Universitat de Girona
Facultat de Medicina

Pathological assessment of the margin status to reduce the recurrence rate in women with DCIS of the breast

A retrospective and multicenter cohort study

END OF TERM PROJECT

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18/01/2016

I would like to thank Dr. Josep Trueta's Hospital Breast Pathology Unit for their time and encouragement: Dra. Ester Vila, Dr. Francesc Tuca, Dra. Alejandra Azkargorta and Dra. Alexandra Bonmatí.

Thank you for opening my eyes to the world of the gynaecology and the breast pathology unit.

I would also like to thank all the people who got involved with this project.

INDEX

1. ABBREVIATIONS

BCS Breast Conserving Surgery

BPU Breast Pathology Unit

CIS Carcinoma In Situ

DCIS Ductal Carcinoma In Situ

ER Estrogen Receptors

HER2 Human Epidermal Growth Factor Receptor 2

HUJT Hospital Universitari Josep Trueta

LCIS Lobular Carcinoma In Situ

LR Local Recurrence

MRI Magnetic Resonance Imaging

PgR Progesterone Receptor

RT Radiotherapy

SM Surgical Margins

US Ultrasound

2. ABSTRACT

Introduction: Breast cancer is a major health problem in women worldwide. Many efforts have been done to develop screening strategies and to improve the treatment in order to reduce its incidence, recurrence rate and mortality. Ductal carcinoma in situ is a pre-invasive breast tumor. Despite being more benign than the invasive tumor, it is more difficult to ensure clear margins after the lumpectomy. Over the last years, margin distance has been studied without reaching a consensus. This margin status is the most important prognostic factor in this type of tumor.

Objective: The goal of this study is to determine the minimum margins that have to be left in ductal carcinoma in situ undergoing conservative surgery. The objective is to reduce the recurrence rate. The aesthetic result is important too, so it is vital to find the best relation between the recurrence rate and the amount of breast tissue removed. Our aim is to compare the recurrence rate obtained depending on the millimetres left (free from tumor cells) around the tumor. We want the pathologist to take our conclusion distance of the study as a reference to define when a margin is free from tumor cells in the future.

Methodology: A longitudinal retrospective and multicenter cohort carried out in the Breast Pathology Unit at eight referral hospitals in Girona. The period of study will be from 2000 to 2017. The sample will be formed by three groups of patients classified by the distance in millimetres of the margins assessment.

Participants: Women diagnosed with ductal carcinoma in situ undergoing breast conservative surgery plus radiotherapy between 2000 and 2015. The patients will be selected from the database of the Breast Pathology Unit or the SAP of eight hospitals in Girona.

Keywords: Breast Cancer, Ductal Carcinoma In Situ, Lumpectomy, Breast Conservative Surgery, Radiotherapy, Margin Status, Millimetres, Recurrence Rate, Pathologist.

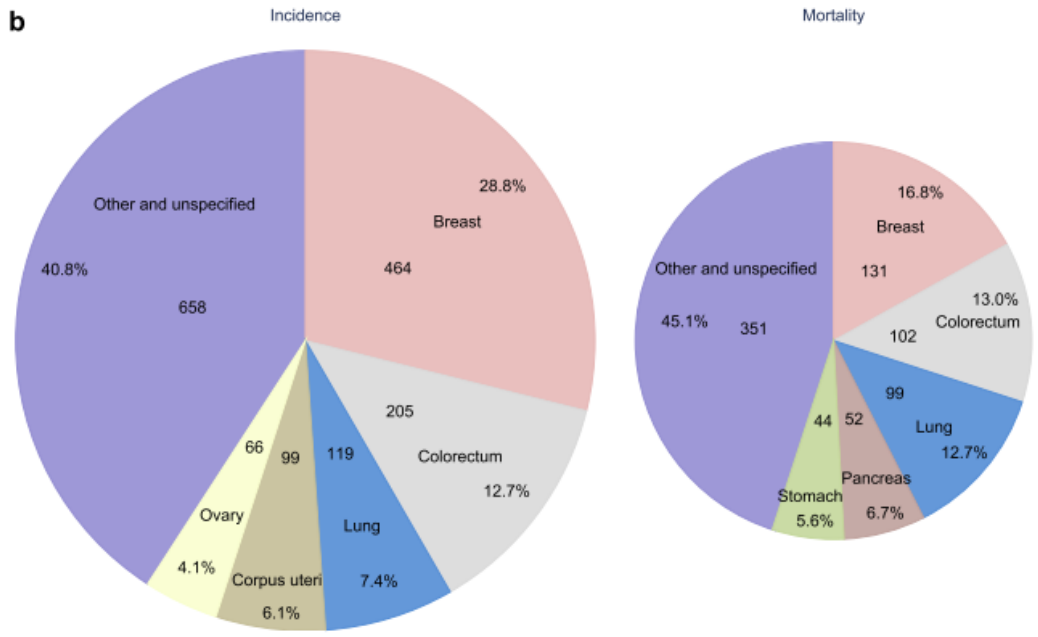
3. INTRODUCTION

3.1. BACKGROUND

3.1.1. EPIDEMIOLOGY

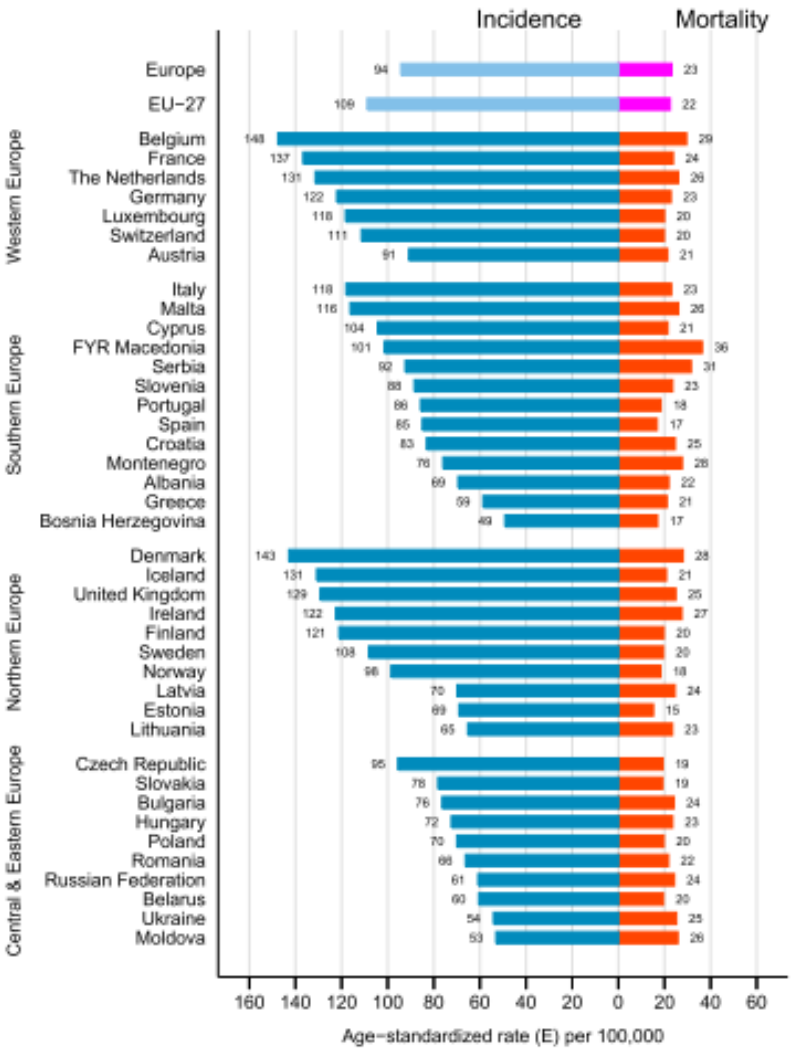
Breast cancer is a very prevalent disease. It has a huge impact in women life, since it is an important cause of death among women. In 2012, the estimated age-adjusted annual incidence of breast cancer in 40 European countries was 94.2/100.000 and the mortality 23.1/100.000 (1,2). The estimated 5-year prevalence of breast cancer in Europe in 2012 was 1.814.572 cases (1).

The diagnosis of breast cancer increased after the introduction of mammography screening, and the incidence continues to grow with the ageing of the population (3). It is higher in women before 50 years. Only the 5% of the breast cancers happen in women under 35 years old (1).



Graphic 1. Distribution of the expected cases and deaths for the most common female cancers in Europe. Each coloured area of the pie chart reflects the percentage of the total number of cases or deaths (2).

In Spain, overall age-adjusted incidence was 83.1 cases per 100.000 women-years in 2000-04. Breast cancer is the most common tumor and the leading cause of cancer death in women in Spain (4). The Spanish regions registering the highest incidence in the period 2000–04 were Girona, Tarragona and Navarre, with age-adjusted rates of 95.5 cases per 100.000 women-years (5,6).



Graphic 2. Age-standardised incidence and mortality rates of breast cancer by area and country in Europe 2012. See the European map in ANNEX 14.1. (2)

Prevalence has risen due to the increased incidence and also because of the improvements in treatment outcomes. In particular, it has improved treatment and early detection that has decreased breast cancer mortality a 19% from 1989 to 2006. Women under 50 years showed the greatest reductions in mortality (7,8). However, breast cancer is still the leading cause of cancer-related deaths in European women (9).

When we talk about the detection of breast cancers at a pre-clinical stage, it has also increased. This is due to the introduction of the population-based mammography screening programmes in women between 50 and 69 years (6,10,11). The evidence for effectiveness in women between 40 and 49 years is limited because this screening also leads to over diagnosis and overtreatment (by 30%) (12).

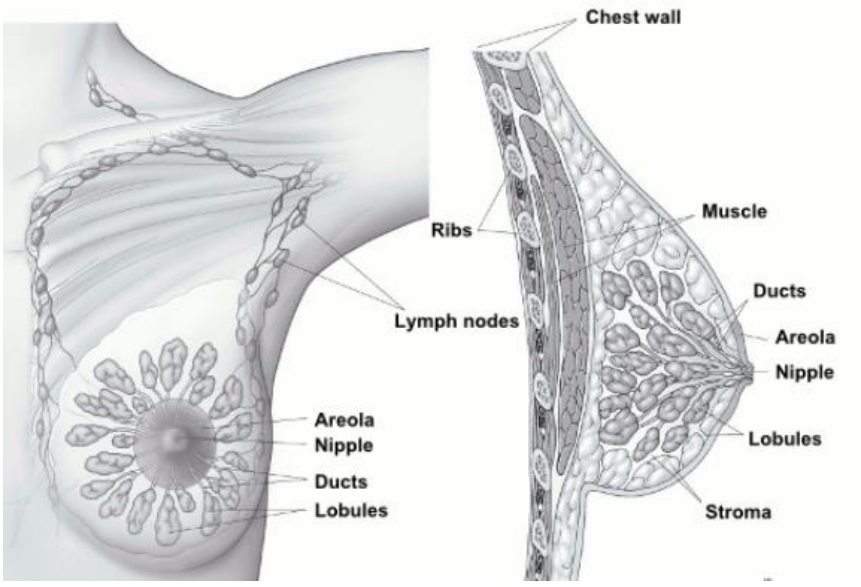
Women with familiar breast cancer, with or without proven BRCA mutations, have to follow a different screening program. It is recommended to carry out an annual MRI concomitantly or

alternating every 6 months with mammography. It has to start 10 years younger than the youngest case in the family (9).

If we take into account the tumor stage there are different types of breast cancer (ANNEX 14.2). We can differentiate between the in situ tumors and the infiltrative ones.

The term "carcinoma in situ" describes abnormal epithelial cells that have not invaded nearby tissues. Conversely, the invasive tumor cells exceed the basement membrane of the ducts into the surrounding breast tissue. Nevertheless, their cells look very similar when viewed under a microscope (13). Inside the in situ carcinoma, there are two types: the lobular and the ductal tumors.

Specifically, we are going to study the ductal carcinoma in situ. It accounts for approximately 20% to 30 % of breast cancers diagnosed by mammography in women (13,14).



Picture 1. Normal anatomy of the breast (15). The female breast is made up mainly of: lobules (milk-producing glands), ducts (tiny tubes that carry the milk from the lobules to the nipple), and stroma (fatty tissue and connective tissue surrounding the ducts and lobules, blood vessels, and lymphatic vessels). Most breast cancers begin in the cells that line the ducts (ductal cancers). Some begin in the cells that line the lobules (lobular cancers), while a small number start in other tissues.

