

Histopathological Study on Conservatively Operated Breast Carcinomas

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ABSTRACT: In this histopathological study we looked at 303 cases of breast carcinomas, managed through conservative breast surgery and later analysed with the help of a classical histopathological technique, paraffin embedding. The carcinomas were assessed in terms of tumor size, lymph node status, histological type, correlation between invasive tumors and an *in situ* carcinoma component, resection margins, grading and patients age. Following assessment, we looked at associations between above morphological and clinical parameters and ipsilateral local recurrences. We concluded that more than half of our cases were carcinomas, measuring between 2 cm and 5 cm, with no associated lymph node involvement, in keeping with pTNM criteria for stage II. By far, in our study, the most frequent histopathological type was type NOS (63.37%) followed by invasive lobular carcinoma (10.56%) and mixed ducto-lobular invasive carcinoma (6.27%). Other types of invasive carcinoma were rarer, each representing less than 4% of cases. In regards to *in situ* carcinomas we noted the most common histological types to be both cribriform intraductal carcinoma and comedocarcinoma, each identified in 1.65% of cases. Amongst invasive breast carcinomas, infiltrating ductal carcinoma not otherwise specified (NOS) was found to be most commonly associated with *in situ* ductal carcinoma lesions. This was seen in 34.9% of cases, and was the only type associated with an extensive *in situ* component. Analysing the grading of mammary carcinomas in our study showed that the vast majority of cases (63.04%) were grade 3 tumors. In regards to surgical resection margins, ¾ of cases were noted to have negative margins. Tumor recurrences were noted in 12 cases. These cases were most commonly noted to reoccur following initial poorly differentiated, infiltrating ductal carcinomas, not otherwise specified (NOS), with positive resection margins, measuring less than 2 cm. Patients tended to be under the age of 40 and had positive lymph nodes. The emergence of local recurrences after conservative surgery for early breast cancer is significantly linked to poorly differentiated primary tumors ($p < 0.05$) but not correlated with histological type, presence of extensive intraductal carcinoma component, size of primary breast tumor or lymph node status ($p > 0.05$). In terms of increasing the risk of ipsilateral recurrence the most important aspect highlighted in our study was the status of the resection margins. Patients with positive resection margins had a significantly high risk to develop recurrences after the conservative surgery, compared to those with negative margins ($p < 0.001$).

KEYWORDS breast cancer, breast conserving surgery, recurrence, histopathological

Introduction

It has taken a considerable amount of time for breast conserving surgery to become the standard way of treating early stage of breast cancer. With an increased number of patients treated for breast cancer with breast conserving surgery we note a corresponding increase in ipsilateral tumor recurrence. Associating postoperative radiotherapy with breast conserving surgery has significantly reduced the incidence of ipsilateral tumor recurrence. There have been a number of other factors reported to have an influence on the incidence of ipsilateral tumor recurrence, for example: patient's age, presence or absence of malignant cells in the resection margins, the size of the primary tumor,

hormone receptor status etc. (Kroman et al, 2004, Park et al, 2000). In this study, histopathological analysis of the 303 cases of breast carcinoma (which included one case of Paget's disease) sought to evaluate the following parameters: size of tumors (pT), status of the lymph nodes, pTNM staging, histopathological type and grading (G), associations with *in situ* carcinoma (intraductal carcinoma, intralobular carcinoma) and resection margins status. Subsequently, we looked at the associations between these parameters and ipsilateral local recurrences.

Material and methods

The analysed material was composed of breast tissue fragments, which were collected

from all 303 cases of diagnosed breast carcinoma all undergoing breast conserving surgery. This covered a 10 years period, with the earliest case dating from 2004 until 2013. Patients in the study were seen and diagnosed with mammary tumors within the Surgery Department at the University Hospital CFR Craiova and the Surgery Department at SCJU, Craiova. In the histopathological study, we employed classical histological techniques of paraffin embedding as well as usual hematoxylin-eosin staining and Van Giesson stain.

The method employed to assess the histological malignancy grading (tumor grading) was the Nottingham combined score (Elston CW, Ellis IO, 1991). According to this grading system, a score between 1-3 is given to the following parameters: tubeleformation, nuclear pleomorphism and mitotic count. The scores for each of the three criteria is added together to give a final overall score and a corresponding grade as follows: score 3-5 = Grade I (G1), score 6-7 = grade II (G2) and score 8-9 = grade III (G3)..

The method used to determine the status of resection margins post breast conserving surgery. Resection margins were defined as positive or negative thusly:

Positive (presence of malignant cells - in situ or invazive in one or more - resection margins)

Negative (absence of malignant cells - in situ or invazive - within resection margins).

Statistical analysis was conducted using mean values and confidence intervals, as well as

comparison tests (chi square) for the groups formed. The software used was SPSS10. Chi square test was used to interpret incidence tables; datas were assessed in terms of dependence between the two classification factors, retaining only the results below 5%, which was though of as a meaningful enough thershold. This test allowed accurate assessment of the relationship (interaction) between two factors. The commands used in the software were Analyze, Descriptive Statistics and CrossTabs.

Quantification of the statistical results obtained using Chi square test was performed as follows:

- $p < 0.05$, the difference between the two means is significant (S).
- $p < 0.01$, the difference between the two means is highly significant (HS).
- $p < 0.001$, the difference between the two means is very highly significant (VHS).
- $p > 0.05$, the difference between the two means is not significant (NS).

This study was conducted in keeping with existing framework for research ethics.

STUDY OF MORPHOPATHOLOGICAL PARAMETERS - RESULTS

Patients, forming part of our study group, were aged between 27 years and 79 years with two age peaks, one between 40-50 years and another one between 60-70 years.

Looking at size of carcinomas (pT), evaluated within our study, we concluded that invasive breast carcinomas varied in size, with an average measured at 3.51 cm +/- SD = 1.8.

Table 1. Distribution of cases according to size of primary tumors (pT)

| TUMOR SIZE | ≤ 2 cm | $2 < \text{tumor} \leq 3$ cm | $3 < \text{tumor} \leq 5$ cm |
|--------------|-------------|------------------------------|------------------------------|
| No. of cases | 143 | 91 | 69 |
| Percentage | 47,19% | 30,03% | 22,77% |

Following this analysis, it was found that the vast majority of tumors (160 cases) were 2 cm to 5 cm in size (pT2), representing 52.8% of all cases. The rest (143 cases) had tumors that were ≤ 2 cm (pT1) in size (Table 1). We note that 7 cases were directly staged as pT4b regardless of the actual size of the tumor in question. This was due to other morphological characteristics.

Analyses of axillary lymph nodes status showed that, out of the 303 patients, a total of 145 cases (47.85%) showed invasion of the axillary lymph nodes (positive lymph node), while 158 cases (51,15%) did not have lymph node involvement (negative lymph node). Out of the 145 cases with positive lymph nodes, 8 cases

(5.52%) were asociated with in situ breast carcinoma whilst the rest were diagnosed with invasive type.

