package of R. Since all quantitative parameters were skewed (apart from age at surgery), medians and IQR were mainly reported as descriptive statistics, though mean and SD values have been added in particular cases as well. Qualitative parameters were summarized by their absolute (N) and relative (%) frequencies. In case of missing values, these were explicitly mentioned for every parameter under investigation.

The differentiation of skewed quantitative parameters according to binary variables, such as dichotomous ER/PR expression (i.e. positive-negative), was tested using the Wilcoxon test, whereas in case of symmetric parameters the independent two-sample t-test was employed instead. Chi-square test was used to check on the potential relation between qualitative parameters of interest. In case of a 2x2 cross tabulation, the two-sample proportion test was also employed in order to produce 95% confidence intervals (95% CI) for the difference in proportions of the characteristic deriving from the first dichotomous variable in the subgroups produced by the second binary variable. Moreover, Spearman’s correlation test was used to measure the correlation between quantitative parameters. A significance level of 5% was used in all the analyses.

Results

According to the medical records of the particular referral breast clinic, 172 out of 2538 breast cancer patients (6.8%) were identified with DCIS and 55 of them (32.0%) underwent a second surgery (either wider excision or mastectomy) due to first surgery positive margins or other histologically derived concerns (multifocality). Figure 1 depicts how the number of newly diagnosed DCIS cases ranged per year starting from 1996 up to 2013 (2014 was excluded from the graph as a non-complete year). Age at first breast surgery indicating DCIS ranged from 27 to 79 years with mean age equal to 50 years (SD=11 years); this is a rough indicator that DCIS frequency is probably not differentiated between pre- and post-menopausal women. The median follow-up period in the sample was 1319 days, i.e. approximately 43 months (IQR=54 months, mean=57 months).

The diameter of the tumor ranged from 1 mm up to 70 mm with a right skewed sample distribution as anticipated; the median tumor size was 10.0 mm (IQR=12.0 mm) and there were 6 missing values. Overall, there were 40 palpable tumor masses (24.1%). For the 73 patients with recorded Ki-67 values, median Ki-67 was 10% (IQR=10%) indicating intermediate levels of overexpression among the patients of this subsample and there were four patients with Ki-67 expression between