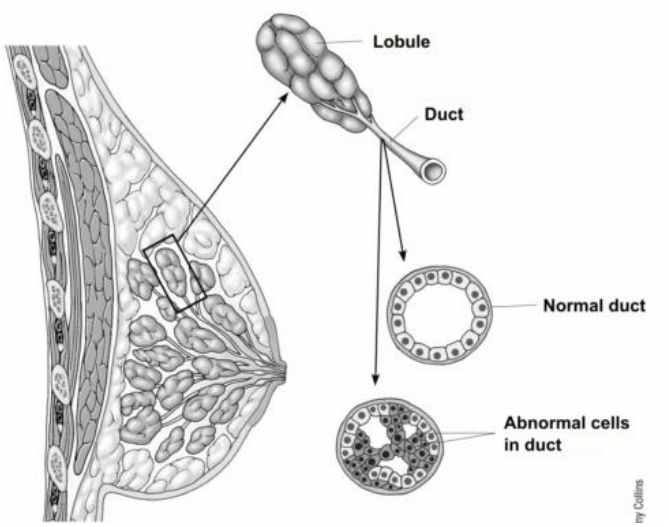
Breast cancer in males is rare, contributing to less than 1% of all male tumors. However, during the last few years there has been an increase in the incidence, along with the increase in female breast cancer (16).

The major male risk factors include: clinical disorders carrying hormonal imbalances (especially gynaecomastia and cirrhosis), radiation exposure and in particular, a positive family history and genetic predisposition (BRCA genes)(17).

About 90% of all male breast tumors have proved to be invasive ductal carcinomas, and this histological type of breast cancer is not the objective of our study. Besides, they are usually treated with mastectomy (17).

3.1.2. DUCTAL CARCINOMA IN SITU

The Ductal carcinoma in situ (DCIS) is a proliferation of malignant-appearing cells of the ducts and terminal lobular units that have not breached the ductal basement membrane. It is a heterogeneous early-stage (stage 0) and pre-invasive disease (14,15,18).



Picture 2. Breast anatomy and DCIS (15). The tree-like branching structure of normal breast ducts.

As explained before, DCIS accounts for 20 to 30% of newly diagnosed breast cancer cases. The majority are asymptomatic, non-palpable and detected primarily by screening mammography, where the 90% are identified by the appearance of microcalcifications (4,19). The microcalcificactions are tiny bits of calcium that appear as clustered white dots in the mammography. They are harmless but indicate the possible presence of in situ or invasive cancer (13).

It is still unknown if this tumor is an inevitable step in the development of invasive breast cancer or merely a marker of risk. It is also unknown the proportion of detected DCIS lesions that will progress into an invasive cancer because almost all women receive some treatment before (4). However, the purpose of treating DCIS is to achieve local control of the disease and to prevent an invasive breast cancer.

The risk of ipsilateral breast cancer has been demonstrated to be highly dependent on the given treatment. 40% to 50% of local recurrences are invasive and represent a potential threat to life translated into lower survival rates. This is due to the higher possibility of distant failure (13). The other recurrences are another DCIS.

As a DCIS has not spread to the surrounding tissue, it is by definition not able to cause death. However, in larger observational studies of patients with DCIS the 10-year overall survival was reported to be 92% (20). The 10-year risk of dying from breast cancer amongst patients with DCIS is four times higher than the risk for women in the general population (21).

3.1.3. RISK FACTORS AND PROGNOSTIC FACTORS

It has been reported that risk factors associated with DCIS are similar to those associated with invasive breast cancer. But there are some differences related to the variety of histological types of DCIS (22).

The most important **risk factors** include: age, female gender, genetic predisposition, exposure to estrogens (endogenous and exogenous), ionising radiation, low parity and a history of atypical hyperplasia. The Western-style diet, sedentary lifestyle, obesity and the consumption of alcohol may also contribute to the rising incidence of breast cancer, but the results have not been conclusive (22,23).

- The *age* is the main risk factor for DCIS. In general, DCIS incidence rates increase with age and peak at ages 70-79 (22).

- *Gender risk factor*: women have more risk than men (100:1). Men have less estrogen and progesterone which can promote breast cancer cell growth (15).

- Risk is also increased among women with a family history of a first-degree relative. The risk is amplified if they had breast cancer or ovary cancer at a young age (22). Genetic predisposition also takes into account genetic mutations: BRCA 1 and 2 genes (24). In BRCA1 mutations the lifetime risk of breast cancer seems to be in the range of 55 to 65%. For BRCA2 mutations the risk is lower, around 45% (15).

- *Reproductive factors*: those patients that have high exposure to estrogens have an increased risk of breast cancer. Such as early onset of the first menstruation (less than 12 years), late menopause (after 55 years) or use of hormone replacement therapy (estrogen and progesterone) after menopause (15,25). Nulliparity and late first child are also associated with more risk (13,22).

- *Previous chest radiation*: Women who had radiation therapy to the chest area as a treatment for another cancer. The risk is highest if the radiation was given during adolescence, when the breasts were still developing. Radiation treatment after age 40 does not seem to increase breast cancer risk (15).

- *High mammographic breast density* (more glandular and fibrous tissue and less fatty tissue) is a risk factor for invasive breast cancer 1.2 to 2 times. It may also increase risk for DCIS. This association is higher in women younger than 55 years. Breast density is also a risk factor for the development of contralateral breast cancer after DCIS

treatment (26). A number of factors can affect breast density, such as age, menopausal status, certain medications (including menopausal hormone therapy), pregnancy, and genetics. It also complicates the diagnosis of a tumor (15).

The most important **prognostic factors** are the following. They are studied to estimate the likelihood of progression or recurrence (9,13):

- *Tumor size*: DCIS very rarely forms a rounded solid mass because the normal breast has a tree-like branching structure. It ramifies within the duct system. It can also extend into the major duct running towards the nipple (27). That's why the measurement of DCIS in two-dimensions underestimates the total size of the in situ tumor. With larger sizes, is more difficult to ensure complete removal too.

- *Extension of the tumor*:
    - Located: one tumor focus, of any size and precise or imprecise boundaries
    - Multiple: Multiple foci of DCIS separated from the principal or located in different quadrants of the breast. It is also called multicentric.

- *Histological grade*: determines the breast cancer management and therefore has a high prognostic value.

  The most accepted method is the Nottingham gradation. It measures three components of the tumor morphology: tubule formation, nuclear pleomorphism and mitotic index. Each one is scored from 1 to 3 and the sum of the overall histologic grade is obtained: Grade I (3-5 score) / Grade II (6-7 score) / Grade III (8-9 score) (18).

  Higher-grade tumors have more cells with abnormal-looking nuclei and have a bigger probability of progression and recurrence.

- *Biological prognostic markers* (ANNEX 14.3.):
    - Expression of ER and PgR: determining hormone receptor is a prognostic marker and a predictor of response to hormonal therapy. It has been described as a positive result: 10% of tumoral cells positive to hormone receptor (28).
    - HER-2: it is necessary for the treatment with trastuzumab in the invasive cancer, but it is not so important in the DCIS because they do not influence the treatment.
    - Other optional markers: Ki-67, p53, bcl-2, cadherina-E

- *Tumor histology*: there are subtypes of DCIS based on how the cells are arranged when viewed under a microscope. However, these groups do not take into account important prognostic features such as nuclear grade, necrosis, and polarization (architectural differentiation). They are generally classified as (14):
    - Papillary: large papillations with fibrovascular stalks

- Solid: ductal filling with neoplastic cells
- Comedo: layer of neoplastic cells surrounding a central area of necrosis. It has aggressive characteristics (high nuclear grade and high proliferation rate).
- Micropapillary: fingerlike papillary projections into dilated ductal spaces
- Cribriform: radially oriented neoplastic cells forming glandular lumina

- The response to *treatment* and the amount of residual disease

- The status of the *surgical margins*. Both last factors will be explained later.

3.1.4. DIAGNOSIS

The diagnosis of breast cancer is based on clinical examination in combination with imaging, and confirmed by pathological assessment. Imaging includes bilateral mammography, ultrasound of the breast and regional lymph nodes and magnetic resonance imaging (MRI) (9).

DCIS is diagnosed primarily via mammography followed by stereotactic needle biopsy (ANNEX 14.4). However, MRI may improve the ability to detect and determine the size and nature of DCIS. It may be particularly useful when evaluating residual disease, occult invasion, and multicentricity (14).

It is recommended to perform annual mammographys in asymptomatic women between 50 and 69 years, because the periodic auto-examination is not enough for the early detection. It is also recommended in women below 50 years with positive clinical exploration or high risk factors (previous thoracic radiotherapy (RT) and genetic predisposition) (16).

The sensitivity of mammography to detect DCIS is 86% (29). The typical image in DCIS' mammography is the linear or multiple grouping of granular microcalcifications with a branching type pattern. It is not seen macroscopically, so we must consider the preoperative localization of the lesion by a harpoon as part of the therapeutic process (22).

Ultrasonography is done in the following cases (16):
- Presence of a nodule or mass on the mammography. To determine its characteristics.
- Palpable nodule or mass on clinical examination but not seen on mammography.
- Study of breast density >90%. And assessing density asymmetries.
- Women under 35 years
- To guide interventional procedures (biopsy location)

Once diagnosed, the disease stage should be assessed according to the TNM system (ANNEX 14.2). And the postoperative pathological assessment of the surgical specimens should be made according to the pathological TNM system too.

The pathological assessment of a DCIS should include: the number, location and maximum diameter of the tumor removed, the histological type and grade of the tumor, the evaluation

of the resection margins including the location and minimum distance of the margin, and a biomarker analysis (9).

Axillary node evaluation with sentinel lymph node biopsy is not required in this tumor. However, it may be reasonable in large and/or high-grade tumours, especially when mastectomy is required (9). The risk of a positive sentinel node with pure DCIS is small (7%–9%) and most of the metastases found are micrometastases or isolated tumour cells, detected by immunohistochemistry (30).

3.1.5. TREATMENT

Is well demonstrated that breast-conserving surgery (BCS) plus radiotherapy (RT) results in an equivalent survival to mastectomy alone for women with pre-invasive breast cancer (3,31). So the preferred surgical procedure for these patients is BCS with wide local excision of the tumor.

To decide the most appropriate treatment for DCIS: excision exclusively, excision plus radiation or mastectomy, the Van Nuys Prognostic Index is used (ANNEX 14.6). It predicts local recurrences for women with DCIS (18).

BCS or lumpectomy. It removes a part of the affected breast, including the area where DCIS is found and a margin of healthy tissue. Clips are used to demarcate the biopsy area, because DCIS may be clinically occult and further surgery may be required. It will depend on the margin status review by the pathologist (24).

A careful histological assessment of margins is essential to achieve acceptable cosmesis in addition to remove the entire tumor with appropriate margins (9). Aesthetic outcomes are substantially affected by the amount of tissue removed and poorer cosmesis has implications for quality of life, so there must be an appropriate balance (32).

Mastectomy. This procedure can also be done, for example in large or high-grade tumors and also if the patient prefers it. The removal of the entire breast is the most common alternative to BCS plus radiation for the treatment of DCIS. Furthermore, the breast reconstruction can be done in the same surgical procedure (16).

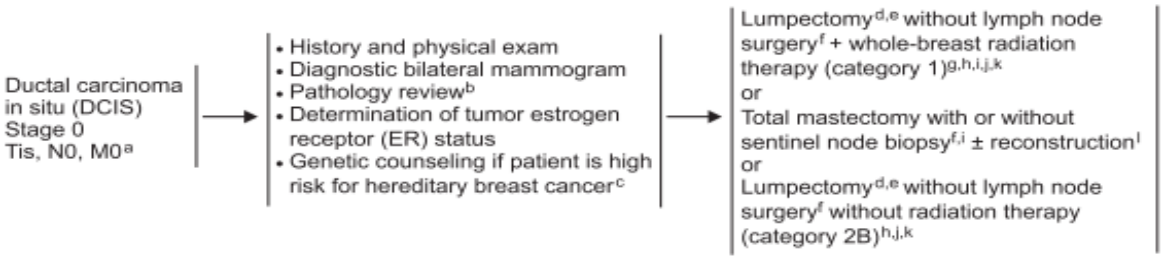
Mastectomy is the recommended treatment for those patients with (16):

- DCIS that involves 4-5 cm with a large tumor-to-breast ratio
- Those patients who should not receive radiation due to certain medical conditions or have received prior radiation therapy
- Patients for whom negative margins could not be achieved with BCS.