Histopathological examination of specimens collected post axillary dissection allowed pTNM staging as follows:

- stage 0** – 20 cases (6,60%)
- stage I** – 114 cases (37,62%)
- stage II** – 139 cases (45,87%)
- stage IIA – 99 cases (32.67%)
- stage IIB – 40 cases (13,20%)
- stage III** – 30 cases (9,90%)
- stage IIIA – 20 cases (6,60%)
- stage IIIB – 10 cases (3,30%)

Following assessment of histopathological type, the following distribution of microscopic subtypes was highlighted:

Ductal carcinoma - 16 cases

- Solid type - 4 cases
- Cribriform - 5 cases
- Micropapillary type - 2 cases
- Comedocarcinoma - 5 cases

Intralobular carcinoma – 3 cases

Paget disease– 1 case

Invasive carcinoma – 283 cases

Invasive ductal NOS carcinoma - 192 cases

Invasive lobular carcinoma - 32 cases

· Joint ducto-lobular carcinoma- 19 cases

· Invasive papillary carcinoma - 7 cases

· Mucinous carcinoma- 12 cases

· Tubular carcinoma - 7 cases

· Medullary carcinoma - 8 cases

· Invasive cribriform carcinoma - 4 cases

· Squamous cell carcinoma - 2 cases

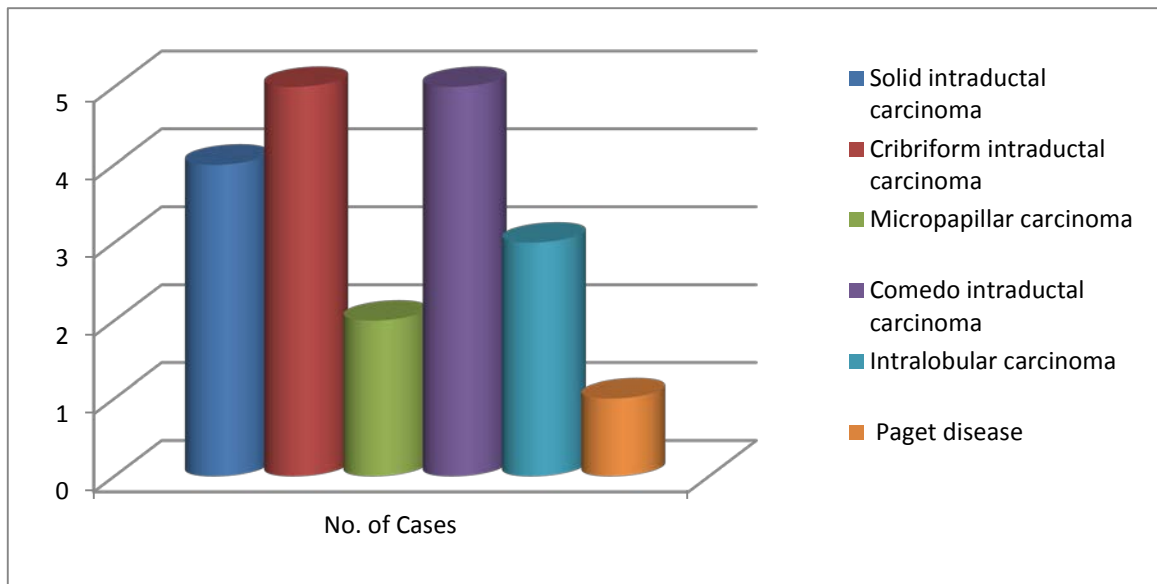


Fig.1. In situ carcinoma – case distribution

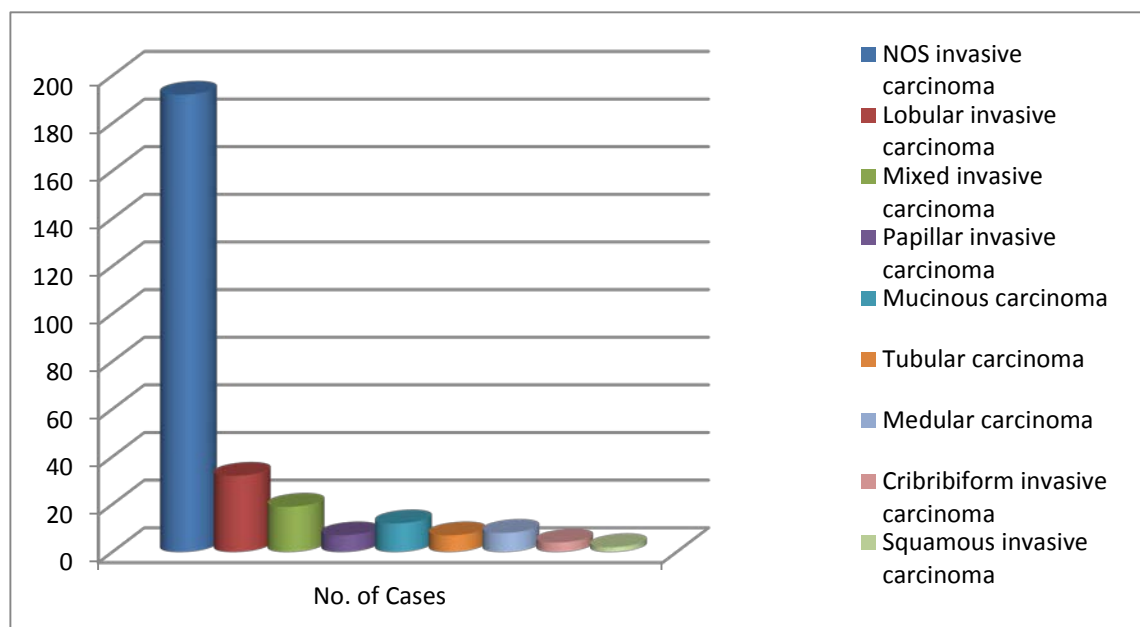


Fig.2. Invasive carcinoma – case distribution

We noted that invasive carcinomas were more frequent compared to in situ ones (93.4% vs 6.6%). Amongst invasive types, not otherwise specified carcinoma (NOS) was the most

commonly found histopathological form (63.37%). This was followed by invasive lobular carcinoma (10.56%) and mixed ducto-lobular invasive carcinoma (6.27%). Other types of

invasive carcinomas (invasive papillary carcinoma, mucinous carcinoma, tubular carcinoma, medullary carcinoma, invasive cribriform carcinoma and invasive squamous cell carcinoma) were rarer, each representing less than 4% of total cases (Fig .1, table 2). Amongst in situ carcinomas, the most common histological types were intraductal cribriform carcinoma and intraductal comedocarcinoma, each diagnosed in 1.65% of cases. The second most frequent one was noted to be intraductal carcinoma, solid type, seen in 1.32% of cases. Other types (micropapilar intraductal carcinoma, intralobular carcinoma and Paget's disease) were present in less than 1% of cases.