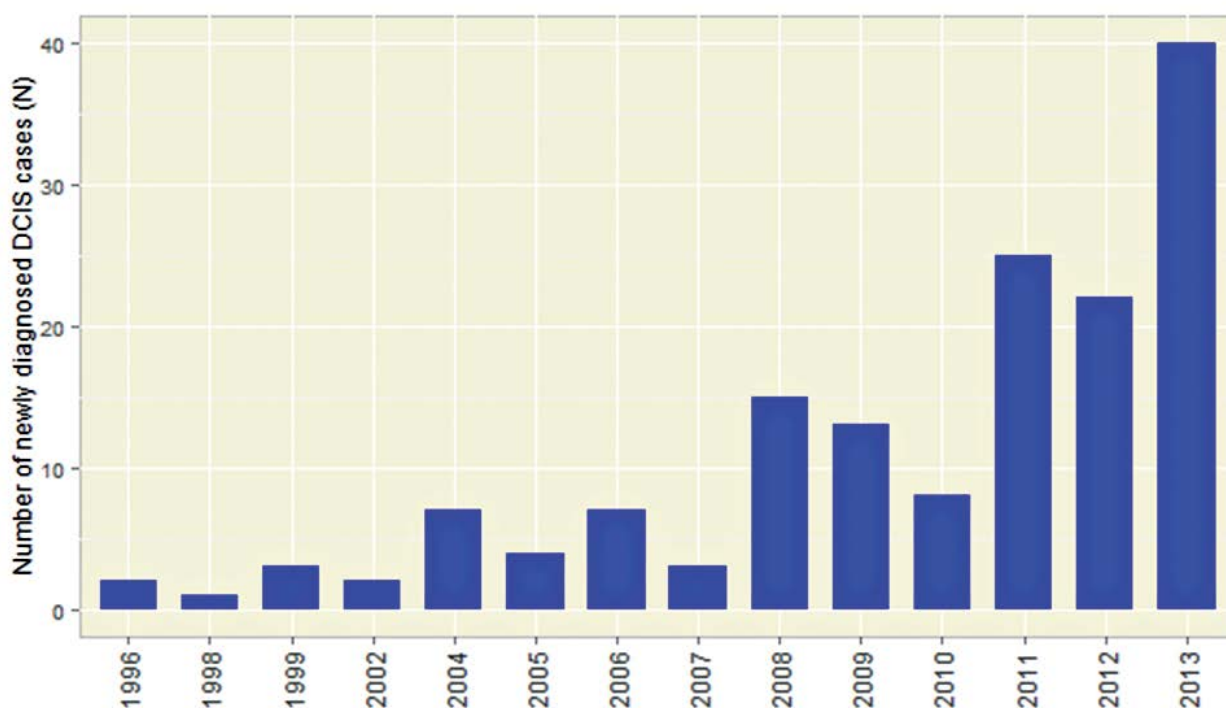


Figure 1. Absolute frequencies of the newly diagnosed DCIS cases per year for the current study (2014 excluded as a non-completed year).

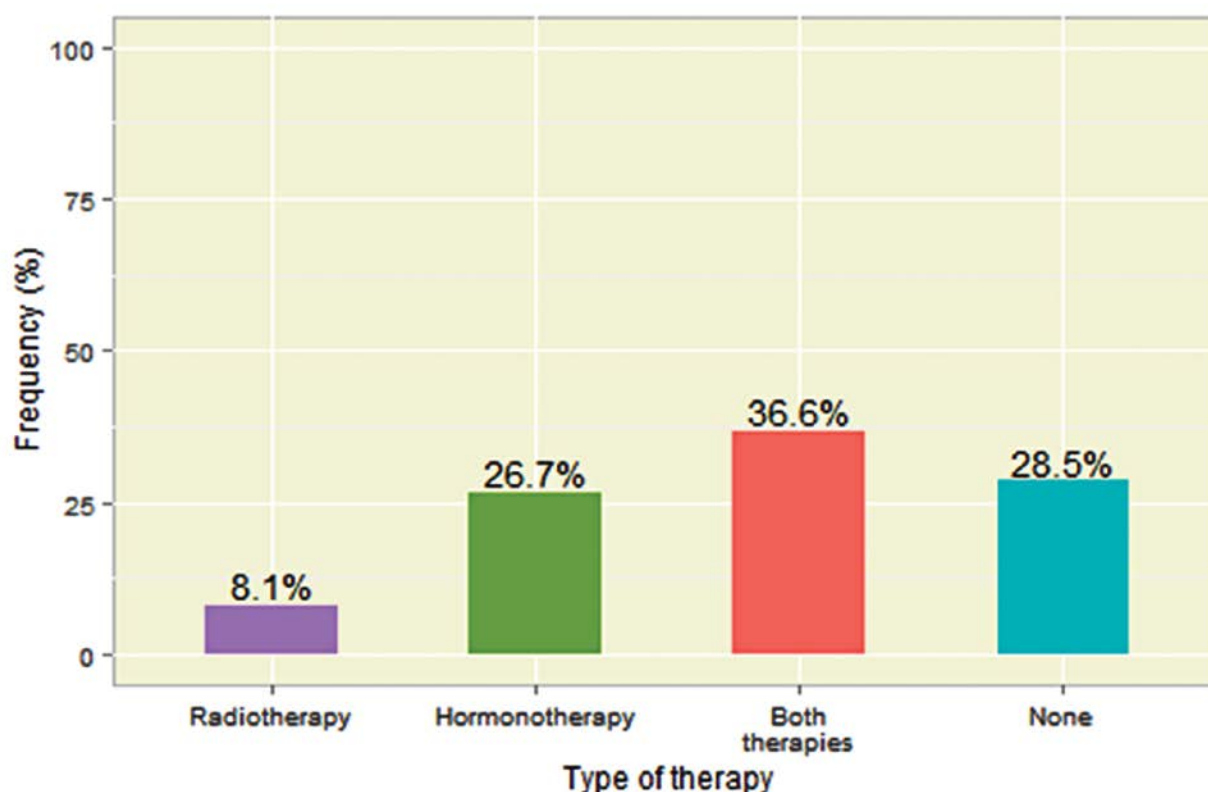


Figure 2. Types of adjuvant therapy given to patients.