- Multicentricity: implies the presence of tumor cells in various foci. It contraindicates conservative surgery. These patients can be offered a simple mastectomy or a skin sparing mastectomy associated with immediate reconstruction in order to obtain a better aesthetic result (18,33). That is why we will not include this type of tumor in the study.

Women who have a mastectomy for DCIS have a very low probability of recurrence in the treated breast (1.45%). However, it remains an increased risk of developing DCIS or invasive breast cancer in the untreated (contralateral) breast (13,34).

The incidence of axillary involvement in DCIS is less than 1% and usually associated with occult microinvasive carcinoma. So the axillar lymphadenectomy is not recommended unless we suspect microinvasion (4,18).



Graphic 3. Diagnosis and surgical treatment possibilities in DCIS (24).

Margins. It is the distance in millimetres from the tumor to the inked surface of the whole surgical piece, healthy tissue included. When we talk about margins we refer to the six sides of a breast: the anterior, superior, lateral, inferior, medial and posterior margin.

The selection of a specific distance to declare negative or clear microscopic margins has been based on long-held opinions. There is still no universal agreement on what is an adequate negative margin for these tumors (31,35). A consensus seems to exist that margins greater than 10 mm are adequate and margins less than 1 mm are inadequate, but no uniform consensus exists for margin status between these values (24).

It is known that no tumor at the inked margin is required and more than 2 mm is preferred (ANNEX 14.5). But it is unknown which is the best relation between margins left and the recurrence rate, taking out the less breast mass as possible (32).

Data synthesis across studies showed that positive and close margins significantly increase the odds of local recurrence (LR) relative to negative margins. And positive margins are associated with 2-fold increase in the risk of local recurrence when compared with negative margins (24). But each study makes its own conclusion on what is a negative margin.

It is demonstrated that the microscopic status of the surgical margin (though not an exact test but reliant on examination of representative tissue sections) is a strong and robust prognostic

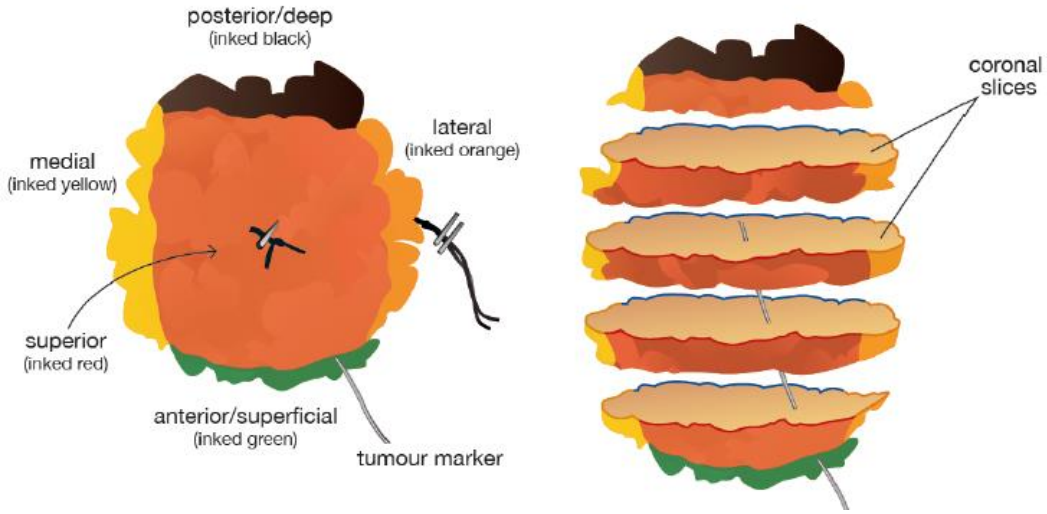
factor for LR (32). New surgical resection is recommended until an adequate margin is obtained.

In published international studies, the range of positive margins in breast cancer conservative surgery is between 20% and 40%. Therefore, approximately 25% of women treated with BCS undergo re-excision to attain more widely clear surgical margins (36).

However, an additional surgery may lead into a deterioration of the cosmetic result and increase surgical complications. It contributes also to emotional stress in both patients and family members, increase medical costs and in many cases, a delay in the beginning of the adjuvant treatments (35). Reductions in re-excision rates would reduce all these factors and may improve cosmetic outcomes following breast conservation therapy. As well, it would reduce the likelihood of mastectomy in these patients (37).

Despite all, negative surgical margins do not guarantee the absence of residual cancer within the breast. Histological studies have shown that additional cancer can be found in a substantial proportion of women despite an adequate surgical resection. Therefore, the goal of margin evaluation is not to ensure that there is no residual tumor in the breast. The aim is to identify those patients more likely to have residual tumor and will require further surgery (32).

A negative margin predicts that residual tumor is minimal and it is likely to be controlled with RT and systemic therapies (32).

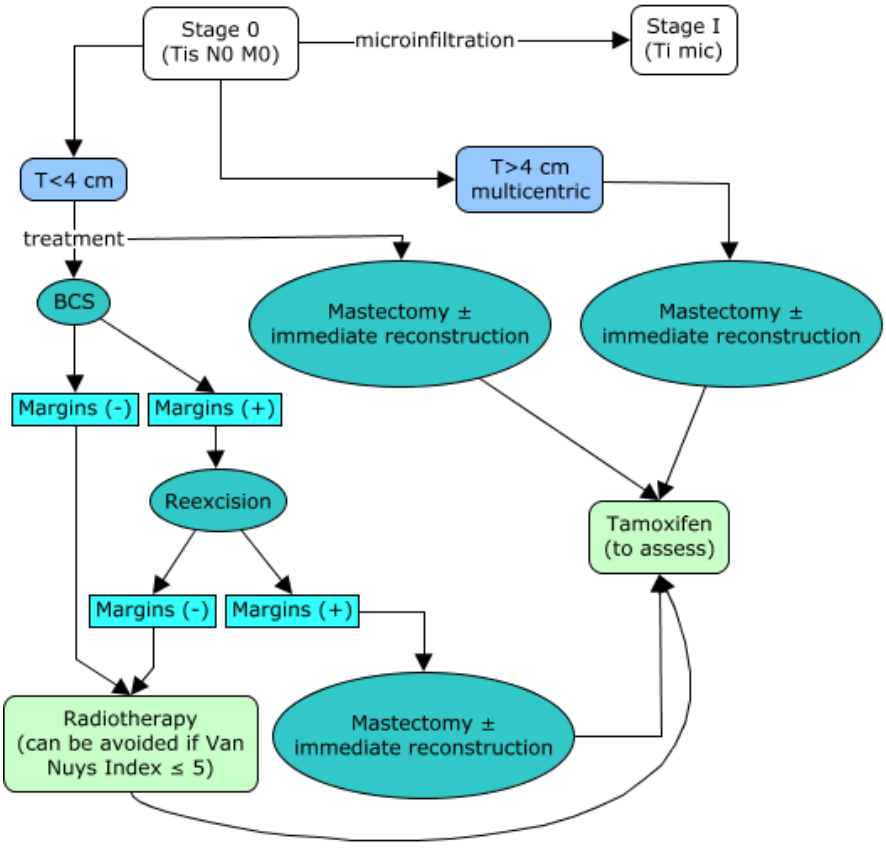


Picture 3. The 6 sides of a breast tumor: anterior, superior, lateral, inferior, medial and posterior. *Mansel, R. Margins-Mistakes to avoid. Curs de controversies en patologia mamaria, HCB. 2015 Jun.*

Radiotherapy. The decrease in the risk of local recurrence by RT is evident in all subtypes of DCIS (3). However, there are also some drawbacks and risks because of the radiation applied. In some patients with low-risk DCIS based on Van Nuys Prognostic Index (ANNEX 14.6) the risk of local recurrence following excision alone is low, and omitting radiation may be an option (9,13,38).

Systemic treatment. The decision on systemic adjuvant treatment should be based on: the predicted sensitivity to particular treatment types, the benefit from their use and the individual's risk of relapse. The final decision should also incorporate: the treatment consequences and the patient's age, general health status, co-morbidities and preferences. Treatment should start within 2–6 weeks after surgery (9).

In patients with ER-positive DCIS treated conservatively, tamoxifen decreases the risk of both invasive and non-invasive recurrences. It also reduces the incidence of a second primary (contralateral) breast cancer (39). This treatment has to be maintained during 5 years (4). Women should be informed of the five years treatment and of the potential toxicities and benefits associated. Besides, women under 50 years and those with positive or unknown resection margins are the most likely to have a positive benefit/risk ratio with tamoxifen (3). Chemotherapy is not indicated in the treatment of DCIS (18).



Graphic 4. DCIS treatment algorithm (18).

3.1.6. COMPLICATIONS AND RECURRENCE RATE

Breast cancer follow-up is very important because of the high risk of ipsilateral and contralateral recurrence. It is recommended to have an anamnesis and physical examination every 3-6 months for the first three years after treatment, every 6-12 months until the fifth year and then, annually. It is also advisable to make annual self-examination (24).

The first mammography screening must be performed 6-12 months after the RT is completed and annually thereafter. The first five years follow up it is done in the Unitat de Patologia Mamaria and the next years are followed up by the general practitioner.

FIRST YEAR	3 months	Physical examination and anamnesis
	6 months	Physical examination , anamnesis and first mammography after radiotherapy
	A year	Physical examination , anamnesis, mammography and gynaecological examination
2-5 YEARS	Every 6 months	Physical examination and anamnesis
	Every year	Physical examination , anamnesis, mammography and gynaecological examination
From the fifth year	Every year	Physical examination , anamnesis, mammography and gynaecological examination

Graphic 5. Follow-up of breast carcinoma patients based on the recommendations from the American Society of Clinical Oncology, the European Society of Medical Oncology and the Steering Committee on Clinical Practice Guidelines for the Care and Treatment of Breast Cancer of Health Canada. (9,18).

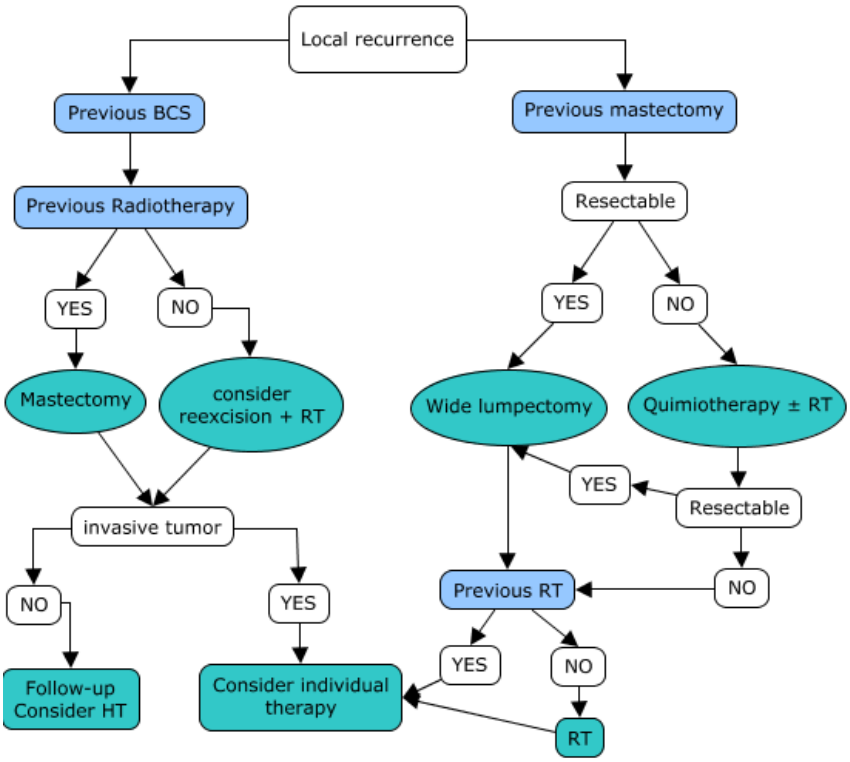
In general, **recurrence rates** after BCS of DCIS go from 8.9-17% to 26-40% with and without radiation therapy, respectively. The expected recurrence rate in the first group in 5 years is between 3-8% and in 10 years is between 10-13%. In those patients treated without RT, the cumulative incidence of any recurrence is around 16% at 5 years and 22.9 % at 10 years. It is more common during the first 3 years, with more possibilities to be found in the area where the primary tumor was located (13,18,20,21,34,40,41).