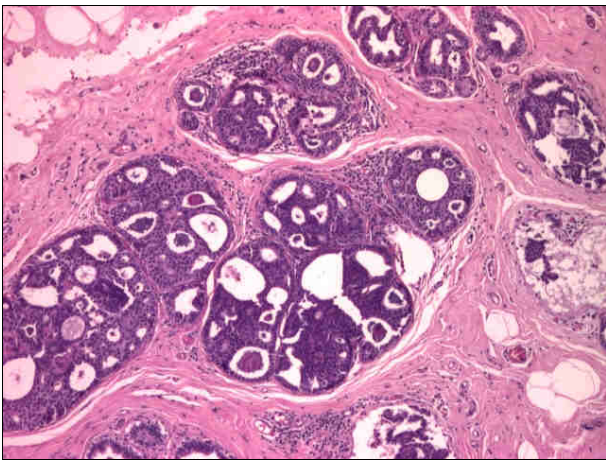


Fig.3. Intraductal carcinoma, cribriform type - HE staining, x40

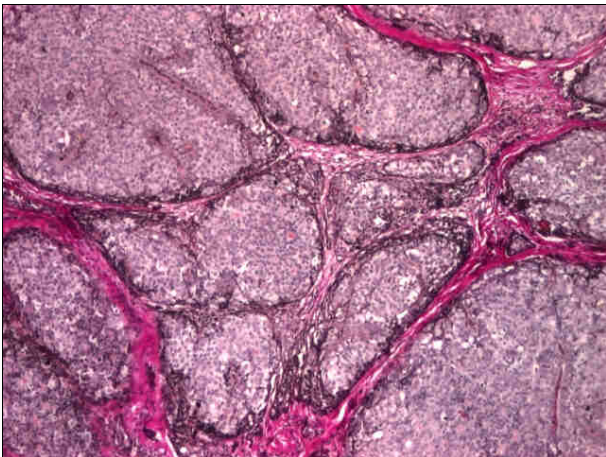


Fig.4. Intraductal carcinoma, solid type – VG staining, x40

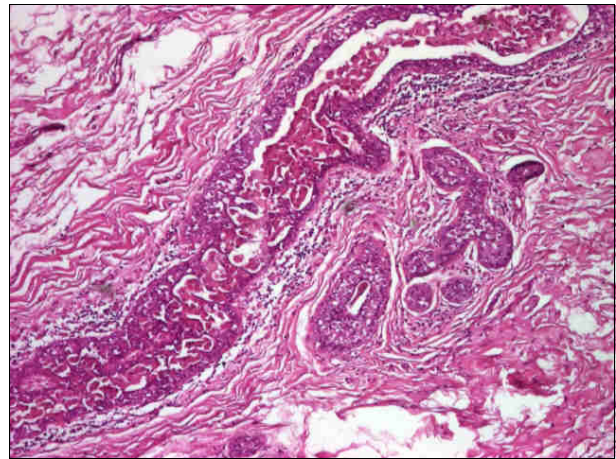


Fig.5. Intralobular carcinoma - HE staining, x40

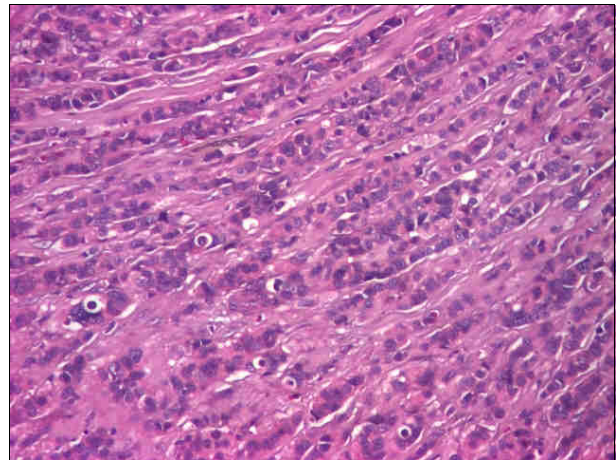


Fig.6. Invasive lobular breast carcinoma – HE staining, x100

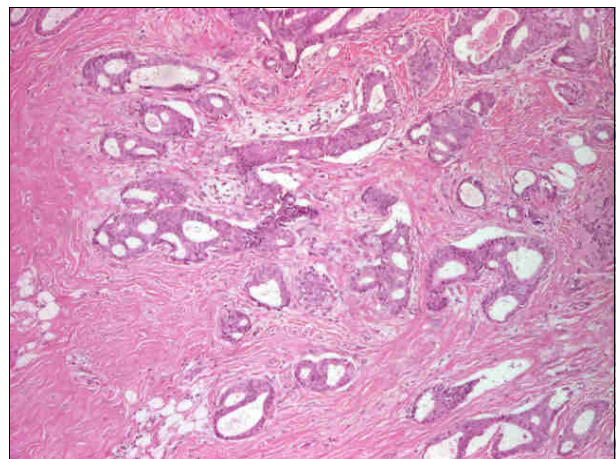


Fig.7. Cribriform invasive breast carcinoma - HE staining, x40

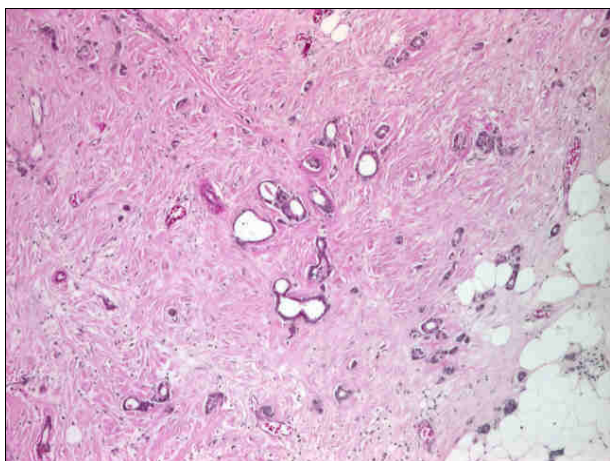


Fig.8. Tubular breast carcinoma -VG staining, x40

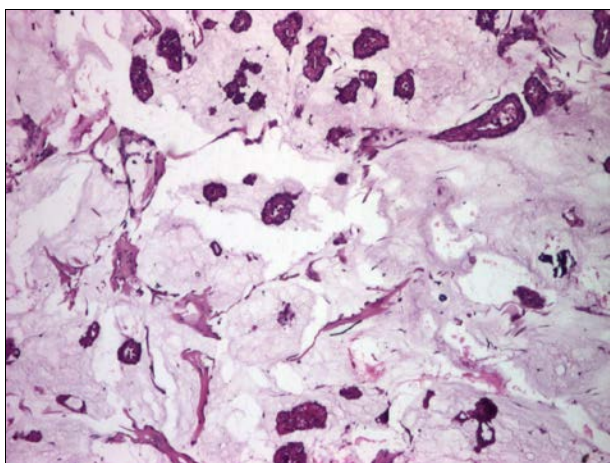


Fig.9. Mucinous breast carcinoma - HE staining, x40

Part of our study, analysis of the extensive intraductal component (EIC) associated with invasive breast carcinomas revealed that the most commonly seen histopathological type of invasive breast carcinoma associated with lesions characteristic of in situ ductal carcinoma was the invasive ductal carcinoma, not otherwise specified (NOS). This was associated with an intraductal component in 67 cases (34.9%). The second most common type showing similar association was the invasive papillary carcinoma, this associated an intraductal component in 2 cases (28.57%). The third one