30 and 60% (maximum sample value). In particular, should the threshold of 10% be considered as indicative for overexpression, 24 patients (32.9%) had Ki-67 values well above this threshold.

CN was identified in 49 cases (28.5%). Regarding the histological grade of the tumor, there were 47 cases (28.1%) of low grade (I), 69 cases (41.3%) of intermediate grade (II) and the rest 51 cases (30.5%) were of high grade (III); there were 5 non-recorded values for this parameter not considered for the calculation of the relative frequencies above. Immunohistochemical tests for C-erbB-2 determination demonstrated that they were negative in the majority of the patients (115 women, 71.4%) and positive in the remaining 46 cases (28.6%); the number of missing values was 11. More specifically, C-erbB-2 was weakly expressed in 21 patients (13.0%), moderately expressed in 14 patients (8.7%) and overexpressed in 32 patients (19.9%). Concerning ER and PR, 36 patients (21.7%) were ER-negative and 69 (41.6%) PR-negative (ER and PR were not recorded in 6 cases). Overall, 28 patients (16.9%) were ER and PR negative, and 15 patients (of whom 9.3% of the total sample with missing values excluded) were triple-negative. (i.e. ER, PR, C-erbB-2 negative). As to receptor positive cases, there were 16 cases (9.6%) with weak ER expression, 25 cases (15.1%) with moderate ER expression and 105 cases (63.3%) with strongly positive ER ex-

pression. Correspondingly, the sample contained 31 women (18.7%) with weak PR expression, 46 women (27.7%) with moderate PR expression and 51 women (30.7%) with strongly positive PR expression. Microcalcifications were observed in 91 patients (53.5%), with two missing values for this parameter, whereas multifocality was present in 21 patients (12.5%) excluding 4 missing values. In 4 cases (2.3%) sentinel lymph node biopsy (SLNB) was carried out, while in 41 cases (23.8%) simple or modified radical mastectomy were performed.

Regarding therapies assigned to the DCIS patients, 77 women (44.8%) underwent RT, while 109 women (63.4%) were given HT. Totally, 63 patients followed a combination of radiotherapy and HT (Figure 2).

Interestingly, only two patients experienced breast cancer recurrence (1.3%). In the first case a relapse of grade III (T = 45 mm, ER (3+), PR (2+), C-erbB-2 (2+)) appeared at the age of 57, 32 months after the first diagnosis which was followed by simple mastectomy. The adjuvant therapy was limited to tamoxifen. The histological findings of the second operation (wide local excision+SLND) revealed the presence of a localized invasive breast cancer and RT was delivered. As for the second case, the first operation took place at the age of 38 years, was a conservative one with no adjuvant treatment and revealed the presence of a DCIS with the following characteristics: grade