In an overview of the randomized trials of **radiotherapy** in DCIS it is demonstrated that RT reduces the absolute 10-year risk of any ipsilateral breast event (recurrence DCIS or invasive cancer) by 15.2%. It is effective regardless the age at diagnosis, the extension of BCS, use of tamoxifen, method of DCIS detection, margin status, grade, comedo-necrosis or tumor size (3).

The proportional reduction in ipsilateral breast events is higher in older than in younger women. The recurrence rate with and without RT is 18.5% vs 29.1% at ages <50 years and 10.8% vs 27.8% at ages ≥50 years. Even for women with negative margins and small low-grade tumors, the absolute reduction in the 10-year risk of ipsilateral breast events is 18.0%. however, after 10 years of follow-up there is no significant effect on breast cancer mortality (40).

Other possible risk factors for malignant recurrences are (42):

- Histopathological tumour characteristics (grade, size, presence of necrosis, multifocality, and surgical margins). Comedo histology has higher risk of another ipsilateral cancer.
- Patients' age: young age at onset
- Having received BCS alone or having neither surgical nor radiation treatment for first DCIS. These factors predict ipsilateral recurrences.



Graphic 6. Algorithm of treatment of the LR (18).

The wider the surgical margin is, the lower the rate of **ipsilateral** relapse is. For example, a margin larger than 10 mm is associated with a recurrence rate of 3-4% at 8 years after conservative surgery plus-minus RT (43).

The risk of **contralateral** invasive breast cancer is also increased in patients with DCIS. The risk of developing it more than 6 months after the initial breast cancer is independent from: surgical or radiation therapy, time since diagnosis, age, histology or anatomic location of the cancer within the breast (21).

Women diagnosed of breast carcinoma in situ are as likely as women diagnosed of non-metastatic invasive breast cancer to develop a contralateral breast cancer. In general, this risk has been reported to increase from 2 to 6 times compared with women without a prior history of breast cancer (21,44).

Between 1% and 6% of women with DCIS have been diagnosed a contralateral invasive breast cancer (42). A study of patients diagnosed with DCIS in 1973-1996 found that the cumulative risk of contralateral invasive or in situ breast cancer was: 3% at 5 years, 6% at 10 years, 9% at 15 years, and 11% at 20 years (13).

If invasive cancer develops after DCIS, the risk of dying of breast cancer increases substantially. Among patients with DCIS, mortality is associated with age at diagnosis, ethnicity, and DCIS characteristics such as estrogen receptor status, grade, size (>5 cm), and comedo-necrosis. But only a small minority of patients will have 1 or more of these high-risk characteristics (19).

The treatment after relapse is re-excision of the tumor and if possible RT. If the recurrence tumor size or the relation between tumor-breast size is high, a mastectomy may be needed (18).

3.2. JUSTIFICATION

Currently, the decision of what is a positive margin in women with DCIS that undergo BCS is complicated. It is not established yet which is the distance that the pathologist has to consider as positive or negative.

In the invasive tumor it is well established that the tumor touching the inked margin is considered positive. Nowadays, the decision around the in situ tumor is taken not only with the margin status but also with the other risk factors to develop a recurrence.

It has been discussed which distance should be the appropriate to avoid recurrence, but any conclusion has been reached. In the in situ tumor is assumed that "no ink on tumor" is not enough to ensure that there are no tumor cells in the remaining breast. This is why in many articles it is taken as reference 1 millimetre, but others prefer 3 or 10 mm as an enough margin. Unfortunately, no one of these opinions has been demonstrated.

The importance of which margin distance should be taken depends on several factors. Firstly, the most important thing is to get enough distance to decrease the recurrence rate to a minimum. Secondly, we must ensure this distance without taking out much healthy tissue. For example, if we took the 10 mm distance as the reference, we would ensure a minimum recurrence rate but a lot of healthy breast tissue would be extracted.

It is important to remember that when we talk about margins we refer to the six sides of a breast: the anterior, superior, lateral, inferior, medial and posterior margin. For this reason, the extraction of 10 mm on each side has an important cosmetic impact, especially in small breasts. We have to remember too, the psychological impact when removing breast tissue for a woman.

Therefore, it is very important to define which the most appropriate volume to remove is. This will prevent to get less tissue than necessary, which could imply a recurrence, and also the removal of overly healthy tissue.

When a reference distance is set, the pathologist reports surgeons the state of the margins depending on this distance (positive/close/negative margins). If one margin is affected, the patient requires reoperation to widen this margin. This implies many consequences:

- Other general anaesthesia and more recovery time
- The emotional involvement that the new surgery implies in two levels: personal and familiar
- Cosmetic implication: the size and aesthetics changes.
- A mastectomy may be needed.
- The delay of the complementary treatment such as RT or hormone therapy.
- Economic implications in the health care system.

4. HYPOTHESIS

The range between 1-2.9mm is a better minimum margin to reduce the recurrence rate when compared with less than 1mm in women with DCIS that undergo breast conserving surgery. There are no significative differences when compared with 3-10mm.

5. OBJECTIVE

The goal is to determine the minimum margins that have to be left in DCIS undergoing conservative surgery to reduce the recurrence rate. Our target is to compare the recurrence rate obtained depending on the millimetre from the tumor to the inked surface of the piece. We want the pathologist to take this distance as a reference to define when a margin is negative (free of tumor cells) in the future.

6. MATERIAL AND METHODS

6.1. STUDY DESIGN

The study design will be a retrospective cohort with a longitudinal and observational design. It will be carried out as a multicentric study in eight referral hospitals in Girona inside the Breast Pathology Unit of each hospital. This unit integrates a multidisciplinary group of health professionals such as gynaecologists, surgeons, pathologists, psychologists, radiologists, oncologists and radiotherapists. The study period will be from 2000 to 2017, taking into account that the sample will be recruited until 2015.

6.2. STUDY POPULATION

The study will identify women of any age who underwent breast conservative surgery plus radiotherapy for ductal carcinoma in situ. Treatment was not randomized. We will take those cases treated from January 2000 to December 2015. The patients will be identified in the maintained database of the Breast Pathology Unit or the SAP database at each hospital.

6.3. INCLUSION CRITERIA

- Women of all ages
- Patients subjected to BCS with or without needle localization procedure in the case of non-palpable lesions.
- Ductal carcinoma in situ
- Palpable and non-palpable lesions
- RT after the surgery
- Systemic treatment (tamoxifen) after the surgery

6.4. EXCLUSION CRITERIA

- Men
- Tumors in which the minimum margin distance is higher than 10mm in the microscopic evaluation
- Multicentric lesions tributary to mastectomy
- Tumor size (relative to breast size) triburary to mastectomy
- Inability to achieve negative surgical margins that require mastectomy
- Most advanced Breast Cancer stage
- History of neo-adjuvant chemotherapy
- History of previous radiotherapy on the chest wall/breast
- Lobular carcinoma in situ
- Patient's choice of mastectomy

6.5. SAMPLE SELECTION

A consecutive non-probabilistic sampling-method will be performed, as we will include women of all ages with DCIS and treated with BCS plus RT who meet the criteria attended. Eight centers will be involved in this project from the province of Girona. The sample recruitment will take place in these health centers as we will work with their previous database or with the SAP. Then, the patients will be codified to analyse the women in the study anonymously.

6.6. SAMPLE SIZE AND SAMPLING METHODS

Sample size calculation is based on the free application GRANMO, and POISSON approximation is used (45). Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 309 subjects are necessary in each group (927 in total) to recognize as statistically significant a relative risk greater than or equal to 1.91. A proportion in the non-exposed group has been estimated to be 0.089 and in the exposed group 0.17 according to our research. It has been anticipated a drop-out rate of 5% because we will work with a previous database and all cases will be analysed retrospectively.

According to the results and taking into account that we have three groups in our study, the total sample size will be 927 patients. So we will have to design a multicenter study with the participation of hospitals from Girona province.

It is estimated that the incidence of breast cancer is 95,5/100.000 women-year in Girona province (5,6). The population was 378.078 women in this province in 2013 (46). As we know that approximately the 20% of breast cancers are DCIS, we can estimate that there are 72,2 cases-year in the province of Girona.

We also know that the referral hospitals in Girona operate breast cancers. So we can estimate that there are about 9 patients-year in each referral hospital: Hospital Universitari Doctor Josep Trueta, Hospital Santa Caterina, Hospital de Figueres, Hospital Comarcal de Blanes,

Hospital de Campdevànol, Hospital de Sant Jaume d'Olot, Hospital de Palamós, Hospital de Puigcerdà.

With the adequate collaboration and coordination, the estimated time of recruitment would be around 13 years. Moreover, we have expanded the collection time of patients to ensure that we reach the 927 we need and also because we are interested in the total following time. If we study these patients from 2000 to 2015 (sixteen years), the median follow-up will be 8 years.

As we are doing a retrospective study, we will collect all the patients from the hospitals database and SAP. We will recruit the patients operated between the years 2000-2015 and we will follow each patient until December 2015. Statistically it should be the same proportion of each study group patients every year, so there would not be problems in studying some patients more time than others.

We are doing this sampling methodology because we are interested in following-up as much years as possible the patients. The availability of the signed informed consent for the surgical procedure and the biobank of tumors consent is needed to carry up the study (ANNEX 14.7).

If more than 927 patients per group are selected, we will include them to our sample too. Since we work with preexisting data no harm to the patients is made by including them to our sample.

6.7. VARIABLES AND METHODS OF DATA COLLECTION:

6.7.1. INDEPENDENT VARIABLE

We will define the margins distance in millimetres of the 6 sides of the tumor. We will take 'no ink on tumor' to 0.9mm, 1mm to 2.9mm and 3mm to 10mm as study distances.

The surgical fresh piece is sent to the pathologist laboratory oriented on a drawing in a polystyrene prepared in the operation room. The correct orientation using metal clips enables individual identification of the surfaces. Therefore, it allows re-excision of only the affected areas (Annex 14.5, picture 1).

First of all the whole tumor is measured with a ruler and pictures are taken (Annex 14.5, picture 1). Then, the tumor is coloured with chinese ink in different colours. Each side has a different colour to help us to identify which side is each one in the microscopic study (Annex 14.5, pictures 2-4).

Moreover, the piece is cut in different antero-posterior slices of 1-2cm thick. Sections are made perpendicular to the axis from the central edge (areolar) to the peripheral edge. This technique facilitates the examination of the extension of intraductal tumors (Annex 14.5, picture 5).

Once this is done, in the invasive tumor, the pathologist evaluates macroscopically each slice to specify if the margins are affected or they are free of tumor cells. They also try to detail the distance between the inked surface and the tumor (Annex 14.5, picture 6). The DCIS needs microscopic examination to assess the margins.

Then, the piece is fixed in formalin for 18-24 hours. After that, the macroscopic study is done and the pathologist reports the localization, treatment done, tumor size, weight and if it involves skin and resection margins with the specification of the distance of each margin.

For the macroscopic study of the DCIS, in case of microcalcifications, previous radiography is needed to evaluate size and margins. Besides this, a microscopic exam is made to specify the histological type, grade, necrosis, in situ component and further information about the margins in millimetres (Annex 14.5, pictures 7 and 8). We will take into account only the smallest distance between tumor and the inked surface to distribute each patient in the study groups.

If a margin is thought to be too close to the tumor, a re-excision is needed. This re-excision is performed in a second surgical procedure, as soon as possible. More tissue is taken from the side the pathologist said the margin was affected.

The extra tissue extracted is also quantified. To measure this final margin we add the distance in millimetres after this surgery to the previous distance of the extended side. In these cases, we will take the smallest margin after the re-excision surgery to distribute the patients in each group of the study.

This variable will be measured as an ordinal qualitative variable (no ink-0.9mm, 1mm-2.9mm, 3mm-10mm).

6.7.2. DEPENDENT VARIABLE

Our outcome variable is the local recurrence rate of the tumor, which most often involves further surgery, so it also modifies the reoperation rate. LR means recurrence of malignant tumor or DCIS anywhere in the treated breast.