was mixed carcinoma seen in 5 cases (26.31%). Considering that an extensive intraductal component (> 25% of primary malignancy) appears to strongly correlate with invasive recurrences in breast malignancies treated with Breast conserving surgery, we paid particular attention to this aspect. In our study we found 5 cases of invasive ductal carcinoma, not otherwise specified (NOS) that had an extensive intraductal component. 9 other cases of invasive lobular carcinoma (28.12%) associated in situ lobular carcinoma areas.

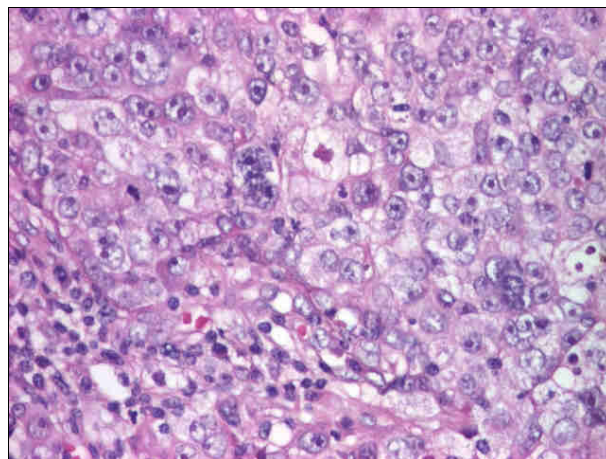


Fig.10. Medullary breast carcinoma - HE staining, x200

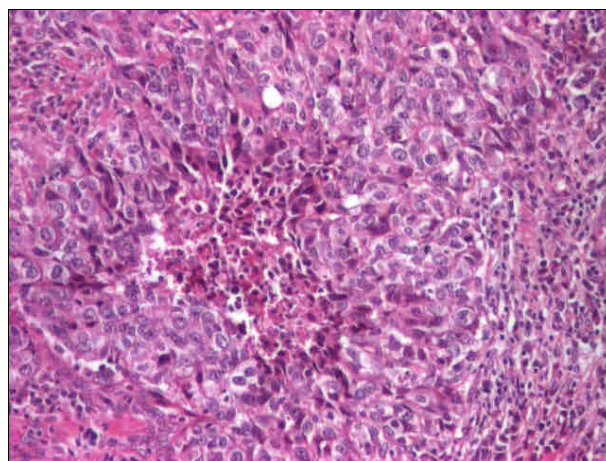


Fig.11. Squamous cell breast carcinoma - HE staining, x100

Table 2. Presence of intraductal component correlated with the histopathologic type of the invasive component

| HISTOPATHOLOGICAL TYPE | No. of cases | No. of cases associated with intraductal component | No. of cases associated with EIC |
|--------------------------------|--------------|--|----------------------------------|
| Invasive ductal carcinoma NOS | 192 | 67(34,9%) | 5 (2,6%) |
| Mixed ductal-lobular carcinoma | 19 | 5(26,31%) | - |
| Invasive papillary carcinoma | 7 | 2(28,57%) | - |

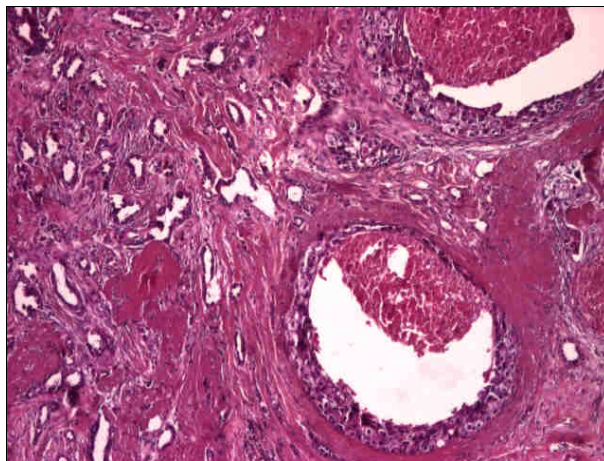


Fig.12. Invasive ductal carcinoma NOS associated with intraductal component, comedocarcinoma type, x40

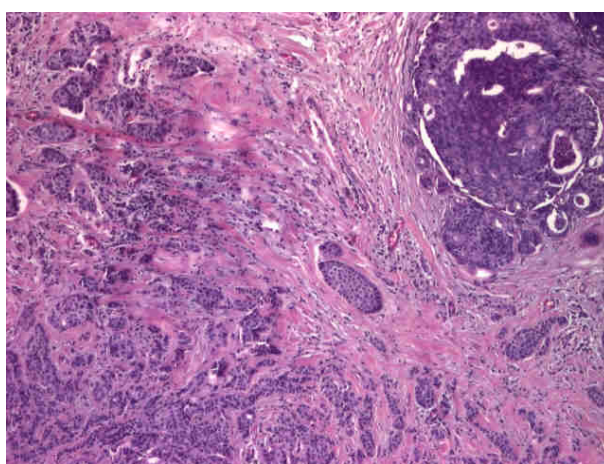


Fig.13. Invasive ductal carcinoma, NOS associated with intraductal component cribriform-type, x40