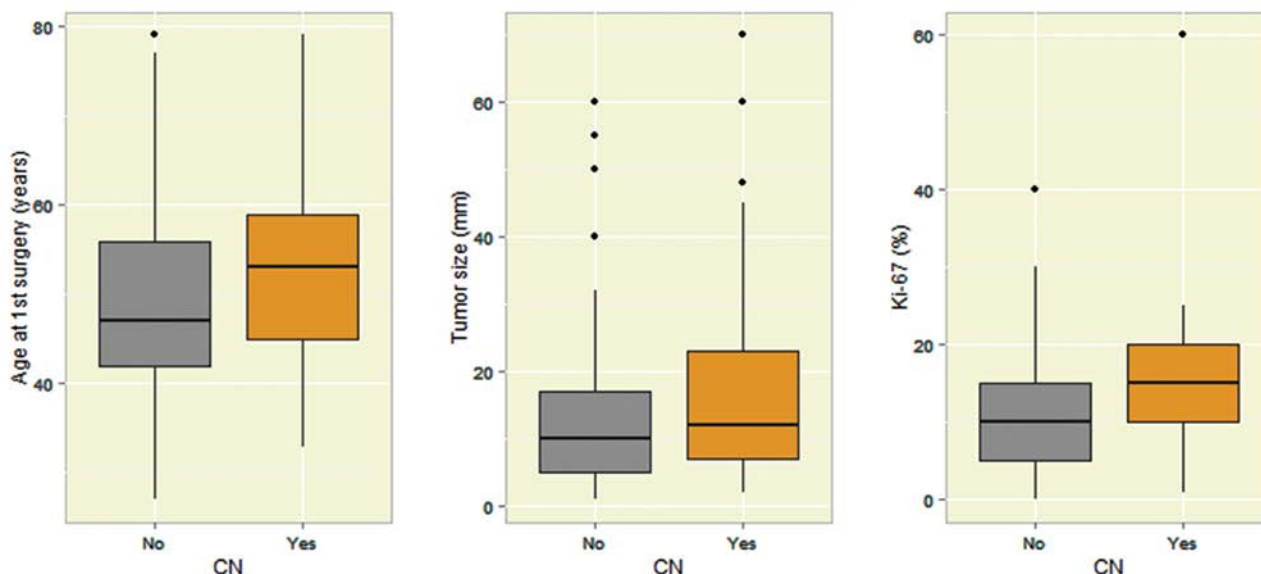


Figure 3. Sample variation of age at surgery, tumor size and Ki-67 expression according to comedo necrosis (CN). Respective p values = 0.038, 0.047 and 0.09. The individual points represent outliers.

II, T = 12 mm, ER (1+), PR (1+), c-erbB-2 (1+). The recurrence occurred two years after the first operation and was treated with wide local excision followed by RT, while the pathology report confirmed the presence of a new small DCIS (T=0.6mm, ER 1+, PR 2+ and C-ErbB-2 1+).

Correlations with comedo necrosis

The total sample was split in two, according to CN ($N_{CN}=49$) and non CN ($N_{NCN}=123$). Figure 3 shows the variation of age at surgery, tumor size and Ki-67 expression in the aforementioned subsamples. Age was found to be significantly correlated with the existence of CN (Median_{NCN}=47 years, IQR_{NCN}=14 years vs Median_{CN}=53 years, IQR_{CN}=14 years; $p=0.038$). Tumor size was also significantly differentiated between CN and NCN groups (Median_{NCN}=10 mm, IQR_{NCN}=12 mm vs MED_{CN}=12 mm, IQR_{CN}=16 mm; $p=0.047$). Ki-67 expression did not significantly vary between these two groups of patients (Median_{NCN}=10%, IQR_{NCN}=10% vs Median_{CN}=15%, IQR_{CN}=10%; $p=0.09$), although one should bear in mind the extended proportion of patients with non-recorded Ki-67 values (respective sample sizes are in this case $N_{CN}=15$ vs $N_{NCN}=58$). Moreover, there were 33.9% of NCN patients who had low grade tumors, 36.4% who had intermediate grade tumors and 29.7% who had high grade tumors, showing that grade was rather uniformly distributed in this subsample. The respective figures for the CN group showed that there was less preference for low grade tumors: 14.2%, 53.1% and 32.7%. The association between grade and CN was statistically significant ($p=0.028$). As for mi-

crocalcifications, their sample frequency within NCN and CN subsamples was 48.7% and 65.3%, respectively. Their association with CN was barely non-significant [95% CI of the difference in microcalcification proportions between NCN and CN groups = (-0.34, 0.01); $p=0.073$]. Finally, CN was significantly correlated with PR [95% CI of the difference in PR-negative proportions between NCN and CN = (-0.38, -0.03); $p=0.023$], though neither with ER [95% CI of the difference in ER-negative proportions between NCN and CN = (-0.26, 0.06); $p=0.19$] nor with C-erbB-2 [95% CI of the difference in C-erbB-2-negative proportions = (-0.10, 0.25); $p=0.43$].