- True recurrence: appears in the area where the primary tumor was located, and corresponds to the area of the surgical scar or radiation boost.
- Marginal recurrence: in the margin area
- Distant recurrence: appears in a different quadrant where the primary tumor was.

The diagnosis of the recurrence is done with the complementary tests performed according to the hospitals protocol for monitoring women with a history of DCIS. It is established that physical examination, mammography and ultrasonography are needed (*3.1.6. Complications and recurrence rate*). The first five years follow up is done in the Unitat de Patologia Mamaria and the next years are followed up by the general practitioner.

This variable will be measured as a nominal dichotomic qualitative variable (recurrence: yes/no).

6.7.3. COVARIABLES

We want to register other variables in order to describe our population in the study and its characteristics. We are also going to use them to make a multivariate analysis to avoid confusion factors.

Personal features:

- *Patient age*: number of years.
  The age of the patients has been collected from their ID card or any other official identification document given to the admission department when arriving to the hospital. This information is reflected in their clinical history when data is collected. It is important to collect this information because in elderly patients, the incidence of breast cancer has an exponential growth.

- *Menopausal status.* It is measured at the time of the carcinoma diagnosis. When 12 months have passed after last patient menstrual period (yes/no).

Tumor characteristics:

- *Localization*: it is defined with the complementary resources like mammography or MRI. Depends on the quadrant of the breast. We will define that the tumor is in the upper inner, upper outer, lower inner or lower outer quadrant.

- *Number of quadrants involved:* it will be measured with the mammography (one, two three or four).

- *Tumor size*: measured in millimetres by the pathologist. It takes into account the biggest diameter of neoplastic cells that contains the surgical piece. It depends on the breast size too. So if the relation tumor-breast size is high, there is more risk of recurrence.

Surgical and pathological procedures:

- *Surgeon and surgical technique:* it implies more or less margins distance. It is measured with the surgeon experience on this type of surgery in number of surgeries.

- *Pathologist experience:* it can vary the margin assessment (Number of years).

Pathological features:

- *Microinvasion*: the extension of cancer cells beyond the basement membrane into the adjacent tissues, with no single focus larger than 1 mm in greatest dimension (T1mic). It is assessed by the pathologist (yes or no).

  DCISM is seen in approximately 14% of DCIS cases. The potential for DCISM should be suspected for DCIS tumors which are large, have comedo-type histology and contain

necrosis. DCISM may also result in axillary lymph node metastases, whereas patients with DCIS should not, by definition, have axillary metastases.

- *Histology*: the pathologist diagnosis of the subtype of DCIS according to the resemblance and tissue pattern: papillary, solid, comedo, micropapillary or cribiforme. Also the subtype of tumor: luminar A, luminar B, Her2 positive and Basal like (ANNEX 14.3).

- *Tumor grade:* is defined by the description of the tumor based on how the cells look under the microscope. It indicates how fast the tumor grows and extends and is based on the resemblance of the tumor to the tissue of origin. It is scored from 1 to 3 being 1 the most differentiated and 3 the most undifferentiated cells, based on the Nottingham system (18).

Treatment features:

- *Time until recurrence:* measured in years and months. From the first day after the last surgery till the day of diagnosis of the recurrence.

- *Systemic treatment.* It can vary the recurrence rate because further treatment is added (Tamoxifen yes/no).

7. STATISTICAL ANALYSIS

The margins distance (in millimetres) will be considered a qualitative variable (no ink on tumor-0.9mm, 1-2.9mm and 3-10mm) and the recurrence rate will be treated as a categorical dichotomic variable. The covariables are defined as quantitative or qualitative.

In the **univariate analysis**, we will define variables as categorical or quantitative.

- Categorical or qualitative variables will be described as percentages and proportions.
- Quantitative variables will be expressed as mean ± standard deviation or with median and interquartile range (25-75) depending on whether or not they were normally distributed.

In the **bivariate analysis**, the independent and dependent variables are categorical. So the comparison between the independent (no ink on tumor-0.9mm, 1-2.9mm and 3-10mm) and dependent (recurrence or not) variables will be carried out with Chi-Square test.

Finally, the **multivariant analysis** will be estimated using a logistic regression test to estimate odds ratio and 95% confidence intervals. It will be used to assess the relationship between the free margins distance from the tumor and the recurrence rate, after adjustment for the potentially confounding effects.

To perform this analysis, we will use the IBM Statistical Package for Social Science (SPSS) 22.0 program. Microsoft Excel tool will be used to manage computed data. P value of <0.05 will be considered to indicate statistical significance.

8. ETHICAL ASPECTS

This research protocol will be presented to the Clinical Research Ethical Committee (CEIC, Comitè d'Ètica d'Investigació Clínica) of Hospital Universitari Dr. Josep Trueta in Girona. They will assess if the study fulfils the required criteria for being approved. Moreover, the recommendations given by the committee will be taken into account to carry out the study.

The project will be carried out according to the ethical principles established by World Medical Association in the *Helsinki Declaration of Ethical Principles for Medical Research Involving Human Subjects* (last actualization October 2013). Furthermore, we will take into consideration the Spanish Organic Law *14/2007, de Investigación Biomédica,* which regulates biomedical investigation involving human beings in Spain.

Since it is a retrospective study, we will depart from a previously constructed database and it will be taken anonymously according to the article from Spanish Organic Law *15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal*. Therefore, this study guarantees the confidentiality of the patient. All data will be only used for the purpose of the research.

All the investigators will have to declare no conflict of interest.

9. LIMITATIONS OF THE STUDY

Analysing our study, we detected and took into account several limitations which interfere in the study. The most relevant limitations are explained below:

- Margin assessment. Technical and methodological limitations:
  - The "pancake phenomenon" contributes to the inaccuracy of margin assessment. It happen when the piece is sliced and the breast specimens are flattened after surgical removal, losing almost 50% of their original height.

  - Breast specimen orientation: it is important to differentiate each side in the microscopic analysis. This limitation can be avoided by orienting the sample in a drawing of the breast with metal clips, which is what is currently done in some hospitals.

  - Due to the fact that it is an operator-dependent technique, it requires pathologists to evaluate the margins distance. This can lead to differences depending on the pathologist that analyses the tumor. It depends on the experience of the professional. This has been taken into account as a covariable.

- As the study population are women from the hospital databases or the SAP, we could have a selection bias. We only include patients that carry out breast cancer screening or that consult due to some finding in the self-examination. Therefore, those women that have no control over their breasts will not be diagnosed early and will present a later stage tumor.

- Another limitation to be acknowledged is related with the sample size. We had to estimate the patients in each hospital based on epidemiology data of DCIS provided by other studies. To ensure we achieve the minimum volume of sample to carry out the study, we have extended the timeframe in order to have patients enough to include to our sample.

- Differences between hospitals, for example in the surgical or pathological protocol. Data from the different hospitals may be analysed separately, so we could evaluate if there was a possible confusion factor.

- It is important to maintain the blindness when we collect the data. The information will be analysed without knowing the name of the patient. A previously assigned code will be used to identify each patient to avoid an observer bias.

- There are different covariables that can modify the recurrence rate causing a confusing bias. This limitation will be minimized by the use of a multivariate analysis to adjust the results for the confusion factors.

- It is also important to consider that it is a retrospective longitudinal cohort, so we could have dropouts. For example if they did not follow the control appointments, which we cannot get back. These losses have been considered when calculating the sample size.

- It is demonstrated that the cosmetic outcome is an important factor to assess the patients' satisfaction of the surgery. Not only the recurrence rate is important, the amount of tissue extracted is significant too. That is why it would be right if we could ask the patients about their satisfaction in this area, so a clinical trial would be the best way to do it in the future.

- We will follow the patients in different periods of years. However, we think this will not be a problem for two reasons. Firstly, other studies have done this methodology before and secondly, we estimate that there will be the approximately same cases in each group study every year.

- It is known that women can have a recurrence many years later from the surgery, and we are studying a median of 8 years. As the information is dynamic and it will be maintained in the SAP program, further studies should be done to have a long-term following-up.

If we carried out a clinical trial, we would avoid these biases improving the level of evidence contributed. However, we believe that the best way to test our hypotheses and achieve our objectives is performing a retrospective cohort.

These results should be considered as preliminary and it will be helpful if the study is repeated using a randomized probabilistic sampling. Additional research will be required to confirm our findings.

10. WORK PLAN

Investigators: The research team will be composed by:

- Study coordinator: Natalia Balot (NB)
- Four gynaecologists on the Breast Pathology Unit: Ester Vila (EV), Francesc Tuca (FT), Alexandra Bonmatí (AB), Alejandra Azkargorta (AA)
- One pathologist: Eugeni Bonet (EB)

Collaborators: statistician

The study will be realized from November 2015 to March 2017, both included (1 year and 5 months). The sequence of activities carried out by the research team is gathered in 6 phases:

STAGE 0. Protocol approval (4 months: November 2015-February 2016)

- Protocol elaboration and evaluation. This stage consisted on literature review, the current protocol's elaboration and then, the protocol will be discussed with the members of the study to make sure that all agree with the procedures. Problem identification, suggestions, and final elaboration and evaluation of the research protocol will be carried out.
- Before getting started, the research protocol will be submitted to the hospital's ethical committee in order to receive its approval for allowing the study to be carried out.

It will involve all the investigators and collaborators.

STAGE 1. Coordination phase (2 months: March - April 2016).
Coordination of the centers and members of the study.

In this phase, organizational and informative meetings will be held between the main investigators and the rest of the research team. The work team will be formed by one main investigator, four gynaecologists that hold the Breast Pathology Unit of the HUJT, one pathologist and one qualified statistician. The objectives of the study will be shared, as well as its methods of data collection and the study chronogram will be scheduled.

Every six months, a coordination meeting (M) will be held and data quality controls will be performed with the aim of evaluating the consistency of the collected data.

STAGE 2. Field work and data collection (5 months: May – September 2016)
The investigators will collect the patients' data from the eight hospitals database and SAP. Patients will be selected with the inclusion/exclusion criteria described before. During the data collection, investigators will be in constant touch and will have one meeting each month to solve possible problems or incidences with the patients' selection process.

The data collection will start when the first participant is recruited and will end after the last patient is included. The patients will be followed a median of 8 years.

STAGE 3. Data analysis (2 months: October – November 2016)
Statistical analysis. It will involve all the investigators and a qualified statistician that will be hired for this purpose.

After processing the database, all data collected will be analysed using the appropriate statistical test. Firstly, a descriptive and bivariate analysis will be conducted and, secondly, a multivariate analysis using a multiple lineal regression to examine the contribution of the confounding variables will be performed.