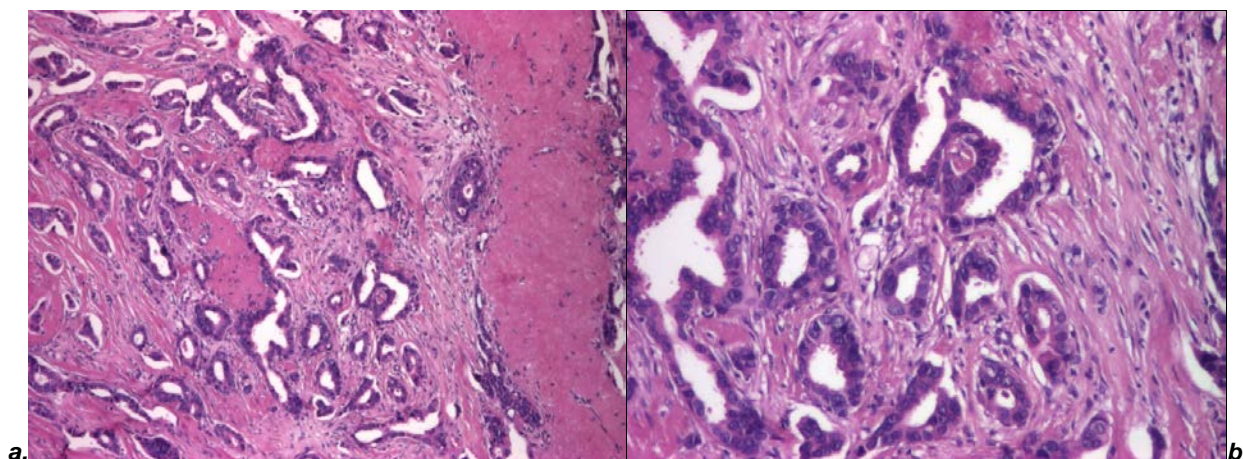


Fig.14. Well differentiated invasive ductal breast carcinoma (G 1), HE staining a) x 40 și b) x100.

The degree of histological malignancy was assessed using the Nottingham grading system. The final overall score of analyzed breast carcinomas allowed the following grading:

- Grade 1 (well-differentiated) score 3-5: 7.26% (22) of cases
- Grade 2 (moderately differentiated) score 6-7: 29.70% (190) of cases

- Grade 3 (poorly differentiated) score 8-9: 63.04% (191) of cases

Based on the grading of mammary carcinomas, it was found that the vast majority of cases (63.04%) were grade 3 tumors (Fig. 14), the least common one (7.26%) were grade 1 (Fig. 12) whilst grade 2 (Fig. 13) was found to be present in 249 cases (70%).

The microscopic study, completed on post-operative pieces, revealed that resection margins were negative in 221 cases (72.93%) and positive in 40 cases (14.85%). The status of margins was not known in 42 cases (13.86%).

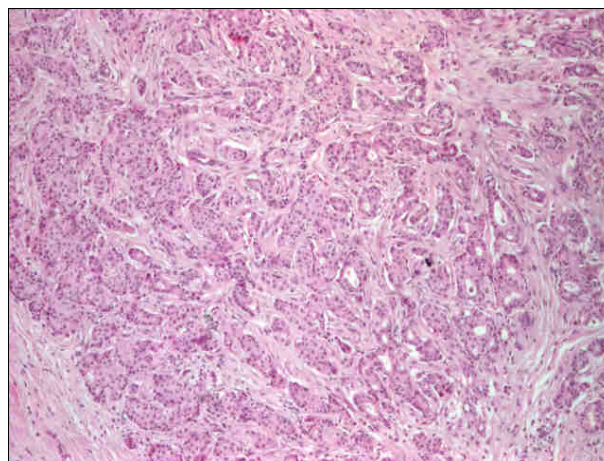


Fig.15. Moderately differentiated invasive ductal breast carcinoma (G 2), HE staining x 40.

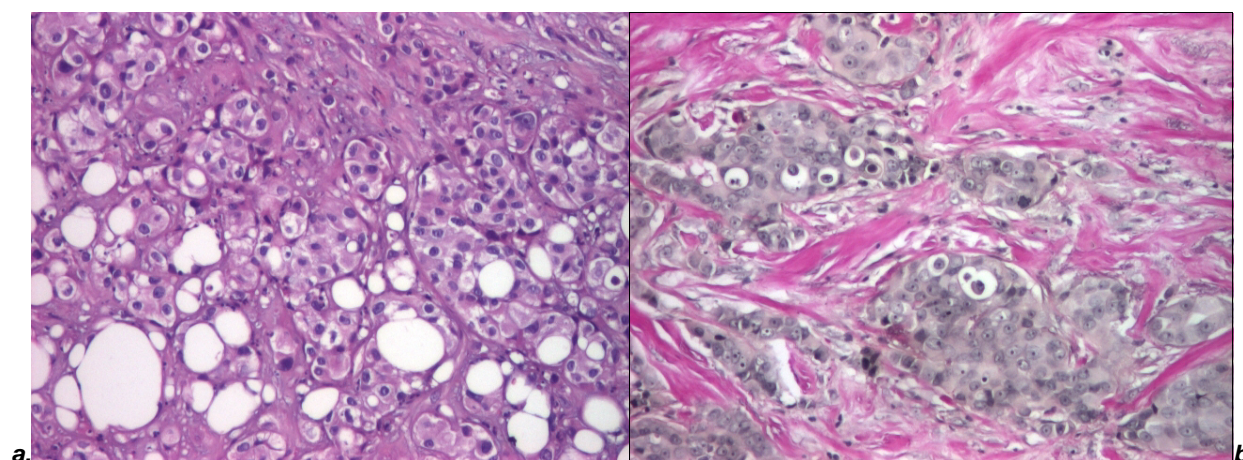


Fig.16. Poorly differentiated invasive ductal breast carcinoma (G 3) a) HE staining x 100 and b) Van Giesson staining x200.

Table 3. Resection margins status in the studied cases

| RESECTION MARGINS STATUS | POSITIVE | NEGATIVE | UNKNOWN |
|--------------------------|----------|----------|---------|
| Number of cases | 40 | 221 | 42 |
| Percentage | 14,85% | 72,93% | 13,86% |

HISTOPATHOLOGICAL ANALYSIS OF CASES PRESENTING WITH IPSILATERAL LOCAL RECURRENCES

Out of 303 patients in our study, all undergoing breast conserving surgery a total of 12 cases (3.96%) had recurrences.

Based on *histological type*, out of the 12 cases of breast carcinomas, operated conservatively, most recurrences were noted to be following originally diagnosed invasive ductal carcinomas, NOS (10 cases, 83.33%), 1 case (8.33%) followed initially diagnosed invasive lobular carcinoma and 1 case (8.33%) recurred after in situ breast comedocarcinoma.

Based on *grading* (malignancy degree), it was noted that most recurrences happened in

poorly differential tumors G3 (11 cases) whilst one case of recurrence followed a moderately differentiated tumor (G2). In our study no recurrences were associated with well differentiated carcinomas (G1).

Extensive intraductal component (EIC) was seen in one case whilst all other 11 malignancy recurrences appeared in cases lacking the extensive intraductal component.

Looking at the status of *resection margins*, assessed via paraffin embedding histopathological exam, it was noted that 6 of recurrences were associated with positive resection margins, 5 recurrences appeared following initial negative resection margins

whilst one case occurred following a primary tumor with unknown status of resection margins.