Other interesting correlations

We investigated the potential correlation between tumor size and Ki-67 expression, but no significance occurred (Spearman's rho = 0.08, $p=0.52$). Another issue to point out is that, although the differentiation of tumor size by grade was non-significant ($p=0.16$), the sample median tumor size for grade I tumors was 8 mm (IQR=12 mm), as opposed to the sample median sizes for grade II and III tumors that were equal to 10 mm (IQR=12 mm) and 10 mm (IQR=17 mm) respectively, thus indicating a slight tendency of the larger tumors being of higher grade. Tumor size was significantly associated with ER ($p=0.04$) and PR ($p=0.002$). More specifically, the median tumor size in ER negative patients was median=15 mm (IQR=17 mm) vs median=10 mm (IQR=11 mm) in ER positive patients and median=10.5 mm (IQR=16.2 mm) in PR negative patients vs median=8.0 mm (IQR=9.5 mm) in PR positive patients (Figure 4).

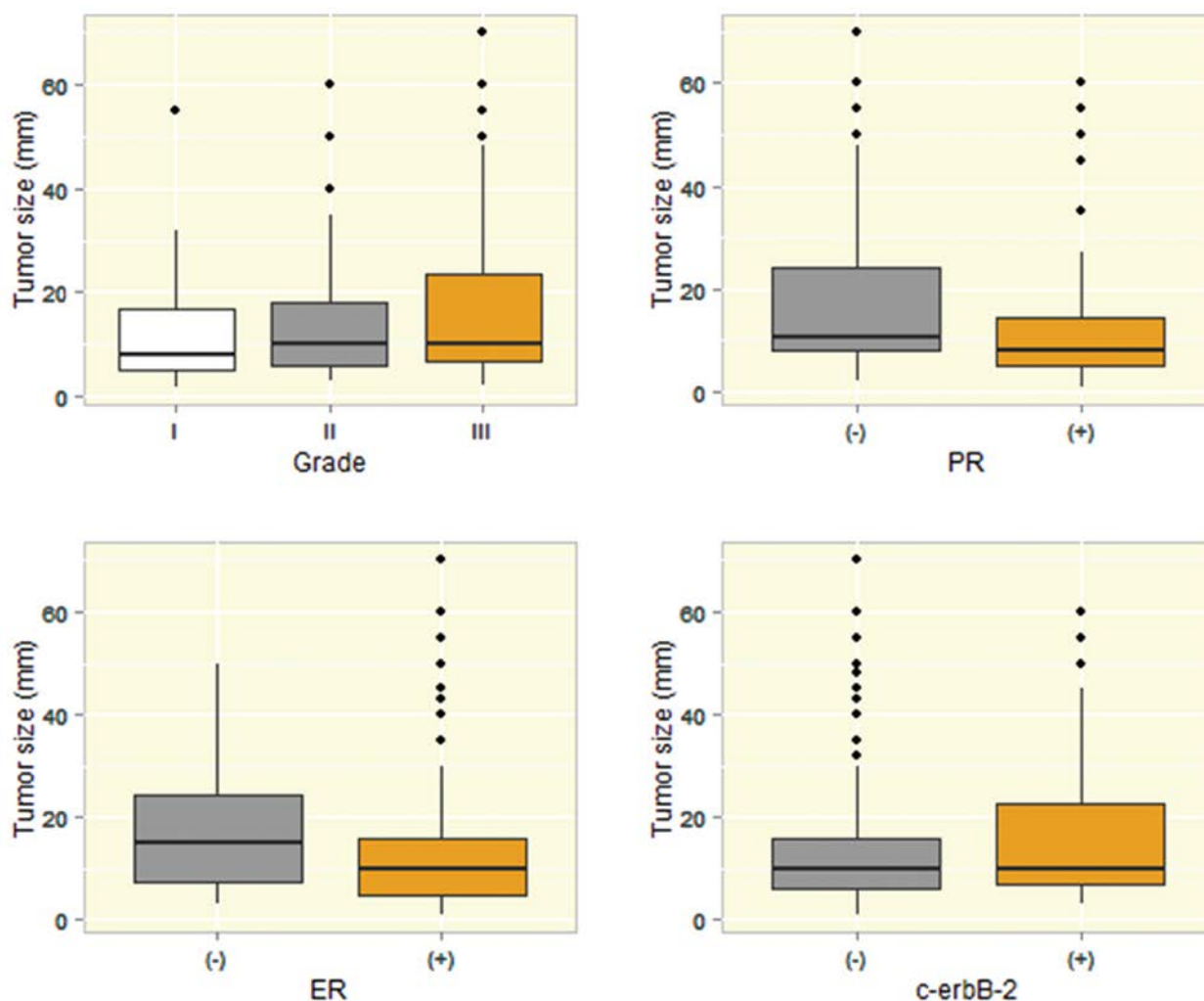


Figure 4. Differentiation of sample tumor size according to several qualitative parameters (grade, ER, PR, C-erbB-2). Respective p values = 0.16, 0.002, 0.04 and 0.42. The individual points represent outliers.

Tumor grade had a significant effect on Ki-67 values ($p=0.004$), with the median Ki-67 values in the three subgroups I, II and III being 10, 10 and 15% (respective IQR=5, 10 and 10%). On the other hand, a significant relation was revealed between tumor grade and PR ($p=0.01$), while the respective result for ER was barely non-significant ($p=0.07$). The percentage of low grade tumors (i.e. grade I) in negative and positive PR cases was 19.1 and 32.9% respectively, and also the percentage of high grade tumors in the same groups was 42.6 and 22.3%. As expected, no correlation was observed between the existence of microcalcifications on one hand and ER, PR, C-erbB-2 and tumor size on the other hand (all $p > 0.05$) (Figure 4).