STAGE 4. Interpretation of the results (2 months: December 2016 – January 2017)

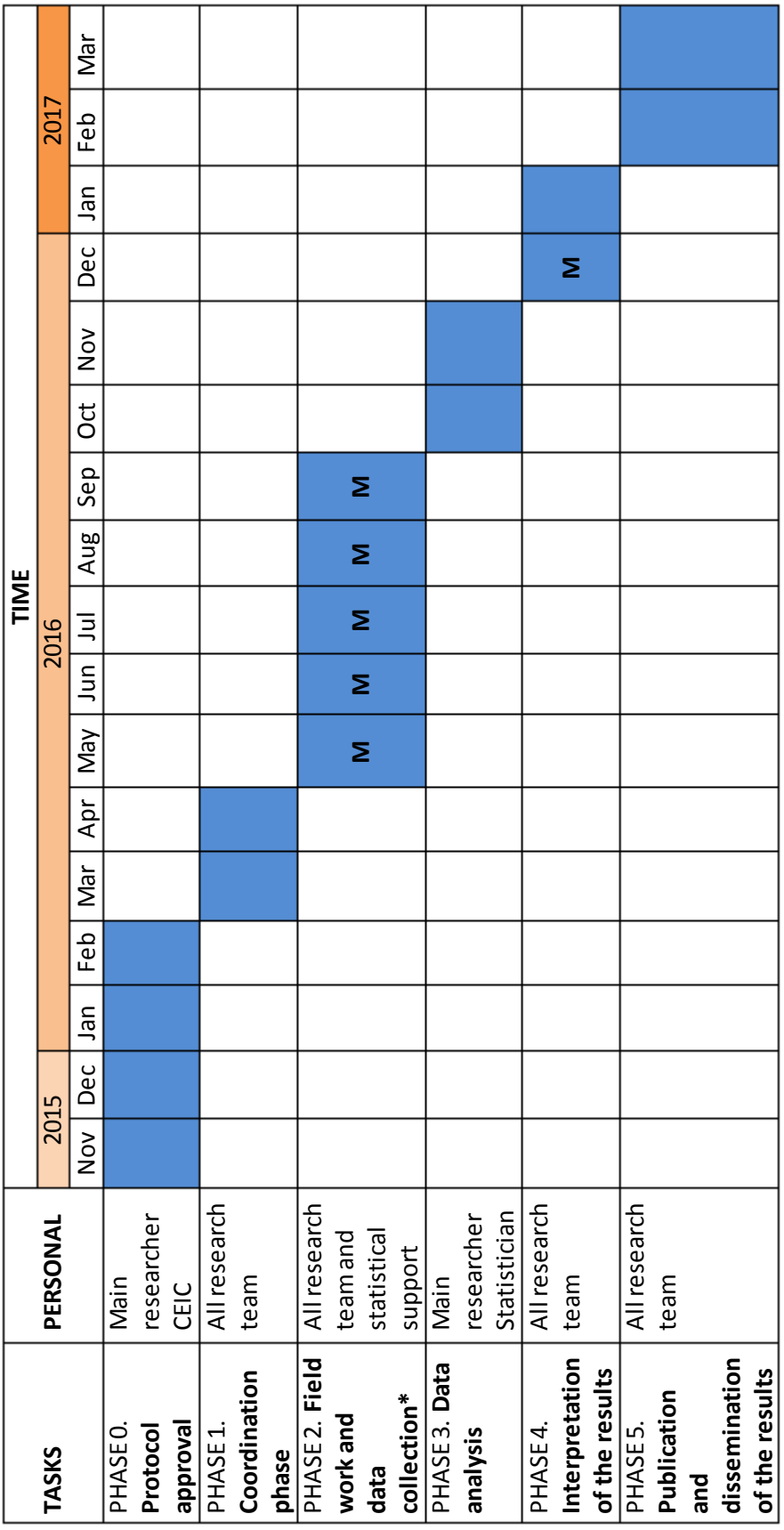
- Analysis and interpretation of results. The team investigators will meet to interpret and analyse the results of the study.
- Final report evaluation

In this moment, the investigators will receive the analysed data from the statistician and will interpret the results. From these results, a final evaluation report interpreting the outcomes will be written and the results will be discussed among all investigators and collaborators.

STAGE 5. Publication and dissemination of the results (2 months: February – March 2017)

- Scientific publications. We will try to spread the evidence-based knowledge by publishing scientific articles in prestigious scientific journals.
- Attendance to congresses. It is important that the findings of this research are widely disseminated. The dissemination strategy includes conference presentations, meetings, and training sessions, among others. For example, we aim to present our findings in the Congreso de la Sociedad Española de Senología y Patología Mamaria (SESPM).

11. STUDY CHRONOGRAM



12. BUDGET

EXPENSES	BUDGET PROPOSAL		
	QUANTITY	COST PER UNIT	COSTS (€)
1. PERSONAL COSTS			
Investigation team	6 investigators	0	0€
2. MATERIAL AND SERVICES			
Statistician	2hours/day x 4 days/week x 8 weeks	35€/hour	2240€
Office material and others	-	-	800€
3. TRAVELS, PUBLICATION AND DISSEMINATION.			
Travel meetings: coordination and analyse of the results	6 persons x 4 meetings	50€/person	1200€
Inscription to Congreso de la Sociedad Española de senología y Patología Mamaria (SESPM)	1	600€	600€
Costs of the trip: - Flights - Accommodation - Subsistence allowance	 2 2 2	 130€ 170€ 200€	 260€ 340€ 400€
Publication. Open access article fees	1	1500€	1500€
TOTAL			**7340€**

Before starting the study it is necessary to ensure its' financing.

The research team will carry out all the tasks related with the selection of the sample from the SAP and database from the BPU, and we do not need to call or visit the patients. We will ask the Technical Secretary of each hospital to codify the patients and to supply to us.

A qualified statistician will be hired in order to perform the statistical analysis and data management services. The cost of this specialist will be 2240€ (35€/hour x 2 hours/day x 4 days/week x 8 weeks). No other human resources are considered. The investigators and the doctors working for the program will not receive a compensation for their work in this study.

The budget does not include material as computers because they are already available in any of the hospitals. Software such as SPSS and Microsoft Access© are not included because they are either available to the statistician or free of charge.

We assume 1000€ for possible costs related with transport and accommodation for attending the meetings for two members of the investigator team. The writing and diffusion of the definitive article will be task of the investigation team. We assume costs for the study publication of 1500€. The study will be presented to the Congreso de la Sociedad Española de senología y Patología Mamaria (SESPM).

13. FEASIBILITY AND IMPACT

Feasibility.

The research study is proposed to be performed from January 2000 to March 2017. The hospitals will provide investigators salaries and instruments.

We have estimated that the duration of the data collection will be 16 years. This will be possible with the participation of eight hospitals in Girona province. Patients from 2000 to 2015 will be selected. The duration of the analysis part of the study will be 1 year and 5 months and the budget is affordable for any hospital.

Impact.

Despite the fact that breast cancer has been studied much, there is no conclusive research on how the margin assessment varies the recurrence rate in DCIS. All studies put much emphasis on the lack of consensus in the decision of re-operating DCIS depending on the margin status. Many studies also explain the importance of margin assessment regarding the recurrence rate.

This study pretends to work as a tool and a resource for the healthcare staff to take better decisions for the treatment of the patient, based in worldwide recognized guidelines.

If the results are relevant and our hypothesis is validated, it would change the treatment procedure in women with DCIS undergoing BCS. The pathologist will have a proven scientific basis to decide which margin is considered positive and which not.

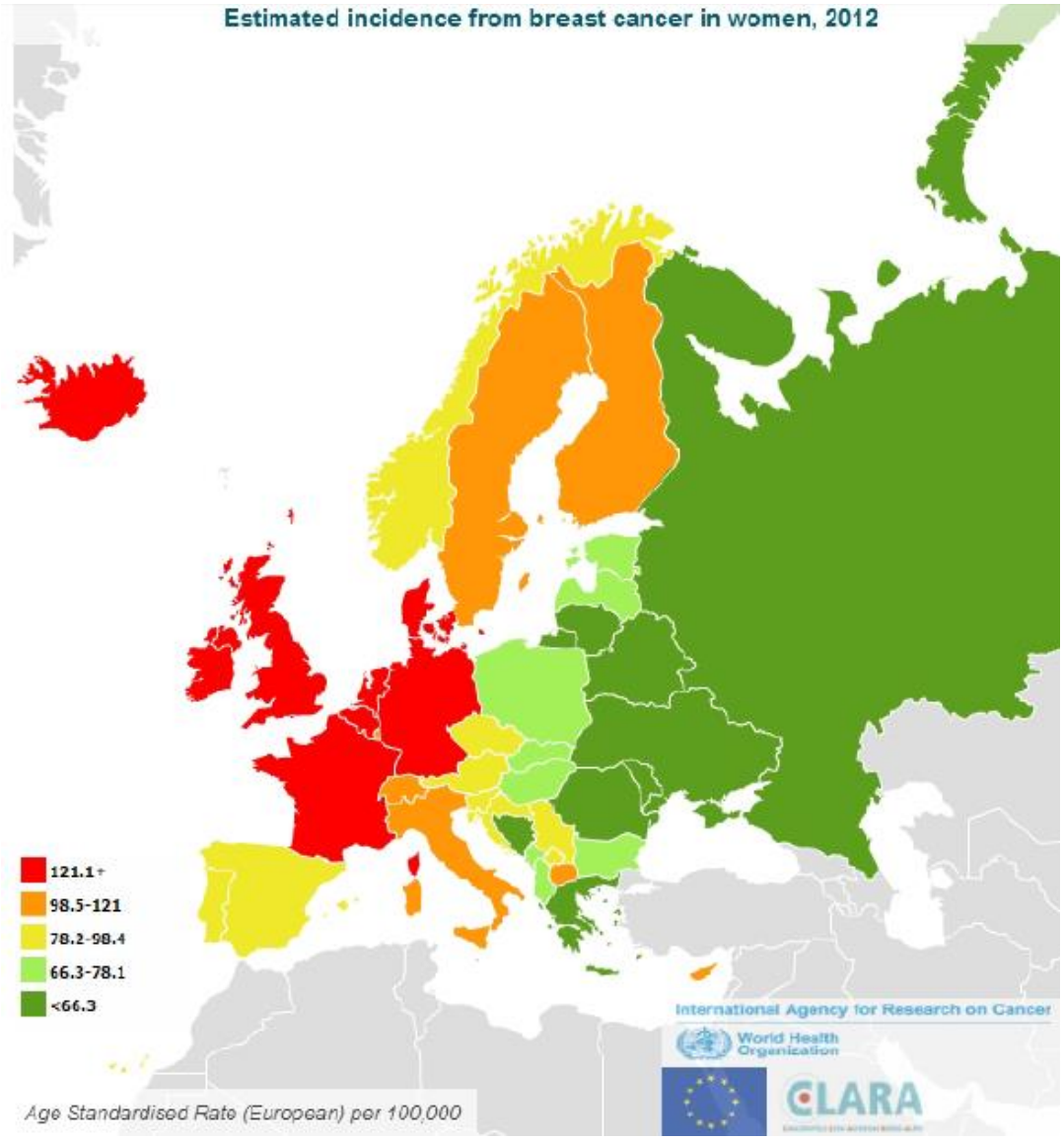
Then, with the results obtained in the study, a woman who may have previously needed reoperation will not need it now because her margin recurrence probability is small. Or it could happen the opposite, a patient who was not going to be operated again; it proves that further treatment is required.

With a better management of the breast DCIS, we expect to avoid some cases of reoperation and reduce recurrence rates. With the management improvement we expect to reduce the number of days of hospitalization per patient too, and also reduce the time needed until further treatment (RT or Tamoxifen).

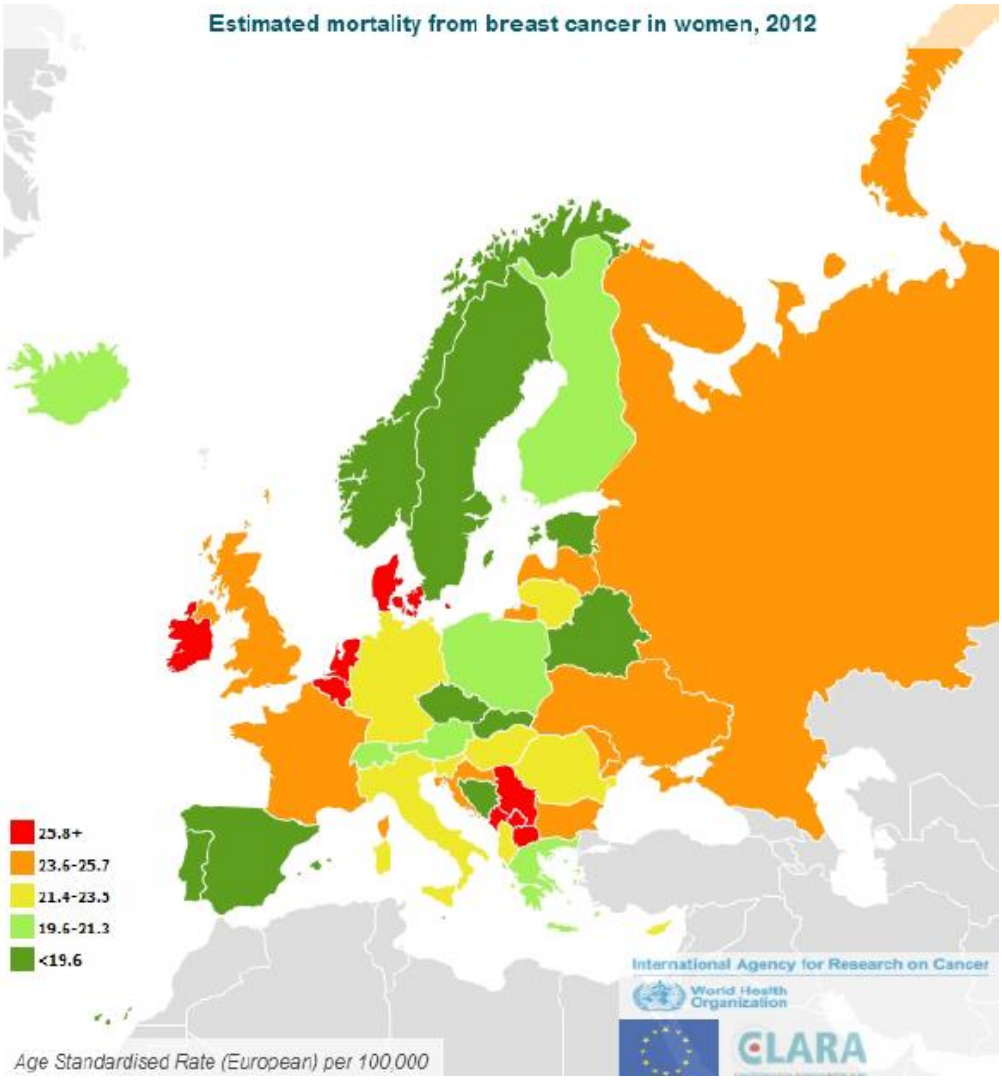
This study should be seen as a basis for future research: further studies focused in applying the research to clinical trials. This would allow the extrapolation of the results for the general population and would confirm our findings.