Based on *tumour size* we concluded that 5 of recurrences followed an initial carcinoma of ≤ 2 cm, 3 of them were associated with breast malignancies > 2 cm but ≤ 3 cm and 4 followed carcinomas > 3 cm but ≤ 5 cm.

According to *lymph node status*, 7 patients that had relapses presented initial positive lymph nodes at the time of diagnosis, whilst 5 relapses were seen in patients with initially negative lymph nodes.

Looking at *patient's age* it was highlighted that 8 relapses happened within the group of patients aged ≤ 40 years whilst only 4 patients aged > 40 relapsed.

The statistical analysis of the data, as shown in the table below, indicated that all results in terms of histology, presence of extensive intraductal component, primary mammary tumor size and lymph node status associated with recurrences following breast conserving surgery ($p > 0.05$, see the table below) are of statistical significance. In regards to histological grade, it was observed that ipsilateral recurrences were more often correlated with poorly differentiated primary tumors (G3) when compared with moderately differentiated tumors and (G1-G2), difference being statistically significant ($p < 0.05$). Also, it was noted, that patients age at the time of primary diagnosis played is very important part in determining future ipsilateral recurrences. Thus, breast carcinoma relapses were more commonly seen in patients aged ≤ 40 years compared to those over 40 years, the difference being of high statistical significance ($p < 0.01$). Based on our observations, the parameter that appears to bear the most significance in determining future risk of ipsilateral recurrence is the status of resection margins. Patients with positive resection margins had a high risk (of high statistical significance) of developing recurrences after breast conserving surgery when compared to those with negative margins ($p < 0.001$, see table below).

Table 4 Statistical analysis of studied parameters in relation to risk of ipsilateral malignancy recurrences

| Analyzed parameter | p |
|-----------------------------|---------|
| HISTOLOGICAL TYPE | |
| In situ carcinoma | 0,23 NS |
| Invasive carcinoma | |
| HISTOLOGICAL GRADING | |

| | |
|--|--------------|
| G1/G2 | 0,042* S |
| G3 | |
| EXTENSIVE INTRADUCTAL COMPONENT | |
| Positive | 0,114 NS |
| Negative | |
| TUMOR SIZE | |
| ≤ 2 cm | 0,706 NS |
| $2 < T \leq 5$ cm | |
| LYMPH NODES STATUS | |
| Positive | 0,476 NS |
| Negative | |
| RESECTION MARGIN STATUS | |
| Positive | 0,00039* VHS |
| Negative -Unknown | |
| PATIENTS AGE | |
| ≤ 40 years | 0,0053* HS |
| > 40 years | |

Discussions

This histopathological study was conducted on a sample of 303 patients, diagnosed with breast cancer, stages 0-IIIb, TNM stages T1-T2 and T4b, measuring less than 5 cm, treated with breast conserving surgery. All cases involved axillary dissection as well as postoperative breast radiotherapy.

Current guidelines, recommend that patients with early stages breast cancer, are treated via breast conserving surgery followed by whole breast irradiation [1], thereby reducing the risk of local recurrence [2,3] and mortality [4,5]. A number of randomized prospective studies and retrospective studies have shown that breast conserving surgical interventions in patients with stages I and II breast cancer is similar to mastectomy in terms of overall disease-free interval and survival [6,7].

Tumor recurrences on the ipsilateral breast occur in about 8-20% of all women undergoing breast conserving surgery. The risk appears to peak at 10 years post breast conserving therapy [8,9]. Recently the National Surgical Adjuvant Breast and Bowel Project (NSABP) reported a cumulative incidence for IBTR (ipsilateral breast tumor recurrence) of 14.3% following breast conserving surgery. The study analysed cases over a period of 20 years [6]. Even if that rate is less than 1% per year, the increasing number of women opting for breast conserving surgery makes IBTR a clinically significant problem [10]. The ipsilateral mammary

recurrence can be defined as a malignancy recurrence on a previously treated breast. There have been a series of studies suggesting that there is a group of ipsilateral recurrences associating a relatively favorable prognosis. We have identified a number of factors linked to a favorable prognosis of a ipsilateral recurrence : older age, longer time interval lapsed between initial treatment and relapse, small sized tumors, non-invasive histology, low histological grading, negative axillary lymph nodes and different site of recurrence compared to the primary tumor site [11,12]. There was one hypotheses that suggested prognosis was based on the existence of two distinct types of IBTR: true recurrences and a new primary ipsilateral malignancy. This distinction was first made by Veronesi et al [13], who defined recurrences as genuine cases malignant cells regrowth, cells that were not surgically removed or were not destroyed by radiotherapy during initial treatment. New ipsilateral primary tumors are, according to the same authors, cases of de novo malignancies that arise from epithelial cells of remaining breast tissue. Therefore, often seen complicated IBTR behaviour may arise from the fact that patients with IBTR can actually be part of two separate entities. Theoretically, IBTR as a new primary tumor is a primary breast cancer and the prognosis of these patients may be more favorable compared to those who suffer a true recurrence. Another hypothesis is that the development of new primary tumors may indicate a genetic predisposition to breast carcinoma and is therefore associated with an increased risk of developing carcinoma in the controlateral breast [14]. Based on these two assumptions, it appears that a de novo tumor and genuine recurrences have a different natural history, a different prognosis and therefore a different therapeutic management. Based on location and histology IBTR usually is divided into two different subgroups. Given that an exact location and a clear relationship between initial and recurring tumor can be difficult to assess, using histological classification as another adjuvant factor may be helpful. It has been reported that when the classification scheme only employs location, the rate of de novo tumors is 37% compared to 51%, when taking into account DNA and histological aspects as well [15]. Huang et al [14] have defined a malignancy as a true malignancy if it is located within 3 cm of the primary tumour bed and if it shows consistent histological similarities to the primary malignancy.

Similarly, a de novo tumor is suggested by its association with in situ carcinoma component and better histological grading of the recurrence. Haffty et al [16] have divided these tumors based on the location and DNA flow cytometry . They concluded that DNA modification through conversion from aneuploid DNA to diploid DNA suggests a de novo type "recurrence". In the current study we focused on the analysis of local recurrences, without classifying them into the two recently proposed subgroups. We noted an overall recurrence rate of 3.96% of all analyzed cases.