Characteristics of the group that received no adjuvant therapy

As illustrated previously in Figure 2, there were 49 (28.5%) out of 172 patients that received neither RT nor hormonotherapy after being di-

agnosed with DCIS. It is interesting to outline the characteristics of this particular group of patients. Tumor size ranged from 2 to 60 mm (median=11 mm, IQR=15 mm, with 4 missing values) and 12 patients (26.7% excluding missing values) had palpable tumors. CN was present in 6 patients (12.2%) and microcalcifications characterized 48.9% of tumors. Regarding tumor grade, the respective frequencies of low, intermediate and high grades were 15 (31.9%), 13 (27.7%) and 19 (40.4%) excluding 2 missing values. Lastly, it was noted that negative ER occurred in 11 cases (23.4%), negative PR in 18 cases (38.3%) and negative C-erbB-2 in 34 patients (73.9%).

Discussion

DCIS is an extremely heterogeneous group of lesions. Most of the newly diagnosed cases are asymptomatic, impalpable and occur as random mammographic findings (e.g. microcalcifications). Nowadays, management of DCIS is focused on

the following treatment modalities: surgery, RT and adjuvant systemic therapy. The former is represented by breast-conserving surgery (BCS) – with or without RT – or mastectomy and can be coupled with SLND. Regarding adjuvant systemic therapy, this more often consists of estrogen blockade with tamoxifen (HT) and aromatase inhibitors. As previously mentioned, DCIS can be viewed as non-obligatory malignant precursor of invasive breast cancer; therefore, it is crucial to estimate which lesions are likely to develop into invasive forms in the future, in order to be able to distinguish which DCIS cases should be treated aggressively and which need not. Unfortunately, to date, there are no such tests developed and it is possible that many of the DCIS lesions are over- or under-treated to a considerable extent.