14. ANNEXES

14.1. INCIDENCE AND MORTALITY MAPS. BREAST CANCER IN EUROPE.



Incidence and mortality maps in Europe from the WHO (8,47).


Estimated mortality from breast cancer in women, 2012
25.8+
23.6-25.7
21.4-23.5
19.6-21.3
<19.6
Age Standardised Rate (European) per 100,000
International Agency for Research on Cancer
World Health Organization
CLARA

14.2. BREAST CANCER STAGING. DEFINITION OF THE TNM CATEGORIES

The AJCC (American Joint Committee on Cancer) breast cancer classification is based on TNM: Primary Tumour; Regional Lymph Node; Distant Metastasis (48).

Primary tumor (T) The T classification of the primary tumor is the same regardless of whether it is based on clinical (physical examination or radiologic) or pathologic criteria, or both. Size should be measured to the nearest millimetre. In general, pathologic determination should take precedence over clinical determination of T size.
TX primary tumor cannot be assessed
T0 no evidence of primary tumor
Tis Carcinoma in situ **Tis(DCIS)** ductal carcinoma in situ **Tis(LCIS)** lobular carcinoma in situ **Tis(Paget)** Paget's disease of the nipple with no tumour (Note: if there is an associated tumor, the disease is classified on the basis of the size of that tumor). Not associated with invasive carcinoma and/or carcinoma in situ in the underlying breast parenchyma
T1 Tumour 2 cm or less in greatest dimension **T1mi** Microinvasion: 0.1 cm or less in greatest dimension **T1a** (If associated with in situ carcinoma, more than 0.1 cm) but not more than 0.5 cm in greatest dimension **T1b** More than 0.5 cm but not more than 1 cm in greatest dimension **T1c** More than 1 cm but not more than 2 cm in greatest dimension
T2 More than 2 cm but not more than 5 cm in greatest dimension
T3 Tumour more than 5 cm in greatest dimension
T4 Tumour of any size with direct extension to chest wall or skin (ulceration or skin nodules) only as described in T4a to T4d *Note: invasion of the dermis alone does not qualify as T4* **T4a** Extension to the chest wall, not including only pectoralis muscle adherence **T4b** Oedema (including peau d'orange), or ulceration of the skin of the breast, or satellite skin nodules confined to the same breast, which do not meet the criteria for inflammatory carcinoma. **T4c** Both T4a and T4b **T4d** Inflammatory carcinoma

Regional lymph nodes (N) **1. Clinical**
Nx Regional lymph nodes cannot be assessed (not removed for study or previously removed)
N0 No regional lymph node metastasis
N1 metastases to movable ipsilateral level I, II axillary lymph node/s.

N2 metastases in ipsilateral level I, II axillary lymph nodes that are clinically fixed or matted; or in clinically detected (imaging studies or clinical examination) ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastases.

N2a Metastasis in ipsilateral level I, II axillary lymph nodes fixed to one another or to other structures.

N2b Metastasis only in clinically apparent internal mammary lymph node(s) in the absence of axillary lymph node metastasis

N3 metastasis in ipsilateral infraclavicular (level III axillary) lymph nodes with or without level I, II axillary lymph node involvement; or in clinically detected ipsilateral internal mammary lymph nodes with clinically evident level I, II axillary lymph node metastasis; or metastasis in ipsilateral supraclavicular lymph nodes with or without axillary or internal mammary lymph node involvement.

N3a Metastasis in ipsilateral infraclavicular lymph nodes

N3b Metastasis in ipsilateral internal mammary lymph nodes and axillary lymph nodes

N3c Metastasis in ipsilateral supraclavicular lymph node(s)

2. Pathologic (pN):

pNx Regional lymph nodes cannot be assessed (not removed for study or previously removed)

pN0 No regional lymph node metastasis identified histologically

pN0(i-) no regional lymph node metastasis histologically, negative by inmunohistochemical (IHC) methods.

pN0(i+) malignant cells in regional lymph nodes no greater than 0.2 mm detected by IHC.

pN1 Metastasis in 1-3 ipsilateral axillary lymph node(s), and/or in ipsilateral internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent (i.e. not detected by clinical examination or imaging studies excluding lymphoscintigraphy)

pN1mi Micrometastasis (larger than 0.2 mm, but none larger than 2 mm in greatest dimension)

pN1a Metastasis in 1-3 axillary lymph node(s), including at least one larger than 2 mm in greatest dimension

pN1b Internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent

pN1c Metastasis in 1-3 axillary lymph nodes and internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent

pN2 Metastasis in 4-9 ipsilateral axillary lymph nodes, or in clinically apparent ipsilateral internal mammary lymph node(s) in the absence of axillary lymph node metastasis

pN2a Metastasis in 4-9 axillary lymph nodes, or including at least one that is larger than 2 mm

pN2b Metastasis in clinically apparent internal mammary lymph node(s) in the absence of axillary lymph node metastasis

pN3 Metastasis in 10 or more ipsilateral axillary lymph nodes; or in ipsilateral infraclavicular lymph nodes; or in clinically apparent ipsilateral internal mammary lymph nodes in the

presence of one or more positive axillary lymph nodes; or in more than 3 axillary lymph nodes with clinically negative, microscopic metastasis in internal mammary lymph nodes; or in ipsilateral supraclavicular lymph nodes

pN3a Metastasis in 10 or more axillary lymph nodes (at least one larger than 2 mm) or metastasis in infraclavicular lymph nodes

pN3b Metastasis in clinically apparent internal mammary lymph node(s) in the presence of positive axillary lymph node(s); or metastasis in more than 3 axillary lymph nodes and in internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent

pN3c Metastasis in supraclavicular lymph node(s).

Distant metastasis (M)
Mx Distant metastasis cannot be assessed
M0 No clinical or radiographic evidence of distance metastasis
cM0(i+) no clinical or radiographic evidence of distant metastasis, but deposits of molecularly or microscopically detected tumor cells in circulating blood, bone marrow, or other non-regional nodal tissue that are no larger than 0.2 mm in a patient without symptoms or signs of metastasis
M1 Distant detectable metastasis as determined by classic clinical and radiographic means and/or histologically proven larger than 0.2 mm.

ANATOMIC STAGE/PROGNOSTIC GROUPS			
Stage 0	Tis	N0	M0
Stage IA	T1 (+T1mi)	N0	M0
Stage IB	T0	N1mi	M0
	T1 (+T1mi)	N1mi	M0
Stage IIA	T0	N1	M0
	T1 (+T1mi)	N1	M0
	T2	N0	M0
Stage IIB	T2	N1	M0
	T3	N0	M0
Stage IIIA	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
Stage IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
Stage IIIC	Any T	N3	M0
Stage C	Any T	Any N	M1

14.3. SUBTYPES OF BREAST CANCER

Intrinsic subtype	Clinico-pathologic definition	
Luminal A	'Luminal A like' ER-positive HER2-negative Ki67 low: around 10% PgR high Low-risk molecular signature	
Luminal B	'Luminal B like' (HER2-negative) ER-positive HER2-negative Ki67 high or PgR low Low-risk molecular signature	'Luminal B like' (HER2-positive) ER-positive HER2-positive Any Ki67 Any PgR
HER2 overexpression	'HER2-positive (non-luminal)' HER2-positive ER and PgR absent	
'Basal like'	'Triple-negative (ductal)' HER2-negative ER and PgR absent There is ~80% overlap between 'triple-negative' and intrinsic 'basal-like' subtype, but 'triple-negative' also includes some special histological types such as (typical) medullary and adenoid cystic carcinoma with low risks of distant recurrence.	

CLASSIFICATION BASED ON HORMONE RECEPTORS AND HER2 STATUS (15,49):

Hormone receptor-positive: the breast cancer cells contain either estrogen or progesterone receptors. They can be treated with hormone therapy drugs. This includes cancers that are ER-negative but PR-positive. These cancers tend to grow more slowly than those that are hormone receptor-negative. Women with these cancers tend to have a better outlook in the short-term, but these cancers can sometimes come back many years after treatment. Hormone receptor-positive cancers are more common in women after menopause.

Hormone receptor-negative: the breast cancer cells do not have either estrogen or progesterone receptors. Treatment with hormone therapy drugs is not helpful for these cancers. These cancers tend to grow more quickly than hormone receptor-positive cancers. If they return after treatment, it is more often in the first few years.

HER2 positive: Cancers that have too much HER2 protein or extra copies of the HER2 gene. These cancers can be treated with drugs that target HER2.

HER2 negative: Cancers that don't have excessive HER2.

Triple-negative: If the breast cancer cells don't have estrogen or progesterone receptors and do not have too much HER2, they are called triple-negative. These cancers tend to occur more often in younger women. They tend to grow and spread more quickly than most other types of breast cancer. Hormone therapy is not helpful in treating these cancers and drugs that target HER2 are not helpful, either. Chemotherapy can still be useful, though.

Triple-positive: This term is used to describe cancers that are ER-positive, PR-positive, and have too much HER2. These cancers can be treated with hormone drugs as well as drugs that target HER2 like trastuzumab and lapatinib.

BASED ON GENE EXPRESSION:

Luminal A and luminal B types: The luminal types are ER-positive.

- Luminal A cancers are low grade, tend to grow slowly, and have the best prognosis.
- Luminal B cancers generally grow somewhat faster than luminal A cancers and their outlook is not as good.

HER2 type: These cancers have extra copies of the HER2 gene. They usually have a high-grade appearance under the microscope. These cancers tend to grow more quickly and have a worse prognosis, although they often can be treated successfully with targeted therapies which are often given along with chemotherapy.

Basal type: Most of these cancers are triple-negative type. They lack estrogen or progesterone receptors and have normal amounts of HER2. This type is more common among women with BRCA1 gene mutations. It is also more common among younger and African-American women. These are high-grade cancers that tend to grow quickly and have a poor outlook. Hormone therapy and anti-HER2 are not effective against these cancers, although chemotherapy can be helpful.

14.4. BI-RADS CLASSIFICATION (mammography, ultrasound and MRI)

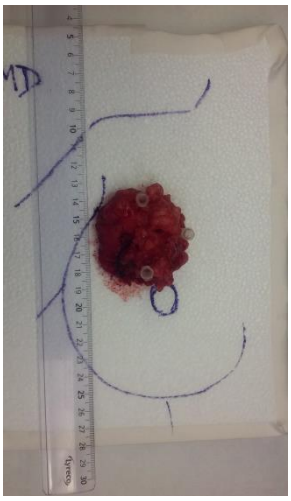
The BI-RADS (Breast Imaging Reporting and Data System) classification includes morphological and kinetic assessment. Five evaluation criteria (each one titrated with 0-2 points) are combined (4,18).