A very important observation, made during our study, was that the age of patients, at time of diagnosis factored heavily in the rate of ipsilateral recurrence. We noted recurrences to be more common in patients aged ≤ 40 years compared to those over 40 years, the difference being of high statistical significance ($p < 0.01$). Other studies have also shown the strong influence of young age on the risk of ipsilateral breast tumor recurrence. Jobsen and collaborators have previously reported that being aged < 40 was the only significant predictor of ipsilateral breast tumor recurrence in women post breast conserving surgery, who had pT1 grade malignancies as well as negative lymph node status [17]. Similarly, Harrold et al showed a correlation of young age with recurrences of ipsilateral breast tumor using the age of 40 as a cut-off point [17]. In another study, Freedman et al also found that age is a risk factor for recurrence of ipsilateral breast tumor but the cut-off age they used was 55 years [19]. In the 2006 statistical analysis by Komoike Y et al, the results were similar showing that young patients have the highest risk of ipsilateral breast tumor recurrence. In their study, Diab and Daidone have shown that older patients have a low risk of recurrence when compared to young patients. Malignancies appearing in older patients were more likely to have be of a biology more compatible with a favorable evolution [20,21]. More recent studies such as the 2011 one conducted by Arista show that the risk of local recurrence decreases with increasing age. The same author shows that age is a protective factor for local recurrences but not for recurrences located within lymph nodes [22].

The axillary lymph nodes status, as assessed in our study, showed that 47.85% of cases had invasion of the axillary lymph nodes, whilst 51.15% of cases had negative axillary lymph nodes. In previously existent literature, we note a reported analysis of a series of 671 elderly

patients with diagnosed breast cancer with non-palpable axillary nodes who underwent breast conserving surgery with axillary lymphadenectomy. The percentage of affected lymph nodes on histological preparations, was reported to be 34% [23]. In the 2006 study of Komoike et al 2006, conducted on a much larger number of patients (1901 cases) undergoing breast conserving surgery following diagnosed early breast carcinoma, 20% of cases had positive lymph nodes. It is noteworthy that the study included a large number of cases with unknown axillary node status. Within univariate analyses, the same study found no significant association between axillary lymph positivity when compared to recurrence rate ($p = 0.141$). Similarly, in our study, we found no significant correlation between recurrences and the status of axillary lymph nodes ($p = 0.476$), although we noted relapses to be slightly more common in patients that had positive nodes at the time of interventions compared to those with unaffected lymph nodes (4, 82% vs 3.16%). On the other hand, Komoike and collaborators showed, in their analyses, that initial status of axillary lymph nodes is significantly associated with distant metastases arising after ipsilateral recurrences. Univariate analysis showed in this 2006 study, that initial metastases within the lymph nodes, lymphovascular invasion, malignancy grading and time lapsed between first intervention and local recurrence were significantly associated with distant metastases. Similar studies have shown that these risk factors appear to reflect an inherent aggressiveness characteristic for the primary tumor [24,25].

When looking at the *histological characteristics* of the analyzed tumors, it was observed that invasive tumors were more frequent compared to in situ tumors (93.4% vs 6.6%). Among invasive carcinomas, the most common histological form was invasive ductal carcinoma, not otherwise specified (NOS) (63.37%) followed by invasive lobular carcinoma (10.56%) and mixed ductal-lobular invasive carcinoma (6.27%). The other particular types of invasive carcinomas were rare, each representing less than 4% of total cases. In regards to in situ carcinomas we noted that the most common histological types were intraductal carcinoma cribriform type and intraductal comedocarcinoma, each being diagnosed in 1.65% of cases. These were followed by solid type intraductal carcinoma present in 1.32% of cases, whilst other types

were seen in less than 1% of the cases. Within the 12 reported cases of recurrences following breast conserving surgery it was noted that most patients had mainly an initially histological diagnose of invasive ductal carcinomas NOS (10 cases), 1 case being that of primary invasive lobular carcinoma and another one presenting with initial in situ comedocarcinoma. Thus, of the total number of recurrences, 83.33% of all cases followed an invasive ductal carcinoma, 8.33% were secondary to invasive lobular carcinoma and 8.33% were subsequent to in situ carcinomas. When analysed statistically the rate of relapse as influenced by histology did not show statistically significant results ($p > 0.05$). However, we note that invasive breast carcinomas are more likely to relapse compared to in situ ones (4.24% vs 1.7%) and of all invasive carcinomas only the lobular and ductal subtypes were followed by malignancy recurrences.

Komoike and coworkers reported similar results in 2006. Following univariate statistical analyses they failed to find statistically significant differences between the risk of local recurrence and the type of breast carcinoma (invasive / non-invasive / other, $p = 0.6053$). In his study NR West showed that from all reported ipsilateral recurrences relapses followed, invasive ductal carcinoma in 79.2% of cases, mixed ducto-lobular carcinomas in 8.3% of cases and other types of carcinomas in 12.5% of cases. These results are consistent with findings highlighted in our study as well [26]. In regards to lobular carcinoma, available literature indicates that invasive type is likely to be associated with an increased risk of developing a new primary malignancy of the contralateral breast when compared to other histological types [27]. Other prospective and retrospective studies have shown that currently, invasive in situ lobular carcinoma and lobular carcinoma co-diagnosed with an invasive carcinoma can be managed successfully allowing breast preservation [28,29].

When talking about in situ carcinomas, and more precisely comedonecrosis, as reported in our study, this was the only type that associated local recurrence. The recurrence rate for this particular type of carcinoma was 5% compared to 0% to all other types of in situ carcinoma. ER Fisher's 2007 study, conducted on 1,456 patients diagnosed with in situ carcinomas showed that after a follow-up period, averaging at 10.5 years, comedonecrosis, in situ carcinoma micropapillary type and multifocal in situ carcinomas develop

into independent risk factors for mammary gland ipsilateral tumor relapse. The same study showed that the risk increases if existent low comedonecrosis vs absent comedonecrosis, as well as existent moderate comedonecrosis vs low comedonecrosis. The same authors found that the micropapillary type malignancies, larger than 1 cm, are independent risk factors for cancer of the contralateral breast [30].

Analyzing of *histologic score*, with the help of the Nottingham grading system, we found that the vast majority of carcinomas in our study group (63.04%) were grade 3 carcinomas. At the opposite end (7.26% of cases) were grade 1 carcinomas whilst grade 2 was found in 29.70% of cases. Regarding recurrences, it was observed that most relapses occurred in poorly differentiated carcinomas - G3 (11 cases, 5.76%), followed by relapse of G2 carcinomas (moderately differentiated) with 1 reported case (1.11%). On the other hand G1 carcinomas (well differentiated) were not accompanied by any recurrences, as reported by our study. Following statistical analysis it was observed that ipsilateral recurrences were more likely linked to primary poorly differentiated carcinomas (G3) when compared with well and moderately differentiated ones (G1-G2). The difference was noted to be statistically significant ($p < 0.05$).