The main aim of this study was the description and further investigation of the characteristics of DCIS patients of a Greek female cohort and the comparison of our clinical practices regarding treatment with international patterns and approaches. Retrospective inspection and analysis of the DCIS cases emerging from the medical records of the participating referral breast clinic, being visited by a large number of patients from all over the country, showed that one in four tumors were palpable. Almost one in three cases had CN and an equivalent rate corresponded to high grade tumors. The detection of CN was significantly associated with older age at diagnosis, higher tumor size and lower probability of highly differentiated tumors (i.e. grade I). Also, the likelihood of a PR negative pattern was significantly higher in CN cases. As for the tumor size, it was statistically larger in ER negative and PR negative cases. Finally, the better differentiated a tumor was, the lower was the probability of a PR negative case and the greater was the probability for a lower Ki-67 value, although the latter finding should be cautiously used due to the large number of Ki-67 missing values.

The post-surgical treatment approach in this sample of DCIS patients was RT or HT or a combination of both in two out of three patients. Mastectomy was carried out in less than one in four cases, showing a tendency to avoid radical surgery solutions. The fact that only two relapses (both invasive) occurred is very encouraging.

In addition, it was observed that the mean age at DCIS diagnosis in the sample was 50 years. Therefore, in our records, DCIS does not seem to be associated with the menopausal status of a woman. In addition, there were 15.1% women younger than 40 years; 46% of these women did

not receive any adjuvant therapy and one in five underwent mastectomy only, approximately one out of 10 underwent mastectomy followed by HT and almost one in four women received RT and/or HT. A little less than one third of the total DCIS patients sample received no therapy at all.

Regarding the triple negative patients in the sample, 40.0% underwent mastectomy (most often followed by no therapy), 46.7% underwent breast conserving surgery and received RT or HT or both, whereas no therapy was given in the rest seven cases. Concentrating our view on the 13 ER/PR negative patients, once again 38.4% were treated with mastectomy (often combined with HT), 61.5% received a combination of RT and HT and no therapy was assigned in one case.

According to the literature, a DCIS patient is regarded as a high risk case if accompanied by the following characteristics: high tumor grade, CN, tumor size over 4 cm and younger age (less than 40 years) [30]. In our sample, there were no patients matching the particular profile characteristics simultaneously.

In our sample, the new DCIS cases were 8.1% of the new breast cancer cases considering data of 2005 onwards. Taking into account data from 1996 up to 2004, the corresponding rate dropped to 3.6%, thus following the international trend of a considerable rise in the associated DCIS rate [31].

Among the well-established tumor related factors (such as tumor size, micropapillary distribution, CN, high grade, multifocality, multicentricity) and biological markers (ER/PR status, HER2/neu+, high Ki-67 index, angiogenesis) [32], we strongly believe that the margin status has the highest impact on the effort to achieve local control of the disease since it is strongly correlated with the local ipsilateral recurrence rate which is responsible for the appearance of invasive disease [33]. Recently, no ink on tumor has been accepted as a standard for clear margin although there is no consensus regarding this subject. In our discipline, we aim at obtaining a free margin of at least 1 mm.

With regard to the Van Nuys Prognostic Index (VNPI) [34,35], patients were not treated according to the VNPI guidelines and RT was carried out at each surgeon's discretion.

Limitations and conclusions

DCIS still remains a controversial issue in terms of its biological mechanisms and clinical evolution. In this study we implemented international practices (surgery, RT and prophylactic

HT) to patients diagnosed with DCIS and have observed only two relapses, one of them invasive. It is our belief that DCIS requires a multidisciplinary approach and patient-tailored treatment which can potentially contribute to the minimization of the local recurrence risk and subsequently to the reduction of the possibility of evolving into invasive life-threatening forms. Limitations of the present study include the medium sample size and some missing values present in the medical records of the patients, especially for the Ki-67 expression whose role could not be sufficiently explored. Sim-

ilarly, immunohistology measurements for ER, PR and C-erbB-2 were not largely conducted in the initial time period considered in this study, which also resulted in a small number of missing values on those parameters. With regard to future research, we aim at applying the Oncotype DX DCIS score with a view of identifying subgroups of DCIS patients who could avoid over-treatment.

Conflict of interests

The authors declare no conflict of interests.

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