Criterion	Result	Punctuation
Signal increase (1-3min post contrast)	Low <50%	0
	Moderate 50-100%	1
	High > 100%	2
Signal behaviour until 9 min	Continuous >10% increase	0
	Plateau +/- 10%	1
	Wash out >10% descent	2
Shape of the lesions that capture contrast	round, oval, lobular	0
	dendritic, starry	1
Lesion margins	Well defined	0
	badly defined	1
enhancement pattern	Homogeneous	0
	Non-homogeneous	1
	Ring	2

With these parameters, punctuation from 0 to 8 is elaborated. It classifies the lesions in five categories:

- Group I: 0 points. Negative. Normal breasts.
- Group II: 1-2 points. Benign lesion.
- Group III: 3 points. Probably benign. Needs monitoring in short interval of time.
- Group IV: 4-5 points. Suspicious for malignancy. Consider biopsy.
- Group V: 6-8 points. High suspect of malignancy.

14.5. PICTURES OF THE PATHOLOGIST PROCEDURE

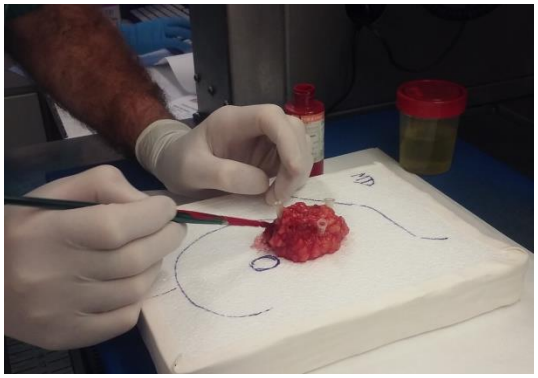


Picture 1.

The surgical fresh piece is oriented and fixed with metal clips on a drawing in a polystyrene. After that, it is sent to the pathologist unit.

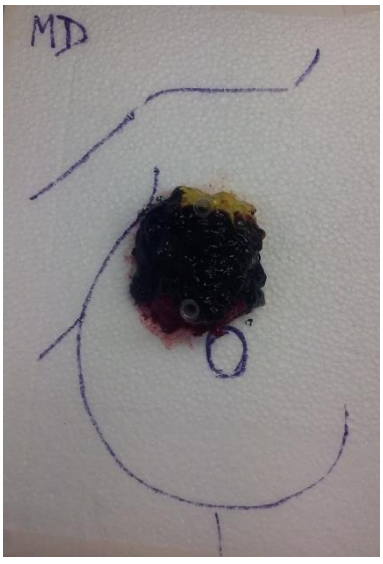
It is also specified in the drawing which breast it is. (MD = mama dreta. Right breast.)

Then, the whole tumor is measured in its three sides with a ruler and pictures are taken.



Picture 2.

The tumor is painted with chinese ink. Each side has a different colour to help us to identify each side once the piece is sliced.



Picture 3.

Example:
Yellow-superior side
Blue-lateral side
Red-inferior side
Green-medial side
Black-anterior side.

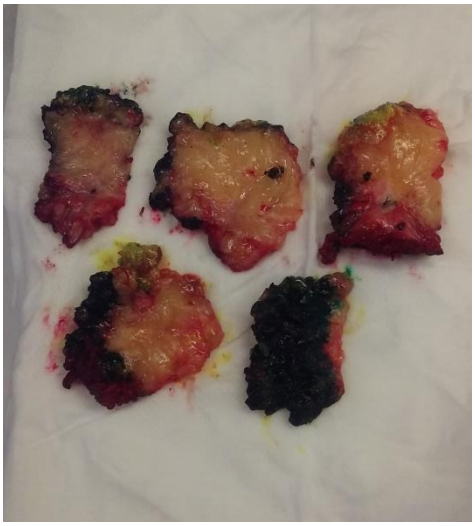
Picture 4.

Chinese ink. Five colours for the five sides of the breast. The posterior side is not painted.

Picture 5.

The piece is cut in different antero-posterior slices of 1-2cm thick.

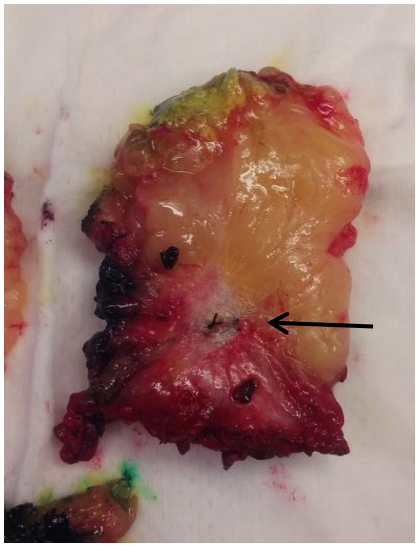
Sections are made perpendicular to the axis from the central edge (areolar) to the peripheral edge.



Picture 6.

All the slices are placed in the proper order and orientation to evaluate them.

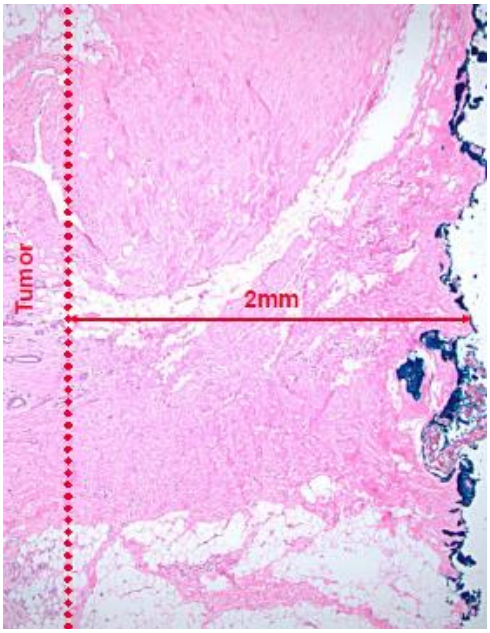
The pathologist evaluates macroscopically each slice to specify if the tumor is close to any margin.



Picture 7.

As it is an example of an invasive tumor, we can see it as a compact mass. In the case of the DCIS, normally it is not observed macroscopically. Microscopic study is needed to define the margins.

In this picture, indicated with the arrow, we can see the tumor in a whiter colour compared with the yellow colour of the normal breast tissue.



Picture 8.

The microscopic exam is made to specify the histological type, grade, necrosis, in situ component and further information about the margins in millimetres.

14.6. VAN NUYS PROGNOSTIC INDEX (VNPI)

Score	1	2	3
Tumor size	≤ 15 mm	16-40mm	≥ 40mm
Status of surgical margins	>10mm	1-10mm	<1mm
Pathologic classification	Low-grade Without necrosis	Low-grade With necrosis	High grade +/- necrosis
Age	≥ 60 years	40-59 years	<40 years

The Van Nuys classification stratifies patients into three groups. Non–high-nuclear-grade cases are placed in group 1 if they lack comedo-type necrosis and in group 2 if they have necrosis, and cases with high nuclear grade are placed in group 3. The 8-year actuarial disease-free survival rates are 93%, 84%, and 61%, respectively (14).

14.7. INFORMED CONTENT. It is given before the surgery.

CONSENTIMIENTO INFORMADO PARA CIRUGÍA CONSERVADORA DE LA MAMA

DATOS DE IDENTIFICACIÓN

Nombre y apellidos del paciente: ..... nº historia: .....

Nombre y apellidos del representante (si procede): .....

SOLICITUD DE INFORMACIÓN

Deseo ser informado sobre mi enfermedad y la intervención que se me va a realizar:

Sí☐ No☐

Deseo que la información de mi enfermedad e intervención le sea proporcionada a:

.....

DESCRIPCIÓN DEL PROCEDIMIENTO

El cirujano/a me ha explicado que, mediante este procedimiento, se me va a extirpar la lesión de la mama con un margen de tejido sano y los ganglios de la axila del mismo lado. Durante la cirugía se realizará un estudio anatomopatológico que valorará la afectación de los márgenes de resección, de manera que si están afectados por la enfermedad es posible que sea necesario la extirpación de toda la mama en la misma intervención o de forma diferida (en otra intervención) por los resultados de estudios posteriores.

Si se extirpara toda la mama, en ocasiones es posible, que se me coloque un dispositivo en la zona de la operación que facilitará la reconstrucción estética posterior.

Es posible que durante la cirugía haya que realizar modificaciones del procedimiento, por los hallazgos intraoperatorios, y siempre con la intención de proporcionarme el tratamiento más adecuado.

El procedimiento requiere anestesia de cuyos riesgos seré informado por el anestesiólogo, y es posible que durante o después de la intervención sea necesaria la utilización de sangre y/o hemoderivados.

Se podrá utilizar parte de los tejidos obtenidos con carácter científico, en ningún caso comercial, salvo que yo manifieste lo contrario.

La realización de mi procedimiento puede ser filmado con fines científicos o didácticos, salvo que yo manifieste lo contrario.

BENEFICIOS DEL PROCEDIMIENTO

El cirujano/a me ha informado que, mediante este procedimiento, se pretende la extirpación de mi lesión evitando su extensión a tejidos vecinos y a distancia.

ALTERNATIVAS AL PROCEDIMIENTO

En su caso particular se considera que esta es la alternativa más eficaz. Existe la alternativa de extirpar toda la mama. En caso de no aceptar la resección quirúrgica, en algunos casos se pueden valorar tratamientos paliativos con quimioterapia, radioterapia, hormonoterapia o una combinación de estas.

RIESGOS GENERALES Y ESPECÍFICOS DEL PROCEDIMIENTO

Comprendo que, a pesar de la adecuada elección de la técnica y de su correcta realización, pueden presentarse efectos indeseables, tanto los comunes derivados de toda intervención y que pueden afectar a todos los órganos y sistemas como otros específicos del procedimiento, que pueden ser:

Riesgos poco graves y frecuentes: Infección, sangrado o alteraciones de la cicatrización de la herida quirúrgica. Colección de líquido en la herida. Flebitis. Edema transitorio del brazo. Alteraciones de la sensibilidad alrededor de la herida. Dolor prolongado en la zona de la operación y dificultad transitoria en la movilidad del brazo.

Riesgos poco frecuentes y graves. Inflamación grave de los linfáticos del brazo. Sangrado importante. Dificultad para la movilidad del hombro y brazo. Reproducción de la enfermedad.

Estas complicaciones habitualmente se resuelven con tratamiento médico (medicamentos, sueros, fisioterapia, etc.), pero pueden llegar a requerir una reintervención, generalmente de urgencia, y excepcionalmente puede producirse la muerte.

RIESGOS PERSONALIZADOS Y OTRAS CIRCUNSTANCIAS:

……..

CONSECUENCIAS DE LA CIRUGÍA

En algunos casos se produce una alteración de la anatomía de la mama……
……

¿DESEA REALIZAR ALGUNA MANIFESTACIÓN EN RELACIÓN CON LA INTERVENCIÓN?:
……

Declaraciones y firmas:

D./Dª: …… con DNI: ……

- DECLARO: Que he sido informado con antelación y de forma satisfactoria por el médico, del procedimiento **(CIRUGÍA CONSERVADORA DE LA MAMA)** que se me va a realizar así como de sus riesgos y complicaciones.
- Que conozco y asumo los riesgos y/o secuelas que pudieran producirse por el acto quirúrgico propiamente dicho, por la localización de la lesión o por complicaciones de la intervención, pese a que los médicos pongan todos los medios a su alcance.
- Que he leído y comprendido este escrito. Estoy satisfecho con la información recibida, he formulado todas las preguntas que he creído conveniente y me han aclarado todas las dudas planteadas.
- Que se me ha informado de la posibilidad de utilizar el procedimiento en un proyecto docente o de investigación sin que comporte riesgo adicional sobre mi salud.
- También comprendo que, en cualquier momento y sin necesidad de dar ninguna explicación, puedo revocar el consentimiento que ahora presto, con sólo comunicarlo al equipo médico.

Firma del médico que informa Firma del paciente

Dr/a: ..... D./Dª: .....

Colegiado nº .....

Fecha: .....

D./Dª: ..... con DNI: .....

en calidad de ..... a causa de .....

doy mi consentimiento a que se le realice el procedimiento propuesto.

Firma del representante

Fecha: .....

Revocación del consentimiento:

D./Dª: ..... con DNI: .....

REVOCO el consentimiento anteriormente dado para la realización de este procedimiento por voluntad propia, y asumo las consecuencias derivadas de ello en la evolución de la enfermedad que padezco / que padece el paciente.

Firma del paciente Firma del representante

Fecha .....

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