In his 2011 study, conducted on 575 patients diagnosed with T1-T2 breast carcinomas treated with breast conserving surgery and axillary dissection, Arista noted that the risk of recurrence within lymph node was higher in patients with G3 poorly differentiated carcinomas compared to G1-G2 carcinomas ($p = 0.005$). One of the most important criterios in determining histologic score according to the Nottingham system is the nuclear grade. According to nuclear grade, Komoike et al showed in a 2006 study that ipsilateral local recurrences are more common in patients with nuclear grade 3 carcinomas compared to those with grade 1 and 2 carcinomas. However, following univariate statistical analysis there was no statistically significant difference detected ($p = 0.0650$). The study conducted in 2010 by Liau SS et al on a group of 563 patients with unilateral invasive breast carcinoma (T0-T3, N0-N1, M0) undergoing breast conserving surgery in Cambridge hospital, showed that in patients receiving appropriate local therapy (adequate resection margins and radiotherapy), tumor biology becomes, probably, the single dominant predictive factor for local ipsilateral recurrence [135]. A number of surrogate

markers for the aggressiveness of the tumor can be obtained via conventional histopathological analysis of excised specimens. Therefore histological grading, tumor size and lymphovascular invasion have been shown to be predictive factors for local ipsilateral recurrences and overall survival rates [32,33].

Association between *Extensive intraductal component* and invasive carcinomas operated with breast conserving techniques, was highlighted in 5 cases, throughout this study, accounting for only 1.65% of total cases. The incidence of an extensive intraductal component as reported by previous studies ranges from 13.3% -39.0% [138,139]. Specialist research showed the presence of an extensive intraductal component in only 5% of cases in a study by Freedman et al in 1999. This was conducted on a sample of 1262 patients with stage I and II breast cancer managed with conservative breast surgery [36]. The intraductal extension of breast carcinoma is considered to be a non-invasive, continuous component of a tumor originating from the ductal or lobular epithelium that interests the terminal ductolobular unit [37] which extends along the ductolobular system towards the nipple, while keeping the integrity of basal membranes [38]. The term of extensive intraductal component, based on routine histological examination has been widely used to describe an intraductal malignancy with marked extension [39].

According to statistical analysis in our study, the association between an extensive intraductal component and invasive breast carcinomas was not significantly correlated with ipsilateral local recurrences ($p = 0.114$), although 20% of carcinomas that had an extensive intraductal component relapsed compared with only 3.96% of tumors without extensive intraductal component. The results were similar to those of Komoike et al (2006) who after univariate analysis noted that the extensive intraductal component was significantly correlated with distant metastases but not with ipsilateral recurrences following breast conserving surgery [40].

However the extensive intraductal component seems to be the main factor associated with positive resection margins following breast conserving surgery, therefore playing a role in postoperative recurrences [41]. In this study, the analysis of resection margins was possible in 261 cases. We remind readers that surgical margins were defined as positive if we noted the presence of malignant cell - in situ

or invasive- in one or more limits of resection and negative if there were no malignant cell noted in any of the resection limits. According to the above criteria it was found that 15% of cases had positive margins following breast conserving surgery, 1.5% of cases had negative margins whilst in 2.38% of cases the status was inconclusive. The statistical analysis showed that positive resection margins, were significantly linked with high incidence of recurrences ($p = 0.00039$). As a result, we concluded that this is likely to be the most important parameter in regards to the risk of ipsilateral recurrence. Patients with positive resection margins were statistically significant at high risk of recurrence following breast conserving surgery compared to those with negative margins ($p < 0.001$).

It should be noted that when it comes to defining the status of surgical resection margins this is not standardized. Some authors define this status similar to the current study as "positive" and "negative" [6,42]. Other studies evaluate the resection margins based on the distance between presence of malignant cells and resection margin. However it is noteworthy that this distance ranges from less than 1 mm to less than 10 mm [25]. It should also be noted that there are some technical difficulties in assessing resection margins [43]. Thus, when more specimens are excised from the same primary tumor, it is difficult to establish the relationship between these specimens in order to establish the resection margins. Based on local characteristics it is noteworthy that when talking about the mammary gland the area of specimen excision is often adipose and irregular, leaving a big and difficult to assess area. Although a special ink is used to mark the edges of the surgical resection specimen, it can diffuse into the gap making it difficult to assess the true resection margins (Schnitt et al, 1994). Other authors have employed cytological evaluation of cells collected from the edges of the resection specimens, assessed sometimes with the aid of immunohistochemical staining and tumour cells markers [44,45]. Despite these limitations, recent studies continue to show that assessing the resection margins status continues to be extremely useful for predicting the risk of local recurrences. In their 2006 multivariate analysis, Koimoke Y et al showed that young age, positive resection margins and omitting radiotherapy are significant predictive factors for ipsilateral recurrences [40].

Some investigators [46] have also found that local recurrences are not particularly common when resection margins are negative. These observations are consistent with the results of our study, which showed that when the margins are negative, the recurrence rate is much lower than in cases with reported positive margins (1.5% vs 15%). According to the analysis of Freedman et al 1999 [36], conducted on 1262 patients that underwent both breast conserving surgery and radiotherapy, negative margins (> 2 mm) identify patients at a very low risk of ipsilateral breast recurrences (7% in 10 years). At the same time, they show that patients with margins measuring ≤ 2 mm have a higher or equal risk of ipsilateral recurrence when compared to patients with positive margins, especially after reexcision. There is no difference reported between positive resection margins of intraductal carcinoma when compared to positive resection margins of invasive carcinoma in terms of risk for ipsilateral recurrence. A re-excision of margins, of an initially positive margin which will lead to a negative final margins, will reduce the risk of ipsilateral recurrence at a level equal to an initial negative excision margin. Positive margins or close malignant positivity to resection margins are associated with an increased risk of ipsilateral recurrences even in patients that have a negative intraductal component or have received a high dose of radiation [36]. Thus, we conclude that the use of breast conserving surgery as a treatment for patients with positive margins is a controversial method especially as this adds another risk factor to the overall chance of local recurrence.

Conclusions

Breast conserving surgery for stages I and II breast cancer is a viable option that offers patients the same chance of postoperative survival as mutilating surgery.

The emergence of local recurrences following breast conserving surgery for early breast cancer is frequently correlated with poorly differentiated primary tumors (G3) compared with well and moderately differentiated tumors (G1-G2), the difference being of statistical significance ($p < 0.05$, S).

The emergence of local recurrences after breast conserving surgery for early breast cancer are not correlated with histologic type, presence of extensive intraductal component, size of primary breast malignancy or lymph node status ($p > 0.05$). However, invasive ductal carcinoma

and in situ comedocarcinoma causes frequent ipsilateral local recurrences consecutive following breast conserving surgery. Invasive tumors associated with an extensive in situ component are five times more likely to relapse as opposed to those without this association.

According to the current study, the parameter that appears to factor more than anything in determining the risk of ipsilateral recurrence is the status of surgical resection margins. Patients with positive resection margins have a statistically significant, very high risk of developing recurrences following breast conserving surgery compared to those with negative margins ($p < 0.001$, VHS).

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